

THE GENETICS OF SELF-INCOMPATIBILITY IN *OENOTHERA ORGANENSIS*¹

STERLING EMERSON

California Institute of Technology, Pasadena, California

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O*OENOTHERA organensis* is endemic to the Organ Mountains of southern New Mexico where the entire population apparently consists of considerably less than one thousand individuals.² The morphology of the seeds indicates a relationship to members of the subgenus *Oenothera* (sometimes called *Onagra*), but the growth habit and ovule placentation resemble types belonging to the subgenus *Raimannia* (the *Euoenothera* of Engler and Prantl). *Oenothera organensis* has proved completely cross-sterile with five species of *Raimannia*. Hybrids with *Oenothera* species are obtained infrequently, and are almost completely sterile, resembling *Oenothera-Raimannia* hybrids. *Oenothera organensis* differs from both these subgenera in being completely self-sterile.

The inheritance of self-incompatibility in *Oe. organensis* proved to be of the *Nicotiana* type, governed by a series of multiple allelomorphs. Interest attaches not so much to the mode of inheritance as to the methods by which genetic analyses can be followed in this material.

MATERIAL

Seeds of *Oenothera organensis* were collected at Modoc Waterfall, at the mouth of Filmore Canyon on the western slope of the Organ Mountains by Dr. E. W. ERLANSON and Dr. A. E. ARCHER in November, 1928. Plants from these seeds were grown in Pasadena in 1929, 1930 and 1931, and were continued from a single open-pollinated capsule from one of these 1931 plants. The culture grown in 1934 from these 1931 seeds was the first to be tested for self- and cross-incompatibility. Ten plants tested proved to be self-sterile. Nine of these plants were pollinated by a tenth, eight being cross-fertile and one cross-sterile. The first experiments to be reported were made on the progenies of these cross-fertile combinations.

In 1935, Professor P. A. MUNZ collected seeds from plants growing in the type locality, Dripping Springs, in the next canyon south of Filmore Canyon. Professor R. E. CLELAND grew plants from these seeds in Balti-

¹ *Oenothera organensis* Munz n. nom. *Oe. macrosiphon* Woot. & Standl., Contr. U. S. Nat-Herb. 16: 155. 1913, not *Oe. macrosiphon* Lehm. in Hamb. Gartenz. XIV: 439. 1858.

² In September, 1937 there were 70 plants in one canyon at Dripping Springs, 42 plants in another canyon at Dripping Springs, less than 20 plants at Modoc Waterfall (June 1937), and about 20 plants in one fork of McAllister Canyon on the north slope of the mountains. Unless there are some unexpected stands of this species in other parts of the mountains, the total population must be less than 500.

more the following year, found that they were self-sterile and made intercrosses between seven of them. Seeds of these crosses were sent me and the progenies were tested at Pasadena in the summer of 1937.

CROSS-INCOMPATIBILITY IN ONE FAMILY

A culture of 21 plants was grown in the summer of 1936 from seeds of a cross between two members of the 1934 culture of the Modoc strain. Fifteen of these (including all then in flower) were intercrossed in nearly all possible combinations. The first results were extremely complicated: reciprocal crosses reacted alike in some instances and not in others. Following repeated pollinations, seeds were finally obtained in both or neither directions in most reciprocal crosses, and the family could be classified into four intra-sterile, inter-fertile groups. The data obtained are summarized in table 1.

TABLE I
Results of cross-pollinations within one family. A set of seeds is indicated by the + sign, the failure of the capsule to set by the - sign.

♀ \ ♂	TYPE A				TYPE B			TYPE C		TYPE D	
	6	8	9	10	1	4	7	18	3	12	
TYPE A	2	---	---	-	-	++	+	+++	-+	+	
	5	---	-	-	-	+	+	+	+	+	++
	6	---	---	-	---	+	+	+++	+++	+	++
	8	---	---	-	-	+	+	+++	+	+	++
	9	-	---	---	-	+	+	+++	+	+	++
	10	---	---	-	-	-	+	+	+	+	++
20	-	---	-	-	+	+	+	---	++	-	
TYPE B	1	-+				-	-	-	+	+	-
	4	++	+	+	++	-		-	+	+	++
	7	--+	++	+	+	-	-	---	-+	+	+
TYPE C	18	-						-	---		
	3	++	+	+	++	+	+	+	-	---	++
	14	-	+			+	+			-	
	15	---	+	++	++	-+	-		-	-	+
TYPE D	12	-+	++	+	++	+	+	++	+	+	--

INCOMPATIBILITY TESTS WITHIN THE STYLAR TISSUE

Flowers which had been pollinated 24 hours earlier were removed from the plant, killed, the styles dissected and stained by the method of BUCHHOLZ (1931) for *Datura*. Following compatible cross-pollination, the

pollen tubes had just reached the ovaries (150 to 180 millimeters below the stigma), whereas following self-pollination the pollen tubes remained in the stigma and were mostly less than one millimeter in length.

On flowers removed from the plant and kept in a moist chamber at 25°C, pollen tubes from compatible pollinations grew at nearly a constant rate. Pollen tubes from self-pollinations apparently stopped growing after the first hour or two (fig. 1).

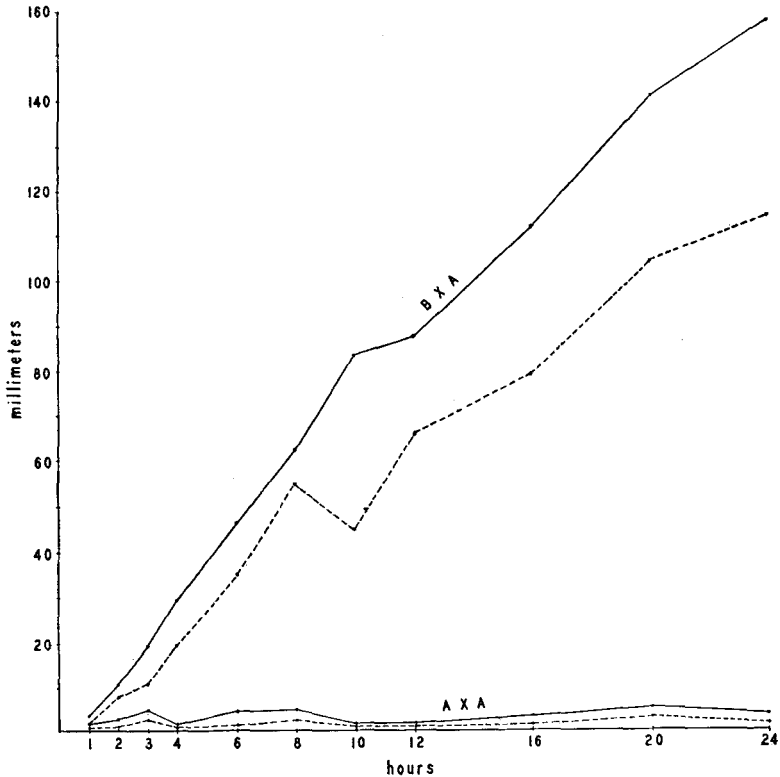


FIGURE 1.—Curves showing the growth of compatible (B×A) and incompatible (A×A) pollen tubes in the styles of flowers removed from the plants. The solid lines show the lengths of the longest pollen tubes, the dotted lines show the mean lengths of all pollen tubes in the styles.

The distinction between compatible and incompatible combinations is clearly evident after four hours, at which time the longest pollen tubes are still within the relatively thick portion of the style (approximately 1 millimeter in diameter) where dissections are readily made. All subsequent tests for cross-incompatibility have been made by this method. In figure 2 the results of tests made in the summer of 1936 are summarized. In these tests the time varied from four to five hours and the temperature from 20° to 27°C. The tests are generally very clear, with the exception of occasional flowers in which incompatible pollen tubes grew longer than is customary,

and in such flowers very few pollen tubes reacted in this way. Consequently, tests made by this method proved much more reliable than those in which the failure of seeds to set was used as a criterion of incompatibility.

The culture which had previously been tested by the set of capsules (table 1) proved to have 8 plants of type A, 5 of type B, 4 of type C and 3 of type D, and there was one plant that failed to flower. Other cultures grown in that year were not tested as completely, but only one culture contained plants belonging to a different incompatibility group. This culture had 8 plants of type C and 3 of type E, with 11 that failed to flower.

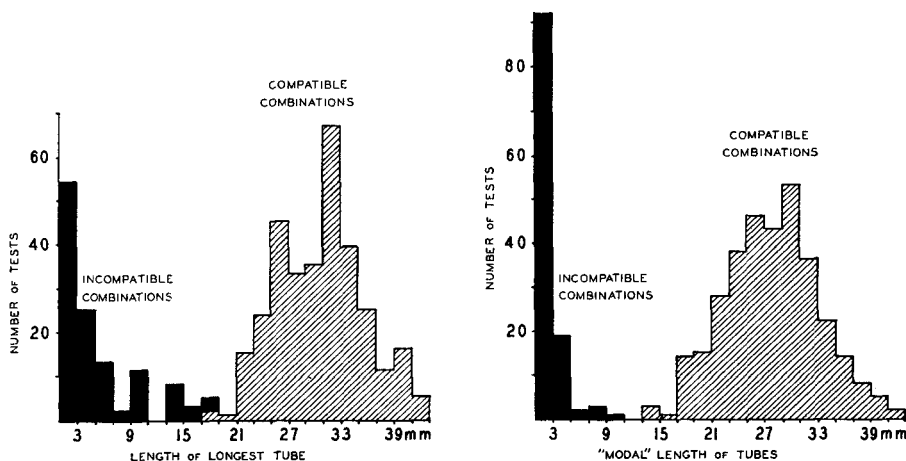


FIGURE 2.—Distribution curves of pollen tubes of compatible and incompatible combinations 4 to 5 hours after pollination. The modal length was determined by inspection, not from measurements of all tubes.

PROGENY TESTS

Intercrosses between the different incompatibility groups were grown in the greenhouse in the winter of 1936-37 where their flowering was induced by artificial illumination (long day treatment). The frequencies of the various incompatibility types appearing in these cultures are summarized in table 2, to which have been added data from similar crosses grown in the summer of 1937.

The data indicate that a series of multiple allelomorphs are responsible for self-incompatibility in *Oenothera organensis*, as in *Nicotiana* (EAST and MANGELSDORF 1925) and many other plants. The action of these allelomorphs is such that pollen carrying any particular allelomorph fails to produce normally developing pollen tubes in styles heterozygous (or homozygous) for that allelomorph. In those crosses in which the parents had completely dissimilar allelomorphs, four sterility types are expected

TABLE 2

Frequencies of different incompatibility types in the progenies of intercrosses between types A, B, C, D and E.

CROSS	GENOTYPES OF PARENTS	TYPE A S_1S_2	TYPE B S_3S_4	TYPE C S_1S_3	TYPE D S_2S_4	TYPE E S_2S_3	TYPE F S_1S_4
A×B	$S_1S_2 \times S_3S_4$	$\begin{cases} 5 \\ 4 \\ 6 \\ 6 \end{cases}$	$\begin{cases} 4 \\ 0 \\ 1 \\ 2 \end{cases}$	$\begin{cases} 3 \\ 0 \\ 3 \\ 9 \end{cases}$	$\begin{cases} 9 \\ 1 \\ 2 \\ 1 \end{cases}$
B×A	$S_3S_4 \times S_1S_2$	$\begin{cases} 1 \\ 1 \end{cases}$	$\begin{cases} 1 \\ 1 \end{cases}$	$\begin{cases} 1 \\ 1 \end{cases}$	$\begin{cases} 1 \\ 1 \end{cases}$
A×C	$S_1S_2 \times S_1S_3$	$\begin{cases} 2 \\ 4 \\ 10 \end{cases}$	$\begin{cases} 3 \\ 2 \\ 16 \end{cases}$
C×A	$S_1S_3 \times S_1S_2$	$\begin{cases} 1 \\ 2 \\ 7 \\ 9 \\ 5 \end{cases}$	$\begin{cases} 1 \\ 1 \\ 3 \\ 9 \\ 4 \end{cases}$
A×D	$S_1S_2 \times S_2S_4$	$\begin{cases} 3 \\ 0 \end{cases}$	$\begin{cases} 2 \\ 1 \end{cases}$
D×A	$S_2S_4 \times S_1S_2$	3	0
B×C	$S_3S_4 \times S_1S_3$	$\begin{cases} 5 \\ 2 \end{cases}$	$\begin{cases} 1 \\ 6 \end{cases}$
C×B	$S_1S_3 \times S_3S_4$	1	2
B×D	$S_3S_4 \times S_2S_4$	1	1
D×B	$S_2S_4 \times S_3S_4$	0	1
C×D	$S_1S_3 \times S_2S_4$	$\begin{cases} 1 \\ 0 \end{cases}$	$\begin{cases} 2 \\ 0 \end{cases}$	$\begin{cases} 2 \\ 2 \end{cases}$	$\begin{cases} 0 \\ 0 \end{cases}$
D×C	$S_2S_4 \times S_1S_3$	2	0	1	3
E×A	$S_2S_3 \times S_1S_2$	12	4
E×B	$S_2S_3 \times S_3S_4$	7	4
E×C	$S_2S_3 \times S_1S_3$	10	12
E×D	$S_2S_3 \times S_2S_4$	13	7

among the offspring, none of which represents either parental type. In those crosses in which the parents had one allelomorph in common, only two sterility types are expected among the offspring, and one of these should be identical to that of the male parent.

The allelomorphs responsible for the present types may be determined by the following method:

The cross A×B yields four types, C, D, E and F, hence A and B can have no allelomorph in common. A may be taken as S_1S_2 , B as S_3S_4 .

The cross A×C produces only two types, C and E, hence A and C have one allelomorph in common and this is taken as S_1 .

The cross B×C also produces two types, C and F, and the allelomorph which B and C must have in common is taken as S_3 . Then C has the constitution S_1S_3 .

In the cross $A (S_1S_2) \times C (S_1S_3)$, only S_3 pollen functions, producing S_1S_3 which is type C and S_2S_3 which must then be type E.

In the cross $B (S_3S_4) \times C (S_1S_3)$, only S_1 pollen functions, producing S_1S_3 which is type C and S_1S_4 which must then be type F.

Since the crosses $A \times D$ and $B \times D$ each produce but two types including that of the pollen parent, D must have one allelomorph in common with type A and one with type B, and since C and D can have no allelomorph in common (the four types A, B, E and F are produced in intercrosses between them). D must be S_2S_4 .

The remaining crosses listed in table 2 serve as a check on this interpretation. The o's included in this table indicate classes that should have occurred in the particular families but which were not observed.

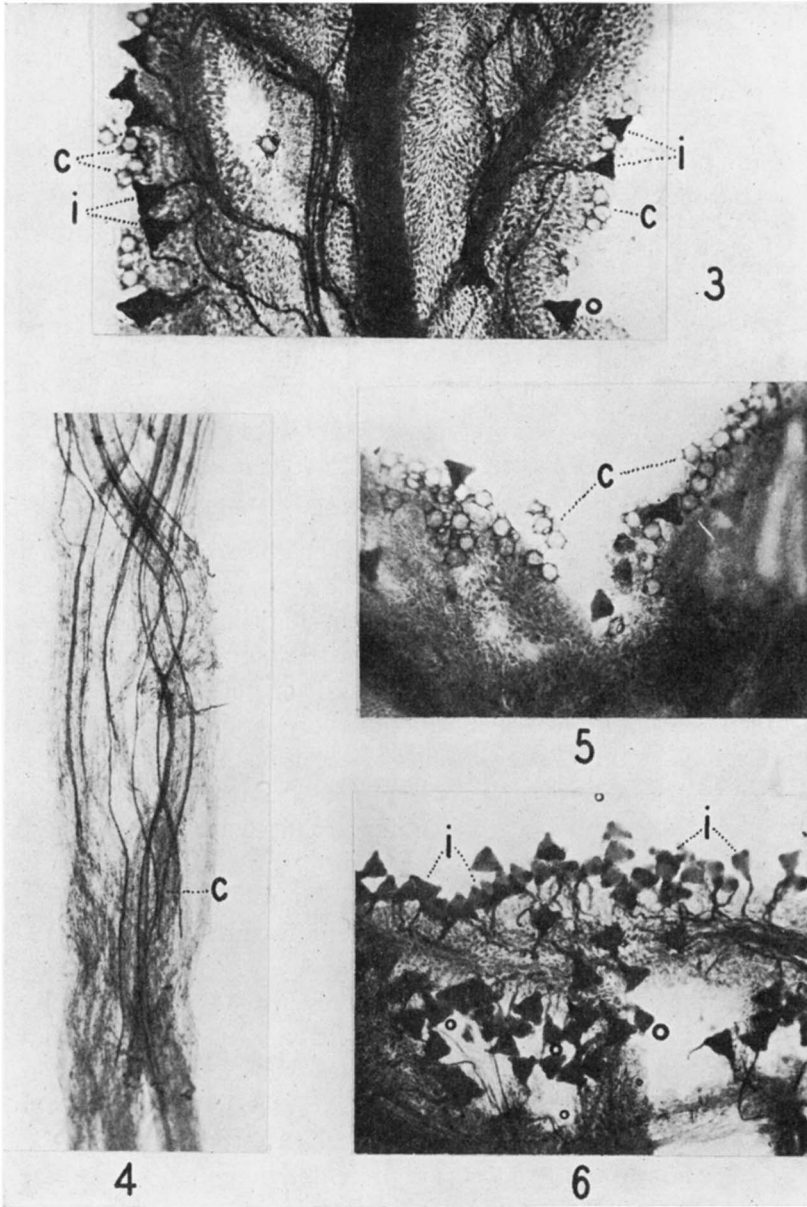
TESTS FOR ADDITIONAL ALLELOMORPHS IN THE GAMETOPHYTE GENERATION

An examination of stigmas prepared four hours after pollination showed that it is possible to distinguish between the two sorts of pollen in crosses between types having an allelomorph in common, for example $S_1S_2 \times S_1S_3$. Figure 3 is a photomicrograph of a stigma following this type of pollination. The incompatible pollen (carrying the allelomorph also present in the stylar tissue) produces very short tubes and the pollen grains retain their contents. The compatible pollen produces very long tubes (fig. 4) and the pollen grains and the upper portions of the tubes are empty and fail to stain. Figure 5 is a photograph of a completely compatible combination in which all of the germinating pollen has produced long tubes, the pollen grains becoming empty. Figure 6 is a photograph of a completely incompatible combination, equivalent to self-pollination, in which all of the germinating pollen produces short tubes and in which there are no empty pollen grains.

By using the presence of two types of pollen, that with long tubes and that with short, to indicate the presence of an allelomorph common to the two parents, it is possible to determine the genetic constitution of any particular plant without progeny tests. This method was used in analyzing the crosses obtained from Professor CLELAND (the Dripping Springs Strain). The data from these tests are presented in figures 7 and 8. Figure 7 is a table showing the behavior of pollen in intercrosses between the various incompatibility types occurring. Figure 8 is a table showing the incompatibility groups expected and the observed frequencies of plants belonging to these groups in the different crosses.

The details of the analysis follow:

Type O has one allelomorph in common with types C (S_1S_3) and E (S_2S_3) none with type A (S_1S_2), hence O has allelomorph S_3 . Type O has no



FIGURES 3 to 6.—Photomicrographs of portions of prepared stigmas or styles four hours after pollination (magnification $22\times$). Figure 3, a partially compatible combination ($S_7S_8 \times S_5S_7$); c, compatible (S_5) pollen; i, incompatible (S_7) pollen. Figure 4, portion of style 20 mm below the stigma from the same preparation as Figure 3 to show ends of compatible pollen-tubes. Figure 5, completely compatible combination ($S_3S_7 \times S_6S_9$); the few stained pollen-grains failed to germinate. Figure 6, incompatible combination ($S_6S_6 \times S_6S_6$) equivalent to self-pollination.

allelomorph in common with type F (S_1S_4), hence the second allelomorph carried by O is different from all previously determined and is taken as S_5 . Type O is S_3S_5 .

Type G has one allelomorph in common with type O (S_3S_5), none with

♂	A	C	E	F	D	B	O	P	G	Q	J	H	M	S	V	T	L	N	K	Z	Γ	Φ		
♀	$\frac{1}{2}$	$\frac{1}{3}$	$\frac{2}{3}$	$\frac{1}{4}$	$\frac{2}{4}$	$\frac{3}{4}$	$\frac{3}{5}$	$\frac{3}{6}$	$\frac{5}{6}$	$\frac{3}{7}$	$\frac{5}{7}$	$\frac{6}{7}$	$\frac{5}{8}$	$\frac{6}{8}$	$\frac{7}{8}$	$\frac{5}{9}$	$\frac{6}{9}$	$\frac{7}{9}$	$\frac{8}{9}$	$\frac{6}{10}$	$\frac{7}{10}$	$\frac{7}{11}$		
C	$\frac{1}{3}$	-	$\frac{1}{2}$	$\frac{1}{2}$	+																			
E	$\frac{2}{3}$		$\frac{1}{2}$	-	+	$\frac{1}{2}$																		
F	$\frac{1}{4}$		$\frac{1}{2}$	+	-	$\frac{1}{2}$																		
D	$\frac{2}{4}$		+	$\frac{1}{2}$	$\frac{1}{2}$	-																		
B	$\frac{3}{4}$	+	$\frac{1}{2}$			$\frac{1}{2}$	-																	
O	$\frac{3}{5}$	+	$\frac{1}{2}$	$\frac{1}{2}$	+	+	$\frac{1}{2}$	-	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	+	$\frac{1}{2}$	+	+		+	+	+		+	+	
P	$\frac{3}{6}$		$\frac{1}{2}$				$\frac{1}{2}$	-	$\frac{1}{2}$		+	$\frac{1}{2}$												
G	$\frac{5}{6}$	+	+	+	+	+	$\frac{1}{2}$		-			$\frac{1}{2}$												
Q	$\frac{3}{7}$	+	$\frac{1}{2}$			$\frac{1}{2}$			+	-	$\frac{1}{2}$	$\frac{1}{2}$	+							$\frac{1}{2}$				
J	$\frac{5}{7}$	+	+	+	+	+	$\frac{1}{2}$		$\frac{1}{2}$	$\frac{1}{2}$	-	$\frac{1}{2}$	$\frac{1}{2}$	+	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	+	$\frac{1}{2}$	+	+	$\frac{1}{2}$	$\frac{1}{2}$	
H	$\frac{6}{7}$	+	+	+	+	+	+		$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	-	+	$\frac{1}{2}$	$\frac{1}{2}$	+	$\frac{1}{2}$	$\frac{1}{2}$	+	$\frac{1}{2}$	$\frac{1}{2}$	+	$\frac{1}{2}$	
M	$\frac{5}{8}$								$\frac{1}{2}$		$\frac{1}{2}$	+	-	$\frac{1}{2}$	$\frac{1}{2}$				$\frac{1}{2}$				+	
S	$\frac{6}{8}$						+		$\frac{1}{2}$		+	$\frac{1}{2}$	$\frac{1}{2}$	-	$\frac{1}{2}$		$\frac{1}{2}$	+	$\frac{1}{2}$					
V	$\frac{7}{8}$						+	+	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	-		+	$\frac{1}{2}$	$\frac{1}{2}$	+	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	
W	$\frac{3}{9}$									$\frac{1}{2}$		+					$\frac{1}{2}$							
T	$\frac{5}{9}$						$\frac{1}{2}$	+	$\frac{1}{2}$		$\frac{1}{2}$	+					$\frac{1}{2}$		$\frac{1}{2}$					
L	$\frac{6}{9}$	+				+			$\frac{1}{2}$	+	+	$\frac{1}{2}$	+	+			-	$\frac{1}{2}$	$\frac{1}{2}$		+			
N	$\frac{7}{9}$	+	+	+	+	+	+		+	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	+	+	$\frac{1}{2}$		$\frac{1}{2}$	-	$\frac{1}{2}$	+		$\frac{1}{2}$		
K	$\frac{8}{9}$			+	+				+		+	+			$\frac{1}{2}$					-			+	
X	$\frac{3}{10}$									$\frac{1}{2}$		+												
Z	$\frac{6}{10}$	+	+	+	+	+			$\frac{1}{2}$		+	$\frac{1}{2}$	+	$\frac{1}{2}$	+		$\frac{1}{2}$	+	+	-	$\frac{1}{2}$	+		
Γ	$\frac{7}{10}$	+	+	+		+	+		+	+			+		$\frac{1}{2}$			$\frac{1}{2}$	+	$\frac{1}{2}$			$\frac{1}{2}$	
Θ	$\frac{3}{11}$									$\frac{1}{2}$		+												
Φ	$\frac{7}{11}$	+	+			+	+		+		$\frac{1}{2}$	$\frac{1}{2}$			$\frac{1}{2}$			$\frac{1}{2}$						

FIGURE 7.—Behavior of pollen-tubes in crosses between the different incompatibility types. The + symbol indicates a completely compatible combination, - a completely incompatible combination and $\frac{1}{2}$ a combination with one allelomorph common to both parents. The letters in the headings refer to the incompatibility types, the fractions under or beside them refer to the allelomorphs of S carried by each type, for example, $\frac{3}{5}$ represents S_3S_5 , etc.

types A to F (S_1 to S_4), hence G has S_5 and no other previously determined allelomorph. Type G is S_5S_6 .

Type H has one allelomorph in common with type G (S_5S_6), none with type O (S_3S_5), hence H carries S_6 and no other previously identified allelo-

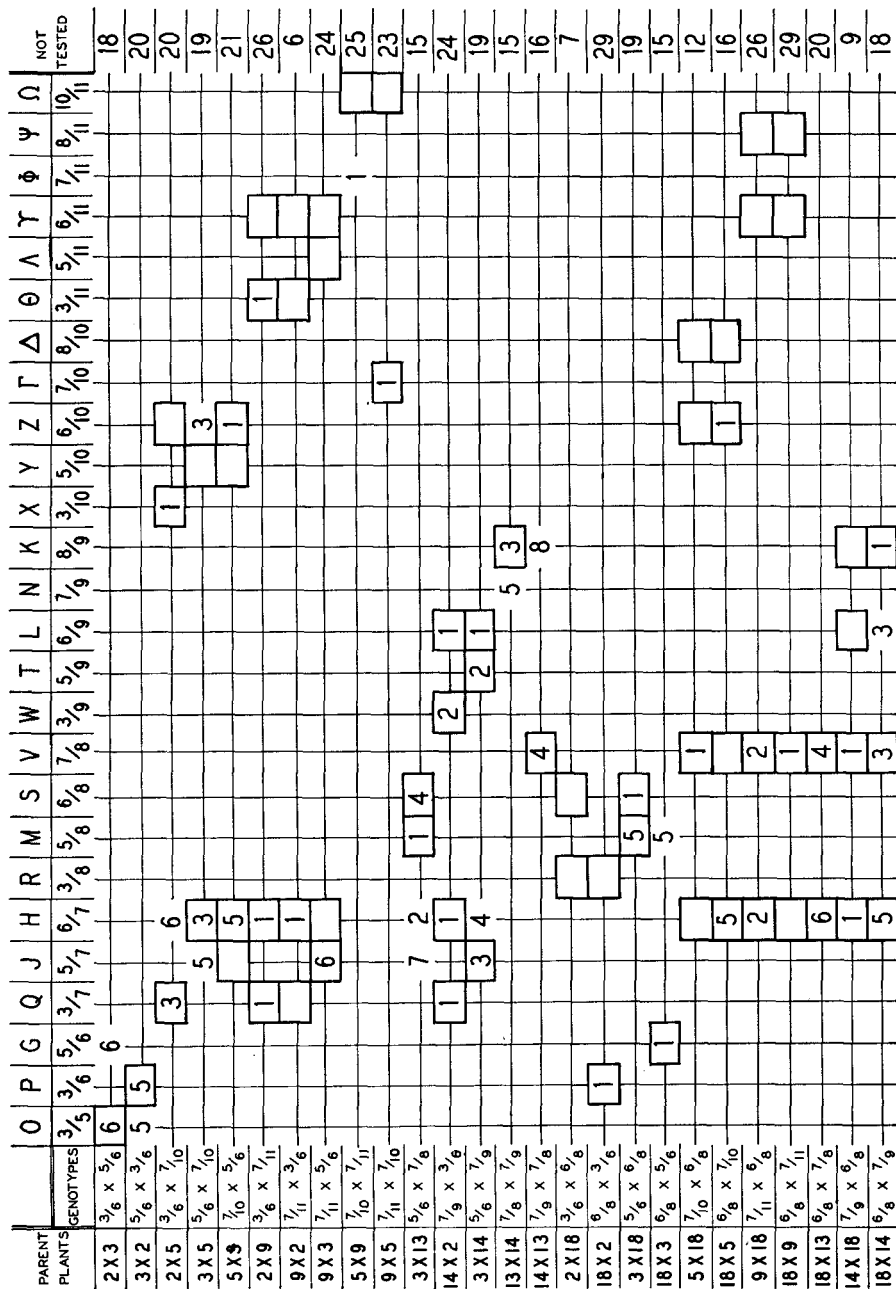


FIGURE 8.—Frequencies of different sterility types in different intercrosses. The heavy-lined squares in the table indicate predicted classes (see text).

morph, as shown by crosses with types A to F (S_1 to S_4). Type H is S_6S_7 .

Type J has one allelomorph in common with each of types O (S_3S_5), G (S_5S_6) and H (S_6S_7). Type J does not have S_3 as it has no allelomorph in common with type C (S_1S_3). Hence J has S_5 in common with types O and G, S_7 with type H. Type J is S_5S_7 .

Type K has no allelomorph in common with types E (S_2S_3), F (S_1S_4), G (S_5S_6) or J (S_5S_7). Type K is S_8S_9 .

Type L has one allelomorph in common with type G (S_5S_6), none with type J (S_5S_7), hence L has S_6 . Type L has one allelomorph in common with type K which can be either S_8 or S_9 . Type L is S_6S_9 .

Type M has one allelomorph in common with type J (S_5S_7), none with type H (S_6S_7), hence M has S_5 . It has one allelomorph in common with type K (S_8S_9), none with type L (S_6S_9), hence M has S_8 . Type M is S_5S_8 .

Type N has one allelomorph in common with types J (S_5S_7) and L (S_6S_9) none with type G (S_5S_6), therefore N has S_7S_9 .

Type Z has one allelomorph in common with type G (S_5S_6), none with type J (S_5S_7), hence type Z has S_6 . Type Z has no allelomorph in common with types A to F (S_1 to S_4) nor with type K (S_8S_9). Type Z is S_6S_{10} .

Type Φ has one allelomorph in common with type J (S_5S_7), none with type G (S_5S_6), hence Φ has allelomorph S_7 . Type Φ has no allelomorphs in common with types A (S_1S_2), B (S_3S_4), K (S_8S_9) or Z (S_6S_{10}). Type Φ is S_7S_{11} .

After these types had been identified, the genetic constitutions of the parents were deduced. It was then possible to predict the types that should occur in all intercrosses. The method of determining the constitutions of the parents is as follows:

Parent no. 3.

$\#2 \times \#3$ produced type G, $\#3$ has S_5 or S_6 or both.

$\#3 \times \#2$ produced type O, $\#3$ has S_3 or S_5 , hence $\#3$ is one of S_3S_6 , S_5S_6 , S_5S_8 .

$\#3 \times \#13$ produced type H, $\#3$ has S_6 or S_7 , hence S_3S_6 , S_5S_6 or S_5S_7 .

$\#3 \times \#13$ produced type J, $\#3$ has S_5 or S_7 not both, hence parent no. 3 is S_5S_6 or type G.

Parent no. 14.

$\#3 \times \#14$ produced type H (S_6S_7) and since S_6 came from $\#3$, $\#14$ has allelomorph S_7 .

$\#3 \times \#13$ produced type H, hence $\#13$ similarly has S_7 .

$\#13 \times \#14$ produced type N (S_7S_9) and since $\#13$ and $\#14$ both have S_7 , the pollen parent must be type N. Parent no. 14 is S_7S_9 or type N.

Parent no. 13.

#13 has been shown above to have S_7 .

#14 × #13 produced type K (S_8S_9) and since S_9 is from #14, parent #13 is S_7S_8 , called type V.

Parent no. 5.

#3 × #5 produced types J (S_5S_7) and Z (S_6S_{10}), and since allelomorphs S_5 and S_6 were from #3, parent no. 5 is S_7S_{10} called type Γ.

Parent no. 2.

#3 × #2 produced type O (S_3S_5) and since S_5 is from #3, #2 must have S_3 .

#2 × #5 produced type H (S_6S_7) with S_7 from #5, hence parent no. 2 is S_3S_6 called type P.

Parent no. 9.

#9 × #2 produced type H (S_6S_7) with S_6 from #2,

#5 × #9 produced type Φ (S_7S_{11}) with S_7 from #9, parent no. 9 is S_7S_{11} or type Φ.

Parent no. 18.

#18 × #3 produced type M (S_5S_8) with S_5 from #3,

#18 × #14 produced type L (S_6S_9) with S_9 from #14, parent no. 18 is S_6S_8 called type S.

In figure 8, the heavy-lined squares indicate the additional classes expected in the various crosses. The numbers enclosed in these squares represent the observed frequencies of these classes. Unfortunately a fairly high proportion of the plants failed to flower during the summer so that the tests are necessarily incomplete. The data are sufficient, however, to serve as a reasonably good check on the above determinations.

CHROMOSOME CONFIGURATIONS

The culture of *Oenothera organensis* grown in 1931 directly from seeds collected at Modoc Waterfall contained plants with different chromosome configurations. The female parent of the 1934 culture had seven chromosome pairs. At least one other plant had a ring of six chromosomes and four pairs. Plants of the 1934 culture were not examined cytologically, but both configurations appeared in the 1936 culture derived from it. The configurations observed in the 1936 culture discussed earlier were:

Type A (S_1S_2)—	{	6 plants with 7 pairs
	{	1 plant with a ring of 4
Type C (S_1S_3)—		3 plants with 7 pairs
Type D (S_2S_4)—		3 plants with ring of 6
Type B (S_3S_4)—		4 plants with ring of 6

The ring of 6 appeared whenever S_4 was associated with either S_2 or S_3 . With the exception of the one plant with a ring of four chromosomes, 7 pairs resulted from associations of S_1 with S_2 and of S_1 with S_3 . The further crosses made to test the association of the ring of 6 with S_4 are reported in table 3. While the tests are not extensive, all of the plants carrying S_4 (a total of 8) had rings of 6 chromosomes, whereas those with combinations of other allelomorphs (a total of 6) had 7 pairs of chromosomes.

TABLE 3

PARENTS	PROGENY
S_1S_2 (7 pairs) \times S_2S_4 (ring 6)	1 S_2S_4 with ring of 6
	1 S_1S_3 with 7 pairs
S_1S_2 (7 pairs) \times S_3S_4 (ring 6)	3 S_1S_4 with ring of 6
	1 S_2S_3 with 7 pairs
	3 S_2S_4 with ring of 6
S_1S_2 (7 pairs) \times S_2S_4 (ring 6)	1 S_1S_2 with 7 pairs
S_2S_4 (ring 6) \times S_1S_2 (7 pairs)	1 S_1S_2 with 7 pairs
S_3S_4 (ring 6) \times S_2S_4 (ring 6)	1 S_2S_4 with ring of 6
S_1S_2 (7 pairs) \times S_1S_3 (7 pairs)	1 S_2S_3 with 7 pairs

The exceptional type A plant with a ring of 4 chromosomes (S_1S_2) was pollinated by a C-type plant (S_1S_3) with 7 pairs. Among the progeny, one S_1S_3 plant had 7 pairs, one S_2S_3 plant had a ring of 4, and three plants of the latter type had 7 pairs of chromosomes. These data, while extremely meagre, indicate that the S locus is independent of the ring of 4 chromosomes.

Both the ring of six chromosomes and the ring of four are invariably associated with the nucleolus. That the nucleolar chromosome does not contain the S locus is shown by the inheritance of the ring of four.

THE INHERITANCE OF MALE-STERILE

The 1936 culture previously discussed also segregated for male-sterile. In the male-sterile plants, meiosis proceeds normally, but the pollen grains produced are small and deficient in starch content and the anthers fail to dehisce.

Of the 20 plants in the 1936 culture, 5 were male-sterile. One of these belonged to type C (S_1S_3), four to type A (S_1S_3). Intercrosses between normal sibs showed that male-sterile is a simple recessive and that several of the normal plants were heterozygous for the gene (ms) involved. A B-type plant (S_3S_4) with a ring of 6 chromosomes and heterozygous for ms was backcrossed to an A-type (S_1S_2) male-sterile. Of the offspring carrying S_3 , 14 were normal and 6 male-sterile; of the progeny carrying S_4 , 2 were normal and 1 male-sterile. These results indicate that ms is independent of the S locus and of the ring of 6 chromosomes associated with S_4 .

SUMMARY AND CONCLUSIONS

Self-incompatibility in *Oenothera organensis* is governed by a series of multiple allelomorphs in the same manner as in *Nicotiana* (EAST and MANGELSDORF, 1925). Pollen carrying any particular allelomorph fails to produce normally developing pollen-tubes in any style also carrying that allelomorph. The scheme was proved to hold for *Oe. organensis* first by progeny tests and secondly by tests of pollen-tube development in the tissues of the style and stigma.

The failure of seed production proved to be an unreliable criterion for determining cross-incompatibility, but direct tests of pollen tube development within the stigma proved entirely satisfactory. These results suggest that certain of the complications observed by SIRKS (1926) in *Verbascum* and by SEARS (1937) in *Brassica* may have been due to the inadequacy of the seed-set test for determining cross-compatibility.

The examination of pollen tube development directly within the stigma has a further advantage in that progeny tests are rendered unnecessary in establishing genetic constitutions.

In the studies to date, eleven allelomorphs have been established. Three of these were found only in material from Modoc Waterfall, seven only in material from Dripping Springs. Only one allelomorph occurred in both samples. An extensive test of the distribution of allelomorphs in the native populations is now under way.

Other characters studied in *Oenothera organensis* were chromosome configurations and male-sterility. Translocations involving three chromosomes are associated with allelomorph S_4 so that all combinations between S_4 and S_1 , S_2 and S_3 have a ring of six chromosomes. All possible combinations between S_1 , S_2 and S_3 have normally pairing chromosomes. A ring of four chromosomes appearing in a single plant proved to be independent of the S locus. Since both the ring of six and the ring of four are invariably associated with the nucleolus, S cannot be carried by the nucleolar chromosome. Male-sterile (ms) proved to be a simple recessive independent of the S locus and of the ring of six.

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