INCOMPATIBILITY STUDIES IN *OENOTHERA*: THE DISTRIBUTION OF S_I ALLELES IN *BIENNIS* 1 POPULATIONS[1]

ERICH STEINER
Department of Botany, University of Michigan, Ann Arbor, Michigan

Accepted March 13, 1964

The studies of the population structure of the North American Euoenotheras which have been carried out largely by Cleland and his associates have analyzed the segmental arrangement and phenotypes of the genomes in plants collected from a wide range of geographical locations (see Cleland, 1958 for a summary of this work). Although some collections have included three or four different plants from the same population, other than the work of Winterheimer (unpublished), relatively little has been done to investigate in more detail a sizable sample from a single stand of plants. Because this type of study requires on the average 15 to 20 hybrids for each collection analyzed, the size of the population sample undertaken for investigation tends to be limited to modest numbers.

The discovery of an incompatibility allele system in the complex heterozygotes of *Oenothera* (Steiner, 1956) suggested another approach to population analysis, which, although providing in part different data, allows an estimate of the diversity of single populations as well as enabling an appreciable number of plants to be included in the study.

The group of oenotheras best suited for this type of analysis is known as the *biennis* group 1 (Cleland et al., 1950). Members of this group are typical complex heterozygotes, composed of two genomes or "complexes" involving seven chromosomes each. These differ completely in the arrangement of their chromosome segments, so that metaphase I of meiosis is characterized by a ring of 14 chromosomes. The adjacent chromosomes of the ring regularly go to opposite poles at anaphase so that only two kinds of spores, and subsequently, gametes, are formed. Although these plants are normally self-pollinated, the homozygous complex combinations do not occur because of a balanced lethal system. Thus, the offspring are, like the parent, complete translocation heterozygotes which continue to breed true in succeeding generations.

When different strains of complex heterozygotes are outcrossed, it becomes apparent that of the two complexes making up a plant, one is usually transmitted more readily through the egg, and the other more frequently through the pollen. On this basis, the two complexes are identified as alpha and beta, the alpha characteristically coming through the egg, the beta through the pollen. In addition, the alpha and beta complexes of a particular strain differ not only in the segmental arrangement of their chromosomes and their transmission behavior, but also produce distinctive phenotypes.

Races differ in the extent of transmission of their complexes through egg and pollen. Some pass on only the alpha through the egg and the beta through the pollen; others may transmit the alpha through the pollen and the egg but only the beta through the pollen. Still others show offspring receiving both alpha and beta through pollen and egg; races also occur which transmit the alpha alone through the egg, but both complexes through the pollen.

By intercrossing appropriate *biennis* group 1 races, alpha·alpha combinations can readily be obtained. These are invariably self-incompatible because of the failure of pollen tubes to develop; when these plants are outcrossed either as male or female parents, they are perfectly compatible. An analysis of the compatibility behavior of *biennis* 1 alpha·alpha showed that the alpha complexes carry an incompatibility allele system of the gametophytic type

[1] Supported by Grant G-23596 from the National Science Foundation.

(Steiner, 1956, 1957). In the naturally occurring complex heterozygote the S allele acts as a pollen lethal. Pollen grains carrying the alpha complex do not germinate on the stigma of the plant producing them. Thus only the pollen with the beta complex can develop and fertilize the eggs.

One of the interesting questions about the incompatibility mechanism in the complex heterozygotes is whether the plants of a single stand all possess identical S alleles or whether a few or many different alleles are present. From the standpoint of its function in the complex heterozygote, i.e., as the pollen lethal of the balanced lethal system, there would appear to be no advantage for the members of a single population to carry different S alleles. This is, of course, in contrast to structurally homozygous species exhibiting a gametophytic incompatibility mechanism in which each population is characterized by a great diversity of alleles. In the complex heterozygote it may even be argued that a selective advantage lies in members of the same population having identical alleles; if some hybridization does occur between members of the population, the presence of identical alleles would exclude the possibility of obtaining alpha·alpha combinations among the hybrid offspring. Because alpha·alphas are generally less viable than alpha·betas, their failure to occur would enhance the survival potential of the population.

The current study is an analysis of three separate *biennis* 1 populations to determine the number and distribution of the incompatibility alleles in the alpha complexes of a representative sample of their members.

Materials and Methods

Plants were collected in early May while yet in the rosette stage or just beginning to form shoots. Population I was gathered on the outskirts of Indianapolis, Indiana, population II 2 miles south of Bloomington, Indiana, and population III in Lexington, Kentucky. Each site was adjacent to railroad tracks. In the first two collections, the plants were growing in cinders forming the roadbed; population III was found on a bluff just above the railroad tracks growing in a light clay. The plants were transplanted to pots or flats and later set out in the experimental garden. In each case a sample of 40 to 50 plants of the actual population was brought into the garden. The plants were cut back severely, first, to enhance the probability of survival after transplanting, and secondly, to delay their development, because the cultures to which they later were to be crossed ordinarily do not bloom until late summer under conditions in southern Michigan.

Each plant was crossed with a hybrid carrying the alpha complex of *Hot Springs* and the beta complex of *Camp Peary L*; both parents are *biennis* group 1 races. The hybrid is true breeding, exhibiting a circle of 14 chromosomes at meiosis, and thus fundamentally no different from the naturally occurring complex heterozygotes. It does possess, however, the important property of transmitting the alpha *Hot Springs* complex through the pollen at a high frequency. Therefore, if it is used as the male parent, its contribution to the offspring is most likely to be alpha *Hot Springs* rather than beta *Camp Peary L*, in spite of the latter being a true pollen complex. By using the plants of the collections as female parents, it is possible to obtain the alpha complex of most of them in combination with the alpha *Hot Springs* complex. These alpha·alphas should be self-incompatible. Because all carry in common the alpha *Hot Springs* complex contributed by the male parent, pollen bearing the S allele of *Hot Springs* will not grow in any crosses between them. Therefore, if pollen tubes are produced in such a cross pollination, the incompatibility alleles in the alpha complexes of the two members of the population must be different. On the other hand, if the pollen fails to develop, the two alleles must be identical. By intercrossing the alpha·alphas derived from crossing each member of a population with the synthetic hybrid, alpha *Hot Springs*·beta *Camp Peary L*, it is thus possible to determine which members of the population carry identical, and which carry different S

alleles. In addition, by intercrossing the compatibility types identified in each population with those of the other populations, one can compare the S alleles found in the different populations.

At the same time, by determining the chromosomal configuration of the alpha·alpha hybrids, some information about the segmental arrangement of each alpha complex of the population can be obtained. A configuration of seven pairs of chromosomes would indicate that the segmental arrangement is identical to that of alpha *Hot Springs,* namely, 1·2 3·4 5·14 7·10 9·8 11·12 13·6. Any other configuration would indicate that the alpha complex was characterized by another arrangement, although in the absence of other hybrid combinations the particular arrangement could not be determined. Because the arrangement found in alpha *Hot Springs* has proved to be widespread among alpha *biennis* 1 complexes, it seemed worthwhile to determine whether in fact the alpha complexes of these populations carried the same or different arrangements. Cytological preparations for determining the chromosome configurations were made according to the method of Cleland et al. (1950).

Although *biennis* 1 alpha·alpha hybrids are in general phenotypically quite uniform regardless of the races from which they are derived, a comparison of the phenotypes of the different alpha·alpha hybrids was made with the hope of gaining additional information about the alpha complex composition of the populations under study.

From the crosses made with each population sample, 25 were selected at random for analysis. Cultures 200 through 225 represented crosses with members of population I; 226 through 250, population II; and 251 through 275, population III. Each culture was tested for compatibility with every other culture of that series. Preliminary tests showed that reciprocal crosses gave identical results, as predicted on theoretical grounds; this enabled a reduction in the number of tests of essentially one-half.

The compatibility tests were carried out as follows: flowers of the female parent were emasculated in early morning in order to obtain stigmas as mature as possible without the anthers of the flower having already shed their pollen. These flowers would ordinarily have opened on the day following emasculation. The emasculated flowers were bagged and allowed to remain on the plant for 24 hours, during which time the stigmas became receptive. The flowers were then collected in vials filled with tap water and pollinated. A single test involved in most cases three flowers to which the same kind of pollen was applied. Pollen was obtained from unopened buds, whose anthers, however, had already dehisced. Following pollination, the flowers were placed in a dark incubator at 26° C for 24 hours. The styles were then removed and fixed in 30% alcohol–iodine–potassium iodide. After 24 hours of fixation the styles were processed by laying those of a single test, numbering usually three, on a microscope slide, covering them with a second slide, and pressing until they were flattened. These preparations were examined immediately under a dissecting binocular to determine whether pollen tubes had developed or not.

Results

Germination and seedling survival were uniformly good. The seed of only two cultures (238, 240) out of the 75 failed to germinate. Only in one case (250) were all of the seedlings of a culture lost. Of the remaining 72 cultures all except five had ten plants of each set out into the field; no culture in the field consisted of less than four plants. Most of the cultures were composed entirely or predominantly of alpha·alpha combination; only four cultures totally lacked alpha·alphas.

Cultures carrying the alpha complexes of population I showed extensive chlorosis, which, however, did not become apparent until the plants had been in the field plot for several weeks. Many of the plants were weak, pale green in color, reaching a height

on the average of only 18 inches and showing little branching. Those plants of a culture which were more nearly a normal green or had only a few chlorotic segments developed into moderately vigorous individuals similar to the alpha·betas, but nevertheless showing a characteristic phenotype.

Among the alpha·alphas derived from population I, two distinct phenotypes were recognized. The predominant one, found in all except four cultures, was characterized by cauline leaves twisted in appearance tending to curl downward from base to tip as well as along the margins; the leaves also frequently showed mottling with irregular albino or pale green patches. The stem tips of these plants were of two types. One developed normally, producing large, glabrous buds. In contrast many of the branches had at their tips a tight cluster of small, reddish buds giving the appearance of a rosette. After reaching a length of 7 to 9 mm, these buds aborted. This phenotype, which for convenience will be designated as type A, also occurred among the alpha·alphas derived from population II, but only in two cultures. It was absent among the cultures stemming from population III.

The second alpha·alpha phenotype (type B), found in the first series in cultures 200, 212, 214, and 225, appeared more nearly normal in comparison to a naturally occurring alpha·beta. The leaves were undistorted and uniformly light green in color. The plants were reasonably vigorous with no abnormal stem tips. The buds were somewhat smaller and with more pubescence than the normal ones of type A.

In the series of hybrids derived from population II chlorotic plants were exceptional in contrast to the series from population I where they were predominant. The alpha·alphas of population II were all of phenotype B with the exception of cultures 242 and 247; these were of type A. The hybrids derived from population III exhibited the B phenotype except that the leaf color tended to be a darker green than that found among alpha·alphas of populations I and II.

Chromosome configurations were determined for alpha·alphas of all except five cultures (203, 242, 252, 258, and 264). The plants of all cultures exhibited seven pairs of chromosomes at meiosis with the exception of four (200, 212, 214, and 225) which instead showed a circle of four and five pairs of chromosomes. Since the alpha *Hot Springs* complex with which each of the alpha complexes of the collections was combined has the segmental arrangement, 1·2 3·4 5·14 7·10 9·8 11·12 13·6, this must be the arrangement of all alpha complexes except those of the four cultures having the circle of four and five pairs. The latter have an arrangement one interchange removed from the typical alpha *biennis* 1 arrangement; in this case it seems safe to predict that the same interchange was involved in all four cultures. No attempt was made in this study to identify the specific chromosomes involved in the interchange giving the circle of four.

No attempt was made to survey in detail the cytological configurations of the original collections. A few plants were sampled in each of the populations to verify the expected configuration of a circle of 14 chromosomes. Populations II and III proved to be phenotypically uniform, but population I showed two phenotypes, the one characterized by buds with erect, glandular hairs and spreading sepal tips and ovaries with appressed pubescence, the other identified by glabrous buds with appressed sepal tips and glabrous ovaries. Cytological determinations revealed that the type with spreading sepal tips was characterized by a circle of ten and a circle of four chromosomes at meiosis. The remaining type showed the expected circle of 14 found in the majority of collections from nature of complex heterozygotes. The four cultures, 200, 212, 214, and 225, that had a circle of four and five pairs instead of seven pairs were all derived from plants showing circles of ten and four. In the original collection there were seven plants showing the two circles.

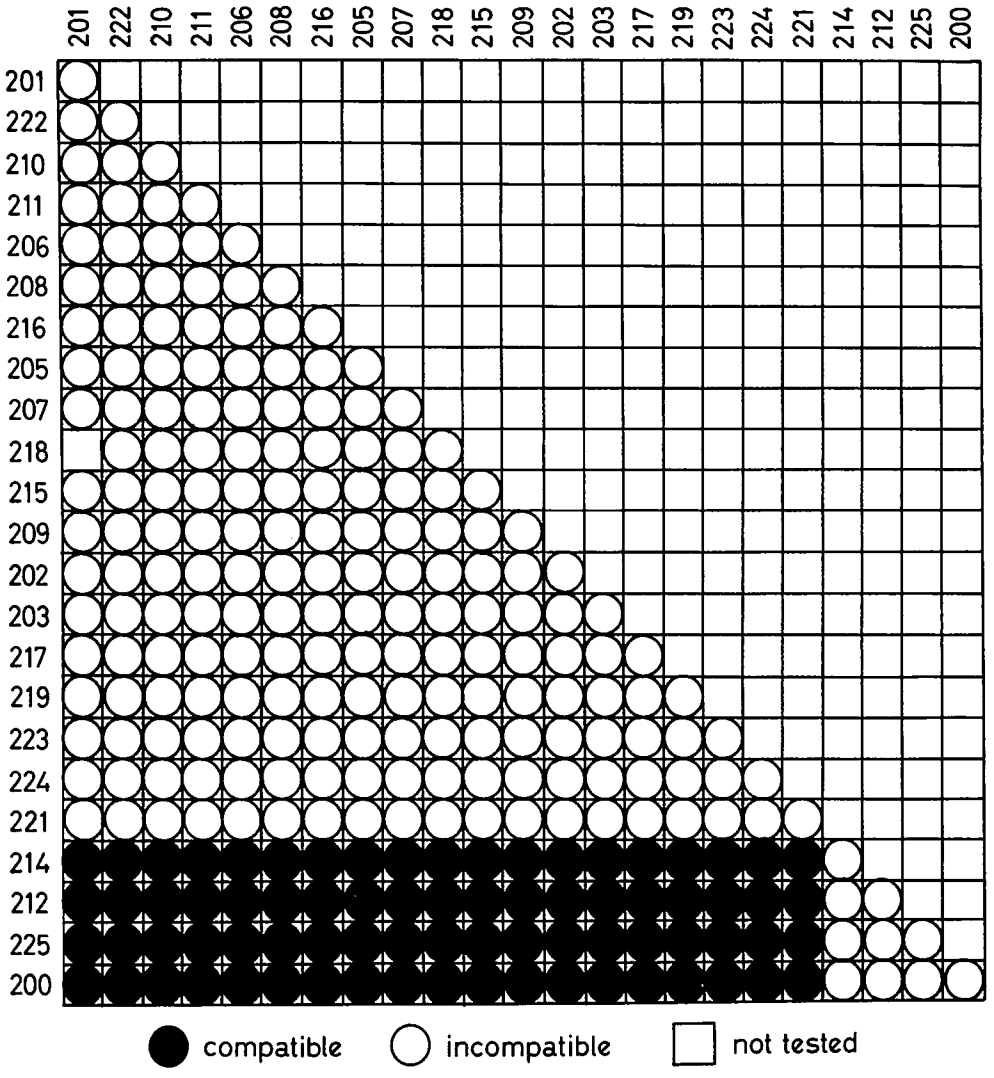

FIG. 1. Compatibility relationships of alpha·alphas derived from population I.

The compatibility relationships between the members within each population are shown in the first three figures. Fig. 4 shows the relationships between each of the types found in the different populations. The rearrangement of cultures in fig. 1 to deviate from the regular numerical sequence was a practical consideration stemming from the fact that the extensive chlorosis and resultant poor vigor of many of the plants made it easier to use the more vigorous plants as the source of emasculated flowers and the less vigorous as pollen parents. In the series from populations II and III the vigor of the plants was no problem and the tests were made merely according to the original sequence of the cultures.

The figures show clearly that each population consists of two compatibility types, that is, each alpha complex in the population carries one or the other of the two S alleles. For convenience the allele of most frequent occurrence in population I can be

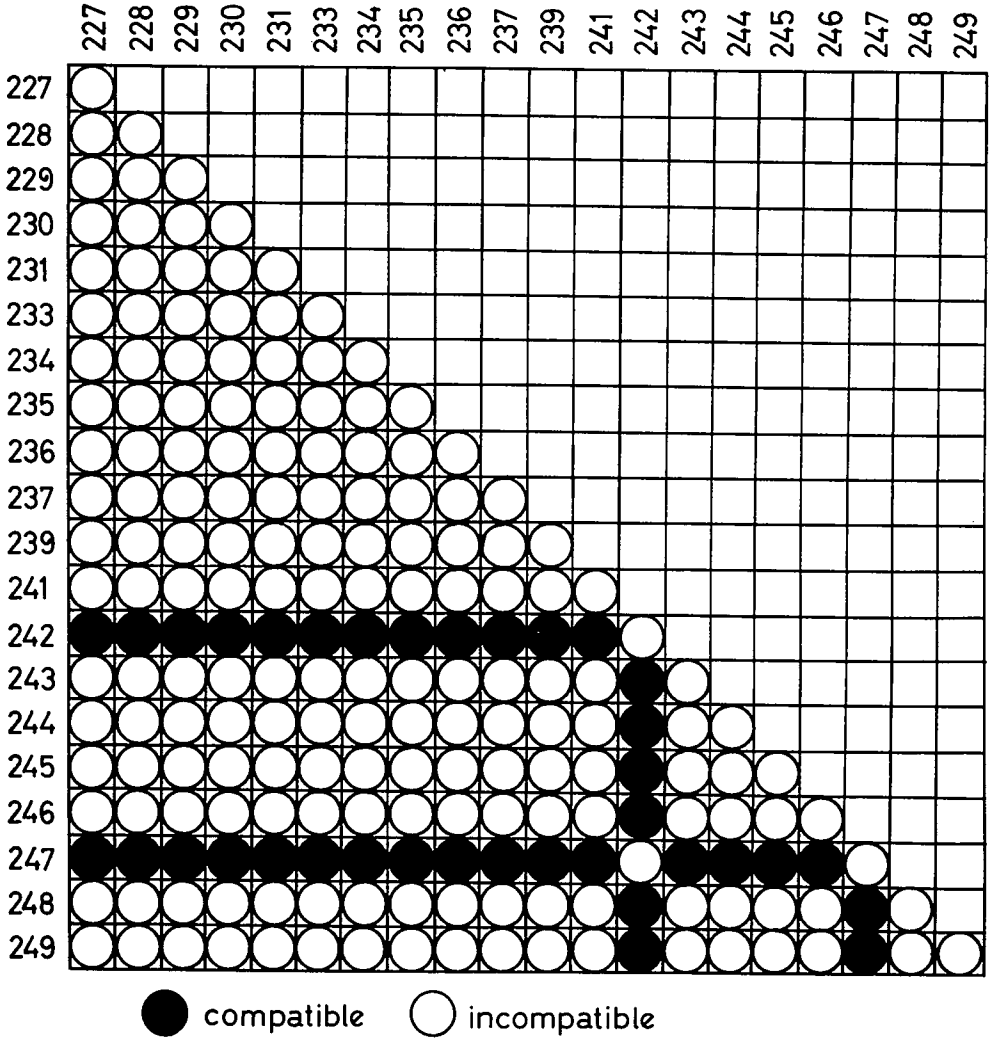

FIG. 2. Compatibility relationships of alpha·alphas derived from population II.

designated as S_a and the less frequent, S_b.[2] From fig. 4 it is apparent that population II carries the same alleles, S_a and S_b, but figs. 1 and 2 show that the frequencies are essentially reversed, with S_a occurring in only two cultures (242 and 247). As might be expected from the phenotypic data, the four cultures of population I which show a different segmental arrangement from the others carry the S_b allele. It is interesting to note, however, that the alpha complexes in population II which carry the S_b allele do not have the atypical segmental arrangement with which the S_b allele is associated in population I.

Fig. 4 also shows that of the two alleles in population III, only one is common to the other populations, namely S_b; thus,

[2] In the earlier studies with S alleles of alpha *biennis* 1 complexes a numerical subscript was used to designate the allele of a particular complex. Rather than assign numbers to the alleles identified in the present study before their identity or dissimilarity with previously designated alleles has been determined, letters are used here temporarily.

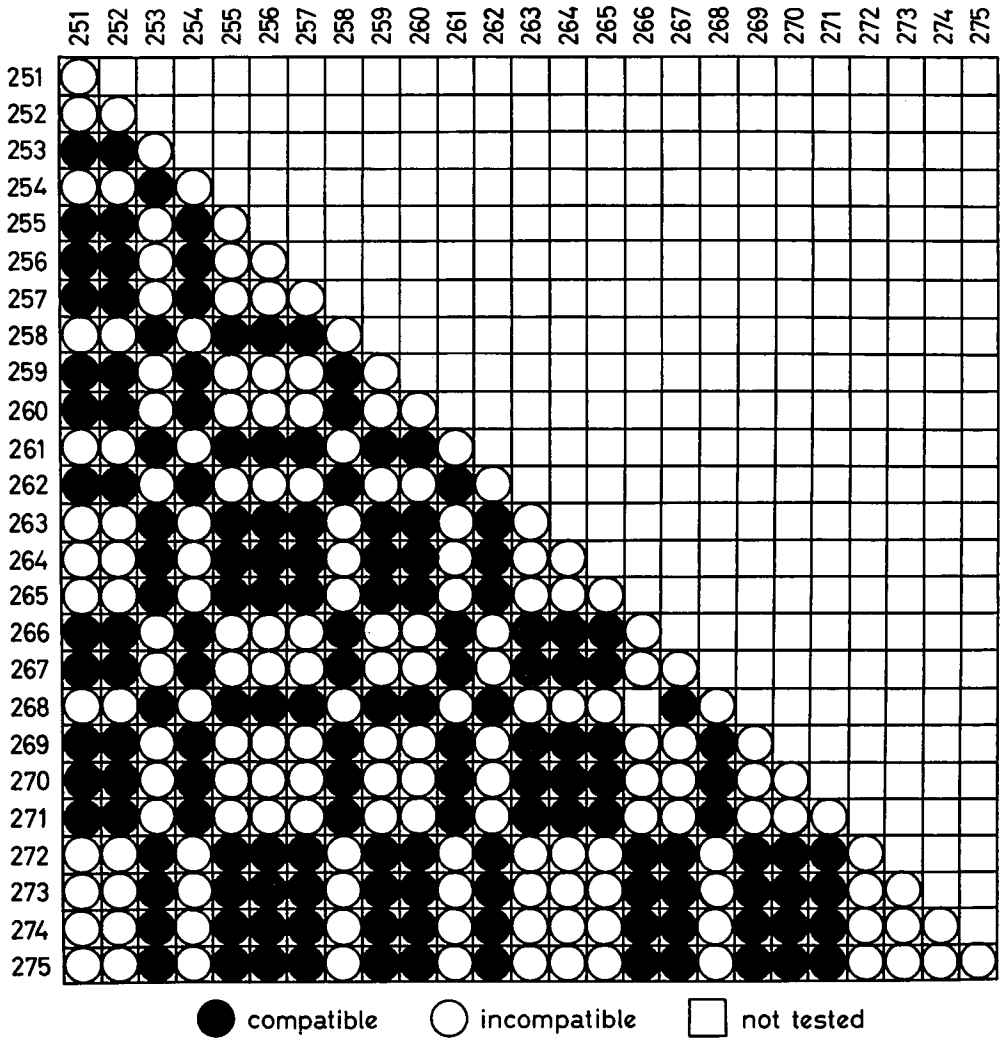

FIG. 3. Compatibility relationships of alpha·alphas derived from population III.

population III carries an allele which can be designated S_c for the time being. In population III the distribution of the two alleles is nearly equal in contrast to the distribution in the other populations.

DISCUSSION

In an earlier study, Schooley (1962) analyzed for identity of incompatibility alleles the alpha complexes of many of the *biennis* group 1 races which had been cytogenetically characterized by Cleland and his associates. Schooley found among 21 different alpha *biennis* 1 complexes a total of 15 different incompatibility alleles. Further, in each of two instances, two races collected in the same locality proved to have different S alleles. As a result, it seemed reasonable to expect some allelic diversity even within the same population of plants. It was thus surprising in the present study that two of the populations which were 50 miles apart carried identical alleles, while a third population 170 miles distant had one allele in common with the other two.

The relative uniformity of the populations under study with regard to S alleles may be explained in the following way. Because complex heterozygotes are inbred lines which nevertheless retain a high level of hybrid vigor, they must be ideal colonizers of new habitats. A single, well-developed plant produces thousands of seeds capable of giving rise to a population which, barring mutations, is essentially clonal. Such a population would possess, of course, only a single incompatibility allele. Thus, if more than one allelic type is found in a population, it may indicate that the population was established by colonizers of more than a single origin.

The considerable allelic diversity which is found among the alpha *biennis* 1 complexes as a whole remains a puzzle. That the different incompatibility alleles arose after the origin of the *biennis* 1 complex heterozygotes is unlikely, if for no other reason than the fact that their role as pollen lethals in these self-pollinating forms is unchanged, whether there be a single or innumerable different alleles in the population. If, as previously suggested (Steiner, 1956), the *biennis* group I arose through the hybridization of two structurally homozygous, but segmentally differentiated ancestral populations, one a *biennis* type which was self-incompatible, and the other a self-compatible *strigosa* type, the allelic diversity suggests that repeated hybridizations must have occurred to form the population of complex heterozygotes. In each case a different plant of the ancestral *biennis* population serving as a parent contributed a particular incompatibility allele to the hybrid. In this way a number of the alleles of the ancestral self-incompatible population came to exist in the complex heterozygotes derived from it.

The variation in the frequencies of the two alleles found in each of the analyzed populations may be the result of differences in the adaptive value of the alpha complexes involved. As a part of the alpha complex of the complex heterozygote, the S allele has no opportunity to segregate. Since selection operates on the total complex, the extent to which the particular incompatibility allele influences the end result is difficult to assess. The frequency of the incompatibility alleles may be determined, therefore, not so much by the alleles themselves, but by the other genes to which they remain effectively linked.

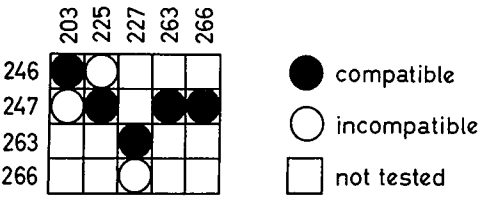

FIG. 4. Compatibility relationships between compatibility types identified in populations I, II, and III.

It is perhaps unfortunate that the collections for these experiments were all made along railroad tracks; it is likely that this type of habitat tends to be more or less continuously occupied by oenotheras because the maintenance of the roadbed prevents succession. In other situations, such as abandoned fields, the *Oenothera* populations are probably largely transient and may represent single colonizations which do not persist more than one or two generations. In this connection it would be interesting to know whether new populations arise largely from freshly distributed seed or whether old seed which has remained in the soil for years germinates in an appreciable frequency when conditions become favorable. If both newly dispersed and old seed contribute to the establishment of a population, one might expect it to be more diverse than if only one type of seed is involved.

The occurrence in population I of plants with a cytological configuration of a ⊙ 10, ⊙ 4 is interesting, because relatively few collections in this part of the country have shown other than a ⊙ 14. The fact that these plants possessed a distinctive phenotype and were represented by a number of individuals suggests that they constituted an inbred line descended from a plant in which the chromosomal interchange had occurred. If this is true, it is difficult to

say how recently the interchange had occurred. Unfortunately, none of these plants showing the two circles was selfed and it is not known whether they were able to breed true.

The segmental arrangement of all alpha complexes in these populations with the exception of those bearing the above-mentioned interchange was that found to be typical of the earlier *biennis* 1 collections analyzed by Cleland and his associates. Similar results have been obtained by Winterheimer (personal communication), who analyzed three populations growing within a radius of 1 mile for segmental arrangement; the alpha complexes all possessed the typical alpha *biennis* 1 arrangement. However, these populations were characterized by three different beta complex arrangements. In the present study it would have been interesting to know what arrangements the beta complexes exhibited, but the analysis would have been a task of major proportions for the number of individuals concerned and thus not feasible from a practical standpoint.

One contribution of the current study is that the feasibility of this type of population analysis is established. Extension of this technique to study a limited geographical area intensively as well as to make a more extensive sampling of the total range of the *biennis* group 1 is unquestionably practical and should lead to a better understanding of the population dynamics of *Oenothera*. One of the striking observations in this work has been the unequivocal nature of the compatibility tests. In every case the pollen tubes clearly either grew, or did not grow; when the result was negative, inhibition always occurred in the stigma under the conditions of the experiment. The perfect correlation between the results and the predictions on the basis of the incompatibility allele hypothesis adds convincing additional support to the view that an incompatibility allele system does operate in *biennis* group 1 complex heterozygotes.

Summary

Three population samples of *Oenothera biennis* (*biennis* group 1 in Cleland's classification), collected in Indianapolis, Indiana; Bloomington, Indiana; and Lexington, Kentucky, respectively, were analyzed for identity and distribution of incompatibility alleles.

Each population sample was characterized by the presence of two different incompatibility alleles.

Population samples I and II possessed identical alleles, but in significantly different frequencies. Population sample III had one allele in common with the other two populations. The two alleles of sample III occurred in essentially equal frequencies.

The significance of these results is discussed.

Acknowledgments

The capable assistance of Mrs. R. Hubert is gratefully acknowledged. Thanks are also due the University of Michigan Botanical Gardens personnel, especially Mr. Walter Kleinschmidt, for the growth and care of the experimental plants.

Some preliminary work for this study was carried out during tenure by the author of a National Science Foundation Senior Postdoctoral Fellowship at the Institut für Entwicklungsphysiologie of the University of Cologne, West Germany. The author is grateful to Professor Dr. C. Harte for the courtesies extended to him during his stay in Cologne.

Literature Cited

Cleland, R. E. et al. 1950. Studies in *Oenothera* cytogenetics and phylogeny. Indiana Univ. Publ. Sci. Ser., **16**: 1–348.

——. 1958. The evolution of the North American oenotheras of the "biennis" group. Planta, **51**: 378–398.

Schooley, James B. 1962. The incompatibility mechanism of the biennis group 1 races of *Oenothera*. Dissertation. Univ. Michigan.

Steiner, Erich. 1956. New aspects of the balanced lethal mechanism in *Oenothera*. Genetics, **41**: 486–500.

——. 1957. Further evidence of an incompatibility allele system in the complex-heterozygotes of *Oenothera*. Am. J. Bot., **44**: 582–585.