A PRELIMINARY NOTICE OF GENETICAL STUDIES OF RESISTANCE TO MILDEW IN OENOTHERA [1]

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Introduction

Ever since the senior writer of this paper commenced the growing of Oenothera cultures for experimental purposes, it has been noticed every year that nothing is more characteristic of the various elementary species and hybrids than the great differences that they show in susceptibility to infection by mildew. Thus, among the recently described species, such ones as *Oenothera stenomeres* and *Oe. pratincola* have been uniformly, year after year, heavily infected. Others, such as *Oe. Reynoldsii*, *Oe. numismatica*, and *Oe. scitula*, have been quite as uniformly immune. Similar facts have come to the attention of Professor de Vries, who, in a recent letter, writes that certain of the types grown by him would have been admirably adapted to a study of the inheritance of immunity.

For several years prior to 1919, more or less adequate notes had been kept in the garden as a whole as to the prevalence of mildew, but it became obvious that the solution of the problem would demand special cultures of forms particularly marked in their resistance or susceptibility, as the case might be, which might be handled with the question of disease resistance paramount. Furthermore, since the differences shown by certain pairs of reciprocal hybrids were so astonishingly definite, the one being white with mildew and the other absolutely free, although both were grown in adjoining rows, under identical conditions, and often with interlocking branches, it seemed that the material offered an excellent opportunity for biochemical studies, to be conducted parallel with the genetical work, and designed to trace, if possible, the relationship of immunity and susceptibility to chemical characters of the forms. Consequently, a biochemical study of carefully selected material from these cultures has been undertaken by Mr. Joaquin Mejorada Marañon. His results cannot be reported in this preliminary notice, which, even on the genetical side, aims to present only part of the results, typical of those which are being obtained.

Most of the previous work on the problem of varietal and specific resistance to mildews has been done by Salmon (8, 10). He has published several short papers on varietal susceptibility to the powdery mildew of

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corn and grasses (*Erysiphe graminis* DC.), and to the hop mildew (*Sphaerotheca Humuli* (DC.) Burr.). He proved the existence of biologic strains among the powdery mildews, especially in his work on *Erysiphe graminis* DC.

That in many cases resistance to mildew is inheritable is without doubt, though little is known of the quality of the resistance or of the genetics of the situation.

No previous work has been reported, as far as we know, on the mildew problem as it is presented by the Oenothera cultures. Atkinson (1, 2) made observations upon immunity and susceptibility to a downy mildew, *Peronospora Arthuri* Farlow, in connection with his genetical studies of *Oenothera pycnocarpa* (susceptible) and *Oe. nutans* (immune). He published his results on the hybrids produced from these crosses, but made only one statement in regard to their susceptibility or immunity to this downy mildew, namely, that the F_1 of the cross *Oe. pycnocarpa* × *Oe. nutans* was susceptible (1). The results, however, suffice to show that the markedly antithetic characters of the Oenotheras, as concerns disease resistance, extend to fungi of other groups than the true mildews with which the present paper is concerned. Of course, the observations of de Vries (12) upon the relative resistance of the mutations of *Oe. Lamarckiana* to infection by Micrococcus are well known, and especially interesting because of the mutational origin of disease susceptibility in the case of mut. *nanella*.

Material

At the outset, for the sake of clarity, it will be well to state that general observation had indicated that susceptible species when crossed reciprocally with immune ones gave only one immune cross. It was not possible to get immune hybrids by crossing susceptible parents, and in the case of crosses between immune strains, both reciprocals might be immune, or one of them immune and the other susceptible. The results could be formulated in accord with the hypothesis of heterogametism, already set forth in several papers (3, 6). Each species of Oenothera is supposed to produce two types of gametes called α and β gametes. The α gametes are generally female and the β gametes generally male, although other conditions occur, as will be shown later in the discussion of the phenomenon of metacliny. If the immune strains carry a factor **I** for immunity (**i** will then represent the absence of the factor for immunity, or presence of a factor for susceptibility) in only one type of gamete, and if only $\alpha\beta$ combinations are viable, then it can readily be seen that such a strain will breed true for immunity, but will give a susceptible hybrid, one way or the other, when reciprocally crossed with a susceptible strain. If **I** were a dominant factor, all the breeding behavior would be clear, providing it were possible for **I** to be an attribute of the α gamete in some strains, and of the β gamete in others. This hypothesis has been borne out by the results obtained, and the following description of the strains used gives their constitutional formulae as established by the various crosses into which they have entered.

A review of the Oenothera cultures at the Botanical Garden of the University of Michigan in the summer of 1919 led to the selection of the following forms as especially likely to give interesting results:

1. *Oenothera pratincola* Bartlett (4). This species is highly susceptible and was chosen because of the long period (seven years) that it had been grown in self-pollinated lines. Susceptibility of the chosen strain ("Lexington C") had been observed for eight generations. This strain (originally from Kentucky) has, according to the above-mentioned hypothesis of α and β gametes, the genetical constitution $\alpha\mathbf{i}\beta\mathbf{i}$.

2. "*Oenothera biennis Chicago.*" This is a hardly distinguishable strain of the preceding, and is referred to under the provisional name assigned to it by de Vries in *Gruppenweise Artbildung* (13). It was chosen because it was essentially identical with the foregoing, but of entirely different provenience. Both forms had been so extensively used in crosses that the opportunity was seized to see if their apparent specific identity would be verified by identical breeding behavior. "*Oe. biennis Chicago*" was received from de Vries in 1912, and had been mildewed every year for seven years. Its genetical constitution has proved to be the same as that of *Oe. pratincola* ("Lexington C"), namely, $\alpha\mathbf{i}\beta\mathbf{i}$.

3. *Oe. mississippiensis* Bartlett (5). This species had been grown for seven seasons under the tentative name "Cartersville," assigned to it by de Vries, who collected it at Cartersville, Mississippi, in 1904, and sent it to one of the writers in 1912. It has always been heavily mildewed. Genetical constitution, $\alpha\mathbf{i}\beta\mathbf{i}$.

4. *Oe. pratincola* hyb. *immunis*. The hybrid which we introduced into our mildew experiments under the name *Oe. pratincola* hyb. *immunis* had an interesting origin. Of the two Oenothera species known from Lexington, Kentucky, and extensively grown in experimental cultures for many years, one, *Oe. pratincola*, is always mildewed, whereas the other, *Oe. numismatica*, is very slightly infected, or not at all. When these species are hybridized, the cross with *Oe. pratincola* as the pistillate parent gives twin hybrids, both of which are mildewed. One of them is strictly like the maternal parent in all characters except one trivial one, namely, the presence of erect, thin-walled, viscid hairs on the flower buds, a character of the paternal parent. This matroclinic cross, known as *Oe. pratincola* hyb. *viscida*, behaves in every respect like *Oe. pratincola*, even to throwing the same mutations. The reciprocal cross, in which *Oe. numismatica* is the pistillate parent, is immune and in all other respects like pure *Oe. numismatica*. According to our hypothesis of α and β gametes we explain these facts as follows:

A. Both the α and β gametes of *Oe. pratincola* are carriers of the factor **i** (susceptibility to mildew).

B. In *Oe. numismatica* the α gamete carries the factor **I** (immunity to mildew), whereas the β gamete carries the factor **i**.

C. The composition of *Oenothera pratincola* hyb. *viscida* is therefore α**i**β**i**, just as is the case in true *Oe. pratincola.*

Now the interesting situation develops. Both true *Oe. pratincola* and hyb. *viscida* have thrown a mutation which closely simulates a certain wild elementary species, namely, *Oe. Reynoldsii* Bartlett (4). However, this mutation coming directly from *Oe. pratincola* is susceptible to mildew, whereas that from hyb. *viscida* is immune. Furthermore, the immunity of the mutation from hyb. *viscida* (called mut. *simulans* because it is indistinguishable from *Oe. Reynoldsii*) is concerned with the β gamete, since the cross *Oe. pratincola* hyb. *viscida* × mut. *simulans* yields an altogether immune hybrid closely resembling *Oe. pratincola* in morphology, but smaller in size, and, perhaps on account of its immunity, very different in coloration. The type comes true from seed, and has been so frequently used in crosses that it has been designated for convenience as *Oe. pratincola* hyb. *immunis.* This hybrid has the composition α**i**β**I**, and, as we interpret the situation, the immunity factor resides in the β gamete by virtue of mutation of the β**i** gamete originally entering into the composition of the line from *Oe. numismatica* to β**I**, this mutation taking place at the time of origin of mut. *simulans.* That the β**i** gamete of *Oe. pratincola* does not undergo such a mutation is shown by the fact that the mutation simulating *Oe. Reynoldsii,* which arises from pure *Oe. pratincola,* and is called mut. *simulans rubricalyx,* because it differs from the otherwise identical mutation from hyb. *viscida* in having red buds, is neither immune itself, nor can its β gamete impart immunity to crosses with the parent type. Thus, *Oe. pratincola* mut. *simulans rubricalyx* is not a type like hyb. *immunis,* but is merely a mildew-susceptible *Oe. pratincola.* It shows neither the immunity nor the small stature of hyb. *immunis,* thus proving that the unique characters of hyb. *immunis* are due to the β gamete from *Oe. numismatica.*

These genetical facts are of no moment to the reader who is interested in the inheritance of the immunity after it has once arisen. They are a necessary part of the present record, however, since hyb. *immunis* has been extensively used in our crosses, being the one available form through which immunity could be transmitted to a cross through the pollen. It should be remarked that hyb. *immunis* breeds quite as true from seed as the other types used as parents. Constitution, α**i**β**I**.

5. *Oenothera cinerescens* Bartlett (5). This species was collected at White Sulphur Springs, West Virginia, in 1912, and has been continuously in culture ever since. It is an outstandingly resistant type, and had been observed to be free from mildew for eight generations up to the time it was used for the crosses described below. Constitution, α**I**β**i**.

The Identity of the Fungus (*Erysiphe Polygoni* DC.)

Salmon (7) has shown that *Erysiphe Polygoni* occurs on a great many different host plants, among which the common garden pea, *Pisum sativum*, is one of the best known. This powdery mildew often bears the name of "mildew of the pea." When the seedling plants of the Oenothera crosses were grown in the greenhouse it was found that the pea mildew did not infect them. The seedling plants were placed in two separate greenhouses, in each about 1,500 plants. In one of the greenhouses there were many pots of garden peas covered with powdery mildew (*Erysiphe Polygoni* DC.). Whenever these plants were moved or shaken, small clouds of spores arose from leaves and stems. None of the Oenothera seedlings standing in the same house showed infection at the time of planting in the field about three months after being placed in the greenhouse. In the other greenhouse a few small plants of *Oenothera nutans* Atkinson & Bartlett, fall seedlings, which were kept during the winter and which were abundantly infected by mildew, were standing near the young seedlings. Before these seedlings were set out in the field, all the susceptible plants among them showed infection. A general exposure to conidia of *Erysiphe Polygoni* from pea did not infect any Oenothera seedlings, while a much less general exposure to conidia of *Erysiphe Polygoni* from *Oenothera nutans* resulted in the infection of a large number. This seems to support the statements of Salmon that, although no distinct morphological differences are found among the strains of *Erysiphe Polygoni* from different host plants, physiological differences may exist, upon which are based the so-called "biologic strains." Searle (11) also proved the existence of biologic strains of *Erysiphe Polygoni* among various hosts.

It was not considered necessary to make any extensive trials at cultivating the Erysiphe of Oenothera on artificial media, since the powdery mildews in general have been amply proved to be obligate parasites by Salmon and others. Salmon (9) found in his experiments that he could grow powdery mildew (oidium) on leaves of *Euonymus japonicus* L. placed on moist filter paper in a damp chamber for as long as 14 days, in which time the leaves were badly affected. In his experiments with biologic strains, Salmon kept his strains growing on living plants.

Examinations were made of prepared slides of leaves from the five different species and strains chosen, in order to determine if any morphological differences might account for the differences in susceptibility and immunity. No such differences were found among the morphological characters of the leaves, suggesting in this case that immunity must have a physiological or chemical basis. Salmon (8) reached the conclusion "*that susceptibility and immunity were due to constitutional* (*physiological*) *peculiarities and not to any structural ones.*"

Description of the Mildew, *Erysiphe Polygoni*

A study of the Oenothera mildew showed that in general it conforms to the description of *Erysiphe Polygoni* given by Salmon (7). It is amphigenous; mycelium very variable, persistent to evanescent, thin and effused; perithecia few and scattered, 85–95 μ in diameter, cells distinct, 11–14 μ wide; appendages simple and long, variable in number (3–6), partly interwoven with the mycelium and colorless; asci few (3–5), small and ovate, 50–60 $\mu \times$ 30–35 μ, containing 3–6 spores, 20–23 $\mu \times$ 9–12 μ. Conidia (oidium stage) cylindrical to ellipsoid, 33–36 $\mu \times$ 15–18 μ. The mildew grows very superficially, feeding by means of haustoria extending into the epidermal cells.

Powdery mildew may infect the host plant at any time. Infection in Oenothera is generally first noticed in leaves approaching maturity. Neither very young nor very old leaves will show any infection when a healthy plant is first attacked. At a later stage in the growth the mildew may cover the entire plant. Infections were found as early as April in the greenhouse, and in the fields as soon as the plants were set out. The heaviest infection in the field is commonly found in the summer from June to September, depending upon weather conditions, rainfall being conducive to the spreading of the disease. It is often found that during the summer time susceptible plants are entirely covered with mildew, so as to appear whitish. No evidence has been found that mildew does any great damage to Oenothera plants, as it does to the pea. Though entirely covered by Erysiphe, infected Oenotheras appear to grow normally, to come to bloom and to ripen seeds in exactly the same way, and presumably in the same amount, as healthy plants. Even though highly susceptible, species of Oenothera seem to be very tolerant to the disease.

In our experiments the plants have been under observation during the whole season, and have been classified as immune to powdery mildew if they have shown no infection at any time. It may be objected that in some cases immunity may have been only apparent and due to a position in the field preventing infection. This objection is easily answered. The plants were set out in the field in rows of from 150 to 160 each. Of each of the parent strains chosen, 25 plants were grown to maturity, and of the hybrids about 100 plants. Cultures differing in their susceptibility to mildew were grown near together, so that in many cases an immune strain or species was grown among highly susceptible strains, often so as to be entirely surrounded by them, and with intertwining branches. When a form remains free of mildew under such favorable conditions for infection, it may be called immune, especially when the disease spreads as easily as in the case of powdery mildews.

A second objection to our experimental procedure has been based on the supposition that somewhere there might exist strains of *Erysiphe Polygoni* which would infect the so-called "immune" strains of Oenothera. This is quite possible but hardly concerns us, since we have not been interested particularly in the production of disease-free Oenothera strains, but rather in the fact that immunity to certain strains of Erysiphe exists, and that such immunity acts as a dominant unit factor in heredity. Our data in regard to the inheritance of immunity or susceptibility concern a *certain biologic strain of Erysiphe Polygoni*, abundant upon Oenothera at Ann Arbor, Michigan. Other strains of Erysiphe might conceivably infect our "immune" types. In general, however, the types immune at Washington, D. C., also proved to be immune in Michigan.

General Statements in Regard to the Crosses Made

Of the five strains of Oenothera selected, each was crossed with the four remaining ones, and each was self-pollinated; together there were five self-pollinations, and 20 cross-pollinations in the first season.

It might be asked if crosses between two immune or two susceptible strains would not be superfluous. The genetical relations of the Oenothera have been proved to be different in so many instances from those of other plants that all the possible crosses were made. The results obtained showed that in one case susceptible plants were obtained by crossing immune strains (in *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*). In no case were immune plants obtained in a cross between two susceptible species.

Before going into detail regarding the crosses made and the F_1 generations produced, it will be well to state that the system used of designating the crosses is the conventional one. The pistillate parent is always named first, followed by the name of the pollen parent.

Since the prevalence of zygotic sterility is surely significant in connection with the explanation of genetical phenomena in Oenothera, it is perhaps of interest to state the germination data for the seeds of the five strains. It should be strongly emphasized, however, that seeds of very low viability are usually those produced too late in the season to ripen normally. In other words, the high proportion of bad seeds is partly due to environmental factors. Abundant seeds were obtained in every case. The highest germination obtained was in *Oe. mississippiensis*, with 43 percent germination; the lowest was in one culture of *Oe. cinerescens*, with no germination, probably an example of the effect of immaturity; another culture of the same species, but from a different individual plant, showed about 10 percent germination. The other three strains germinated as follows: "*Oenothera biennis Chicago*," 23.6 percent; *Oe. pratincola* hyb. *immunis*, 29 percent; *Oe. pratincola* ("Lexington C"), 29.4 percent.

From each of the crosses, whenever possible, about 500 seeds were sown, and of the plants obtained 100 were potted off and later planted in the field. The data included in this paper extend to the F_2 generations obtained by the self-pollination of typical F_1 plants.

A few words in regard to metacliny will not be out of place at this time. As has been said, an Oenothera hybrid is an $\alpha\beta$ combination and usually similar in most of its characters to one of its parents. Sometimes in hybrid progenies a few plants are observed like the other reciprocal cross. These are metaclinic plants and are interpreted as $\beta\alpha$ combinations. In these cases the β gamete is the female (comes from the pistillate parent) and the α gamete is male (from the pollen parent).

Groups of Crosses

I. Crosses between *Oenothera mississippiensis* (susceptible) and *Oenothera cinerescens* (immune).

The F_1 plants produced in the cross *Oe. mississippiensis* × *Oe. cinerescens* were, in each of two crosses made, all of the *mississippiensis* type and showed abundant infection with mildew, except that in one of the progenies there was one metaclinic plant of the type of *Oe. cinerescens*, which was immune. The F_2 plants, obtained by self-pollination of typical F_1 plants from both crosses, were entirely similar to the F_1 plants, both in external morphological characteristics and in the degree of susceptibility.

In the reciprocal cross (*Oe. cinerescens* × *Oe. mississippiensis*) twin hybrids of the *Oe. cinerescens* type were obtained, both types immune to mildew. There was one metaclinic plant, of the type of *Oe. mississippiensis*, which was susceptible. The self-pollinated matroclinic plants of the F_1 gave no seeds, and the most essential data on the F_2 are therefore lacking. The single metaclinic plant, however, gave seeds by self-pollination and produced, in the F_2, susceptible plants similar to itself and to those of the cross *Oe. mississippiensis* × *Oe. cinerescens.*

II. Crosses between *Oenothera mississippiensis* (susceptible) and *Oenothera pratincola* hyb. *immunis* (immune).

In the cross *Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis*, all the plants of the F_1 produced were of the *mississippiensis* type, and immune towards powdery mildew. The F_2 plants were again of the *mississippiensis* type, with some slight morphological differences between two cultures coming from two different individuals of the same F_1 culture, which, however, were not detected as different when self-pollinated. All the plants obtained in both cultures were immune.

In the F_1 of the reciprocal cross (*Oe. pratincola* hyb. *immunis* × *Oe. mississippiensis*), all the plants with one exception were matroclinic, except for lack of mildew resistance. (The one exceptional plant was a mutation.) All the plants were susceptible and of the type of *Oe. pratincola.*

The F_2 plants from the reciprocal cross, *Oe. pratincola* hyb. *immunis* × *Oe. mississippiensis*, were of three types, all closely resembling *Oe. pratincola*. All the plants were susceptible, as in the F_1.

III. Crosses between *Oenothera mississippiensis* (susceptible) and "*Oenothera biennis Chicago*" (susceptible).

In the cross *Oe. mississippiensis* × "*Oe. biennis Chicago*," all F_1 hybrids, with the exception of one, proved to be matroclinic, while the exceptional plant was metaclinic and of the *Oe. pratincola* type. (See description of "*Oe. biennis Chicago.*")

Among the F_1 hybrids of the reciprocal cross ("*Oe. biennis Chicago*" × *Oe. mississippiensis*), three plants of the *mississippiensis* type were produced, while the rest were of the "*Oe. biennis Chicago*" type, again showing matroclinic inheritance with a tendency toward metacliny. One mutation (of the *latifolia* type) was produced in one of these reciprocal crosses.

All the plants of this pair of reciprocals were mildewed. No difference seemed to exist in the degree of susceptibility, and, because both parents are susceptible in the same degree, no other data could be obtained on this point. The F_2 plants of both reciprocals were respectively of the same general type as the F_1, and all plants were susceptible.

IV. Crosses between *Oenothera mississippiensis* (susceptible) and *Oenothera pratincola* ("Lexington C") (susceptible).

The F_1 and F_2 generations of the cross *Oe. mississippiensis* × *Oe. pratincola* consisted of only one matroclinic type. All the plants were susceptible.

The F_1 of the reciprocal cross, *Oe. pratincola* × *Oe. mississippiensis*, was likewise of one general type, similar to *Oe. pratincola*, another illustration of matroclinic inheritance. A part of the plants, however (28 out of 100), showed a distinct yellowish-green coloring and mottling of the leaves, in some cases going over to white, especially at the margins of the leaves. On this account the culture might be interpreted as consisting of very closely similar twin hybrids, both, however, resembling the pistillate parent (*Oe. pratincola*) in external characters, and probably only slightly different in genetical constitution. All the plants were susceptible, somewhat more so than those of the reciprocal. The F_2 repeated the two types of the F_1, with some slight segregation in morphological characters, but all plants were susceptible.

V. Crosses between "*Oenothera biennis Chicago*" (susceptible) and *Oenothera cinerescens* (immune).

All the plants of the F_1 produced from the cross "*Oe. biennis Chicago*" × *Oe. cinerescens* were of the *Oe. pratincola* type. (See description of "*Oe. biennis Chicago.*") All were susceptible. No seeds were obtained by self-pollination, and consequently no F_2 can be reported.

The F_1 plants of the reciprocal (*Oe. cinerescens* × "*Oe. biennis Chicago*") were all of the *cinerescens* type, with the exception of two metaclinic plants of the *pratincola* type. All *cinerescens*-like plants were immune, in both the F_1 and the F_2. Both metaclinic plants were resistant.

VI. Crosses between "*Oenothera biennis Chicago*" (susceptible) and *Oenothera pratincola* hyb. *immunis* (immune).

The cross "*Oe. biennis Chicago*" × *Oe. pratincola* hyb. *immunis* gave an F_1 generation of one type (similar to *Oe. pratincola*). All the plants were immune to mildew. No F_2 was obtained.

The F_1 plants of the reciprocal cross (*Oe. pratincola* hyb. *immunis* × "*Oe. biennis Chicago*") were likewise of one type (*Oe. pratincola*). All the plants obtained were susceptible to mildew, and gave an identical, susceptible F_2.

In these crosses the factors determining immunity are in the β gametes, and immunity is therefore a patroclinic character.

VII. Crosses between "*Oenothera biennis Chicago*" (susceptible) and *Oenothera pratincola* ("Lexington C") (susceptible). These crosses offer the same difficulty as the former in regard to the differentiation of types in the progenies, since the parents are themselves doubtfully distinguishable.

In the cross "*Oe. biennis Chicago*" × "Lexington C," all F_1 plants with the exception of three were *pratincola*-like, the three exceptions being mutations. Two of these were similar (probably of one type) and showed a slight susceptibility towards mildew. The third was of a different type and was very susceptible towards mildew. All the typical plants were highly susceptible. The F_2 was the same as the F_1. In the reciprocal cross ("Lexington C" × "*Oe. biennis Chicago*") all plants of the F_1 and F_2 generations were of one type and very susceptible.

The only statement that can be made in regard to inheritance of susceptibility in these crosses in which both parents are highly susceptible is that the offspring are likewise highly susceptible. The two slightly susceptible mutations show that a marked degree of resistance may be acquired as a result of mutational change.

VIII. Crosses between *Oenothera cinerescens* (immune) and *Oenothera pratincola* hyb. *immunis* (immune).

The F_1 generation of the cross *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis* consisted of two *cinerescens* types, one of them being similar to *Oe. cinerescens* in nearly all respects, the other a smaller or dwarf type. All plants were immune, and gave an immune F_2 like the F_1, with a few mutations.

In the reciprocal cross (*Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*) the F_1 generation consisted of only one type of plants (a small *pratincola* type), all of which were susceptible to mildew. The F_2 showed a splitting into two types, one more delicate than the other, but both *pratincola*-like and both heavily mildewed.

These crosses show conclusively that the female α gametes of the hyb. *immunis* do not carry factors for immunity, but that the male β gametes do. Consequently, any combination to which hyb. *immunis* contributes the egg will be immune only providing the pollen parent produces male gametes with the immunity factor.

IX. Crosses between *Oenothera cinerescens* (immune) and *Oenothera pratincola* ("Lexington C") (susceptible).

All the F_1 plants produced from the cross *Oe. cinerescens* × "Lexington C" were of the *Oe. cinerescens* type and similar to the larger one of the twin hybrids produced in the cross *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*.

All plants produced were immune to mildew. The F_2 plants were similar to the F_1 and immune.

In the reciprocal cross, "Lexington C" × *Oe. cinerescens*, all F_1 plants were of the *Oe. pratincola* type and susceptible. The F_2 split into two types, both in general like *Oe. pratincola*. All the plants of both types were susceptible.

X. Crosses between *Oenothera pratincola* hyb. *immunis* (immune) and *Oenothera pratincola* ("Lexington C") (susceptible).

All F_1 and F_2 plants of the cross *Oe. pratincola* hyb. *immunis* × "Lexington C" were of the type of *Oe. pratincola* and susceptible, while all plants in the F_1 of the reciprocal cross, *Oe. pratincola* × *Oe. pratincola* hyb. *immunis*, were of the hyb. *immunis* type and immune. Among the F_2 plants of the reciprocal were several mutations and a few metaclinic plants, the latter being highly resistant. All other plants, including the mutations, were immune.

Conclusions

In several of the foregoing cases in which metaclinic plants were produced in crosses between immune and susceptible parents, the immunity-factor combination which would insure susceptibility or immunity in one particular type seems not to insure the same effect in another type. Investigations are now started to prove, in these cases, whether or not the expression of the immunity factors is influenced by morphological characters. In other words, is it possible that types may exist in which susceptibility is so great that one **I** factor will not confer immunity, whereas in other types the factor complex, without **I**, is so highly resistant that the addition of **I** confers complete immunity? The explanation of the phenomena presented by metaclinic plants must be deferred. As far as the normal hybrids are concerned, the results are all consistent and lead to definite conclusions.

The results accord perfectly with the following hypotheses:

1. The factor for immunity (**I**) is dominant. If it enters the zygote from either side, the plant produced is immune.

2. In all the five strains involved in the experiments, the eggs are different from the sperms. The former are α gametes, the latter β gametes. A few exceptions to this general rule are indicated by the rare appearance of metaclinic plants in the progenies. Whereas a normal hybrid is an $\alpha\beta$ combination, the metaclinic hybrid is $\beta\alpha$.

3. In both the immune strains, the immunity is due to an unbalanced factor for immunity in the zygote. In *Oe. cinerescens* this factor is strictly associated with the α gamete, and in *Oe. pratincola* hyb. *immunis* with the β gamete.

4. Representing immunity and susceptibility by capital **I** and small **i** respectively, the zygotic composition and reaction to mildew of the five strains are as follows:

Oe. pratincola hyb. *immunis*, $\alpha i\beta I$, immune.
Oe. cinerescens, $\alpha I\beta i$, immune.
Oe. mississippiensis, $\alpha i\beta i$, susceptible.
"*Oe. biennis Chicago*" (a strain of *Oe. pratincola*), $\alpha i\beta i$, susceptible.
Oe. pratincola ("Lexington C"), $\alpha i\beta i$, susceptible.

5. The composition and reaction to mildew of the several F_1 hybrids must therefore be as formulated below:

Oe. mississippiensis × *cinerescens*, $\alpha i\beta i$, susceptible.
Oe. cinerescens × *mississippiensis*, $\alpha I\beta i$, immune.
Oe. mississippiensis × *Oe. pratincola* hyb. *immunis*, $\alpha i\beta I$, immune.
Oe. pratincola hyb. *immunis* × *Oe. mississippiensis*, $\alpha i\beta i$, susceptible.
Oe. mississippiensis × "*Oe. biennis Chicago*," $\alpha i\beta i$, susceptible.
"*Oe. biennis Chicago*" × *Oe. mississippiensis*, $\alpha i\beta i$, susceptible.
Oe. mississippiensis × *pratincola*, $\alpha i\beta i$, susceptible.
Oe. pratincola × *Oe. mississippiensis*, $\alpha i\beta i$, susceptible.
"*Oe. biennis Chicago*" × *cinerescens*, $\alpha i\beta i$, susceptible.
Oe. cinerescens × "*Oe. biennis Chicago*," $\alpha I\beta i$, immune.
"*Oe. biennis Chicago*" × hyb. *immunis*, $\alpha i\beta I$, immune.
Oe. pratincola hyb. *immunis* × "*Oe. biennis Chicago*," $\alpha i\beta i$, susceptible.
"*Oe. biennis Chicago*" × *pratincola*, $\alpha i\beta i$, susceptible.
Oe. pratincola × "*Oe. biennis Chicago*," $\alpha i\beta i$, susceptible.
Oe. cinerescens × hyb. *immunis*, $\alpha I\beta I$, immune.
Oe. pratincola hyb. *immunis* × *Oe. cinerescens*, $\alpha i\beta i$, susceptible.
Oe. cinerescens × *Oe. pratincola*, $\alpha I\beta i$, immune.
Oe. pratincola × *Oe. cinerescens*, $\alpha i\beta i$, susceptible.
Oe. pratincola hyb. *immunis* × *pratincola*, $\alpha i\beta i$, susceptible.
Oe. pratincola × hyb. *immunis*, $\alpha i\beta I$, immune.

In every case the reaction of the hybrid to mildew conformed exactly to expectations, according to the formulation above.

6. On account of their peculiar type of heterogametism, immunity due to a single factor must breed as true as that due to a factor pair. Of the total number of 20 hybrids, 13 were susceptible, 3 had a single factor for immunity, derived from the maternal parent, 3 had a single factor for immunity, derived from the paternal parent, and one only had double immunity, derived from both parents.

7. In accord with the hypothesis of immunity advanced above, combined with the hypothesis of heterogametism, the F_2 generation by self-pollination of F_1 plants should be the same, in regard to immunity or susceptibility, as the F_1. This conclusion has been amply proved.

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