THE INCOMPATIBILITY MECHANISM OF THE BIENNIS GROUP I RACES OF OENOTHERA

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NEARLY all of the North American euoenotheras east of the Rocky Mountains remain heterozygous generation after generation even though they have the self-pollinating habit. A specialized cytogenetic mechanism involving multiple reciprocal translocations and a balanced lethal system accounts for this paradox. These *Oenotheras* possess 14 chromosomes of which each set of seven, because of reciprocal translocations, is characterized by a completely different chromosomal end arrangement. As a result of pairing specificity at meiosis the 14 chromosomes become arranged in a circle with adjacent chromosomes in the circle moving toward opposite poles at anaphase I. Thus the entire maternal genome goes to one pole and the entire paternal genome to the other; ultimately only two kinds of gametes are produced which are genetically identical with the two which united to form the plant. These genomes are passed from generation to generation intact. Such genomes are known as "Renner complexes".

Because these forms are self-pollinating, the offspring should include homozygotes for each of the complexes. These do not occur, however, because of a balanced lethal system. The lethals may be zygotic or gametophytic. In the case of zygotic lethals each complex carries a different recessive lethal; in the complex heterozygote the effect of each lethal is masked. On the other hand, the complex-homozygotes do not survive because the lethals are homozygous and able to express their effect. When gametophytic lethals are present, one complex of a complex-heterozygote carries a lethal which prevents the functioning of the male gametophyte, while the lethal of its associated complex does not allow the normal development of the female gametophyte. Therefore, only one complex is transmitted through the pollen and the other through the egg. Yet the functioning of these gametophytic lethal factors is not always so sharply defined. In some races of *Oenothera* the complex, which is usually transmitted only through the egg, is transmitted through the pollen in a certain percentage of cases. In other cases the complex, which is usually transmitted only through the pollen, may be transmitted through the egg. It has long been the practice to designate the complex ordinarily transmitted through the egg as the alpha complex and the complex ordinarily transmitted through the pollen as the beta complex.

CLELAND (1931) has classified the North American *Oenotheras* into phylogenetic groups on the basis of morphology, segmental arrangement of the chromo-

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somes, and their geographical distribution. One of these, the biennis group 1, is composed of complex-heterozygotes which range throughout the midwestern, mid-Atlantic, and southeastern states. The alpha complexes of the biennis group 1 produce the so-called biennis phenotype characterized by broad, dark green, crinkly leaves and slender buds which are glabrous or with erect hairs, and with appressed sepal tips. The beta complexes, on the other hand, produce narrow, gray-green leaves and thick flower buds with appressed pubescence and spreading sepal tips, characters of the *strigosa* type. Races of the biennis group 1 vary widely with respect to the frequency of transmission of their complexes through pollen and egg.

When races of biennis 1 which transmit the alpha complex through a portion of the pollen are used as the male parent in crosses between different races, hybrids are obtained among the offspring which carry the alpha complexes of both parents. Such alpha-alpha hybrids are self-incompatible, although, when alpha-alpha combinations derived from different parents are intercrossed, they are generally compatible. The self-incompatibility of the alpha-alphas results from the failure of pollen tube growth. STEINER (1956) has presented evidence in the alpha biennis 1 complexes of an incompatibility system of the gametophytic oppositional type as is well known from the work of EAST and MANGELS-DORF (1925) on *Nicotiana sanderae*. Such a system involves a single gene with a large number of allelic forms ($S_1, S_2, S_3 \dots$). When a particular allele is present in the style, it inhibits the growth of a pollen grain possessing the same allele.

In the biennis 1 races the incompatibility allele occurs in the alpha complex, while at the homologous locus in the beta complex an S_c (self-compatibility) allele is found. The latter conclusion is supported by the observation that beta-beta hybrids are self-fertile. In self-pollinations of a complex-heterozygote, pollen grains carrying the alpha complex bear the same incompatibility allele that is present in the style and therefore fail to develop. The pollen carrying the beta complex develops normally. It thus becomes clear that in these forms, when self-pollination occurs, the incompatibility allele acts as a gametophytic pollen lethal.

The results of the initial studies of STEINER (1956) suggested that most biennis 1 races carry different incompatibility alleles. It seemed of interest to make a more extensive survey of the races of this group for identity and distribution of incompatibility alleles. It was hoped that this information would contribute to a better understanding of the role of incompatibility in the group and possibly provide additional evidence of the evolutionary relationships between the different biennis 1 forms.

MATERIALS AND METHODS

Twenty-three of the biennis group 1 races whose complexes had previously been cytogenetically analyzed (CLELAND 1958) were crossed as female parents with each of three races known to transmit the alpha complex through the pollen. The races chosen as pollen parents were Hot Springs, Birch Tree 1, and Camp Peary E. Table 1 shows the races which were pollinated and their geographical origin.

Thus three series of self-incompatible alpha-alpha hybrids were obtained. One series com-

TABLE 1

Biennis group 1 races used in this study

Race	Geographical origin	Race	Geographical origin
Baltimore	Maryland	Iowa 1	Iowa
Bestwater 1	Arkansas	Lake	Virginia
Bestwater 2	Arkansas	Magnolia	Kentucky
Birch Tree 1	Missouri	Paducah	Kentucky
Birch Tree 2	Missouri	Poplar Bluff	Arkansas
Camp Peary E	Virginia	Princeton	Indiana
Camp Peary L	Virginia	Rich Mt.	Arkansas
Chicaginensis	Illinois	Walkerton	Indiana
Friendship	Indiana	Warrenton	Virginia
Hopkinsville	Kentucky	Warwick A	Virginia
Hot Springs	Arkansas	Whartonae	Kentucky
Indianapolis	Indiana	Williamsburg	Virginia

bined alpha Hot Springs with the alpha complexes of the biennis group 1 races listed. Another had alpha Birch Tree 1, and a third series possessed alpha Camp Peary E as a common complex. Since the alpha complex is not transmitted as frequently through the pollen as the beta complex, 50 plants of each cross were grown with the hope of obtaining at least one alpha-alpha of each combination.

The alpha-alphas which were obtained were then intercrossed in all combinations within each series. Since each member of the series had one alpha complex, and hence one *S* allele, in common, the compatibility or incompatibility of the cross would indicate whether the other alpha complexes, which were not common to both the parents in the cross, possessed identical *S* alleles. An incompatible reaction would indicate that they did have identical *S* alleles, and a compatible reaction would show that their *S* alleles were different.

Alpha-alphas can be readily distinguished from alpha-betas in the late rosette stage by the pale green color of their leaves. Alpha-alphas also often show chlorotic segments. In the flowering stage they can be recognized by their glabrous flower buds, clasping bracts, and green stem tips. Further, self-incompatibility serves as an additional criterion for identifying the alpha-alpha combinations.

For the compatibility tests mature buds were emasculated, cut from the plants, and placed in small vials containing water. When the stigmatic lobes become receptive, they were pollinated with fresh pollen brought directly from the field. The preparations were allowed to stand in the vials for an additional 12 to 18 hours at room temperature and then processed.

The style was cut at its base and placed for 3 minutes in a solution composed of one part absolute alcohol, one part concentrated hydrochloric acid and two parts of a 1% solution of iodine:potassium iodide in lactic acid in order to stain the pollen tubes. The styles were then placed on a slide and pressed out with a second slide. Pollen tubes are filled with starch grains which are clearly visible when stained with iodine. The presence or absence of pollen tube development was recorded and the slide discarded. A magnification of 35 $\times$ was found excellent for this work.

RESULTS

The results of the three series of intercrosses are shown in Tables 2, 3 and 4. In the Hot Springs series only two crosses failed to yield alpha-alphas. In the Birch Tree 1 series six crosses failed to yield alpha-alphas. Alpha-alphas failed to appear in eight of the Camp Peary E series. Of the three races used as pollen parents Hot Springs transmits the alpha complex most frequently, Camp Peary

TABLE 2

Crosses between alpha-alphas having alpha Hot Springs as a common complex

The egg Parent	The Pollen Parent																		
	♂ Baltimore	♂ Bestwater 1	♂ Bestwater 2	♂ Birch Tree 1	♂ Birch Tree 2	♂ Camp Peary E	♂ Camp Peary L	♂ Chicaginensis	♂ Friendship	♂ Hopkinsville	♂ Hot Springs	♂ Indianapolis	♂ Lake	♂ Magnolia	♂ Paducah	♂ Poplar Bluff	♂ Princeton	♂ Rich Mt.	♂ Walkerton
♂ Baltimore	—											○							
♂ Bestwater 1		—																	
♂ Bestwater 2			—																
♂ Birch Tree 1				—															
♂ Birch Tree 2					—														
♂ Camp Peary E						—													
♂ Camp Peary L							—												
♂ Chicaginensis								—											
♂ Friendship									—										
♂ Hopkinsville										—									
♂ Hot Springs											—								
♂ Indianapolis												—							
♂ Lake													—						
♂ Magnolia														—					
♂ Paducah															—				
♂ Poplar Bluff																—			
♂ Princeton																	—		
♂ Rich Mt.																		—	
♂ Walkerton																			—
♂ Warrenton																			
♂ Warwick A																			
♂ Whartoniae																			
♂ Williamsburg																			

Stippled squares=compatible pollination. Pollen tubes reach the base of the style in 12 hours or less.
Minus sign=incompatible pollination. Pollen tubes fail to grow beyond the stigma.
Zero=incompatible pollination. Pollen tubes grow several millimeters into the style but fail to reach the base of the style.
Empty square=cross not made.

E least often. The results of the study make it possible to arrange most of the compatibility reactions into classes: (1) *Crosses always compatible*.—This includes the alpha complexes of Baltimore, Bestwater 2, Birch Tree 2, Camp Peary E, Paducah, and Warrenton. (2) *Pollinations compatible except in one or two cases where the crosses and their reciprocals are incompatible*.—This includes the alpha complexes of Magnolia, Friendship, Chicaginensis, Whartoniae, Walkerton, Poplar Bluff, Lake, Hot Springs, and Birch Tree 1. (In other literature, the alpha complex of Chicaginensis is called *excellens*.) (3) *Crosses incompatible in the Birch Tree 1 and Camp Peary E series but generally compatible in the Hot Springs series*.—This includes the alpha complexes of Chicaginensis, Hopkins-

TABLE 3

Crosses between alphas having alpha Birch Tree 1 as a common complex

The egg parent	The pollen parent															
	♂ Baltimore	♂ Bestwater 1	♂ Bestwater 2	♂ Birch Tree 1	♂ Birch Tree 2	♂ Camp Peary E	♂ Camp Peary L	♂ Chicaginensis	♂ Friendship	♂ Hopkinsville	♂ Hot Springs	♂ Indianapolis	♂ Lake	♂ Magnolia	♂ Paducah	♂ Poplar Bluff
♂ Baltimore	1															
♂ Bestwater 1																
♂ Bestwater 2																
♂ Birch Tree 1																
♂ Birch Tree 2																
♂ Camp Peary E																
♂ Camp Peary L																
♂ Chicaginensis																
♂ Friendship																
♂ Hopkinsville																
♂ Hot Springs																
♂ Indianapolis																
♂ Lake																
♂ Magnolia																
♂ Paducah																
♂ Poplar Bluff																
♂ Princeton																
♂ Rich Mt.																
♂ Walkerton																
♂ Warrenton																
♂ Warwick A																
♂ Whartoniae																
♂ Williamsburg																

Stippled squares = compatible pollination. Pollen tubes reach the base of the style in 12 hours or less.

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Empty square = cross not made.

ville, Poplar Bluff, Rich Mt., Warwick A, and Williamsburg. Alpha Princeton, which is nonfunctional in the Hot Springs series, should be classified here.

DISCUSSION AND CONCLUSIONS

Groups 1 and 2 above include complexes whose compatibility reactions are governed by a gametophytic oppositional system with no gene interaction. The results obtained in these three series of intercrosses are mainly in agreement wherever data are available. The compatibility reactions are always positive except in a few cases which can readily be explained by assuming identity of S alleles. The inability of the pollen to function in the crosses Magnolia × Friend-

TABLE 4

Crosses between alpha · alphas having alpha Camp Peary E as a common complex

The egg parent	The pollen parent															
	♂ Baltimore	♂ Bestwater 1	♂ Bestwater 2	♂ Birch Tree 1	♂ Birch Tree 2	♂ Camp Peary E	♂ Camp Peary L	♂ Chicaginensis	♂ Friendship	♂ Hopkinsville	♂ Hot Springs	♂ Indianapolis	♂ Lake	♂ Magnolia	♂ Paducah	♂ Poplar Bluff
♂ Baltimore																
♂ Bestwater 1																
♂ Bestwater 2																
♂ Birch Tree 1																
♂ Birch Tree 2																
♂ Camp Peary E																
♂ Camp Peary L																
♂ Chicaginensis																
♂ Friendship																
♂ Hopkinsville																
♂ Hot Springs																
♂ Indianapolis																
♂ Lake																
♂ Magnolia																
♂ Paducah																
♂ Poplar Bluff																
♂ Princeton																
♂ Rich Mt.																
♂ Walkerton																
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ship, Friendship × Whartoniae, Magnolia × Whartoniae, Chicaginensis × Walkerton, Lake × Poplar Bluff, and their reciprocals is interpreted as resulting from identity of *S* alleles in these complexes. Although alpha Poplar Bluff and alpha Lake appear to have the same *S* alleles so far as the Hot Springs series is concerned, these conclusions are not supported in the Birch Tree 1 and Camp Peary E series.

When the presence of identical *S* factors in the several races of the biennis 1 *Oenotheras* is compared to other criteria which are used to show phylogenetic relationship, little correlation exists. In the case of alpha Poplar Bluff and alpha Lake which have in common the allele *S*₁₀, there is a great discontinuity in geographical origin. Poplar Bluff is a race from Arkansas, and Lake comes from Virginia. The races Friendship, Magnolia and Whartoniae, which bear the allele

S_7 , likewise do not come from contiguous geographical areas. Inasmuch as those races which have identical S alleles are not from contiguous geographical areas, the occurrence of these identical alleles appears not to make any significant contribution to an understanding of the phylogeny of the group.

Certain combinations gave inconclusive results. In the Birch Tree 1 series the pollen of alpha Hot Springs alpha Birch Tree 1 grows normally in all crosses except on the stigma of alpha Poplar Bluff-alpha Birch Tree 1. The reciprocal cross is also negative, but since the pollen of alpha Poplar Bluff is regularly nonfunctional in this series, the significance of the incompatible reactions in these crosses is obscured. Identity of S alleles in alpha Hot Springs and alpha Poplar Bluff cannot be the explanation for this behavior, since alpha-alphas of Poplar Bluff-Hot Springs were obtained in the Hot Springs series. The inability of alpha Hot Springs to grow in the cross alpha Poplar Bluff-alpha Birch Tree 1 $\times$ alpha Hot Springs-alpha Birch Tree 1 cannot be attributed to an interaction between the alpha Hot Springs and alpha Birch Tree 1 complexes in the pollen mother cell because the pollen of alpha Hot Springs is functional in all other crosses where alpha Hot Springs-alpha Birch Tree 1 is used as a pollen source. No case can be made to support any hypothesis relative to the deleterious effect of Hot Springs cytoplasm in the pollen mother cell. The negative results of the cross mentioned above remain unexplained.

Certain other combinations proved to be incompatible. The alpha Birch Tree 1 pollen in the Hot Springs series of crosses is mainly positive except in the cross with alpha Princeton. The reciprocal is likewise negative, but the failure of pollen tube growth, where alpha Princeton-alpha Hot Springs pollen is used, occurs in all combinations. Again it is postulated that alpha Princeton and alpha Birch Tree 1 may carry identical S alleles, but the inability of alpha Princeton pollen to function in any of the crosses prevents confirmation of this hypothesis.

The occurrence of gene interaction in certain alpha-alpha combinations.—The alpha complexes of Warwick A, Poplar Bluff, Chicaginisensis, Hopkinsville, Rich Mt., and Williamsburg, while giving uniformly positive reactions in the Hot Springs series, gave uniformly negative reactions in the Birch Tree 1 and Camp Peary E series. The alpha complex of Princeton in the Hot Springs series also regularly fails to function. These negative reactions suggested at first that the pollen might be inviable, yet most of these alpha complexes give compatible reactions in the Hot Springs series, and the pollen in the Birch Tree 1 and Camp Peary E series occasionally produces short tubes which grow part way down the style. They grow sufficiently to attest to their viability but not enough to give a compatible reaction. These short tubes are not produced by those alpha complexes which are common to both pollen and style since these are governed by the gametophytic oppositional mechanism and are completely suppressed. Such grains do not produce tubes which leave the stigma.

Several possible explanations for these results were examined, and of the several possibilities gene interaction prior to meiosis seemed to be the most plausible explanation. It is postulated that certain gene combinations interact in such a way as to inhibit subsequent pollen growth. Thus, as an example, the S allele of alpha Chicaginisensis and that of alpha Birch Tree 1 interact so that alpha Chica-

ginensis pollen cannot grow to the base of the style in any cross. Yet in the Hot Springs series the alleles of alpha Chicaginensis and alpha Hot Springs do not so interact. This kind of gene interaction appears in only one combination in the Hot Springs series, with alpha Princeton, but in several combinations in the Birch Tree 1 and Camp Peary E series.

While alpha Birch Tree 1 and alpha Camp Peary E possess the ability to suppress pollen tube growth through gene interaction prior to meiosis in the several races indicated, neither of them possess the ability to suppress the other. Alpha Birch Tree 1 expresses no inhibitory effect on alpha Camp Peary E, and alpha Camp Peary E does not suppress the growth of alpha Birch Tree 1 pollen.

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SUMMARY

Twenty-three races of *Oenotheras* of the biennis group 1 were crossed with three races known to transmit the alpha complex through the pollen. The races used as pollen parents were Hot Springs, Birch Tree 1, and Camp Peary E. Alpha-alphas were obtained in 21 of the crosses with Hot Springs, 17 of the crosses with Birch Tree 1, and 14 of the crosses with Camp Peary E. The alpha-alphas of each series were intercrossed in all combinations to determine compatibility relationships.

The pollen tube behavior of the alpha complexes of the six races Baltimore, Birch Tree 2, Bestwater 2, Camp Peary E, Paducah, and Warrenton is governed by a gametophytic oppositional system with no gene interaction and no identity of *S* alleles among them. The alpha complexes of the races Magnolia, Friendship and Whartoniae have the same factor, S_7 . The S_9 allele is common to both alpha Chicaginensis and alpha Walkerton. Alpha Poplar Bluff and alpha Lake have identical *S* alleles, S_{10} . The occurrence of identical *S* alleles in the alpha complexes of several races is considered to be without phylogenetic significance.

It is postulated that gene interaction occurs prior to meiosis in a number of combinations in the Birch Tree 1 and Camp Peary E series, and in one combination in the Hot Springs series, which then later prevents the pollen from growing in any combination even when the gametophytic oppositional mechanism is not involved.

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