NEW ASPECTS OF THE BALANCED LETHAL MECHANISM IN OENOTHERA[1]

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CERTAIN kinds of Oenothera are well known as naturally occurring translocation heterozygotes whose heterozygosis is maintained through successive generations by self-pollination and a balanced lethal mechanism. In recent years, especially through the work of CLELAND and his associates, natural populations of Oenothera have been analyzed cytogenetically for chromosomal end arrangement to obtain evidence of their phylogenetic relationships (see CLELAND, *et al.* 1950). In connection with such studies, an investigation has been undertaken by the author to determine whether members of a particular phylogenetic grouping carry the same lethal factors, and whether, therefore, lethals can be used as an additional index of phylogenetic relationship. The results of this investigation, which has been of an exploratory nature, reveal some interesting and new aspects of the balanced lethal mechanism in Oenothera which seem of sufficient interest to warrant a preliminary report; complete analysis will require several additional generations

The phylogenetic grouping selected for this study is one which has been tentatively designated by CLELAND as the "biennis group 1". In some respects at least, its members show a much closer relationship to one another than is true in most of the other groups which have been studied; thus it did not seem unlikely that some of the forms might possess identical lethal factors. The geographic range of the biennis group 1 extends from Missouri and Arkansas throughout the central Midwest to the East Coast in the region of Virginia and southward into a large part, if not most, of the Southeast. Plants belonging to this category are typically structural or "complex"-heterozygotes showing a circle of fourteen chromosomes at meiosis. Adjacent chromosomes of the circle regularly go to opposite poles so that only two kinds of genomes or "complexes", each with a different chromosomal end arrangement, occur among the spores. Although these forms are normally self-pollinating, homozygous combinations of the complexes do not occur among their progenies. When there is evidence from outcrossing that both complexes occur among the viable pollen as well as the eggs, the failure of the homozygotes to appear has been attributed to zygotic lethals. RENNER (1917, 1925, 1933) has shown this to be the case in *Oenothera lamarckiana* as well as many other forms. There are other races which, as far as one can determine from outcrosses, transmit only one of the two complexes present in the heterozygote through the pollen, and the other only through the egg. This has been interpreted as evidence of gametophytic lethals.

Situations intermediate between the two outlined above are also found; in fact,

the majority of the races of the biennis group 1 behave in this way. Some races in outcrosses transmit both complexes through the egg and only one through the pollen. In others, both complexes may come through the pollen and only one through the egg. Furthermore, in different outcrosses the percentage of progeny which receives a particular complex may vary widely. In this paper the convention will be followed in which the complex which is transmitted entirely or predominantly through the egg is called the alpha complex, and the beta complex designates the genome which comes mainly through the pollen.

The races of the biennis group 1 are characterized by possessing alpha complexes having for the most part identical arrangements of chromosome ends. Of the forty-two alpha biennis group 1 complexes so far analyzed, thirty-two possess the chromosome end arrangement, 1·2 3·4 5·14 7·10 9·8 11·12 13·6.[2] Moreover, the alpha complexes of this group all show basic phenotypic similarities such as broad, thin, dark green, crinkly leaves and thin-sepaled, glabrous flower buds with clasping bracts. The beta complexes of the biennis group 1 races display, in contrast, the strigosa phenotype, characterized by thick, narrow, grayish-green leaves and buds with a heavy appressed pubescence. Their segmental arrangements are much more variable than those of the alpha complexes. The alpha complexes are unusual in their segmental and phenotypic uniformity.

Evidence regarding the identity of zygotic lethals among the alpha biennis 1 complexes can be obtained from the results of crosses between different biennis group 1 races. If among the progeny of such crosses viable hybrids combining the alpha complex of each parent occur, the conclusion follows that the two alpha complexes do not possess identical zygotic lethal factors. This is pointed out by RENNER (1925): "Durch Kreuzung zweier komplexheterozygotischer Arten ist eine homozygote Sippe natürlich nur dann zu gewinnen wenn ihre Letalfaktoren nicht homolog sind." Alpha·alpha combinations can be expected, of course, only in crosses in which the pollen parent is known to be capable of producing functional pollen carrying the alpha complex; races of this type cannot possess a male gametophytic lethal. Thus, the method outlined here can be expected to give only information about zygotic lethals. Nevertheless, evidence regarding the identity of zygotic lethals among the alpha biennis 1 complexes, which on the basis of identical segmental arrangements and similar phenotypic effects are closely related, might give insight into the phylogeny of the biennis group 1.

Alpha·alpha combinations in which each complex possesses the same segmental arrangement show at meiosis seven pairs of chromosomes. It was hoped that through inbreeding the structural homozygotes obtained from these crosses, it would be possible to select among succeeding generations segregates in which lethals were no longer present. Thus we would have obtained from a complex-heterozygote a derivative with a return to the original type of chromosome behavior. This would be of interest in the light of STINSON's (1953) conclusion that such derivatives are not

[2] Several hundred collections of North American Oenotheras have been analyzed and the end arrangements of their complexes determined in terms of a standard arrangement. Many of these determinations, carried out by CLELAND and his associates, have not been published. For further details regarding the methods of analysis, see CLELAND et al. 1950.

likely to occur in nature. It would also indicate that the highly specialized complex-heterozygote still retains some evolutionary plasticity.

MATERIALS AND METHODS

The races of the biennis group 1 used in this study are the following:

Race	Geographic origin
Baltimore	Maryland
Birch Tree 1	Missouri
Birch Tree 2	Missouri
Camp Peary E	Virginia
Camp Peary L	Virginia
Friendship	Indiana
Hopkinsville	Kentucky
Hot Springs	Arkansas
Lake	Virginia
Magnolia	Kentucky
Paducah	Kentucky
Princeton	Indiana
Rich Mountain	Arkansas
Warwick A	Virginia
Warrenton	Virginia
Williamsburg	Kentucky
Winslow	Arkansas

The alpha complexes of these races all possess the typical alpha biennis 1 chromosome end arrangement. Thus hybrids between the races which carry the alpha complex of each parent must show seven pairs of chromosomes at meiosis. In order to obtain an alpha·alpha hybrid, the race used as the male parent must, of course, transmit the alpha complex (normally the egg complex) through the pollen. Where earlier data indicated conclusively that a race did not transmit the alpha through the pollen, the race was not used as male parent in any of the crosses. Where previous data were limited, crosses were made to determine whether a particular race transmitted the alpha complex only through the egg.

A preliminary series of biennis group 1 hybrids was studied during the summer of 1952. A much more extensive set of crosses was grown in 1955. The size of the hybrid progenies ranged from five to sixty-nine plants, although most cultures in the first series consisted of fifty or more plants, while in the second series the majority of cultures contained from thirty-five to forty individuals. All cultures were observed for the appearance of distinct classes at the seedling stage, at the rosette stage, and periodically after being placed in the field. Identifications of the different hybrid combinations among the progeny were made on the basis of phenotype and confirmed by cytological observations.

Controlled self-pollinations were carried out on the majority of the cytologically confirmed alpha·alpha combinations of both series. The flowers of the alpha·alphas

proved to be naturally self-pollinated; therefore, an entire inflorescence was simply bagged and the bag allowed to remain long enough for ten to twenty flowers to undergo pollination. As an added safeguard, a few flowers of each alpha·alpha were also pollinated by hand.

EXPERIMENTS AND RESULTS

The results of all crosses between biennis 1 races are shown in table 1. Part A includes the first series studied in 1952, while Part B consists of those analyzed during the summer of 1955. The races Baltimore, Camp Peary L, Friendship, Hopkinsville, Magnolia, Princeton, Rich Mountain, Warrenton, Williamsburg and Winslow do not transmit the alpha complex through the pollen. Crosses were made with some of these races as pollen parents, but since they have no pertinence to the problem other than to establish that the alpha complex does not come through the pollen, they are not listed in table 1. The races Lake and Warwick A transmit their alpha complexes through the pollen only rarely. Of a total progeny of 459 obtained in crosses with Lake as the male parent, only two alpha·alphas occurred, while Warwick A used as a pollen parent gave only one alpha·alpha among 502 offspring. Crosses with these races as pollen parents have not been included in table 1.

Structural homozygotes of alpha·alpha constitution appeared among most of the crosses listed. In the 1955 series, for example, Hot Springs transmitted the alpha complex through the pollen to some of the progeny in every cross, Camp Peary E in all except one cross, Birch Tree 2 in every cross except two, and Birch Tree 1 in all but three crosses. In crosses with Paducah as pollen parent, however, seven out of fifteen cultures failed to show alpha·alpha combinations. Table 2 summarizes the data for each of these races with regard to the frequency of alpha·alphas among their total progenies.

Identifications of the alpha·alpha combinations could be made with certainty in most cases from the phenotype of the mature plants. The glabrous buds and clasping bracts serve as the most definitive characters; in addition, the structural homozygotes tend to possess somewhat thinner, broader leaves than the heterozygotes. Although recognition of the alpha·alpha combinations in general presents no difficulties, the differences between a plant with two biennis-type complexes (alpha·alpha) as contrasted with one carrying a biennis and a strigosa-type complex (alpha·beta) are not always as striking as might be expected. In many cultures the classes could be distinguished shortly after the rosettes were growing in the field. The distinctive characteristics of the alpha·alpha combinations at this stage in contrast to the alpha·betas are the darker green, broader, more strongly crinkled, but less wavy leaves often showing bronze margins and tips. These rosettes were usually slower to develop than the alpha·betas. In some cases an approximately correct classification was possible while the plants were still in the greenhouse. Many of the alpha·alphas were recognizable at an early stage because they were chlorotic. This characteristic was not reliable, however, since usually it was not found in all of the alpha·alphas of any one culture; alpha·betas of some cultures showed it as well.

Cytological confirmation was made in all except nine of the seventy-four different alpha·alpha combinations obtained. In all cases they showed regular pairing at

TABLE 1

Part A.—*Occurrence of alpha·alpha complex combinations among crosses between different races of biennis group 1*

Culture no.	Cross	α·α	α·β	β·β	Total progeny	% α·α	Cytologically confirmed
87	Birch Tree 1 × Birch Tree 2	—	43	—	43	0	
88	Birch Tree 1 × Hot Springs	1	34	—	35	2.8	+
90	Warrenton × Birch Tree 1	12	28	—	40	30	+
91	Warrenton × Birch Tree 2	15	35	—	50	30	+
94	Warrenton × Hot Springs	31	19	—	50	62	+
97	Friendship × Birch Tree 1	36	30	1	67	54	+
98	Friendship × Birch Tree 2	13	54	—	67	19	+
100	Friendship × Hot Springs	40	28	—	68	59	+
104	Birch Tree 2 × Birch Tree 1	—	36	—	36	0	
107	Birch Tree 2 × Hot Springs	—	57	—	57	0	
110	Hopkinsville × Birch Tree 1	39	21	—	60	65	+
111	Hopkinsville × Birch Tree 2	2	63	—	65	3	+
113	Hopkinsville × Hot Springs	38	28	—	66	57	+
115	Hot Springs × Birch Tree 1	38	19	—	57	67	+
116	Hot Springs × Birch Tree 2	23	33	—	56	41	+
118	Paducah × Birch Tree 1	4	1	—	5	80	+
119	Paducah × Birch Tree 2	1	62	—	63	1.5	+
122	Paducah × Hot Springs	42	27	—	69	61	+
124	Winslow × Birch Tree 1	55	12	2	69	80	+

Part B.—*Occurrence of alpha·alpha complex combinations among crosses between different races of biennis group 1*

Culture no.	Cross	α·α	α·β	β·β	Total progeny	% α·α	Cytologically confirmed
100	Baltimore × Birch Tree 1	27	13	2	42	64.2	+
102	Baltimore × Birch Tree 2	4	31	3	38	10.5	+
103	Baltimore × Camp Peary E	18	15*	5	38	47.5	+
105	Baltimore × Hot Springs	20	16	0	36	55.5	+
107	Baltimore × Paducah	9	29	0	38	23.6	+
111	Birch Tree 1 × Birch Tree 2	0	21	0	21	0	
112	Birch Tree 1 × Camp Peary E	1	19	0	20	5.0	+
114	Birch Tree 1 × Hot Springs	7	24	0	31	22.6	+
116	Birch Tree 1 × Paducah	2	37	0	39	5.1	+
120	Birch Tree 2 × Camp Peary E	0	14	1	15	0	
121	Birch Tree 2 × Hot Springs	6	16*	0	22	27.2	+
122	Birch Tree 2 × Paducah	2	34	1	37	5.4	+
123	Camp Peary E × Birch Tree 1	14	21	0	35	40.0	+
124	Camp Peary E × Birch Tree 2	25	12	1	38	66.0	+
127	Camp Peary E × Hot Springs	30	7	0	37	81.1	+
129	Camp Peary E × Paducah	7	34	0	41	17.1	+
133	Camp Peary L × Birch Tree 1	13	25	0	38	34.2	+
134	Camp Peary L × Birch Tree 2	12	22	0	34	35.3	+
136	Camp Peary L × Hot Springs	14	22	1	37	37.8	+
138	Camp Peary L × Paducah	0	24	0	24	0	
142	Friendship × Birch Tree 1	13	2	0	15	86.7	+
143	Friendship × Birch Tree 2	4	27	0	31	12.9	+
144	Friendship × Camp Peary E	37	4	0	41	90.3	+
146	Friendship × Hot Springs	37	0	0	37	100	+
148	Friendship × Paducah	6	33	0	39	15.4	+
152	Hopkinsville × Birch Tree 1	26	9	0	35	74.4	+
153	Hopkinsville × Birch Tree 2	7	12	2	21	33.3	+
154	Hopkinsville × Camp Peary E	28	10	0	38	73.8	+
156	Hopkinsville × Hot Springs	33	5	0	38	87.0	+
158	Hopkinsville × Paducah	0	39	0	39	0	
163	Hot Springs × Birch Tree 1	21	27	0	48	43.8	+
164	Hot Springs × Birch Tree 2	12	28	0	40	30.0	+
165	Hot Springs × Camp Peary E	16	16	0	32	50.0	+
168	Hot Springs × Paducah	2	35	0	37	5.4	+

TABLE 1. *Continued*

Culture no.	Cross	$\alpha \cdot \alpha$	$\alpha \cdot \beta$	$\beta \cdot \beta$	Total progeny	% $\alpha \cdot \alpha$	Cytologically confirmed
172	Lake × Birch Tree 1	0	17	2	19	0	
173	Lake × Birch Tree 2	0	33	0	33	0	
174	Lake × Camp Peary E	2	24*	1	27	7.4	+
176	Lake × Hot Springs	10	18	1	29	34.5	+
177	Lake × Paducah	0	28	1	29	0	
181	Magnolia × Birch Tree 1	15	2	1	18	83.4	+
182	Magnolia × Birch Tree 2	15	21	0	36	41.7	+
183	Magnolia × Camp Peary E	28	1	0	29	96.6	+
184	Magnolia × Hot Springs	5	28*	0	33	15.1	+
186	Magnolia × Paducah	4	35	0	39	10.2	+
189	Paducah × Birch Tree 1	33	7	0	40	82.5	+
190	Paducah × Birch Tree 2	12	29	0	41	29.2	+
191	Paducah × Camp Peary E	33	3	0	36	91.6	+
193	Paducah × Hot Springs	26	15	1	42	62.0	+
200	Princeton × Birch Tree 1	0	14	0	14	0	
202	Princeton × Camp Peary E	19	17*	0	36	52.8	
204	Princeton × Hot Springs	18	18	1	37	48.6	
205	Princeton × Paducah	0	34	0	34	0	
208	Rich Mt. × Birch Tree 1	29	11	0	40	72.5	+
209	Rich Mt. × Birch Tree 2	4	29*	4	37	10.8	+
210	Rich Mt. × Camp Peary E	31	2	0	33	94.0	
212	Rich Mt. × Hot Springs	25	11	0	36	69.3	+
214	Rich Mt. × Paducah	0	35	3	38	0	
225	Warrenton × Birch Tree 1	32	9	0	41	78.0	
226	Warrenton × Birch Tree 2	14	21	0	35	40.0	
227	Warrenton × Camp Peary E	11	27	0	38	29.0	+
229	Warrenton × Hot Springs	35	2	0	37	94.8	
231	Warrenton × Paducah	5	48	0	53	9.4	+
217	Warwick A × Birch Tree 1	29	10	0	39	74.4	+
218	Warwick A × Birch Tree 2	10	29	0	39	25.6	
219	Warwick A × Camp Peary E	13	13	0	26	50.0	
221	Warwick A × Hot Springs	22	19	0	41	52.6	
223	Warwick A × Paducah	0	34	0	34	0	
235	Williamsburg × Birch Tree 1	0	42	0	42	0	
237	Williamsburg × Camp Peary E	21	21	0	42	50.0	+
239	Williamsburg × Hot Springs	22	14	0	36	66	+
240	Williamsburg × Paducah	0	31	0	31	0	

* The figures given include beta·alphas as follows:

Culture	beta·alphas
103	3
121	11
133	23
174	2
184	1
202	1
209	3

meiosis. No evidence was found of abnormal behavior during meiosis in any of the material.

The feature of the alpha·alpha combinations which is most striking and of central importance in further investigation is the fact that all of them are self-incompatible. No normal seeds were obtained from any of the controlled self-pollinations. In the second series (table 1), for instance, alpha·alpha combinations from thirty-three different cultures were selfed; in each case this involved usually three or four hand

TABLE 2

Frequency of alpha·alpha combinations in crosses of each race used as male parent

	% of $\alpha \cdot \alpha$	
	Range	Average
1955 series		
Paducah	0–17.1%	6.1%
Birch Tree 1	0–86.7%	52.4%
Birch Tree 2	0–66.0%	25.8%
Camp Peary E	0–96.6%	52.6%
Hot Springs	15–100.0%	56.6%
1952 series		
Birch Tree 1	0–80%	53.7%
Birch Tree 2	0–41%	15.7%
Hot Springs	0–62%	40.6%

pollinations and from ten to twenty natural self-pollinations. None of them produced viable seed.

On the other hand, when an alpha·alpha is outcrossed, either as a male or female parent and either to normal races or to other alpha·alphas, abundant and normal seeds are produced. The pollen as well as the megaspores must, then, be viable and capable of producing normal gametophytes and gametes. Alpha·alpha combinations have been outcrossed successfully to the following races:

Bestwater 2	Friendship
Birch Tree 2	Hopkinsville
Camp Peary E	Hot Springs
Camp Peary L	Magnolia
Chapultepec	Paducah
Chicaginensis	Warwick A

The following alpha·alphas were intercrossed in all possible combinations:

α Hot Springs · α Birch Tree 2
α Camp Peary E · α Birch Tree 2
α Paducah · α Birch Tree 2
α Hot Springs · α Camp Peary E
α Paducah · α Camp Peary E
α Paducah · α Hot Springs

All crosses proved to be fertile.

Crosses between alpha·alphas which carry the same complexes but which were obtained from reciprocal crosses between races, are listed below. These, like the self-pollinations, were uniformly sterile.

α Birch Tree 1 · α Camp Peary E $\times$ α Camp Peary E · α Birch Tree 1
α Camp Peary E · α Birch Tree 1 $\times$ α Birch Tree 1 · α Camp Peary
E

α Birch Tree 1 · α Hot Springs   × α Hot Springs · α Birch Tree 1
α Hot Springs · α Birch Tree 1   × α Birch Tree 1 · α Hot Springs
α Birch Tree 1 · α Paducah   × α Paducah · α Birch Tree 1
α Paducah · α Birch Tree 1   × α Birch Tree 1 · α Paducah
α Hot Springs · α Paducah   × α Paducah · α Hot Springs
α Paducah · α Hot Springs   × α Hot Springs · α Paducah
α Hot Springs · α Camp Peary E   × α Camp Peary E · α Hot Springs
α Camp Peary E · α Hot Springs   × α Hot Springs · α Camp Peary E
α Paducah · α Camp Peary E   × α Camp Peary E · α Paducah
α Camp Peary E · α Paducah   × α Paducah · α Camp Peary E

When an alpha·alpha is crossed to an alpha·beta of the same culture in either direction, seeds are always obtained. Such crosses are shown below:

α Paducah · β Hot Springs   × α Paducah · α Hot Springs
α Paducah · α Hot Springs   × α Paducah · β Hot Springs
α Paducah · β Birch Tree 1   × α Paducah · α Birch Tree 1
α Paducah · α Birch Tree 1   × α Paducah · β Birch Tree 1
α Magnolia · β Hot Springs   × α Magnolia · α Hot Springs
α Magnolia · α Hot Springs   × α Magnolia · β Hot Springs
α Magnolia · α Camp Peary E   × α Magnolia · β Camp Peary E
α Magnolia · α Birch Tree 2   × α Magnolia · β Birch Tree 2
α Magnolia · β Birch Tree 2   × α Magnolia · α Birch Tree 2
α Magnolia · β Birch Tree 1   × α Magnolia · α Birch Tree 1
α Magnolia · α Birch Tree 1   × α Magnolia · β Birch Tree 1
α Lake · β Camp Peary E   × α Lake · α Camp Peary E
α Lake · α Camp Peary E   × α Lake · β Camp Peary E
α Lake · β Hot Springs   × α Lake · α Hot Springs
α Lake · α Hot Springs   × α Lake · β Hot Springs
α Lake · α Hot Springs   × β Lake · α Hot Springs

A study was undertaken to determine whether the self-incompatibility might involve the failure of pollen tube growth. One to several styles from flowers of the following series of crosses were fixed twenty-four hours after pollination with alpha· alpha pollen:

Bestwater 2 × α Hot Springs · α Paducah
Chapultepec × α Hot Springs · α Paducah
Chicaginensis × α Hot Springs · α Paducah
Hopkinsville × α Hot Springs · α Paducah
Hot Springs × α Hot Springs · α Paducah
Magnolia × α Hot Springs · α Paducah
Paducah × α Hot Springs · α Paducah
Warwick A × α Hot Springs · α Paducah

Pollen tube growth extended to the base of the style in every case. Similar preparations, however, made in following self-pollination of alpha·alpha plants revealed an inhibition of pollen tube growth. Most pollen tubes either stopped growing shortly

after germination or did not proceed more than a few millimeters down the style. Occasionally, however, one or two tubes were observed at the base of the style. When the ovaries of such self-pollinated flowers were examined, it was noted that a few of the ovules had enlarged. The number of enlarged ovules varied from ovary to ovary; they ranged from one to as many as twenty-six, although usually there were fewer than a dozen. Gross dissection of such ovules revealed an embryo which at a later stage disintegrated to form a soupy liquid within a normal appearing seed coat. At the time the seeds would normally be mature, the capsule contained the dried undeveloped ovules and a few empty seed coats. The ovary usually enlarged slightly after pollination and persisted until the seeds would normally be mature. The self-incompatibility of the alpha · alpha combinations, therefore, can be attributed primarily to the failure of pollen tube growth. When a pollen tube occasionally does reach an embryo sac, the fertilization which occurs is unsuccessful because of the deterioration of the partially developed embryo. A more detailed study of the development of such an embryo has not yet been carried out.

Inbreeding of the seven-paired alpha · alpha combinations was originally attempted with the hope of obtaining an alethal, homozygous segregate which thus would be derived from a complex-heterozygote, but would show a return to the ancestral type of chromosome behavior. When self-pollination of the alpha · alphas proved unsuccessful, it was thought that a type approaching homozygosis might be obtained through a series of backcrosses. At the time, of course, the nature of the self-incompatibility was little understood. One of the alpha · alpha combinations, alpha Paducah · alpha Hot Springs, was backcrossed as the male parent to Hot Springs as the female parent. The progeny from this cross consisted entirely of structural homozygotes, since Hot Springs transmits only the alpha complex through the egg. Four plants from this progeny were crossed to Hot Springs, using the latter again as the female parent. All offspring from each of these crosses were structural homozygotes. A third backcross generation gave again only structural homozygotes. All offspring of the three backcross generations were self-incompatible.

Among the cultures grown during the 1955 season a number of beta · beta combinations also appeared. As will be noted in table 1, the frequency of this type of hybrid is small compared to that of the alpha · alpha. Since this material was at hand, however, MISS MARY SEAMAN began studies similar to those carried out with the alpha · alpha forms.

Only in one culture was the beta · beta a structural homozygote and thus strictly comparable. Interestingly enough, the plants of this combination were perfectly self-fertile. What the progeny will be like must await the coming growing season. Likewise, all other beta · betas, showing large or small circles at meiosis, proved to be self-fertile.

<div align="center">DISCUSSION</div>

The most interesting feature of these results is the self-incompatibility of the alpha · alpha combinations. The consistency with which this behavior is shown by all alpha · alpha combinations so far produced points to its being a phenomenon of fundamental significance in the oenotheras of the biennis group 1. The pattern of self-

incompatibility strongly suggests that the alpha complexes possess an incompatibility allele system of the Nicotiana type. Such a system is already well known in *Oenothera organensis* (EMERSON 1938).

Conclusive evidence for the existence of an incompatibility allele system in the alpha complexes of the biennis group 1 will require an additional generation which will be grown during the coming summer. The results of a number of crosses which have already been carried out, however, are consistent with this hypothesis. Furthermore, predictions which on this basis can be made concerning the constitution of the next season's progenies, will serve as critical tests of the hypothesis.

The first line of evidence supporting the incompatibility allele hypothesis comes from the results of crosses between different alpha·alphas. If one assumes that the alpha complex of each race carries a distinctive incompatibility allele (designated by the letter S), then the crosses among different alpha·alphas should all be fertile, as the results indicate. Further, the compatibilities of the offspring from these crosses should be predictable. For example, assume that alpha Hot Springs carries S_1, alpha Birch Tree 2, S_2, and alpha Camp Peary E, S_3. Then, the cross: alpha Hot Springs (S_1)·alpha Birch Tree 2 (S_2) × alpha Camp Peary E (S_3)·alpha Birch Tree 2 (S_2) should produce both S_1S_3 and S_2S_3 progeny. The cross of S_1S_3 with alpha Hot Springs (S_1)·alpha Camp Peary E (S_3) should be incompatible, while S_1S_3 crossed with alpha Camp Peary E (S_3)·alpha Birch Tree 2 (S_2) should give seed. Likewise, the S_2S_3 offspring should give seed when crossed with alpha Hot Springs (S_1)·alpha Camp Peary E (S_3) but not with alpha Camp Peary E (S_3)·alpha Birch Tree 2 (S_2). This kind of analysis of the offspring from the crosses between different alpha·alphas should constitute, therefore, one test of the incompatibility allele hypothesis.

A second test is provided by the reciprocal crosses which have been made between alpha·alphas and alpha·betas of the same culture. On the basis of an S allele system, the results of these crosses are predictable. In the example which is given below, the race Paducah is involved; let us assign to the alpha Paducah complex the S_4 allele. Also, since our data show that beta·betas from the biennis 1 crosses are self-fertile, it must be assumed that S alleles are absent in the beta complexes. The corresponding locus in the beta complex can, then, be represented by the symbol, $(+)$.

Parents: Paducah × Hot Springs

F_1: α Paducah (S_4) · α Hot Springs (S_1) × α Paducah (S_4) · β Hot Springs $(+)$
  Progeny should consist only of alpha·betas.
  α Paducah (S_4) · β Hot Springs $(+)$ × α Paducah (S_4) · α Hot Springs (S_1)
  Progeny should include alpha·alphas.

A third line of evidence comes from a series of crosses in which alpha·alphas were hybridized as female parents with races known to transmit their alpha complexes through the pollen. All of these crosses were fertile. According to the incompatibility allele hypothesis, if the female parent possesses an alpha complex in common with the race used as male parent, only the beta pollen should grow and the progeny will consist entirely of alpha·beta combinations. On the other hand, if the alpha·alpha used as the egg parent was derived from races other than that used as the male parent,

then alpha·alpha combinations should also appear among the progeny. Likewise, in crosses of races as female parents with synthesized alpha·betas, it should be possible to predict whether the progenies will contain only alpha·betas or both alpha·alpha and alpha·beta combinations. The validity of these predictions will be tested in the next growing season.

The incompatibility allele hypothesis provides an explanation of the results of backcrossing alpha Paducah·alpha Hot Springs to the Hot Springs parent for three generations. These crosses can be represented as follows:

F_1 * α Hot Springs (S_1) · β Hot Springs $(+)$ × α Paducah (S_4) · α Hot Springs (S_1). Progeny all S_1S_4

F_2 α Hot Springs (S_1) · β Hot Springs $(+)$ × S_1S_4. Progeny all S_1S_4

F_3 α Hot Springs (S_1) · β Hot Springs $(+)$ × S_1S_4. Progeny all S_1S_4

The 1955 planting included three crosses in which an alpha·alpha (S_1S_4) from the second backcross generation described above was crossed as a female parent to the races Birch Tree 1, Hot Springs, and Paducah respectively. Since alpha Birch Tree 1 cannot carry the same S allele as either alpha Hot Springs or alpha Paducah, the S_5 allele can be assigned to alpha Birch Tree 1. The above crosses may then be represented with regard to S alleles as follows:

I (S_1S_4) × α Birch Tree 1 (S_5) · β Birch Tree 1 $(+)$
   alpha·alphas (S_1S_5), or (S_4S_5)
   alpha·betas (S_1+), or (S_4+)
   Both alpha·alphas and alpha·betas appeared among the progeny.

II (S_1S_4) × α Hot Springs (S_1) · β Hot Springs $(+)$
   Only alpha·betas are possible on the hypothesis and only alpha·betas appeared.

III (S_1S_4) × Paducah (S_4) · β Paducah $(+)$
   Only alpha·betas are possible on the hypothesis and only alpha·betas appeared.

Each of the races used as male parent has been shown to transmit the alpha complex through the pollen, yet in these crosses alpha·alpha progeny occurred only in the Birch Tree 1 cross where, according to the hypothesis, one would assume that the incompatibility allele differs from those present in the female parent. Thus in crosses II and III the alpha pollen carrying either S_1 or S_4 apparently cannot grow in the style where these alleles are present.

It will be noted that in both the 1952 and 1955 series of hybrids, the crosses between Birch Tree 1 and Birch Tree 2 races have not produced any alpha·alpha combinations even though both races are known to transmit their alpha complexes through the pollen. Since these races were collected in the same locality, it is possible that their alpha complexes carry identical incompatibility alleles. If the incompatibility alleles in the alpha Birch Tree complexes are identical, then a cross between two alpha·alpha combinations which differ only in one possessing the alpha Birch

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* Hot Springs transmits only the alpha complex through the egg.

Tree 1, the other the alpha Birch Tree 2 complex, should be sterile. Three such crosses, which test the identity of the incompatibility alleles in the alpha Birch Tree 1 and the alpha Birch Tree 2 complexes, are listed below; all produced seed.

α Camp Peary E $\cdot$ α Birch Tree 2 $\times$ α Camp Peary E $\cdot$ α Birch Tree 1
α Hopkinsville $\cdot$ α Birch Tree 1 $\times$ α Hopkinsville $\cdot$ α Birch Tree 2
α Birch Tree 2 $\cdot$ α Hot Springs $\times$ α Birch Tree 1 $\cdot$ α Hot Springs

The absence of alpha·alpha combinations among the progeny when these two races are crossed must not, therefore, result from the action of the incompatibility alleles. It should be noted that in the crosses between these races the number of seedlings which survive has been low. For example, in the cross of the 1955 series, Birch Tree 1 $\times$ Birch Tree 2, forty germinated seeds were planted; all of these were growing a week later, but after two additional weeks only nineteen survived. It may well be that some alpha·alphas were among the non-survivors. These results lead to the interpretation that alpha Birch Tree 1 and alpha Birch Tree 2 possess a common zygotic lethal. Thus zygotic lethals may be present in these complexes in addition to incompatibility alleles.

If an incompatibility allele system is present in the alpha biennis 1 complexes, it may have played a significant role in the origin and phylogeny of the biennis group 1. Earlier work (CLELAND, *et al.* 1950) has led to the belief that this group of complex-heterozygotes arose through the hybridization of previously isolated forms which had undergone cytogenetic differentiation. The alpha complexes presumably have been derived from an ancestral group of structural homozygotes not too greatly different from the present day grandifloras of the Southeast (STEINER 1952). On the other hand, this ancestral population must have acquired an incompatibility allele system which *Oenothera grandiflora* does not possess. The population from which the beta biennis 1 complexes have been derived, failed to develop such a self-incompatibility mechanism. When contact between the two populations occurred, hybrids were produced which possess $\odot$ 14 chromosomes at meiosis because of the fact that the two populations had developed differences in chromosome end arrangement. Such forms, when self-pollinated, could not produce alpha·alpha segregates because of the incompatibility alleles. Thus the progeny presumably would consist of alpha·betas and beta·betas. To arrive at true-breeding heterozygotes such as the biennis group 1 oenotheras are, beta·beta offspring must not occur. RENNER (1940) has shown that in many forms a competition between megaspores exists, the genetic constitution of the megaspore determining which succeeds in developing into the embryo sac. Since the original hybrids between the progenitor groups here brought together involved two quite different genomes or complexes, it does not seem unlikely that megaspore competition occurred in the original hybrids, with the alpha complex succeeding. With only the alpha coming through the egg and only beta pollen functional, a true-breeding complex-heterozygote thus could have arisen simply by the act of hybridization between the two progenitor groups. Such a form would possess upon its inception a "balanced lethal mechanism", consisting of the incompatibility allele system on the one hand, and the megaspore competition on the other. "Balanced lethal mechanism" is, therefore, interpreted to include *any* mechanism which prevents the occurrence

of homozygotes of either type. This differs from the point of view of RENNER, who considers "lethal" in an absolute sense and makes a point of emphasizing that megaspore competition cannot be considered to fall into the category of a "lethal" (RENNER 1929, 1940).

Once such complex-heterozygotes became established, however, recessive lethal mutations which occurred in either of the complexes would be preserved because of the nature of the cytogenetic mechanism. After a period of time one would expect both the alpha and beta complexes of these forms to carry recessive lethal or deleterious genes which would reinforce the mechanism by which the complex homozygotes were originally eliminated from the progenies of the heterozygotes. The results of the crosses between the Birch Tree races provides evidence that the alpha complexes may carry zygotic lethals. The presence of deleterious recessives has been shown in alpha Beaufort, a complex occurring in one of the collections from the southeastern United States (STINSON and STEINER 1955). The fact that alpha·alphas can be obtained in so many of the crosses between different biennis 1 races does not deny the presence of zygotic lethals or deleterious genes. Since these complexes each stem from a different inbred line, during their history recessive lethal or deleterious factors which have arisen and have been preserved, are not likely to be identical in the different inbred lines. It should be emphasized, however, that the accumulation of recessive lethal or detrimental genes is a consequence of the cytogenetic mechanism characteristic of the true-breeding complex heterozygote; lethals of this type could, therefore, not have factored in the original establishment of the true-breeding complex heterozygote.

It is interesting to note that the self-incompatibility which is described here as a characteristic of the alpha·alpha combinations was reported by ATKINSON (1917) in a hybrid obtained by crossing *Oenothera nutans* Atkinson and Bartlett and *Oenothera pycnocarpa* Atkinson and Bartlett. From ATKINSON's description he unquestionably had encountered the same behavior described above. It is difficult to say from the descriptions of the two species whether they belong to the biennis 1 group. CLELAND (personal communication) has stated that collections which came to him under the names *Oe. nutans* and *Oe. pycnocarpa*, upon cytogenetic analysis turned out to belong to the biennis group 2. Thus there is some indication that the self-incompatibility mechanism may not be restricted to only one phylogenetic group.

Another interesting feature of some of the crosses made in this study which requires further analysis has been called to my attention by DR. STERLING EMERSON. When races which are known to transmit the alpha complex through the pollen are crossed reciprocally, one generally obtains a much larger percentage of alpha·alpha combinations in one direction than in the other. A group of such crosses is listed in table 3. Where data are available for two seasons, it is apparent that wide variation in the percentages of the alpha·alphas may occur. Only in one case, namely, the crosses between the Birch Tree 2 and Hot Springs, is the percentage of alpha·alpha combinations roughly equal in reciprocal crosses. Thus failure to obtain alpha·alphas in one direction cannot be attributed to zygotic lethals, but must result from some other mechanism which may also account for the variation in the frequency of transmission of the alpha through the pollen in different crosses.

TABLE 3

Alpha·alpha combinations produced in reciprocal crosses between races of the biennis group 1 which transmit the alpha complex through the pollen

Cross	1952		1955	
	Ratio	Percent	Ratio	Percent
Birch Tree 1 × Birch Tree 2	0/43	0	0/21	0
Birch Tree 2 × Birch Tree 1	0/36	0	—	—
Birch Tree 1 × Hot Springs	1/35	2.8	7/31	22.0
Hot Springs × Birch Tree 1	38/57	67.0	21/48	43.8
Birch Tree 2 × Hot Springs	0/57	0	6/22	27.2
Hot Springs × Birch Tree 2	23/56	41.0	12/40	30.0
Birch Tree 1 × Paducah	1/15*	6.6	2/39	5.1
Paducah × Birch Tree 1	4/5	80.0	33/40	82.5
Birch Tree 2 × Paducah			2/37	5.4
Paducah × Birch Tree 2	1/63	1.5	12/41	29.2
Birch Tree 1 × Camp Peary E			1/20	5.0
Camp Peary E × Birch Tree 1			14/35	40.0
Birch Tree 2 × Camp Peary E			0/15	0
Camp Peary E × Birch Tree 2	2/30†	7	25/38	66.0
Hot Springs × Camp Peary E			16/32	50.0
Camp Peary E × Hot Springs			30/37	81.1
Hot Springs × Paducah			2/37	5.4
Paducah × Hot Springs	42/69	61.0	26/42	62.0
Camp Peary E × Paducah			7/41	17.1
Paducah × Camp Peary E			33/36	91.6

* Data from CLELAND.
† Data from STINSON.

It is fully recognized that the proposed explanation of the above data still remains essentially a working hypothesis. Many of the predictions which have been made will be tested with the coming season's progenies. Studies are also under way to determine why a few pollen tubes function in fertilizations when the alpha·alphas are selfed, and what the significance of the degenerating embryos may be. Other groups are being surveyed for the presence of self-incompatibility among their hybrids. It is likely that EMERSON's technique (EMERSON 1938) for testing compatibilities by determining pollen tube growth may be utilized here. With further work a future report should allow a more complete interpretation of these results in the light of our present understanding of the entire Oenothera picture.

SUMMARY

1. Oenotheras belonging to the biennis 1 phylogenetic grouping are true-breeding heterozygotes which are composed of two genomes, the alpha and beta complexes, each of which possesses a distinctive arrangement of chromosome ends.

2. In crosses between many of the biennis group 1 races it is possible to obtain viable hybrids which carry the alpha complex from each parent.

3. Such alpha·alpha combinations are self-incompatible, but fertile in outcrosses.

4. Beta·beta combinations which also occur among some of the hybrid progeny at a low frequency are self-fertile.

5. The hypothesis is proposed that the alpha biennis 1 complexes carry an incompatibility allele system of the Nicotiana type, while such a system is absent in the beta biennis 1 complexes.

6. If an incompatibility mechanism is operative and if megaspore competition occurs, it is possible to explain the origin of the true-breeding complex-heterozygote simply by the act of hybridization. The self-incompatibility system together with megaspore competition constitutes a balanced lethal mechanism.

7. Recessive lethal or detrimental genes which may exist in the biennis 1 complexes appear to have arisen after complex-heterozygotes occurred; they played no role in the original establishment of true-breeding structural heterozygotes.

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