

QUADRUPLE HYBRIDS IN THE F₁ GENERATION FROM *OENOTHERA NUTANS* AND *OENOTHERA PYCNO- CARPA*, WITH THE F₂ GENERATIONS, AND BACK- AND INTER-CROSSES¹

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INTRODUCTION

One of the many interesting and remarkable features of the evening primroses of the genus *Oenothera* (section *Onagra*), is the large number of distinct genetic types in the species *Oenothera "biennis"*, as generally understood in North America. In many localities a superficial examination will reveal distinct types in the population of this "species".

¹ An account of three of the hybrids was presented before the American Phil. Soc. at its general meeting in April, 1914 (see ATKINSON 1914). This paper was accepted by the *Zeitschr. f. ind. Abst. u. Vererb.*, and the proof was read in October, 1914, but the European war has probably prevented its publication. An account of the F₂ generations, and back- and inter-crosses, was presented at the general meeting of the Am. Phil. Soc. in April 1916. Only very brief abstracts of any portion of this work have as yet been published.

These types differ either in the character of the flowers, stems, foliage or rosettes, or in all combined. Certain types manifest great resistance to the attacks of certain fungi, especially to *Peronospora*. The juices of certain types are preferred by the tarnished plant bug. The seeds in some forms present striking differential characters.

In the vicinity of Ithaca, N. Y., as the result of a partial survey of a very small portion of territory, I have found a dozen different forms in the so-called "*biennis* alliance", which in cultivation have proven to be distinct types. The uniformity of character shown in the rosettes, as well as in the mature plants, in the population bred from each of these types, is remarkable, and dispelled the suspicion at first entertained that the different forms recognizable in the field might be merely fluctuating modifications in a very "variable" species. Some of them differ by so many characters and manifest such strikingly different genetic constitutions, that they evidently represent different specific types. Sev-

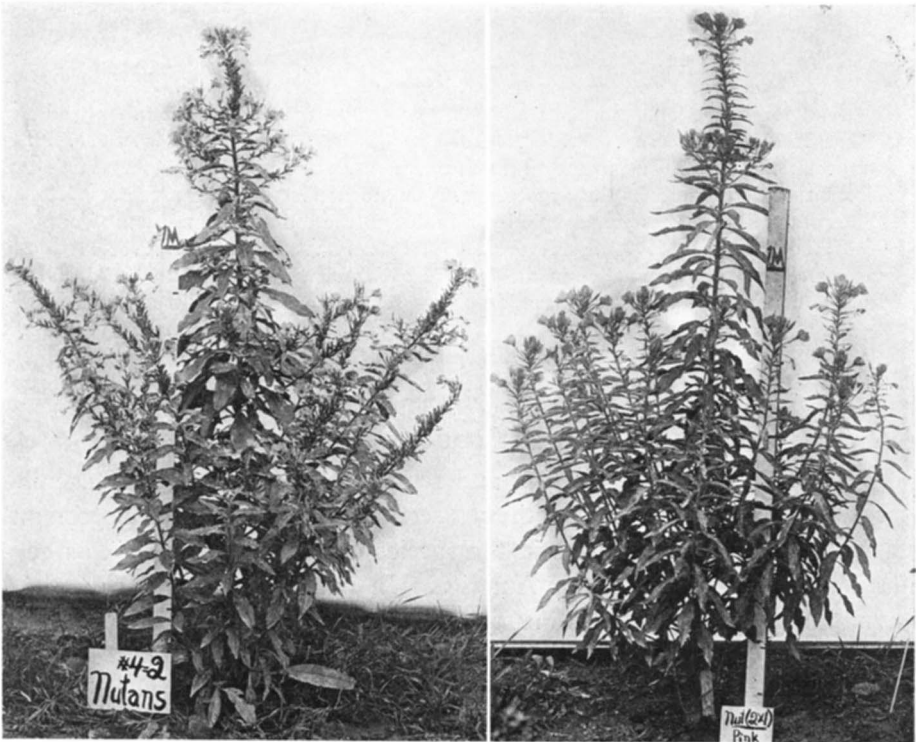


FIG. 1

FIG. 2

FIGURE 1.—*Oenothera nutans* Atkinson and Bartlett.

FIGURE 2.—The blend hybrid, *hybrida nutella* ($n \times p$).

eral such distinct specific types have been isolated from the *Oenothera "biennis"* population in the vicinity of Ithaca. BARTLETT (1911, 1913) has isolated a number from several different localities in the United States, and DE VRIES (1913) had previously observed and gathered similar distinct types in this country.

This composite constitution of the "*biennis* alliance" in North America is reflected in the different concepts of this species in some of the manuals on North American botany, thus rendering it quite impossible, in some localities at least, to correlate any of the forms with the descriptions of *Oenothera biennis* in the manuals. The situation is further complicated by the fact that the true *Oenothera biennis* is probably that referred to by LINNAEUS from the sand dunes of Holland (see BARTLETT 1913, p. 48), where it is believed to be an immigrant from North America, though so far as I know the Holland type of *biennis* has never been observed in our country.

Besides the great number of morphologically different species and varieties in the "*biennis* alliance" in North America, with their characteristic genetic constitutions, there exists another remarkable peculiarity, noted also in other species of the genus, viz., the marked power which some of these types have to produce splitting in the F_1 progeny of certain crosses, while others lack it, or possess it only to a very limited degree. DEVRIES (1913) has clearly demonstrated this variability in splitting power of certain species of *Oenothera*.

During the summer of 1909, on a small area of an abandoned pasture, which I was developing as a private wild garden, just within the limits of the city of Ithaca, there were a number of self-sown evening primroses. No critical examination was made of them, but as I passed among them almost daily I finally became aware, perhaps by a process of unconscious differentiation, that certain individuals had red stems and red mid-veins of the leaves, while other individuals had green stems and white mid-veins. These were the only differential characters which attracted my attention that season, as I was not aware at that time of the fact that *Oenothera "biennis"* was a composite species embracing a large number of elements which breed as distinct species and forms. It occurred to me that it might be interesting to hybridize these forms for the purpose of observing the distribution of color in the hybrids and in their progeny. Cross pollinations were then made between two selected individuals, one of each type. But seed was obtained that year only from the green-stemmed individual fertilized by the use of pollen from the red-stemmed one.

Being absent from Ithaca during the summer of 1910, the seed was not sown until the spring of 1911. Seed from each parent, saved from protected flowers, was also sown. The young seedlings were transplanted from the seed pans to flats in the usual way and when of suitable size they were transplanted to the garden. They were not forced by planting early, and were therefore grown as biennials. During mid-summer marked differences began to appear in the rosette leaves, which became very striking during the autumn. The rosette leaves of the parents differed by five or six characters, and it was evident that there were at least two hybrid types. The hybrid types, however, could not be very well determined because many of the rosettes were more or less severely attacked by the downy mildew (*Peronospora Arthuri*), while many of those of the green-stemmed parent were more severely injured.

The following season (1912) the plants came into flower. A critical study revealed further striking differences between the two parents. The differential characters relate to habit, leaves, stems, inflorescence, flowers, and seed pods, altogether some 25-30 characters being noted by which the two forms differed. The two forms were very distinct from *Oenothera biennis* as recognized by DeVRIES, BARTLETT and others, and were regarded as undescribed species. They were submitted to BARTLETT who confirmed this opinion and they were described as *Oenothera nutans* Atkinson & Bartlett, and *Oe. pycnocarpa* Atkinson & Bartlett. For a complete diagnosis of the two species the original descriptions (see BARTLETT 1913, p. 81) should be consulted, but a brief synopsis of the characters is given below.

All of the cultures were carried out in fairly rich garden soil and some tillage was given, enough to keep down weeds and to stir the soil several times, particularly during rosette development and during the spring of the second season. Under these conditions the full expression and strength of characters are realized. These characters relate to the habit and coloration of the adults; features of the rosettes, foliage and inflorescence. The habit and morphological characters are well shown in the photographs here reproduced. The measurements given are for the garden cultures.

Oenothera pycnocarpa. *Habit*: tall, 1—1.5 m; lower stem branches numerous, strict, not widely spreading, reaching about the middle of the main stem, main stem therefore with high over-top, tips nearly the same level; axillary rosettes over the middle and upper part of the main stem, or short flowering branches just below the main inflorescence.

Autumnal rosettes compact, the larger leaves (late summer and early autumn leaves) narrow, deeply cut over the basal half, furrowed, repand, white-veined, plain or only slightly buckled, no reddish spots. *Stems* green, (rarely tinged red on a portion of the sunny side), tubercles green. *Stem leaves* drooping, narrow, white-veined, slightly sinuate-toothed (lower leaves strongly so over basal portion), furrowed, plain, no red spots. *Inflorescence* long and dense; bracts green, slightly sinuate-toothed, longer than the flower buds, persistent, basal ones longer than the pods. *Petals* lemon yellow, cuneate, strongly emarginate, not plicate, edge plain, medium size, firm, not quickly wilting. *Fertility* high (pods with many viable seeds). *Plant* flowering for a long period and maturing late. See figures 5, 8 and 13.

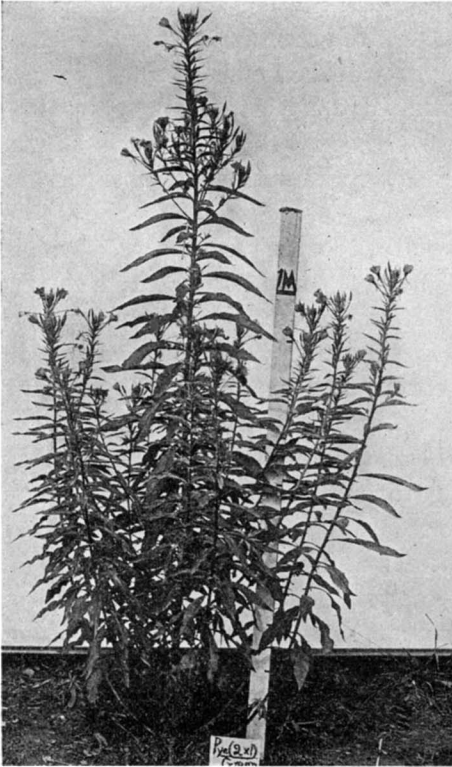


FIG. 3



FIG. 4

FIGURE 3.—The selective hybrid, *hybrida pycuella* ($n \times p$).

FIGURE 4.—The selective hybrid, *hybrida tortuosa* ($n \times p$). The main stem is fasciated and has split into two branches, the height or over-top of the main stem is therefore probably not so great as it would have been if it were not for the fasciation.

Oenothera nutans. *Habit*: medium height, .75—1.00 m; lower stem branches numerous, spreading irregularly, reaching far above the middle of the main stem, main stem therefore with low over-top, tips at unequal heights, not terminating at the same level; axillary rosettes over the middle portion of the stem; short flowering branches at base of the main inflorescence. *Autumnal rosettes* compact, the larger leaves broad, sinuate-toothed over the basal portion, convex, not repand, red-veined, strongly crinkled, reddish spots in the autumn. *Stems* dark red, sometimes also the base of the inflorescence axis is red, otherwise the inflorescence axes are green; tubercles red, even over the green parts of the stem, rarely red on the young pods. *Stem leaves* broad, very slightly sinuate-toothed over the basal portion, flat or convex, red-veined, plain or somewhat crinkled, few or no red spots. *Inflorescence* medium length, dense, sometimes lateral; bracts usually pale green and caducous, except sometimes a few of the basal ones are green and persistent, small and shorter than the flower buds, edge plain. *Petals* chrome yellow, obovate, or scarcely emarginate, plicate, edge eroded, weak, soon withering, medium size, but larger than in *Oe. pycnocarpa*. *Fertility* high. *Plant* maturing early (in late summer or early autumn). See figures 1, 8 and 9.

Besides several minor deviations, of little consequence here, there is an important variation which should be taken into account and clearly understood. This variation consists in the production of annual individuals by biennial species. This is particularly liable to happen in artificial cultures, and sometimes is encouraged by the investigator for the express purpose of saving time. Where seeds are sown during the winter and the seedlings have reached some size by the time the growing season permits transplanting to the garden, the influence of the warm summer season on these more advanced seedlings may stimulate them to early stem growth so that they flower and fruit in a single season. In some cases a large percentage, or the total, of the culture may form annuals, while a small percentage, or none, pass on to the rosette form. Where the species contrasted have autumnal rosettes with strikingly different character composition, the annuals, in the case of some species, fall far short of presenting the full complexion of the specific character. This is true of the two species here studied as well as of their hybrids. The annual forms of *pycnocarpa*, produced in culture in 1913, and again in 1914 and 1915 came into flower early except those of one lot from a seven-carpeled pod in 1914. Those grown as annuals formed no mature rosettes. The later leaves of the rosettes, and the lower

stem leaves of these annual forms, were very strongly toothed over the basal portion, but did not approach the cut condition of the later leaves of mature autumnal rosettes.

If cultures are started in March or April, and the seedlings grown in 2-inch pots until they become pot-bound, all or a percentage may develop as true annual forms, depending on the way the cultures are handled. In the cultures of 1914, some individuals of *nutans* came into flower early in July, others in August, and a very few formed only rosettes which were quite well formed early in August, and characteristically mature in September.

Since the rosette leaves developed in late summer and early autumn attain a higher degree of morphological differentiation than the spring and early summer leaves, the complete life cycle and full expression of the species is not obtained when these species are grown as annuals. The annuals of biennial species reach the flowering and fruit period by short-circuiting the complete life history. They are really

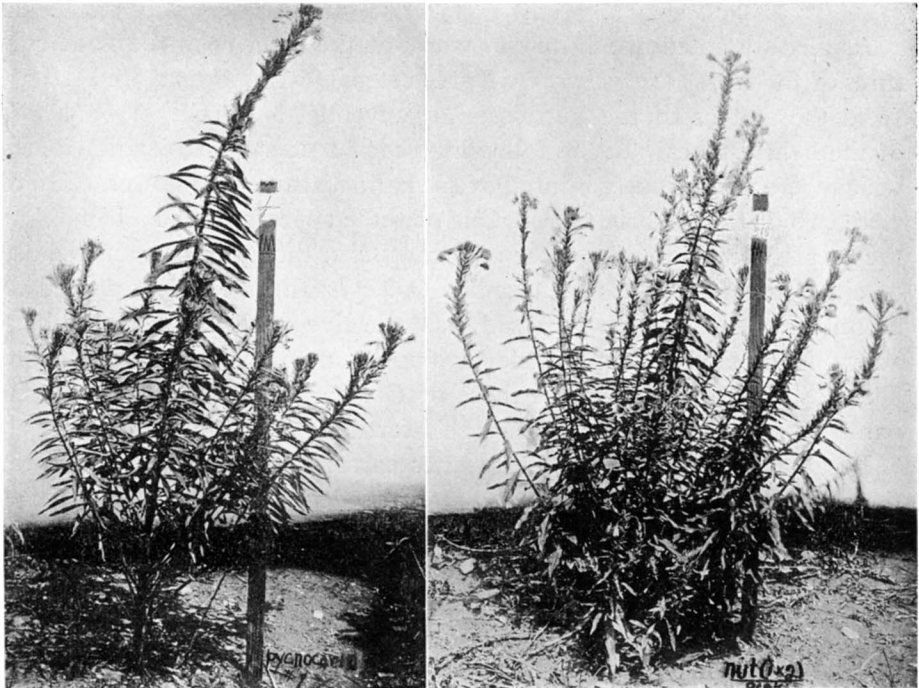


FIG. 5

FIG. 6

FIGURE 5.—*Oenothera pyncocarpa* Atkinson and Bartlett.

FIGURE 6.—The blend hybrid, *hybrida nutella* ($p \times n$).

short-circuit forms with paedogenic rosettes. Sometimes in greenhouse cultures seeds falling from ripe pods of a potted plant during the winter, germinate, and under the warm conditions of hothouse culture begin stem development early, omitting the rosette stage. Such short-circuit forms, in species where late summer and early autumn leaves of autumnal rosettes are strongly cut over the basal portion, and the stem leaves are only slightly cut, or toothed, suggest a variation in the leaves due to the influence of the changed environment, and thus may be misleading. The cycle has been shortened from early rosette leaves slightly cut to lower stem leaves slightly cut, omitting the late-formed strongly cut leaves of the mature rosette stage.² All gradations sometimes appear between the extreme short-circuit forms and those with the rosette stage complete, the degree of rosette development reached depending on the time at which stem development begins. So far as I am aware the conditions have not yet been analyzed which determine the time of stem development in these annual forms of biennial species.

THE F₁ HYBRIDS FROM *OENOTHERA NUTANS* AND *OE. PYCNOCARPA*

As already noted, the injury to many of the rosettes in the 1911 cultures of the cross *Oe. pycnocarpa* (green stem) × *Oe. nutans* (red stem), from the attacks of the *Peronospora* prevented a satisfactory analysis of the hybrid types. In the following year (1912) these biennial forms which were not seriously injured by the parasite came into flower. There were two distinct hybrid types. One of these appeared to be a blend, but the petals were more like those of *Oe. nutans*, though larger than those of either parent. It has been named *Oe. hybrida nutella*. It proved to be self-sterile. The other hybrid was selective, receiving certain characters from each parent and developing them to their full expression. The flowers were exactly like those of *Oe. pycnocarpa*, and it has been named *Oe. hybrida pycnella*. It is self-fertile to a very high degree.

Because of the injury to the rosettes parasitized by the *Peronospora*, the experiments were repeated. It was also desirable to study the reciprocal crosses between the two species. Therefore reciprocal cross-pollinations of the parents in the first generation of the garden cultures were made in 1912.

The seed of these reciprocal crosses, as well as of the two parents, was

² Exactly such short-circuit annual forms appeared in an undescribed species, No. 17 of my 1913 cultures, about 50 percent being short-circuit forms, while the remainder which came into flower in 1914 formed mature rosettes with leaves strongly cut over the basal half.

sown in seed pans during March 1913. When the seedlings were furnished with 4 or 5 leaves they were transplanted to flats, and then when of suitable size were transplanted to the garden in May. Some developed as annuals and began to flower in July, while others formed autumnal rosettes and did not flower until the season of 1914. Of the hybrids which came into flower during July and August it was evident that there were two distinct types, and these two types were identical with the two hybrid types obtained the previous year. Furthermore, these two hybrid types appeared on each side of the reciprocal crosses.



FIGURE 7.—The selective hybrid, *hybrida pycnella* ($p \times n$).

In the seedling stage, as well as in the early rosette stage it was quite impossible to differentiate the parents except for occasional red spots in the leaves of the red-stemmed parent, and as the leaves became larger the mid-veins of this parent also began to show the red color. Likewise it was impossible to distinguish the hybrid types, either from each other or from either parent. But when the annuals began stem development it was possible to differentiate the parents and hybrid types from the character of the lower stem leaves, but not with such certainty as at the flowering period or in the well developed autumnal rosettes.

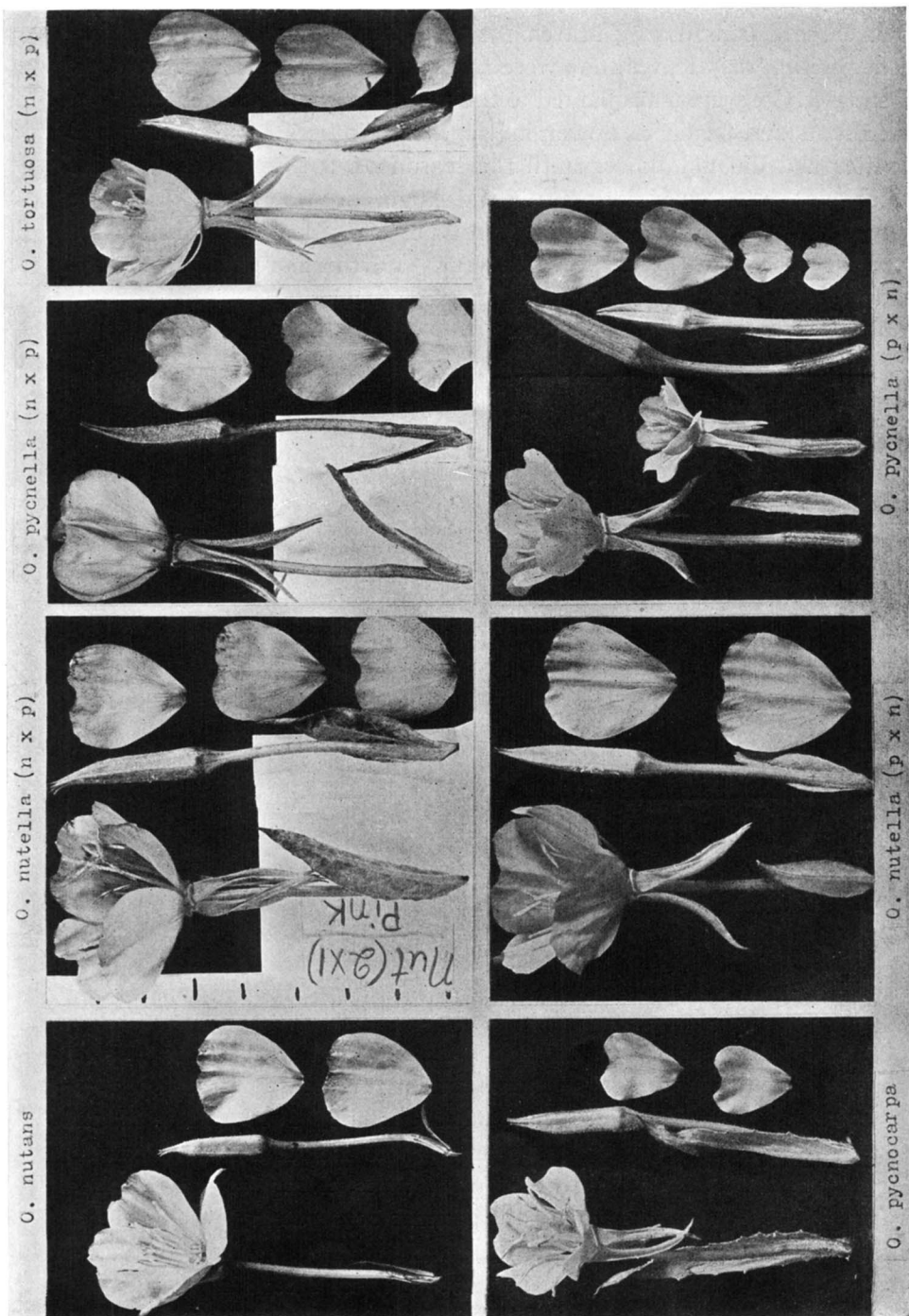


FIGURE 8.—Details of flowers and buds. The upper row contains *Oe. nutans* and three of the hybrid types when *nutans* is the mother. The lower row contains *Oe. pycnocarpa* and the two hybrid types when *pycnocarpa* is the mother. The parents are in the first column; the blend, *hybrida nutella*, in the second column; the selective, *hybrida pycnella*, is in the third column; and

The remaining hybrid rosettes, those which did not proceed to stem development that season, could not be differentiated into types during July or early August. But as they approached the autumnal stage the new and larger leaves began to display the differential characters which permitted sorting into distinct types with a fair degree of accuracy, and when mature in September three distinct hybrid types were easily recognizable, the third type not producing annual forms that season. This third hybrid type is named *hybrida tortuosa*, because the mature autumnal rosette leaves are often more or less strongly twisted due to their narrow, convex, crinkled form. The following season (1914) the biennial individuals, those with the mature autumnal rosettes in 1913, came into flower, and four hybrid types in the F_1 generation of the cross were distinguished.³ The fourth type had the rosette characters of the third but the stem color of the mature plant was green instead of red as in *tortuosa*. It is named *hybrida tortuella*.

*Characters of the four hybrids of the F_1 generation compared
with the parents*

The principal characters of the F_1 hybrids may now be given. For the purpose of easy comparison the principal contrast characters of the parents are repeated.

Oenothera nutans. Rosette leaves broad, with a few rather prominent teeth over the basal part of the blade, convex, crinkled, red-veined; stems red; stem leaves spreading; petals obovate, overlapping, fluted, distal margin eroded and plain, quickly wilting; pods short, usually crowded; plants maturing early, very fertile; progeny very uniform.

Oenothera pycnocarpa. Rosette leaves comparatively narrow, deeply cut over the basal half of the blade, furrowed, repand, plain or somewhat buckled, white-veined; stems green; stem leaves depressed; petals cuneate, not meeting, not fluted, distal margin not eroded, notched, rather firm and wilting late; pods comparatively long, often crowded; plants maturing late, very fertile; progeny very uniform.

Oenothera hybrida nutella. A blend⁴ hybrid. Rosette leaves flat, intermediate in width and edge characters between the two parents, the basal part of the mature autumnal leaves cut about half as deeply as in the *pycnocarpa* parent; stems pink; stem leaves spreading; petals larger than those of either parent, sometimes showing more of the *nutans* char-

³ For the proportional numbers of these hybrid types in the reciprocal crosses see the tables 2 to 9.

acter, more rarely the *pycnocarpa* character dominates; absolutely self-sterile so far as tested, though pollen and egg cells are both functional in crosses with other forms; pods rather long, lax; plants maturing late, flowering for a long period.

Oenothera hybrida pycnella. A selective⁴ hybrid. Rosette leaves broad, with a few rather prominent teeth over the basal part of the blade, furrowed, repand, plain; stem leaves spreading; stems green; flowers exactly like those of *Oe. pycnocarpa*; pods rather long, moderately dense; plants maturing early, very fertile; type fixed in the F_1 generation, breeds true, progeny very uniform.

Oenothera hybrida tortuosa. A selective hybrid. Rosette leaves

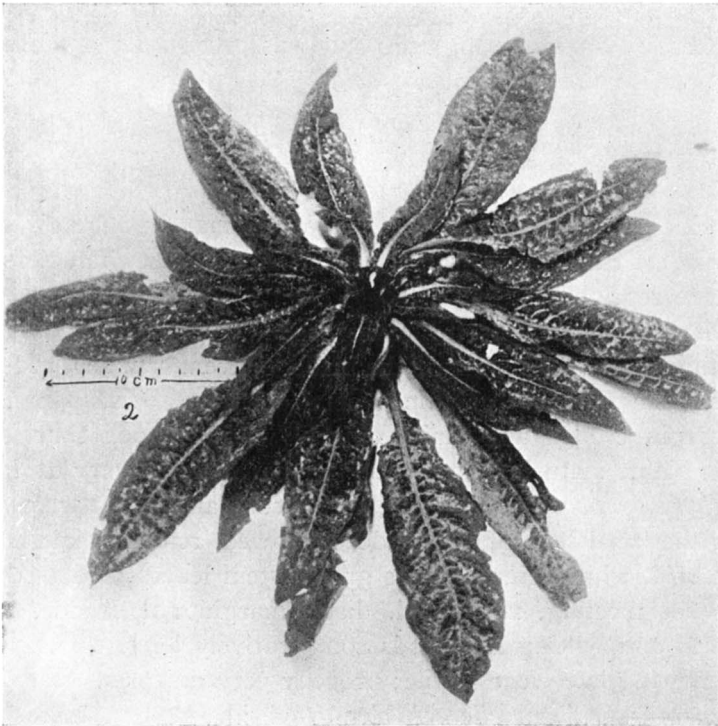


FIGURE 9.—Autumnal rosette of *Oenothera nutans*.

⁴The term "blend" hybrid is here used in the usual acceptance of the term. The allelomorphous factor pairs blend so that a form intermediate between the two appears. The term "selective" hybrid applied to the three other hybrids of the F_1 , has reference to the fact that only one factor of each homologous pair is selected for expression in a given hybrid, from those in the F_1 zygote, the other factors being subordinated. The factors selected come from both parents.

comparatively narrow, deeply cut over the basal half of the blade, convex, crinkled, red-veined, often more or less twisted (probably a result of the tension from strong convexity and crinkledness of the narrow leaves); stems red; stem leaves convex, depressed, a few rather strong, distant teeth over base of blade; flowers exactly like those of *nutans*; pods short, dense (or crowded) like those of *nutans*; plants maturing late, very fertile; type fixed in the F_1 generation, breeds true; progeny very uniform.

Oenothera hybrida tortuella. A selective hybrid. This hybrid appears to have all the characters of *hybrida tortuosa* in the first generation, except the red stem, a *nutans* character, *hybrida tortuella* having the green stem of the parent *pyncocarpa*; very fertile; type not fixed in the F_1 generation, since in the F_2 generation it breaks into a number of types



FIGURE 10.—Rosette of blend hybrid *nutella* ($n \times p$). Note the intermediate width and edge character of the leaves.

showing great variations. This fourth hybrid appears in very small numbers compared with the others.⁵

A single individual of *hybrida tortuella* appeared in the 1913-14 cultures. In the autumn of 1913 its rosette was classed as a *tortuosa*. It caused considerable surprise in 1914 when it came into flower that its stem should be green, since I had forecast a red stem for all the plants with *tortuosa* rosettes.

The photographs reproduced here present in graphic form nearly all



FIGURE 11.—Rosette of selective hybrid, *hybrida pycnella* ($n \times p$). Observe the broadness and toothedness of the *nutans* leaves.

⁵ The plants were not grown in large enough numbers to determine whether or not there is any regularity in the ratios of the different hybrid types appearing in the first generation. In the cross *nutans* \times *pycnocarpa* (1913 culture) there were 108 plants in the garden culture. Of these 35 were annual *nutella*, and 10 were annual *pycnella*, 21 *tortuosa* and 1 *tortuella*. Of the reciprocal cross (*pycnocarpa* \times *nutans*) only 51 plants were grown in the garden. Of these 4 were annual *nutella*, 9 were annual *pycnella*. There were 38 which formed autumnal rosettes, 36 *pycnella* and 2 *nutella*.

the characters of the two parents and their four hybrids. The fact that the parents differ by so many strong contrast characters, the majority of which under normal culture are so clear-cut and strikingly different in the two parents, permits a very satisfactory study of the transmission of the characters, and their composition in the F_1 hybrids.

The habit of the plants is shown in figs. 1 to 7, all from biennials. Figure 1 represents *nutans*, and figures 2 to 4 show three of the hybrid types of the cross *nutans* \times *pyncocarpa*; figure 2 is the F_1 blend hybrid,

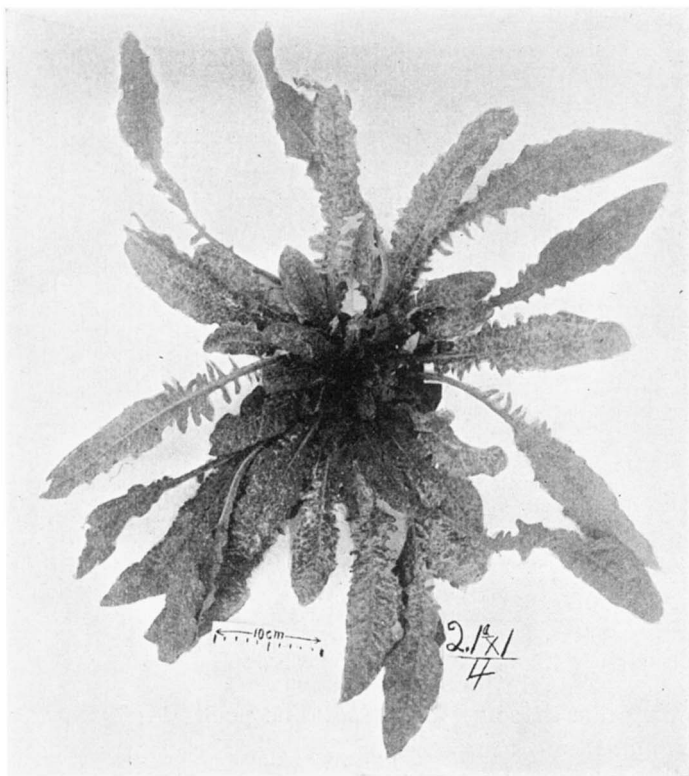


FIGURE 12.—Rosette of selective hybrid, *hybrida tortuosa* ($n \times p$). Note the narrowness and cutness of *pyncocarpa* and the crinkledness and convexity of *nutans* in the leaves.

nutella; figure 3 the green-stemmed F_1 selective hybrid, *pyncella*, and figure 4 the red-stemmed F_1 selective hybrid, *tortuosa*.

In figure 8 are presented details of the inflorescence.⁶ The differences

⁶ It was rather late in the season when the flowers of *nutans* and *pyncella* ($p \times n$) were photographed, and the bracts were not quite so large as the earlier ones.

in the petal characters are striking. The upper row contains *nutans* at the left, and then follow in order three of the hybrid types (*nutella*, *pycnella* and *tortuosa*) when *nutans* is the mother. The lower row contains *pycnocarpa* at the left, and two of the hybrid types (*nutella* and *pycnella*) when *pycnocarpa* is the mother. *Tortuella* is not shown, its

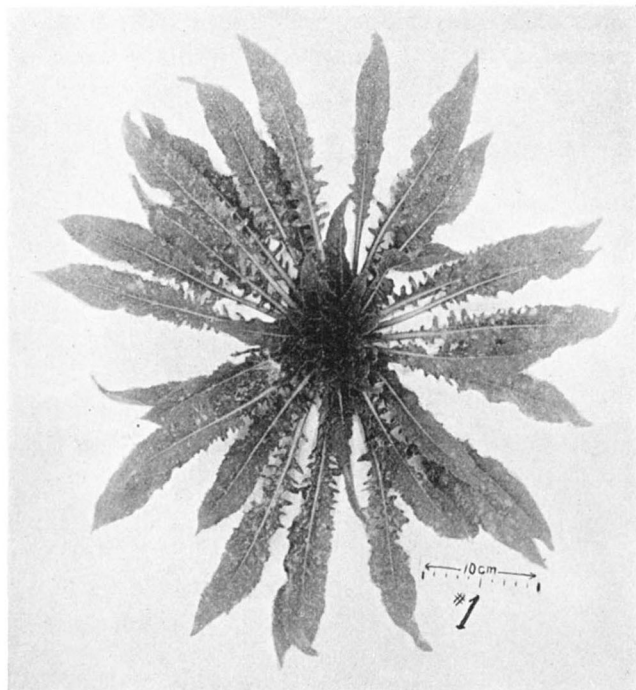


FIGURE 13.—Rosette of *Oenothera pycnocarpa*.

flowers being like those of *tortuosa*. The petals of *tortuosa* are clearly those of *nutans*, and the petals of *pycnella* are clearly those of *pycnocarpa*. The petals of *nutella* are often very like those of *nutans*, particularly as regards the plaited character and oboval form. They are, however, larger than those of *nutans*. The emargination is more pronounced, showing more of the *pycnocarpa* character. Sometimes the plaited character is not so pronounced, as is evident in the photograph of *nutella* ($n \times p$). But in many examples the plaited character is very evident. While, therefore, the petals of this blend hybrid (*nutella*) more strongly resemble those of *nutans*, they are modified by those of *pycnocarpa*, the blend in the petal characters being less striking than in the vegetative characters.

The rosettes of *nutans* \times *pycnocarpa* are reproduced in figures 9 to 12; those of *pycnocarpa* \times *nutans* in figures 13 to 15. Figure 9 is the mother *nutans*; figure 10 is the F_1 blend, *hybrida nutella*. The *hybrida nutella* rosette of the reciprocal cross (*pycnocarpa* \times *nutans*) is shown in figure 14. By a comparison with the rosettes of the parents (for the

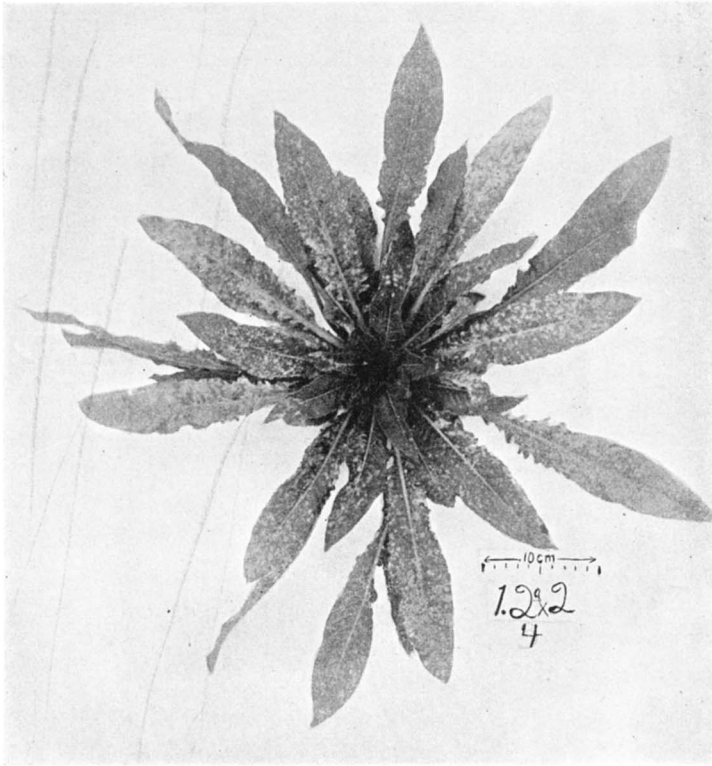


FIGURE 14.—Rosette of the blend hybrid, *hybrida nutella*, from the cross *pycnocarpa* \times *nutans*.

rosette of *Oe. pycnocarpa* see figure 13), the blending of the characters, wideness with narrowness, and cutness with toothedness, is very evident.

In figure 11 is shown a rosette of the F_1 selective, *hybrida pycnella*, from the progeny when *nutans* was the mother, and in figure 15 the same form when *pycnocarpa* was the mother. The two have the same composition of characters, but *pycnella* ($p \times n$) has fewer leaves, selected because the leaf detail could be better represented in the photograph. The photograph shows the selection of the width and toothed character from *nutans*. Furrowedness and repandness come from *pycnocarpa*, but are

not shown well in the photograph. At the time the photographs were made furrowedness had not been recognized as a character and considerable effort was made to flatten the leaves down so that the edge characters would appear to better advantage.

In figure 12 is the F_1 selective *hybrida tortuosa*. This is as yet represented only from the progeny when *nutans* is the mother. It has not yet appeared in the cultures of *pycnocarpa* \times *nutans*. This is quite a remarkable rosette. The larger leaves have the narrowness and cutness of *pycnocarpa*, the crinkledness and convexity of *nutans*, all these characters being clearly represented in the photograph. It has also the red-veinedness of *nutans*.



FIGURE 15.—Rosette of selective hybrid, *hybrida pycnella*, from the cross *pycnocarpa* \times *nutans*.

In diagram 1 the principal characters which have been studied, are tabulated in such a way as to show the blending of the contrast characters of the two parents in the F_1 *hybrida nutella*, and also the sorting of

characters from the two parents with their recombination into new complexes, or mosaics,⁷ in the F₁ selectives, *hybrida pycnella* and *hybrida tortuosa*. In the upper line are arranged the characters of *nutans*, in the lower line those of *pycnocarpa*, while in the middle line are arranged those of *hybrida tortuosa*, *hybrida nutella* and *hybrida pycnella*. The lines indicate the source and distribution of characters.

The abbreviations in the diagram are explained in table 1. *Hybrida tortuella* had not been detected when this diagram was made. But its phenotype in the F₁ is so like that of *hybrida tortuosa* (but having a green stem instead of the red stem of *tortuosa*) it is better to not complicate the diagram by its introduction.

As seen by an examination of diagram 1 there is a distinct linking or association, of certain factors in their transmission to the F₁ selective hybrids, *pycnella* and *tortuosa*. There is a splitting of the total factor composition of certain members of the plant body. The splitting, however, occurs almost entirely *between* factors, not *through* them, so that certain factors, or groups of factors, are transmitted entire to one or the other of the F₁ selective hybrids. Examples of this linking or association of characters are as follows: First, color characters; second, petal characters; third, broadness and toothedness of rosette leaves; fourth, narrowness and cutness of rosette leaves; fifth, crinkledness, convexity and red-veinedness of rosette leaves; sixth, plainness, furrowedness and white-veinedness of rosette leaves (in *hybrida pycnella* the mid-veins of the leaves are often tinged with red). The linked characters are inherited as follows by the two F₁ selectives, *pycnella* and *tortuosa*:

In *hybrida pycnella* the habit is taken from *nutans*; the rosettes take the broadness and toothedness of the leaves from *nutans*; the furrowedness, repandness and plainness of the leaves come from *pycnocarpa*; the green stem and green tubercles come from *pycnocarpa*; width and edge character of stem leaves come from *nutans*; the size and persistency of the bracts are derived from *pycnocarpa*; all the petal characters come from *pycnocarpa*. It will be noted that the green stem and petal characters come from *pycnocarpa*, but it may not be a case of linking between petal characters and stem color, other than that of their association in the parent *pycnocarpa*.

In *hybrida tortuosa* the habit comes from *pycnocarpa*; the rosette leaves take the narrowness and cutness from *pycnocarpa*; the convexity, crinkledness and red-veinedness from *nutans*; the red stem and red tubercles from *nutans*; width of the stem leaves comes from *nutans*, but

⁷ These mosaics are of a different kind from those described by BLARINGHEM (1913).

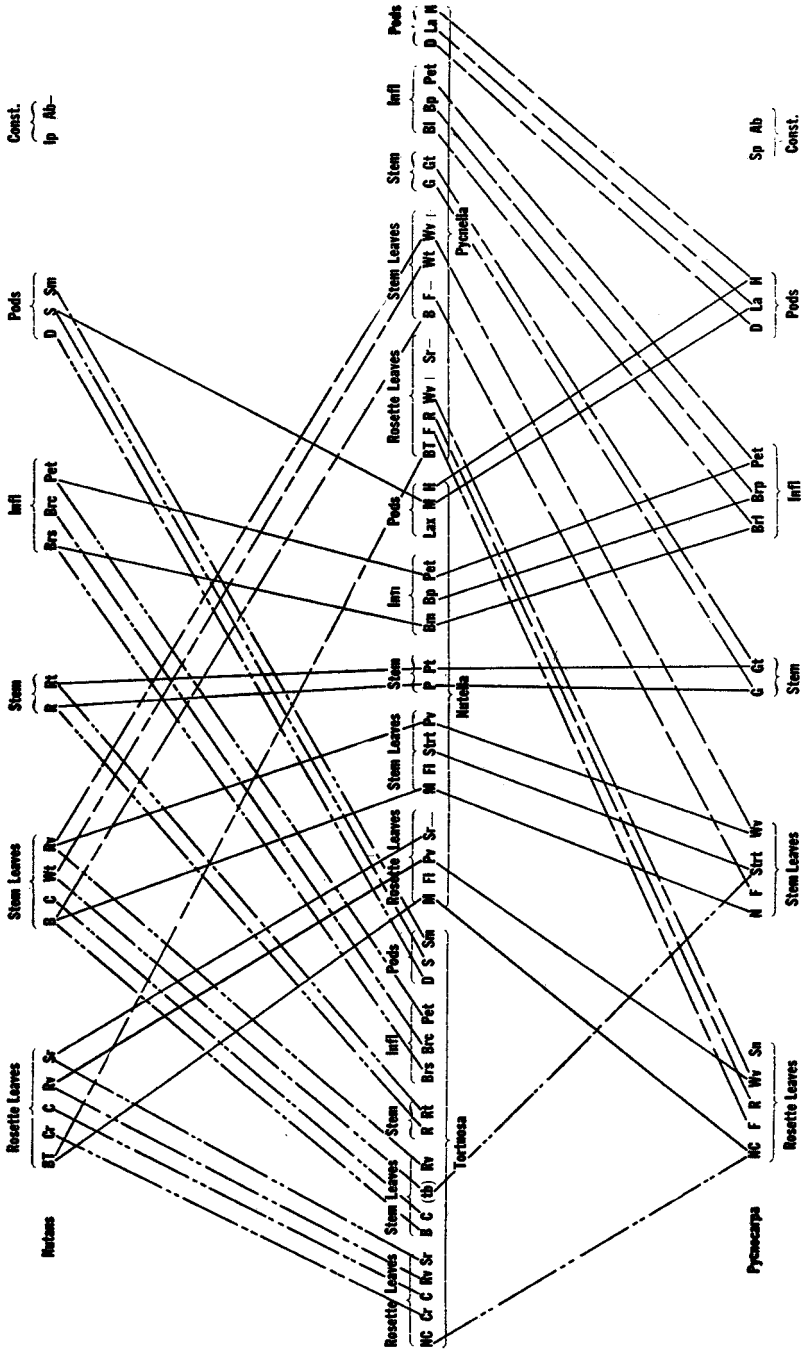


DIAGRAM I.

TABLE I

Key to the abbreviations used in diagram 1

	<i>Nutans</i>	<i>Tortuosa</i>	<i>Nutella</i>	<i>Pycnella</i>	<i>Pycnocarpa</i>
Rosette leaves	<ul style="list-style-type: none"> BT = broad and toothed Cr = crinkled C = convex Rv = red-veined Sr = spots red 	<ul style="list-style-type: none"> NC = narrow and cut Cr = crinkled C = convex Rv = red-veined Sr = spots red 	<ul style="list-style-type: none"> M = medium broad Fl = flat Pv = pink-veined Sr = some red spots 	<ul style="list-style-type: none"> BT = broad and toothed F = furrowed R = repand Wv⁺ = white-veined or pink-veined Sr = sometimes red spots 	<ul style="list-style-type: none"> NC = narrow and cut F = furrowed R = repand Wv = white-veined Sn = spots none
Stem leaves	<ul style="list-style-type: none"> B = broad C = convex Wt = weakly toothed Rv = red-veined 	<ul style="list-style-type: none"> B = broad C = convex (tb) = strongly and distantly toothed over base Rv = red-veined 	<ul style="list-style-type: none"> M = medium broad Fl = flat Strt = strongly toothed Pv = pink-veined 	<ul style="list-style-type: none"> B = broad F = furrowed, but not usually strongly so Wt = weakly toothed Wv⁺ = white-veined or pink 	<ul style="list-style-type: none"> N = narrow F = furrowed Strt = strongly toothed Wv = white-veined
Stem	<ul style="list-style-type: none"> R = red Rt = red tubercles 	<ul style="list-style-type: none"> R = red Rt = red tubercles 	<ul style="list-style-type: none"> P = pink Pt = pink tubercles 	<ul style="list-style-type: none"> G = green Gt = green tubercles 	<ul style="list-style-type: none"> G = green Gt = green tubercles
Inflorescence	<ul style="list-style-type: none"> Brs = bracts small Brc = bracts caducous Pet = petal characters 	<ul style="list-style-type: none"> Brs = bracts small Brc = bracts caducous Pet = petal characters 	<ul style="list-style-type: none"> Bm = bracts medium Bp = bracts persistent Pet = petal characters 	<ul style="list-style-type: none"> Bl = bracts large Bp = bracts persistent Pet = petal characters 	<ul style="list-style-type: none"> Brl = bracts large Brp = bracts persistent Pet = petal characters
Pods	<ul style="list-style-type: none"> D = dense S = small Sm = smooth 	<ul style="list-style-type: none"> D = dense S = small Sm = smooth 	<ul style="list-style-type: none"> Lax = lax M = medium size H = hairy 	<ul style="list-style-type: none"> D = fairly dense La = large H = hairy 	<ul style="list-style-type: none"> D = dense La = large H = hairy
Constitution	<ul style="list-style-type: none"> Ip = immune to Peronospora Ab = attacked by bugs slightly 				<ul style="list-style-type: none"> Sp = susceptible to Peronospora Ab⁺ = attacked by bugs

the edge character is different from either parent; the size and caducous character of the bracts are derived from *nutans*; all the petal characters come from *nutans*. In the mature state, when rosette leaves are not present, *tortuosa* resembles *nutans* very strongly, the habit, drooping of the leaves, and edge character of the stem leaves being the only differential characters in this stage. There are a few rather prominent and distant teeth on the base of the blade,⁸ a very different character from the rather regularly serrate leaves over the middle and upper part of the stem of *pycnocarpa*, and the nearly plain edge of the stem leaves of *nutans*.

In *hybrida tortuella* the phenotypic characters of the F_1 are derived as in *tortuosa*, but instead of receiving the red stem of *nutans*, it takes the green stem of *pycnocarpa*. There is here a crossing over of stem color and flower character as compared with the two other selective hybrids.

While all the contrast vegetative characters possessed by the two parents are transmitted, each in its entirety, to the two F_1 selectives, some to *pycnella*, the others to *tortuosa*, the F_1 blend, *hybrida nutella*, inherits all, or a very large proportion of the contrast characters of the two parents. In such a case it is impossible for both characters of the "allelomorphic pair" to express themselves in their completeness. Each is modified by the other member of the pair so that an intermediate state between the two contrast characters is attained. The "allelomorphs" blend. In the rosettes, the broadness and toothedness of *nutans* blend with the narrowness and cutness of *pycnocarpa*; the convexity and crinkledness of *nutans* blend with the furrowedness of *pycnocarpa* and the leaves are flat or nearly so. There are no real intergrading forms between the three hybrids, though each one shows slight fluctuations. The red color of the stems and tubercles of *nutans* blend with the green of *pycnocarpa* and an intermediate state of coloration (pink) results. The small bracts of *nutans* blend with the large ones of *pycnocarpa* and bracts of an intermediate size appear on *nutella*.

The F_1 blend hybrid (*nutella*) presents a very interesting case of sterility. When the inflorescence is covered with paper bags, or other screen, to prevent insects from bringing foreign pollen to the stigma, no seeds are developed. Though the pods often attain a considerable size and give the impression that seed is being formed, they are found to be

⁸ The few rather prominent teeth over the base of the stem leaves recalls the same feature of the lower stem leaves of the parent *pycnocarpa*, but the stem leaves of *tortuosa* with this character have a more extensive distribution on the stem than is the case in *pycnocarpa*.

hollow, and finally die. This is not due to a failure in pollination, for, like the parents, and others of the smaller-flowered oenotheras, pollination takes place in the bud. In the summer of 1912, when it was discovered that protected flowers were not setting seed, a large number were hand-pollinated. Not only was pollen from the same flower used in some cases, and in others pollen from different flowers of the same individual, but cross-pollinations between different individuals were also made. No seed formed. During the summer of 1913 a much larger number of hand-pollinations were made. Several inflorescences were also protected by covering with paper bags. In no case did seed develop. A large number of hand-pollinations were again made in 1914, using not only *nutella* ($n \times p$) but *nutella* ($p \times n$) in reciprocal cross-pollinations. No seed was formed. The same results were obtained in 1916.

The sterility of the blend hybrid (*nutella*) is not due to sterility of the pollen or egg cells. Flowers which are unprotected, if visited by bees which have access to the parents or other species, growing in proximity, set an abundance of viable seed. When hand-pollinated, using pollen from either parent, from *hybrida pycnella*, *hybrida tortuosa*, from *Oe. grandiflora*, *Oe. Lamarckiana*, and some other species, an abundance of viable seed is formed. The pollen is well formed, showing a small, and no greater, percentage of poor grains than either of the parents. It is effective when placed on the stigma of either parent, on *hybrida pycnella* and *hybrida tortuosa*, on *Oe. grandiflora*, *Oe. Lamarckiana*, and other species.

The cause of the sterility of *hybrida nutella*, therefore, has not been determined. But it may be due to a lack of correlation in certain of the physiological processes among the blended "factors," or characters, inherited from the two parents. Or it may be due to a lack of blending among some of the homologous characters, structures or physiological processes or parts of the inflorescence. If a complete and equal blending of all homologous factors for the inflorescence took place, it may be that the reproductive processes would present as high a degree of efficiency as the vegetative processes have. That the phenomenon of blending of all the homologous factors for the inflorescence does not take place is shown by the petals, which are far more like those of *nutans* than of *pycnocarpa*. The blending of certain parental characters in the inflorescence, the taking over of others in their entirety from one parent with the exclusion of their homologues, and possibly the incomplete blending or unequal sharing in the union of others may produce an organization ineffective for the reproductive processes. Certain of the "qualities" or

properties combined in the egg, or pollen, or both, may lack the reciprocal influences necessary to a union of sperm and egg, although sperm and egg are effective in other combinations. It is also possible that certain physiological properties may retard the growth of the pollen tube when the flowers are selfed, but may not be effective against the growth of the tube from foreign pollen. Some preliminary studies indicate that the pollen tube does not grow rapidly enough down the style to reach the egg in time.

Whatever may be the nature of the lack of a reciprocal working of the reproductive mechanism in *hybrida nutella* it can not be attributed to irregularities in the meiotic division preceding the formation of the pollen, such as have been shown by JUEL (1900, p. 639-641) to take place in *Syringa Rothomagensis* (a hybrid of *S. vulgaris* and *S. Persica*, according to FOCKE 1881, p. 255), where typical mitotic figures are rare in the first division of the gonotokonts. In the atypical cases a process intermediate between mitosis and constriction occurs, a number of chromosomes appear to go to the daughter nuclei undivided, some are left in the cytoplasm. He suggests that there is a lack of mixing of the nuclear substance. Irregularities have also been shown by ROSENBERG (1903, 1904, 1909) in a hybrid between *Drosera rotundifolia* and *D. longifolia*, where there is a difference in the number of chromosomes in the two parents; by CANNON (1903, p. 133) in a *Gossypium* hybrid (*G. Barbadense* × *herbaceum*), where some pollen mother cells divide normally, others abnormally; and by METCALF (1901, p. 109) in *Gladiolus* hybrids, where two spindles were observed, presumably due to a repulsion between the paternal and maternal chromosomes, so that they remain in separate groups. These and similar abnormalities in meiotic division may explain the sterility of pollen in certain hybrids. STRASBURGER (1904, p. 609) makes the interesting suggestion that the difficulties resulting in the formation of imperfect pollen occur in synapsis of the heterotypic division.

But, as stated above, this can not account for the sterility of *hybrida nutella*, unless during synapsis (or other critical moments in the formation of the gones) there is an association of physiological "properties" or "tendencies" in the pollen and embryo sac of such a nature as to prevent the reciprocal working of the sperm and egg of *hybrida nutella* when brought together, but permits reciprocal working when either sperm or egg mates with a germ cell of related forms.

The F₁ hybrids of *Nicotiana Forgetiana* crossed with *N. alata grandiflora* are individually self-sterile according to EAST (1915, p. 80) as are

the F_2 , F_3 and F_4 generations. But cross-fertility is high between different individuals of each generation, or between individuals of different generations. The rate of growth of the pollen tubes down the style is so slow that they do not reach the ovary before the flower wilts, when individual plants are selfed. But in cross-pollinations between different individuals of any generation, the growth of the pollen tube is accelerated and reaches the ovary in time to carry the sperm cell to the egg. The self-sterility of individuals is explained by EAST on the ground that the protoplasts of the sex members are alike; and the pollen therefore lacks the kind of enzymes needed to call forth the secretion of a hexose sugar in the style which gives the direct stimulus to the pollen tube in those cases where its growth is accelerated.

The blend hybrid, *Oe. hybrida nutella*, is thus a striking instance of an intermediate hybrid which is totally self-sterile so far as determined. Not only are individual plants sterile, but all members of the race, whatever their origin (i.e. from either side of the reciprocal crosses of the parents), show cross-sterility when bred *inter se*.⁹ But this sterility is not due to an impairment of the fertile condition of either the pollen or egg cells. The self-sterility may be due to a too great likeness of the protoplasm of the sex members as EAST suggests for the individual self-sterility of the Nicotiana hybrids. The rate of growth of the pollen tube in *nutella* has not been definitely determined. Some preliminary studies suggest that it grows too slowly to reach the ovules in time.

It does not seem clear how the protoplasm of the sex members of *hybrida nutella* could be any nearer alike than those of each of the parents since the parents are very likely what DEVRIES calls isogamous species. Furthermore the parents, like most small-flowered oenotheras, have a very long historical background of close inbreeding, which according to EAST's suggestions should result in protoplasmic identity in the sexual members. Close and long-continued inbreeding in plants at least, does not in all cases lead to complete sterility resulting in an incompatibility between the germ cells, though it may largely account for partial sterility of the pollen. The somatic cells carry all the factors of the two germ cells, but at the time of the development of the reproductive structures there is a differentiation into maleness and femaleness, manifest in many cases by differentiation in form. Probably also physi-

⁹In 1916 I had in culture a new race of *nutella* from a cross between *Oe. nutans* of the third generation and a feral individual of *Oe. pycnocarpa*. Cross-pollinations were made between this race of *nutella* and that from crosses between the 3rd generation of the original parents. No seed was formed.

ological differentiation takes place resulting in the formation of different enzymes, etc. The mere fact then, of the coming together of the protoplasm of *nutans* and *pycnocarpa* into the blend *hybrida nutella* would not make the protoplasm of its sex members any more alike than is the case in either of its parents. But the lack of complete blending in certain features of the inflorescence and flowers may prevent certain physiological differentiations necessary to establish a state of compatibility sufficient to permit the coming together and union of the germ cells.

There is another feature which should be considered. The pollen and egg cells may be overloaded with active factors since the sperm and egg cell each carry all or nearly all the factors of both parents of the cross in an active state. If there is physiological incompatibility between sperm and egg, as I am inclined to believe, can it be that this overload of factors, all tending to express themselves as they do in this blend hybrid, has anything to do with this incompatibility?

Correlation between hybrid constitution and relative state of sterility or fertility

The three hybrids present an interesting correlation between their hybrid constitution and their relative state of sterility or fertility. *Hybrida nutella* inherits all the factors of its parents. All the factors are active and only a mean between the homologous characters results. The vegetative characters are a blend. Certain flower structures are combined in such a way as to produce an inefficient reproductive machine and sterility results, although both pollen and egg cell are efficient in other combinations. In *hybrida pycnella* on the other hand, while "inheriting" in its egg cell all of the same factors, there is selected an effective combination of active factors which do not blend. It reaches a very high state of efficiency in its reproductive structures for it possesses a very high degree of fertility. The same can be said of *hybrida tortuosa* in which different factors are selected from the same "inheritance" in its egg cells,—factors which are complementary to those present in *hybrida pycnella*.

Another feature in which the annual forms of *pycnella* differ from either parent is the less dense inflorescence. Consequently the pods are not so crowded as they are in either parent grown as biennials, though the pods are large and crowded with seeds. So far as the parents are concerned, the biennial forms are stocky, and there is a great tendency to fasciation in the stocky individuals on rich soils, much more so in *pycnocarpa* than in *nutans*. No fasciation has been as yet observed in

pycnella, whether annual or biennial forms. While the petals of *pycnella* are usually of the same size as those of *pycnocarpa*, and smaller than those of *nutans*, under certain conditions, not yet determined, they are larger even than those of *nutans*, in size sometimes approaching those of *nutella*.

In the F_1 blend hybrid, *hybrida nutella*, besides the sterility resulting from the lack of correlation in some part of the reproductive machinery, there are some striking modifications in the inflorescence. As already stated, the petals are larger than those of *nutans*, which in turn has larger petals than *pycnocarpa*. The flower buds also average longer, and the spread of the flower is greater.¹⁰ The cause of this progression in the size of the flower has not been determined. It is not accompanied by increased size of other members of the plant body. Possibly it may bear some correlation to the self-sterility, since certain forms of sterility in plants are sometimes accompanied by more showy flowers. It is known also that some hybrids are larger than either parent, but the increase in size here relates only to parts of the flower. The stigma does not over-top the stamens as in many of the large-flowered open-pollinated oenotheras.

Another striking modification in *hybrida nutella* is the lax inflorescence and the consequent lax relation of the pods. This is a characteristic of the annual as well as of the biennial forms.

While *hybrida pycnella* matures early, the annual forms very early, *hybrida nutella* matures late. The annual forms of *nutella*, even though they flower early in some seasons, usually continue to flower until killed by frost in November. It would seem therefore that the earliness of *nutans* is not only inherited by *pycnella*, but also that a progression in this quality has taken place so that the earliness of maturity is intensified. On the other hand, the lateness of *pycnocarpa* is inherited by *nutella* and there appears to have occurred a progression of the quality of lateness in this hybrid.

In *hybrida tortuosa* the edge character of the stem leaves is peculiar, the basal half having fewer teeth than either parent, over the same extent. *Tortuosa* is the only one of the hybrids thus far which may be fasciated, all of the biennial individuals, except one, presenting this character in a marked degree, so that the over-top of the main stem is

¹⁰ In *Oe. pycnocarpa* the spread of the open flower is 20-35 mm, the petals average about 15 × 15 mm. In *Oe. nutans* the spread of the open flower is 35-42 mm; the petals are 18-32 mm long × 15-20 mm broad. In *Oe. hybrida nutella* the spread of the open flower is 50-55 mm; the petals are 20-25 mm × 20-22 mm broad.

not what it otherwise would be. *Tortuosa* also continues to flower longer than *nutans*, this character as well as that of fasciation probably being drawn from *pycnocarpa*.

Difference in habit between annual and biennial forms

The habit of the annual forms of the two hybrids, *pycnella* and *nutella*, is shown by my cultures thus far to be quite different. The plants are lower in stature, and this is true of annual forms of *pycnocarpa* and *nutans*. The lower branches are more spreading, especially those arising in the axils of the rosette leaves, but to some extent also, the lower stem branches. The wider-spreading branches with the lower stature gives to the plant quite a different habit from that of the biennial forms. This variation in form must be taken into account in any comparison of the hybrids with their parents. The habit varies also according to the time in the season at which stem development begins. The earlier in the season stem formation and branching begin, the more nearly normal will the habit be, according to my observations. If stem development is postponed until August or September the lower branches diverge at a wider angle, and often grow for some distance nearly or quite parallel with the surface of the ground, the free end usually curved more or less strongly upward. Many of these branches may be as long or longer than the central axis, but the upward curving tip may not reach the same height as the main stem. This peculiarity has been observed in *hybrida pycnella*, *hybrida nutella* and the cultures of *pycnocarpa*.

Among the 1913 cultures of *pycnocarpa*, where seeds were sown in the greenhouse during March, and the seedlings later transplanted to the garden, in one lot from a seven-carpeled pod only 2 out of 120 plants formed stems and flowers during the first season. Stem development began late in the season, toward the middle or last of August. In one plant the main stem was inclined at an angle of about 45° , and the branches arising from the base of the stem were wide-spreading. In the other plant the main stem grew parallel with the ground and was so rigid that it could not have been brought to the erect position without breaking.

In another race of *Oenothera* from Ithaca, No. 17 of my cultures, a large percentage of annual forms occurred in transplanted seedlings in 1913. Some of these began stem development early enough to mature seed. The branching of these forms was near the normal. But those which began stem development from about the middle of August presented a wide departure from the normal, due to growth of the lower

branches nearly or quite parallel with the surface of the ground, and at a distance from the main axis of 3-5 dm then curved upward. In another species from Ithaca, *Oenothera angustissima* GATES (1913), in my cultures of 1913 a very small percentage began stem development. Two plants came into flower in September. The branches were somewhat more wide-spreading than in the normal forms. Two others began stem development in September forming three or four stems each. None of these stems grew erect. All were prostrate and applied closely to the ground. During the latter part of September and October a dense rosette was formed at the tip of each of these prostrate branches, but no roots had formed. The plants with a large central rosette, and several smaller ones on prostrate stems 2-3 dm from the central rosette presented a very peculiar appearance. This same phenomenon has been observed in other species where stem development begins late.

These variations are epigenetic. They indicate a wide range in the morphological complex, among the individuals of certain species, races or hybrids. The amplitude of this variation is linear, i.e., it extends along the line of the life cycle which becomes short or long, simple or complex, according to epigenetic conditions. But the lateral variations or fluctuations do not meet nor transgress the limits between the species, races or hybrids.

What all of the conditions are which influence this variation in the length of the life cycle, and the lesser or greater degree in the full expression of the maximum characteristics, is difficult to determine. For example, it is difficult to judge the stimulus which determines the beginning of the stem development in the annual forms, which in some cases may occur at almost any time during the season. But when stem development begins, it appears to serve as a stimulus which checks the further development of the rosette, so that the rosettes are arrested in development at different periods. Soil conditions such as fertility, moisture content, etc., probably play a part. The formation of wide-spreading branches, particularly branches which grow for a considerable distance nearly parallel with, or on the surface of the ground, appears to bear some relation to temperature, especially to the seasonal cold of late summer and autumn.

In addition to the morphological evidence that there are no intergrading forms between the hybrids, very strong evidence is furnished by the high state of fertility in *hybrida pycnella* and *hybrida tortuosa*, which practically show no variability in this respect; and the quite complete self-sterility of *hybrida nutella*.

THE F₂ GENERATIONS

The behavior of these hybrids in the F₂ generation has been studied in all except *hybrida nutella*, which is self-sterile. Thus far the F₂ is obtained only when the hybrid is pollinated by some other form or species. *Hybrida pycnella* and *hybrida tortuosa* are fixed in the first generation, and therefore breed true, the F₂ generation being exactly like the F₁. They will probably continue to breed true in succeeding generations (*pycnella* has been tested to the 3rd generation). In this respect these two hybrids follow the general rule applying to most of the hybrids in the genus *Oenothera* (section *Onagra*) as discovered by DEVRIES (1903, 1911, 1913).

The behavior of the fourth hybrid in the 2nd generation is very peculiar. The F₂ generation of *hybrida tortuella* was first obtained in the summer of 1915 in a number of plants which were grown as annuals: the mature rosette stage was therefore not obtained. The plants presented great variations, apparently none of them assignable strictly to any of the types represented by the two parents or the other three hybrids. No special study was made of these different types partly because the number of other cultures requiring attention made impossible the necessary critical examination of these new types. The result was such a surprising contrast with the behavior of the other three hybrids, it seemed desirable that the culture should be repeated. This was deemed important not only for the purpose of eliminating the possibility of an error in the 1915 cultures, but also that the complete life cycle might be studied in cultures of the plants grown as biennials. Several individuals of the *hybrida tortuella* appeared in the 1915 F₁ generations of reciprocal crosses between *nutans* and *pycnocarpa*, so that there was an opportunity of studying the behavior of this hybrid from both sides of the cross. Flowers were not bagged to save protected seed until quite late in the season. The amount of seed saved was small. As a result of this together with rather scant germination, probably because of immaturity, the number of seedlings obtained was small.

The young plants in 2½-inch pots, bearing 4-5 leaves, were transplanted to the garden near the middle of July 1916. During August the rosettes began to show differences. By September the rosettes were well formed and the variations were very striking. The variations relate to size of the rosette, width of the leaf, edge character, color of the mid-vein, form, crinkledness, etc. In 23 rosettes 9 different types are represented, only one of them approaching the rosette character of *hybrida tortuosa*. These different types of rosette have been photographed and

noted. The further account of them is reserved until the mature plants can be studied in 1917.

BACK- AND INTER-CROSSES OF THE HYBRIDS *PYCNELLA*, *NUTELLA* AND *TORTUOSA*

Further experiments on the interaction of the "factors" present in *Oenothera nutans* and *Oe. pycnocarpa* were undertaken by making all the possible back- and inter-crosses of the three hybrids, *pycnella*, *nutella* and *tortuosa*. The fourth and rare hybrid, *tortuella*, was not included in these experiments for the reason that *tortuella* was at first supposed to be merely a green-stemmed form of *tortuosa*, in which the factor for red stem color was temporarily suppressed, and that it might appear again in the F_2 . It was only after the completion of the back- and inter-crosses of the three other hybrids, that the discovery was made, that the genotypic constitution of *tortuella* was fundamentally different from that of *tortuosa*, resulting in a breaking up into numerous types in the F_2 generation. Some preliminary cultures were made in 1914. These were repeated in 1915 along with the cultures of the remaining back- and inter-crosses.

From the cultures of 1914 it was learned that in the reciprocal back-crosses of *pycnella* with *pycnocarpa*, only one type appeared in the progeny. When *pycnocarpa* was the mother and *pycnella* the pollen parent, the entire progeny was identical with *pycnella*. When *pycnella* was the mother and *pycnocarpa* was the pollen parent, all the progeny was identical with *pycnocarpa*. That is, in the reciprocal crosses between *pycnocarpa* and *pycnella* patrocliny prevailed. But when *nutella* was used in any of the crosses, splitting into two or more types usually occurs. The selective hybrid *pycnella* inherits the greater number of its characters from the *pycnocarpa* parent (furrowedness and lack of crinkledness of the leaves, green stem and all flower characters). This in a large measure, perhaps, accounts for patrocliny as a result of the reciprocal crosses. The segregate hybrid *tortuosa* inherits the greater number of its characters from the *nutans* parent (convexity and crinkledness of the leaves, red stem and all flower characters). The expectation was that reciprocal crosses between *nutans* and *tortuosa* would also result in patrocliny and this expectation was confirmed in the result. The results of all the back- and inter-crosses will not be described in detail, but are given in tables 2 to 9. The F_1 cultures of 1913-1914, and 1915, are combined in tables 2 and 3. The back- and inter-crosses listed in tables 4 to 9 were grown in 1915 unless otherwise indicated.

TABLE 2

*F*₁ generation of *Oenothera nutans* × *Oe. pycnocarpa* showing the splitting into the 4 hybrid types (1 = *pycnocarpa*; 2 = *nutans*).

2 × 1 (1913-1914) Total number plants *F*₁ = 118

	<i>Nutella</i>	<i>Pycnella</i>	<i>Tortuosa</i>	<i>Tortuella</i>
Annuals	35	10	0	0
Biennials	27	24	21	1
Total	62	34	21	1

2 × 1 (1915) Total number plants *F*₁ = 154

Annuals	140	10	2	2
Grand total	202	44	23	3 = 272

TABLE 3

*F*₁ generation of *Oe. pycnocarpa* × *Oe. nutans*, showing the splitting into 3 hybrid types. (1 = *pycnocarpa*; 2 = *nutans*.)

1 × 2 (1913-1914) Total number plants *F*₁ = 51

	<i>Nutella</i>	<i>Pycnella</i>	<i>Tortuosa</i>	<i>Tortuella</i>
Annuals	4	9	0	0
Biennials	2	36	0	0
Total	6	45	0	0

1 × 2 (1915) Total number plants *F*₁ = 213

Annuals	49	163	0	1
Grand total	55	208	0	1 = 264

TABLE 4

Back-crosses of hybrida nutella with the parents (1 = pycnocarpa, 2 = nutans)

74 = <i>Nutella</i> (2 × 1) × 2 =	$\left\{ \begin{array}{l} \textit{Nutans} \\ \textit{Pycnella} \end{array} \right.$	$\left. \begin{array}{l} 69 \\ 53 \end{array} \right\}$	Total 122
75 = <i>Nutella</i> (1 × 2) × 2 =	<i>Nutans</i>		Total 128
58 = 2 × <i>Nutella</i> (2 × 1) =	$\left\{ \begin{array}{l} \textit{Nutella} \\ \textit{Pycnella} \end{array} \right.$	$\left. \begin{array}{l} 50 \\ 2 \end{array} \right\} \begin{array}{l} *8 \\ 6 \end{array}$	Total 66
57 = 2 × <i>Nutella</i> (1 × 2) =	$\left\{ \begin{array}{l} \textit{Nutella} \\ \textit{Pycnella} \end{array} \right.$	$\left. \begin{array}{l} 90 \\ 8 \end{array} \right\}$	Total 99
Mutant =	<i>Gracilis dwarf</i>	1	
73 = <i>Nutella</i> (2 × 1) × 1 =	$\left\{ \begin{array}{l} \textit{Pycnocarpa} \\ \textit{Nutella} \\ \textit{Tortuosa} \end{array} \right.$	$\left. \begin{array}{l} 56 \\ 2 \\ 7 \end{array} \right\} \begin{array}{l} *22 \\ \\ 9 \end{array}$	Total 96
Toothed stem leaves			
52 = 1 × <i>Nutella</i> (2 × 1) =	<i>Nutella</i>		Total 118
51 = 1 × <i>Nutella</i> (1 × 2) =	$\left\{ \begin{array}{l} \textit{Nutella} \\ \textit{Tortuosa} \end{array} \right.$	$\left. \begin{array}{l} 25 \\ 1 \end{array} \right\}$	Total 26

*The numbers in the second column of 58 and 73 were cultures of 1914.

TABLE 5

Back-crosses of hybrida pycnella with the parents (1 = pycnocarpa 2 = nutans)

61 = <i>Pycnella</i> (2 × 1) × 1 =	<i>Pycnocarpa</i>	156	Total 156
60 = <i>Pycnella</i> (1 × 2) × 1 =	<i>Pycnocarpa</i>	52	Total 52
53 = 1 × <i>Pycnella</i> (2 × 1) =	<i>Pycnella</i>	75	Total 75
(1914) 1 × <i>Pycnella</i> (1 × 2) =	<i>Pycnella</i>	25	Total 25
62 = <i>Pycnella</i> (2 × 1) × 2 =	$\left\{ \begin{array}{l} \textit{Nutella} \\ \textit{Pycnella} \end{array} \right.$	$\left. \begin{array}{l} 13 \\ 93 \end{array} \right\}$	Total 106
(1914) <i>Pycnella</i> (1 × 2) × 2 =	$\left\{ \begin{array}{l} \textit{Nutella} \\ \textit{Pycnella} \end{array} \right.$	$\left. \begin{array}{l} 3 \\ 18 \end{array} \right\}$	Total 21
54 = 2 × <i>Pycnella</i> (2 × 1) =	$\left\{ \begin{array}{l} \textit{Nutella} \\ \textit{Pycnella} \\ ? \\ \textit{Tortuosa} \end{array} \right.$	$\left. \begin{array}{l} 90 \\ 6 \\ \\ 26 \end{array} \right\}$	Total 122
55 = 2 × <i>Pycnella</i> (1 × 2) =	$\left\{ \begin{array}{l} \textit{Nutella} \\ \textit{Pycnella} \end{array} \right.$	$\left. \begin{array}{l} 20 \\ 1 \end{array} \right\}$	Total 21

TABLE 6

Back-crosses of hybrida tortuosa with the parents (1 = pycnocarpa, 2 = nutans).

68 = <i>Tortuosa</i> (2 × 1) × 2 = <i>Nutans</i>	108	Total	108
56 = 2 × <i>Tortuosa</i> (2 × 1) =	$\left\{ \begin{array}{l} \textit{Tortuosa} \\ \textit{Tortuella} \end{array} \right.$	$\left. \begin{array}{l} 23 \\ 2 \end{array} \right\}$	Total 25
67 = <i>Tortuosa</i> (2 × 1) × 1 =	$\left\{ \begin{array}{l} \textit{Nutella} \\ \textit{Pycnocarpa} \\ \text{or} \\ \textit{Pycnella} \end{array} \right.$	$\left. \begin{array}{l} 28 \\ 1 \end{array} \right\}$	Total 29
50 = 1 × <i>Tortuosa</i> (2 × 1) =	$\left\{ \begin{array}{l} \textit{Nutella} \\ \textit{Pycnella} \end{array} \right.$	$\left. \begin{array}{l} 63 \\ 85 \end{array} \right\}$	Total 148

TABLE 7

Inter-crosses of hybrida nutella and hybrida tortuosa (1 = pycnocarpa, 2 = nutans).

76 = <i>Nutella</i> (2 × 1) × <i>Tortuosa</i> (2 × 1) =	$\left\{ \begin{array}{l} \textit{Tortuosa} \\ \textit{Pycnocarpa} \end{array} \right.$	$\left. \begin{array}{l} 17 \\ 46 \end{array} \right\}$	Total 63
72 = <i>Nutella</i> (1 × 2) × <i>Tortuosa</i> (2 × 1) =	<i>Pycnocarpa</i>	5	Total 5
(Numbers too small)			
69 = <i>Tortuosa</i> (2 × 1) × <i>Nutella</i> (2 × 1) =	$\left\{ \begin{array}{l} \textit{Nutella} \\ \textit{Pycnocarpa} \\ \textit{Nutans} \end{array} \right.$	$\left. \begin{array}{l} 120 \\ 5 \\ 2 \end{array} \right\}$	Total 127
70 = <i>Tortuosa</i> (2 × 1) × <i>Nutella</i> (1 × 2) =	<i>Nutella</i>	10	Total 10

TABLE 8

Inter-crosses of hybrida nutella and hybrida pycnella (1 = pycnocarpa, 2 = nutans).

78 = <i>Nutella</i> (1 × 2) × <i>Pycnella</i> (2 × 1) =	$\left\{ \begin{array}{l} \textit{Pycnella} \\ \textit{Nutans} \\ \textit{Nutella} \end{array} \right.$	$\left. \begin{array}{l} 90 \\ 29 \\ 1 \end{array} \right\}$	Total 120
65 = <i>Pycnella</i> (2 × 1) × <i>Nutella</i> (2 × 1) =	<i>Nutella</i>	18	Total 18
(1914) — <i>Pycnella</i> (1 × 2) × <i>Nutella</i> (2 × 1) =	<i>Nutella</i>	34	Total 34

TABLE 9

Inter-crosses of hybrida tortuosa and hybrida pycnella (1 = pycnocarpa, 2 = nutans).

72 = <i>Tortuosa</i> (2 × 1) × <i>Pycnella</i> (1 × 2) =	$\left\{ \begin{array}{l} \textit{Nutella} \\ \textit{Tortuosa ? or} \end{array} \right.$	$\left. \begin{array}{l} 23 \\ 16 \end{array} \right\}$	Total 39
71 = <i>Tortuosa</i> (2 × 1) × <i>Pycnella</i> (2 × 1) =	$\left\{ \begin{array}{l} \textit{Nutella} \\ \textit{Tortuosa or} \end{array} \right.$	$\left. \begin{array}{l} 62 \\ 43 \end{array} \right\}$	Total 105
64 = <i>Pycnella</i> (2 × 1) × <i>Tortuosa</i> (2 × 1) =	$\left\{ \begin{array}{l} \textit{Nutella} \\ \textit{Pycnocarpa} \end{array} \right.$	$\left. \begin{array}{l} 54 \\ 58 \end{array} \right\}$	Total 112
63 = <i>Pycnella</i> (1 × 2) × <i>Tortuosa</i> (2 × 1) =	$\left\{ \begin{array}{l} \textit{Nutella} \\ \textit{Tortuosa ? or} \\ \textit{Nutans} \\ \textit{Tortuella} \end{array} \right.$	$\left. \begin{array}{l} 83 \\ 25 \\ 3 \\ 1 \end{array} \right\}$	Total 112

This result is remarkable in that in the F_1 generation from a cross between two feral, non-mutating species, quadruple hybrids appear in the F_1 generation; one is a blend and self-sterile, but its pollen and egg cells are fertile; two of the selectives are fixed types and breed true, while the fourth hybrid (also selective) breaks up into different types in the second generation. The back- and inter-crosses show, either striking examples of patrocliny, or splitting into two types in some cases, three types in other cases and four types in one case.

In the back-crosses there are 9 cases of patrocliny, but probably only 5 of these are real (numbers 53, 60, 61, 62, 68). The other cases would probably show splitting if the numbers were larger (52, 70, 72, 75). But the position of the parents in the formula for No. 75, on the principle of an iterative cross (see DEVRIES, 1913, p. 94) may influence the predominance of *nutans* in the cross *nutella* (1 × 2) × 2 = *nutans*. In the formula in Nos. 60 and 68 the dominance of the extremes is in accordance with the expectation in double reciprocal crosses of DEVRIES, and so, in No. 61 for the expectation of the iterative cross. In other cases, however, the result, even where patrocliny is present, is not in accordance with the laws discovered by DEVRIES. This is in great part due to the fact that the F_1 splitting into several distinct types occurs on both sides of the reciprocal crosses, rather than patrocliny.

In the back-crosses there are at least 5 cases of patrocliny, 10 cases of splitting into 2 types, and 4 cases of splitting into 3 types. In the inter-crosses there are 2 cases of patrocliny, 3 cases of splitting into 2 types, 1 of splitting into 3 types, and 1 of splitting into 4 types.

But in all of the back- and inter-crosses no new types (with a single exception) appear, they all conform to one or other of the six types, the primary parental types, or one or more of the four F_1 hybrid types. The single exception is a mutant of the dwarf *gracilis* type.

THE BEHAVIOR OF *OENOTHERA NUTANS* AND *OE. PYCNOCARPA* COMPARED WITH OTHER WILD SPECIES OF *OENOTHERA*

DEVRIES (1913, pp. 30-59) has shown that the production of but one form of hybrid in the first generation of a cross between old, or wild species of *Oenothera* (section *Onagra*) is the general rule. But the hybrids from reciprocal crosses are often different in type (DEVRIES, 1903, p. 471; 1913, p. 30). The hybrids in these reciprocal crosses often resemble the pollen parent strongly, i. e., they are strongly patroclinous (*Oe. biennis* \times *muricata*, etc.). The parents of such crosses he terms *heterogamous* species. A few of the wild species he has shown to be *isogamous*, i.e., the single hybrids of reciprocal crosses are identical (*Oe. Hookeri*, *Cockerelli* and *strigosa*, see DEVRIES, 1913, p. 59). The behavior of *Oe. nutans* and *pycnocarpa* in reciprocal crosses does not conform to either of the types of behavior found by DEVRIES to be characteristic of the heterogamous or isogamous species which he has studied. So far as the vegetative characters are concerned patrocliny can not be ascribed to either of the hybrids, for the characters are either blended (in *hybrida nutella*) or selected and distributed about equally from the parents to two of the selective hybrids (*pycnella* and *tortuosa*).

In the selectives, while certain characters resemble those of one parent, other characters resemble those of the other parent and it is difficult to say which set of characters dominates. But even if one hybrid should be judged to indicate patrocliny in one cross, the same hybrid appears in the reciprocal cross where it would be a case of matrocliny for the same hybrid. While the behavior of *pycnocarpa* and *nutans* in reciprocal crosses is similar to that of isogamous species, in that the hybrid production in one cross is the same in the reciprocal cross,¹¹ there are three to four different hybrids instead of one, as in the wild isogamous species like *Oe. Hookeri*, *Cockerelli* and *strigosa*. Nevertheless, since the same types of hybrids are produced on each side in reciprocal crosses, it seems to indicate that the same heritable characters are transmitted in the pollen and egg cells. In this respect the parents (*pycnocarpa* and *nutans*) behave like isogamous species, but differ from such isogamous species as *Oe. Hookeri*, *Cockerelli* and *strigosa* in the production of several distinct

¹¹ Only 3 hybrid types have thus far appeared in the cross *Oe. pycnocarpa* \times *nutans*.

hybrid types, all of which, in their vegetative characters are blends or recombinations of the parental characteristics.

INTERPRETATIONS OF SPLITTING IN THE FIRST GENERATION

Selection of characters in the zygote versus Mendelian segregation

The selection and blending of the factors which make up the constitution of the two parents (*nutans* and *pycnocarpha*), into the three selective hybrids (*pycnella*, *tortuosa*, and *tortuella*, and the blend hybrid (*nutella*), occurs in the zygote or fertilized egg. Therefore it is of a very different type from that which takes place in Mendelian segregation. No such qualitative division as occurs in the gonotokonts, providing for the segregation of factors in Mendelian hybrids, is known to take place in the fertilized egg, and can not be invoked to explain zygotic selection.

Very little is known of the cytological processes in the fertilized egg of plants, so far as it relates to the more critical stages in the organization of the nuclear figure for the first division, and the behavior of the paternal and maternal chromosomes in the organization of a working relation in the new diploid nucleus. GUIGNARD (1890) describes and figures the spindle for the first division of the fertilized egg in *Lilium Martagon*. It is preceded by a double gnarl stage representing the paternal and maternal nuclear chromatin skeins. But the formation and association of the paternal and maternal chromosomes in the zygote was not observed. In *Pinus Strobus* MARGARET C. FERGUSON (1901, 1904) has shown that while the sperm and egg nuclei are in close contact the spindle is organized between them, while the paternal and maternal chromosomes form from the chromatin net-work of the sperm and egg respectively and move to the nuclear plate of the spindle. In two successive divisions the paternal and maternal chromosomes are formed in separate groups. But their final arrangement and relation, as cell wall formation and the morphogenic processes begin in the embryo, were not determined. STRASBURGER (1904, p. 20) attempted to study the relation of the paternal and maternal chromosomes in the fertilized egg of *Funkia* and *Galtonia*, where the chromosomes are of different sizes, but was unable to accomplish the desired result because of the rarity of seed formation. In the fertilized egg of *Iris Siberica* and *Triticum vulgare*, he discovered nothing unusual. The difficulties met with in the study of the cytological processes of the first division of the fertilized egg in plants appear to be very great. But it is an important and critical stage in the life cycle of plants, which deserves investigation.

Is the production of these quadruple hybrids due to the mutating condition of one of the parents?

The appearance of quadruple hybrids in the F₁ generation of a cross between two wild, non-mutating species is a rather unique phenomenon. Twin hybrids regularly appear in crosses of *Oe. Lamarckiana* or certain of its derivatives, with certain wild, non-mutating species of *Oenothera*, as demonstrated by DEVRIES (1907, 1909, 1913). These crosses are termed by DEVRIES "mutation crosses." Triple and even quadruple hybrids appear in certain mutation crosses. But heretofore triplets or quadruplets in the F₁ generation have only appeared when the mutant parent is an inconstant race and tends to repeat itself in crosses.

Oenothera nutans and *Oe. pycnocarpa* are constant, non-mutating feral species. The progeny is remarkably uniform, has been grown in several successive generations, presents a high degree of seed and pollen fertility, breeds true and the gametes are uniform. The two species have appeared each year, in typical form, in the same locality where they were discovered in 1909.

The number of plants grown in successive generations is shown in the following table.

TABLE 10

	<i>Nutans</i>	<i>Pycnocarpa</i>
1911-12	100	50
1913-14	75	198
1915-	152	76
1916	15	20

In 1916 very few of the seedlings grown in the pans were transplanted to the garden, since only a few plants were desired to raise seed and for a few pollination experiments. Each year the percentage of seed germination was high though the actual percentage was not determined by counting. The seeds were sown in good potting-room soil in seed pans 4-6 inches in diameter. The seeds were then covered with a rather thick layer of pure sand or fine gravel, so that the cover would not cake. Gravel and coal ashes are favorite substrata for *oenotheras*. The soil was kept well watered and a favorable temperature was maintained. Where an abundance of seed was sown, large numbers of seedlings developed so that in most cases they were closely packed in the pans. In such cases not all of the seedlings were transplanted to the garden, but great care was taken to select from among all sizes of seedlings in the

pan, in order to be certain that different types were not represented by the smaller and younger seedlings. The evidence therefore is very strong that *Oenothera nutans* and *Oe. pycnocarpa* in cultures are pure lines, notwithstanding the unwarranted assumption by DAVIS (1916, p. 245) that they are of "doubtful genetic purity."

This assumption appears to be based on the very questionable hypothesis that, since twin, triple or quadruple hybrids appear in the F_1 generation of a cross, one or both of the parents are mutating species and that a mutating species is *per se* genotypically impure.¹² Numerous cases are known in pure line work, where mutations have arisen from a pure line. N. H. NILSSON of the Swedish Experiment Station at Svalöf has described a mutant with stiff straw and stout heads from a pure line of wheat (see JOHANSEN 1909, p. 443). According to DEVRIES (1907 b, p. 85) the workers at "Svalöf are satisfied that real mutations as well as accidental crosses are occurring in their pure pedigree-cultures, from time to time." JOHANSEN (1909, p. 457) relates the appearance of a mutation from one of his pure lines of beans. He also cites other cases of mutations from pure lines in animals as well as in plants. The evidence of their appearance in pure lines is abundant.

No mutations in the pure lines of *Oenothera nutans* and *Oe. pycnocarpa* have appeared, though several mutants have been thrown in my cultures of *Oenothera Lamarckiana* where the number of individuals in the culture varied from 75 to 100. Among all the crosses (parental, back- or inter-crosses) only one mutant has appeared in the hybrid progeny of *nutans* and *pycnocarpa*. This was a *gracilis* dwarf mutant in the back-cross *nutans* \times *nutella* (1 \times 2).¹³ But the appearance of a mutant is not an indication that the line is impure, and furthermore this mutant did not appear in the direct line of either parent, but in one of the back-crosses.

The appearance of triple and quadruple hybrids in the F_1 generation of reciprocal crosses between *Oe. nutans* and *Oe. pycnocarpa* can not be explained on the basis of the interesting theory of "mutation crosses" in the same sense as interpreted by DEVRIES. We have no evidence that the species are mutating, and furthermore the four hybrids of the F_1 generation are very different from the twin hybrids of DEVRIES's mutation crosses. In mutation crosses, according to DEVRIES, there is a splitting of the entire constitution (gametic) so that all the vegetative char-

¹² JOHANSEN says (1909, p. 448) that the oft repeated conjecture that mutations indicate the hybrid nature of the parent has little worth.

¹³ See table 4.

acters are split, not selected as in *pycnella*, *tortuosa* and *tortuella*, nor equally blended as in *nutella*.

The four different hybrid types in the F_1 generation of crosses between *Oe. pycnocarpa* and *Oe. nutans* differ from the mutation crosses described by DEVRIES in another respect. In the mutation crosses triplets and quadruplets are formed only when the mutant parent is an inconstant race, for example, *Oe. lata*, which is nearly pure female, though rarely *lata*-like forms which produce pollen appear in the F_1 when *lata* is crossed with other species (DEVRIES 1913, p. 245). When *Oe. lata* is pollinated with *Oe. Hookeri*, *Cockerelli*, *biennis Chicago*, etc., the splitting in the first generation produces the twins, *laeta* and *velutina*, and in addition a third form which is *lata* repeated, but slightly modified. The most common modified form of this *lata* arising by splitting in the first generation of a cross shows a resemblance to *laeta* forms and is called *lata-laeta*. Besides the splitting in the F_1 generation into twins, *laeta* and *velutina*, the inconstant race repeats itself. The repeated form is modified by *laeta* characters (the "triplet") and rarely a small percentage is modified by the *velutina* characters ("quadruplet"). This is strong additional evidence that the triple and quadruple hybrids in the first generation of crosses between the wild species *Oe. pycnocarpa* and *Oe. nutans*, represent a type of splitting different from that manifested in the mutation crosses of DEVRIES, and does not appear capable of interpretation on the same hypothesis.

The three hybrids, *pycnella*, *tortuosa* and *tortuella*, have been spoken of as selective hybrids, since certain parental factors are selected in the zygote, and the characters they represent are developed to their full expression, other factors being either eliminated or subordinated, so that they do not enter into the composition, at least the phenotypic composition, of the new types. If they were true segregates in their genotypic constitution, the interesting question would arise as to how this segregation of certain factors in the zygote, and the elimination or subordination of others, takes place. In Mendelian segregation the mechanism of allotypic division in the gonotokonts is generally accepted as furnishing a means for the segregation. But no such mechanism of nuclear divisions occurs in the zygote. The stage of fecundation and the first nuclear division in the zygote is a critical period in the ontogeny of the individual, and particularly so in the F_1 zygote of crosses.

If there is an elimination of certain factors in the composition of these selective hybrids, we may conceive that it takes place by the shunting to one side, during nuclear division in the zygote, of some of the factor-

bearing material, a phenomenon, perhaps, similar to merogony as recently defined by GOLDSCHMIDT (1916). GOLDSCHMIDT (1912) sought to find a cytological basis for patrocliny exemplified in DEVRIES'S crosses of *Oenothera biennis* and *Oe. muricata*, etc., where the F_1 hybrid in its vegetative characters is nearly or quite identical with the pollen parent. His first cytological interpretation of this phenomenon predicated the degeneration of the egg cell, there being no fusion of the sperm and egg, so that the embryo was developed exclusively from the male cell. According to his recent studies on material of reciprocal crosses of *Oe. atrovirens* and *Oe. venosa* there is a fusion of the sperm and egg cells, but during the first division of the zygote the chromosomes from the egg are eliminated from the daughter nucleus of the embryo cell and degenerate in the cytoplasm. Such a process, if the hereditary factors are carried by the chromosomes alone, would result in the complete reproduction of the pollen parent in the F_1 , not modified by the ovule parent. But in cases of patrocliny among the *Oenotheras*, where the flower and inflorescence characters are different in the two parents, the F_1 plants, while very strongly resembling the pollen parent in vegetative characters, often resemble the ovule parent more or less strongly in the inflorescence.

The fact that the patroclinous F_1 generation in such *Oenothera* crosses is more or less modified by the maternal characteristics, especially in the inflorescence, indicates that factor-bearing material is contributed to the F_1 generation by the mother plant of the cross. It should be said, however, that GOLDSCHMIDT (1912) does not commit himself unreservedly to the merogony theory as an explanation of patrocliny in the *Oenotheras*. It appears to me that these cases of patrocliny in the *Oenotheras*, where the hybrid is fixed in the F_1 generation, are examples of *permanent dominance*, such hybrids being *physiological homozygotes*.

Stable and unstable dominance in the hybrids pycnella, tortuosa and tortuella

I have sometimes spoken of these hybrids as "segregate" hybrids, because they at least appear to be segregates in their phenotypic constitution. But I have been led to believe from a study of my *Oenothera* cultures during the past two seasons, that these three hybrids can be classed as cases of selective dominance, though perhaps not in the strict Mendelian sense. If there were a splitting in the F_2 generation, releasing the parent forms in 50 percent of the progeny and forming 50 percent heterozygotes there would be no question that *hybrida pycnella* and *hybrida tortuosa* in the F_1 generation were true Mendelian dominants, for the F_1 hybrids in Mendelian crosses are often intermediates. In each of

these hybrids dominance of parental characters is complete in relation to nearly all the visible characters. But not all of the dominant characters of either hybrid come from a single parent. There is a selection of factors from each parent, a "crossing over" between the linked factors. A certain group of factors is dominant in *pyncnella*, while another group of factors is subordinate. The group of subordinate factors in *pyncnella* becomes the dominant one in *tortuosa*. Since these *hybrids* are fixed in the F₁ generation they may be considered as cases of fixed or permanent dominance.¹⁴ They are physiological homozygotes.

In support of this interpretation of the genotypic constitution of *pyncnella* and *tortuosa* is the fact that in certain of the back-crosses where splitting occurs in the zygote, *nutella* is one of the "segregates." In these back-crosses *nutella* is not one of the parents. This behavior is shown in the following tables; compiled from tables 5 and 6.

TABLE II

Back-crosses of hybrida *pyncnella* with parent *nutans* (2 = *nutans*, 1 = *pyncnocarpa*).

62 = <i>Pyncnella</i> (2 × 1) × 2 =	$\left\{ \begin{array}{l} \textit{Nutella} \\ \textit{Pyncnella} \end{array} \right.$	$\left. \begin{array}{l} 13 \\ 93 \end{array} \right\}$	Total 106
(1914) <i>Pyncnella</i> (1 × 2) × 2 =	$\left\{ \begin{array}{l} \textit{Nutella} \\ \textit{Pyncnella} \end{array} \right.$	$\left. \begin{array}{l} 3 \\ 18 \end{array} \right\}$	Total 21
54 = 2 × <i>Pyncnella</i> (2 × 1) =	$\left\{ \begin{array}{l} \textit{Nutella} \\ \textit{Pyncnella} \\ ? \\ \textit{Tortuosa} \end{array} \right.$	$\left. \begin{array}{l} 90 \\ 6 \\ 26 \end{array} \right\}$	Total 122
55 = 2 × <i>Pyncnella</i> (1 × 2) =	$\left\{ \begin{array}{l} \textit{Nutella} \\ \textit{Pyncnella} \end{array} \right.$	$\left. \begin{array}{l} 20 \\ 1 \end{array} \right\}$	Total 21

TABLE I2

Back-crosses of hybrida *tortuosa* with parent *pyncnocarpa* (2 = *nutans*, 1 = *pyncnocarpa*).

67 = <i>Tortuosa</i> (2 × 1) × 1 =	$\left\{ \begin{array}{l} \textit{Nutella} \\ \textit{Pyncnocarpa} \\ \textit{Pyncnella} \end{array} \right.$	$\left. \begin{array}{l} 28 \\ 1 \end{array} \right\}$	Total 29
50 = 1 × <i>Tortuosa</i> (2 × 1) =	$\left\{ \begin{array}{l} \textit{Nutella} \\ \textit{Pyncnella} \end{array} \right.$	$\left. \begin{array}{l} 63 \\ 85 \end{array} \right\}$	Total 148

¹⁴ The term *permanent*, or *stable*, *dominance*, relates to the fixity of the type in the F₁ generation of a cross so that if selfed, the type appears in successive generations unchanged. It implies also the permanent recessiveness, latency or subordination of the other factors in the F₁ zygote of the cross which were not activated. In *unstable dominance*, the type appearing in the F₁ generation is not fixed, but splits or shows extraordinary fluctuation in the F₂. *Selective dominance* relates to the dominance of the active factors in a selective hybrid.

In table 11 it is seen that from the back-crosses of *hybrida pycnella* with parent *nutans*, 270 in all, there appear 126 individuals of *hybrida nutella*. Parent *nutans* and *hybrida pycnella* it will be remembered, both have broad rosette leaves somewhat toothed over the basal portion. The rosette leaves of *hybrida nutella* are intermediate in width and edge character between the broad and toothed leaves of parent *nutans* and the narrow cut leaves of parent *pycnocarpa*. The factor for this character (narrowness and cutness of rosette leaves, and some others also) of *pycnocarpa* must, therefore, be present in *hybrida pycnella* of these back-crosses, but in a latent or "recessive" state. When *hybrida pycnella* is back-crossed with parent *nutans* these latent factors become active in a large percent of the zygotes and the blend *hybrida nutella* appears.

In like manner, as seen from table 12, in the back-crosses of *hybrida tortuosa* with parent *pycnocarpa*, 177 in all, there appear 91 individuals of the blend, *hybrida nutella*. Since both *tortuosa* and *pycnocarpa* have narrow, cut leaves, the factor for width and edge character of the rosette leaves of parent *nutans* (other factors also) must be present in *tortuosa*, but in a subordinate, or "recessive" state.

These back-crosses, therefore, present evidence that in each of the hybrids, *pycnella* and *tortuosa*, certain factors, selected some from one parent, some from the other, are active or dominant¹⁵ in the zygote of the F_1 and the characters they represent are developed to their full expression. The alternative factors are subordinate or "recessive" in each hybrid and become active in a certain percentage of the zygotes of back-crosses. The result of this analysis of the hybrids indicates quite clearly that the gametes of *Oenothera nutans* and *Oe. pycnocarpa* are uniform. It is not necessary to resort to the rather overworked hypotheses of dissimilar gametes in the parents of twin hybrids, nor of those giving a larger number of distinct hybrids in the F_1 generation, except where one of the parents is an inconstant race, as in *Oenothera lata*, etc. The evidence from *Oenothera* cultures points more and more to the conclusion of SHULL (1914) that "a hereditary mechanism must exist in *Oenothera* fundamentally different from that which distributes the Mendelian unit-characters."

¹⁵ SWINGLE (1898, 1913) has proposed a hypothesis for the different types of hybrids appearing in the first generation of interspecific crosses which he calls "zygotaxis." It assumes the chance arrangement of the parental chromosomes in different positions in different zygotes. These positions are maintained throughout the ontogeny. Those chromosomes situated nearer the cytoplasm are better fed and exercise a greater influence on the formative processes in the cell than those more distant. In consequence of the different arrangement in different zygotes, different hybrid types appear.

In *tortuella*, which differs from *tortuosa* in having a green stem instead of a red one, the dominance of the factor composition in the F_1 is not fixed. A breaking-down takes place in the F_2 generation. The complete composition of the individuals of this second generation can not be determined until they come into flower in 1917; but from the types of rosette present it is clear that in *tortuella* certain factors are present in its genetic constitution which are subordinated in the F_1 and only come into play in some of the individuals of the F_2 (breadth and edge character of the rosette leaves for example).

We do not know the behavior of *hybrida nutella* in the F_2 generation, and cannot say whether or not it is a physiological homozygote. It is almost a perfect blend of the two parents. Besides certain features of the inflorescence and fruit spike which differ to some extent from each parent and do not represent an intermediate condition, the petals are more like those of *nutans*, and it might be considered a dominant in the F_1 favoring *nutans*.

*Multiple dominance*¹⁶ in the F_1 generation of crosses between *Oenothera nutans* and *Oe. pycnocarpa*

On this interpretation, then, there are four types of dominance in the F_1 of crosses between *Oe. nutans* and *pycnocarpa*. All four appear when *pycnocarpa* is the pollen parent. Only three have thus far appeared when *nutans* is the pollen parent. Two of these, *pycnella* and *tortuosa* are fixed dominants, and are physiologically homozygous. The genotypic constitution of the F_1 dominant *tortuella* is dissolved in the F_2 . The later history of *nutella* is unknown.

In many cases interspecific crosses are difficult or impossible to obtain. In general this difficulty is greater in proportion to the remoteness of the relationship between the species used in the attempted cross. There is apparently great difficulty in establishing a working relation between the sperm and egg nuclei after their association, whether of a chemical or mechanical nature. The disturbance caused by the entrance of a foreign sperm into the egg should not be overlooked. Chemical, enzymatic and mechanical disturbances result, the effect of which may vary according to the differences in the constitution of the parents used in the cross. The reciprocal shock on the germ cells when they meet in the egg may be sufficient in many cases to break some of the remoter

¹⁶ Multiple dominance relates to the appearance of several distinct hybrid types in the F_1 of a cross, certain factors being completely or partially dominant in each type, the other factors being subordinate, latent, or recessive.

linkages of factors in the parents. Chance then partly determines the new association in the four different hybrid types in the F_1 of *Oe. nutans* \times *pycnocarpa*.¹⁷

All, or nearly all, the active factors of the two parents enter equally active and with the same association, into the constitution of the zygote of the blend, *hybrida nutella*. The effect of the shock merely retards somewhat the organization of a working relation between the sperm and egg nuclei.

In the three other hybrids the effect of the shock is such, that, when the organization of a working relation between the germ cells is formed, certain factors are rendered subordinate or inactive. The shock, to use a figurative expression, shakes up the elements of the composition, "crossing over" occurs, and they fall into new combinations, or alliances, or systems. To some extent chance probably determines the new combinations but there are varying degrees of linkage between certain factors. Among the factors relating to the leaves, linkage is strong in couplets, as width and edge character, convexity and crinkledness, furrowedness and lack of crinkledness. In the case of the flowers the linkage among all the factors of each parent is strong. While the shock breaks the general bond of linkage of the sum total of factors in each species, it does not destroy the linkage present in lesser associations of factors. To some extent chance determines whether one or the other of the "selective" hybrids is formed. In two of these hybrids (*pycnella* and *tortuosa*) the activity or inactivity of the factors is fixed so long as the hybrids are self-fertilized. They breed true in the F_2 and following generations, since they are physiologically homozygous. But patrocliny and splitting occurs in the back- or inter-crosses. In *hybrida tortuella*, when the working relation is established between the factors of the germ cells in the F_1 zygote, the activity or inactivity of certain factors is fixed only for the F_1 generation. The organization of the working relation between the factors is unstable, and breaks down in the second generation into, perhaps, numerous types, as in certain other species hybrids (see BAUR 1911, p. 207). Since some of the types presented in this F_2 generation have the breadth and edge character of the leaves of the parent *nutans*, a character not present in the phenotype of *hybrida tortuella*, its appearance in the F_2 generation of *tortuella* indicates that the *nutans* factor for breadth and toothedness of the rosette leaves was present in *tortuella* but in a subordi-

¹⁷ The result would vary in different species crosses according to the firmness with which the association of factors in either parent held to its genotypic type.

nate condition. In the fixed "selective" hybrids the color of the stem is linked with flower characters in the same way as in the parents.

In *hybrida pycnella* greenness of stem is linked with *pycnocarpa* flowers, in *hybrida tortuosa* redness of the stem is linked with *nutans* flowers. In *hybrida tortuella* this link between color of the stem and flower characters is broken. It is possible that this may be evidence present in the first generation that many of the linkages among factors in the parents are broken, thus rendering the composition of *tortuella* very unstable. This splitting of *hybrida tortuella* in the second generation does not appear to be of the Mendelian type.

One cause of the peculiar behavior of the *Oenotheras* may be that the association, or linkage of factors, is not very strong in the germ plasm, but varies in that respect in different species. The germ plasm is peculiarly sensitive to shock from the meeting of sperm and egg, particularly when there is a genotypic difference between the two germ plasms. This results more or less in interchange, crossing over, dominance, as well as blending, of factors in the zygote, often accompanied by selection of factors into different associations in different zygotes giving rise to more than one hybrid type in the F_1 generation of crosses.

SUMMARY

1. In the F_1 generation of the cross *Oenothera nutans* \times *Oe. pycnocarpa*, four different hybrid types appear which are named as follows: *Oe. hybrida nutella*; *Oe. hybrida pycnella*; *Oe. hybrida tortuosa*; and *Oe. hybrida tortuella*. In the F_1 generation of the reciprocal cross, *Oe. pycnocarpa* \times *Oe. nutans* three hybrid types have appeared which are identical with three of the types named, viz., *nutella*, *pycnella* and *tortuosa*. If the number of the individuals of the F_1 *pycnocarpa* \times *nutans* was very large it is probable that *tortuella* also would appear.

2. *Nutella* is a *blend* hybrid; all the homologous factors of the parents are active and the phenotype is a mean between the two parents, with some exceptions in the inflorescence. Thus far *nutella* has proven absolutely self-sterile, but the pollen and egg cells are highly fertile in back- and inter-crosses, and in reciprocal crosses with other species.

3. *Pycnella* and *tortuosa* are *selective* hybrids. For example, in the F_1 zygote which develops into *pycnella*, all of the factors of the two parents are present. Certain of these factors from each parent are selected in the organization of the working relation between them, and the characters they represent are developed to their full expression, while the other members of the homologous factors remain subordinate.

Tortuosa is organized in a similar way, but the factors which are subordinate in *pycnella* are active in *tortuosa*, and *vice versa*. In phenotypic constitution *tortuosa* and *pycnella* are counterparts of each other.

4. *Pycnella* and *tortuosa* are physiological homozygotes; they are fixed in the first generation, and when selfed they are repeated in the F_2 and succeeding generations.

5. *Tortuella* is also a selective hybrid, but it is not fixed in the F_1 ; when selfed it dissolves in the F_2 into numerous types, some of which show that certain factors which were subordinate in the F_1 are activated in the F_2 .

6. The production of 4 hybrid types in the F_1 is an example of multiple dominance i.e., many dominant types appear. *Pycnella* and *tortuosa* are examples of permanent or stable dominance of factors.

7. In back-crosses there are clearly 5 cases of patrocliny, 10 cases of splitting into 2 types, and four cases of splitting into 3 types.

8. In the inter-crosses there are 2 cases of patrocliny, 3 cases of splitting into 2 types, 1 of splitting into 3 types, and 1 of splitting into 4 types.

9. In all of the back- and inter-crosses no new types (except a dwarf *gracilis*) appear; they all conform to one or other of the six types, the primary parental types, or one or more of the four F_1 hybrid types.

10. In the back-crosses of the selective hybrid *pycnella* with parent *nutans*, and of the selective hybrid *tortuosa* with parent *pycnocarpa*, the blend hybrid *nutella* appears in a high percentage of the progeny. A number of the phenotypic characters of parent *pycnocarpa* are not present in the phenotype of *pycnella*. The factors for these characters, however, must be subordinate or latent in *pycnella* for when crossed with parent *nutans* these subordinate *pycnocarpa* characters are activated in a high percentage of cases and make possible the appearance of the blend *nutella*. Likewise in back-crosses of *tortuosa* and *pycnocarpa*, the subordinate *nutans* factors in *tortuosa* are activated in a high percentage of cases and make possible the appearance of the blend hybrid *nutella*.

11. The analysis summarized in Nos. 5 and 10 indicates that the gametes in the parents *nutans* and *pycnocarpa* are uniform.

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