CHAPTER 5 OENOTHERA CYTOGENETICS

The genus Oenothera, subgenus Euoenothera, the evening primrose, * has been important in genetics and cytogenetics ever since deVries' first publication in 1901. The work will be described in considerable detail because of its fundamental aspects and also its possible applications.

In his book on the mutation theory (Vol. I, 1901), deVries described the monstrosities and new types that had been reported in many species, and then presented the results of his own observations on Oenothera. In 1886, deVries had begun a search in the country around Amsterdam for material suitable for investigating the origin of species. He looked for species which were throwing off-types including monstrosities. Only one showed this behavior among more than 100 species that he brought into cultivation. This was Oenothera lamarckiana. It had begun to spread (about 1875) from a neighboring park to an abandoned potato field near Hilversum, Holland. By 1885 it had extended over about half the field, and in succeeding years multiplied still more rapidly. In 1887 he noted two distinct new types, O. brevistylis and O. laevifolia, each of which bred true from seed. Nine rosettes of lamarckiana were moved to the botanical garden in Amsterdam, and seeds from two other types were collected. From these he established three families which he studied in succeeding generations from seed produced by self-pollination. The results obtained in the lamarckiana family as listed by deVries, are presented in Table 41. He included only the more distinct types.

Table 41. The origin of new species from the type, lamarckiana.

Generations	gigas	albida*	oblonga*	rubri-nervis	lam-arck-iana	nanella	lata	scin-tillans*
VIII		5	1	0	1700	21	1	
VII			9	0	3000	11		
VI		11	29	3	1800	9	5	1
V		25	135	20	8000	49	142	6
IV	1	15	176	8	14000	60	73	1
III				1	10000	3	3	
II					15000	5	5	
I					9			
TOTALS	1	56	350	32	53500	158	229	8

* Later studies showed these were trisomics, plants with an extra chromosome.

As shown in Table 41, lamarckiana bred relatively true, but produced some exceptions, most of which recurred in later generations. These differed from lamarckiana in several characters, and he concluded that new species had arisen in his cultures as mutations. We know now that most of these "mutations" arose as a result of its unusual cytological and genetical constitution. In spite of this, the ideas he expressed about mutation come close to the general facts. How remarkable this is, will be evident as the ways in which Oenothera differs from most species are described. Cytological studies of different races of Oenothera showed that various numbers of chromosomes were associated in rings or chains at meiosis. Gates (1908) was the first to observe a ring in O. rubrinervis. It was confirmed in further studies by him and by Lutz, Geerts, and B. M. Davis in the period through 1911. For a considerable period, cytologists believed that pairing could be by parasynapsis (side-by-side from the beginning of pairing) or by telosynapsis (end-to-end followed by side-by-side pairing) (see Fig. 116, page 266 in the 2nd edition of Sharp's Cytology, 1926). One by one the supposed examples of telosynapsis were shown to pair by parasynapsis until Oenothera became the last remaining example. It was finally removed when it was shown that the apparent end-to-end associations were the result of interchanges. Studies of pairing showed it was parasynaptic (Emerson 1931a, Catcheside 1931).

Breeding behavior

Details of the breeding behavior were reported by Renner, beginning in 1914; by deVries (1907), Davis (1909), Bartlett (1915), LaRue and Bartlett (1917) and others. Sturtevant (1926a) summarized the earlier results of Renner, and Renner's 1946 paper is a later summary. Renner's papers of 1914 and 1917 reported the breeding behavior of O. lamarckiana. When this species was self-pollinated about half the seeds would not germinate, but when out-crossed with biennis or muricata, there was little or no inviable seed. Also these crosses produced twin hybrids in F_1, that is, two phenotypes whereas selfing produced nearly all lamarckiana (deVries 1907). Most other wild races of Oenothera behaved similarly. To explain these results, Sharp (1934, p. 336) states that Renner "advanced the hypothesis that in Oenothera there are several different genetic factor complexes which are combined in pairs in the various species and that these complexes segregate as wholes in meiosis, each gamete carrying one or the other. Moreover, certain complexes are lethal in ~~gametes~~ spores, while some combinations are lethal in zygotes." Renner assigned special names to each complex and they are now called Renner complexes. O. lamarckiana bred true because each of its two complexes, called gaudens and velans, was lethal when homozygous (zygotic lethals). DeVries had supposed at first that lamarckiana was a pure species, but it proved to be a 'complex heterozygote'. (See Table 48, page 134, for a list of the characters associated with certain complexes).

At about the same time the breeding behavior of Beaded wings, a dominant character in Drosophila was recognized by Muller (1917) as being similar to that in Oenothera, including the production of occasional mutations. When first found, Beaded did not breed true but finally Morgan established a race which did. Crossing tests showed it was not homozygous. Muller found that the major dominant gene was lethal when homozygous; and further that in the true breeding race the homologous chromosome carried a lethal closely linked with the locus of the normal allele of Beaded. It was an example of what he

$$\frac{Bd \quad +}{+ \quad l} \times \frac{Bd \quad +}{+ \quad l} = 1 \frac{Bd \quad +}{Bd \quad +} \text{ lethal}$$

$$2 \frac{Bd \quad +}{+ \quad l} \text{ viable}$$

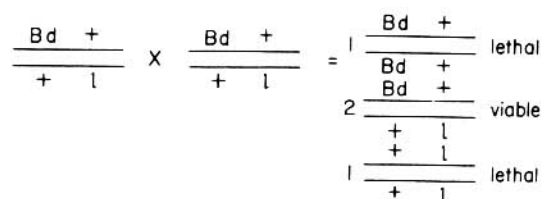$$1 \frac{+ \quad l}{+ \quad l} \text{ lethal}$$

FIG. 36. Diagrams illustrating the effect of balanced lethals on the breeding behavior of Beaded wings (Bd). Beaded is lethal when homozygous and the gene l is a recessive lethal. Barring crossing over between the two loci, the heterozygote is expected to breed true.

referred to as "balanced lethal factors", as shown in Figure 36.

Crossovers between the Bd and l loci account for the occasional normal offspring. By planned crosses with other mutants and lethals, Muller was able to produce twin and multiple hybrids. He concluded that the "so-called mutations in Oenothera lamarckiana are but the emergence into a state of homozygosis, through crossing over, of recessive factors constantly present in the heterozygous stock". This would apply to a single pair of chromosomes. The situation in lamarckiana where the velans complex of chromosomes carried one zygotic lethal and the gaudens complex carried a different one is similar; but the explanation for the fact that for the entire diploid set of chromosomes there were only two kinds of gametes came from subsequent cytological studies.

Chromosome behavior at meiosis

Cleland's 1922, 1923 and later studies showed that for a given race there was at meiosis a circle or chain of chromosomes attached end to end (as shown in Figure 37A), that the number of chromosomes in the configuration was constant for a given race, and that at anaphase I adjacent chromosomes regularly passed to opposite poles as shown in Figure 37B. He had concluded by 1926 (Published in 1929) that each chromosome had a definite position in the ring.

These observations and Belling's theory of interchange of segments between non-homologues as applied to cases of semisterility (1925, 1927), all laid the foundation for the interpretation of the cytological findings and the breeding behavior in Oenothera. Belling was the first to see the application of this hypothesis to Oenothera. Sterling Emerson (1928) used it in an analysis of the breeding behavior in Oenothera, following a suggestion made by Belling in conversation (cf. Emerson and Sturtevant 1931). At about the same time, Håkansson (1928) applied Belling's hypothesis to Oenothera. Then Darlington (1929a) published a general discussion and Cleland and Blakeslee (1930) and Cleland and Oehlkers (1929a) applied it to certain crosses in Oenothera. Based on further

FIG. 37. A. Chromosome configuration at diakinesis of meiosis in O. Lamarckiana. Note the ⊙ 12 plus one pair.
B. Configuration at early metaphase of Division I. Note the zig-zag arrangement (after Emerson, 1935, Fig. 1, p. 547, Amer. Nat. 69).

analyses of the results of crosses, Emerson and Sturtevant (1931), Cleland and Blakeslee (1930, 1931), and Darlington (1931a) concluded that Belling's interchange hypothesis could explain the cytological behavior.
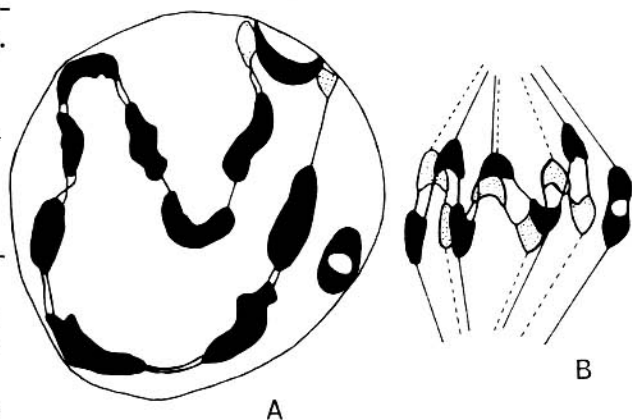
Based on that hypothesis, in the large ring the associated ends of adjacent chromosomes are homologous. For each chromosome, therefore, the two ends are homologous with the ends of different chromosomes. At least every alternate chromosome in the ring, in some cases other chromosomes also, must have resulted originally from interchange between non-homologues. This is illustrated in Figure 38 for lamarckiana with a ⊙ 12 where the chromosome ends are numbered to indicate homologies.

As shown in Figure 38, the v (velans) chromosomes alternate with the g (gaudens) chromosomes. If alternate chromosomes regularly pass to the same pole, this mode of
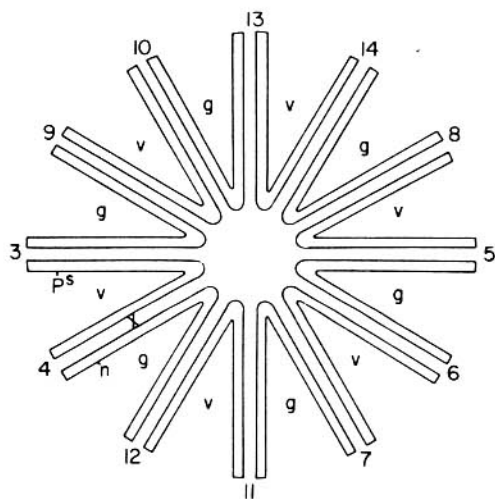
FIG. 38. Diagram illustrating the homologies of the end segments of the 12 chromosomes in the ring in O. lamarckiana, and the pachytene configuration expected if all the centromeres were median and the interchange breaks had occurred adjacent to the centromeres, consequently no differential segment in any of the chromosomes. The ends of the chromosomes are numbered to indicate the homologies, and v and g designate the chromosomes belonging to the velans and to the gaudens chromosome complexes respectively. The crossover shown in the number 4 arm between the gene for nanella (n) and the interchange point would transfer n from the gaudens to the velans complex (modified from Emerson, 1935, Fig. 4, p. 551, Amer. Nat. 69).

separation would produce only two kinds of spores with respect to the chromosomes in the ring, one with the six <u>velans</u>, the other with the six <u>gaudens</u> chromosomes. These spores are identical with the gametes that fused to produce that plant. The members of the separate chromosome pair would be free to pass to either kind of spore. How a crossover might transfer the gene <u>n</u> from the <u>gaudens</u> to the <u>velans</u> complex is shown in chromosome arm number 4 of Figure 38. The crossover gamete carrying the <u>velans</u> complex with the newly acquired <u>n</u>, when fertilized by a non-crossover <u>gaudens</u> gamete carrying <u>n</u>, would result in a nanella (<u>n n</u>) mutant. Emerson (1935) stated that this crossover of <u>n</u> into the <u>velans</u> complex must occur in about .5% of the spore mother cells of <u>lamarckiana</u>. In a similar manner, any other gene present in a chromosome in a ring might be transferred to a different complex, the frequency depending on its crossover distance from the interchange point. Lethals might be lost in a similar manner, and produce a viable homozygote for one of the complexes. These have occurred occasionally and will explain many of the "mutations" observed by deVries (Cleland 1935a). As pointed out earlier any genetic factors located in the separate pairs would be inherited independently of any factors located in the ring. In <u>lamarckiana</u> with a ⊙ 12 and 1 pair, when the red-vein factor <u>R</u> segregates, it is independent of the complexes. It is located in the separate pair. In one race the F_2 progeny from the <u>Rr</u> heterozygote segregated in a ratio of 2 <u>Rr</u>: 1 <u>rr</u> (Nilsson 1915). The homozygous dominant appears to have been lethal, probably because of a recessive lethal linked with <u>R</u>.

The zigzag arrangement at metaphase I is a mechanism for separating paternal and maternal chromosomes into different gametes. All other combinations of six chromosomes in the ring would be inviable, since they would be deficient for one or more chromosome segments.

Thus, Belling's hypothesis furnished the basis for understanding why only alternate chromosomes in such a ring could form viable combinations with the gametic number of chromosomes, and hence only two fixed complexes. The important step which made it possible for true-breeding heterozygotes to be established was the accumulation of enough interchanges to involve all or most of the chromosomes. Directed segregation is important as a mechanism for reducing the sterility.   = alternate

Survey of the genetical peculiarities of Oenothera
(effects on breeding behavior)

This survey of the behavior in <u>Oenothera</u> is based mainly on reviews by Sturtevant, (1926a), Cleland and Oehlkers (1929), Renner (1946), Cleland (1935, 1936, 1944 and 1950a); and Emerson (1935), Emerson and Sturtevant (1931).

Several hundred collections of North American and European Oenothera have been studied in detail by Renner at the University of Jena, later Munich, Oehlkers at Darmstadt, Germany, and Cleland at Indiana University, and their groups of students. Studies of the breeding behavior in selfs and in outcrosses were combined with cytological studies of parents and offspring. Several modifications of or additions to the balanced lethal mechanism that will account for the abnormal breeding behavior were found. There are four main types, namely; lethals or sub-lethals linked with the exchange points, certation, megaspore competition, and self-incompatibility. Various combinations of these are found in different races. *also plastid incompatibility.*

1. *Lethals*

To be effective, the lethals must be linked with one or more interchange points. The lethals may be zygotic or gametophytic (Renner 1933a), as shown in Figure 39; or both may be present.
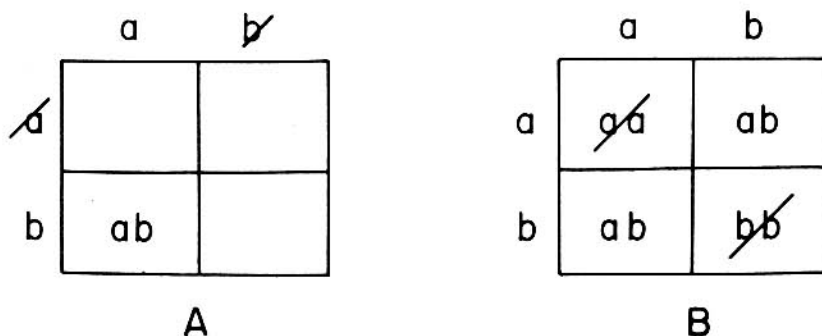

FIG. 39. Diagrams showing the two kinds of balanced lethal situations in Oenothera.
A. Gametophytic lethals. One complex of genes has a lethal which usually prevents the functioning of the male gametophyte, the other complex of genes has a lethal which usually prevents the functioning of the ♀ gametophyte. Most complexes are not rigidly confined to egg or sperm, though mostly so.
B. Zygotic lethals. Each complex of genes has a lethal which kills any zygote homozygous for the lethal. Either kind of balanced lethal situation makes it impossible usually for an individual to exist which has received the same set of genes through sperm and egg. (Cleland, 1949, re-drawn from Scientific Monthly by permission).

If zygotic, as in the velans and gaudens complexes of O. lamarckiana, the first race to be studied, both complexes could be recovered through the ♂ and ♀ in the proper outcrosses. On the other hand, if a gametophytic lethal, for example a pollen lethal, were present, the complex carrying that lethal would not be transmitted through the pollen in selfs or outcrosses. It would be received through the egg and transmitted through the egg. A pollen lethal in one complex and an embryo sac lethal in the other, as shown in Figure 39A, is a frequent combination in races of Oenothera that have large rings and are true-breeding on self-pollination. Most complexes showing such behavior are not rigidly confined to egg or sperm. Certation and megaspore competition to be discussed below may be the methods by which certain of them operate. A complex usually transmitted through the egg may be transmitted occasionally through the pollen and vice versa.

The resultant exceptional combinations, ordinarily expected only in the reciprocal cross, are called "metacline hybrids". One example is from the cross of O. chicaginensis ♀ with O. lamarckiana. In chicaginensis the excellens complex normally functions through

the eggs, the punctulans complex through the pollen. In the r- lamarckiana, velans and gaudens were transmitted through eggs and pollen. For the cross of chicaginensis ♀ x lamarckiana, Renner and Cleland (1933) reported the following offspring:

> 34 plants carried excellens and velans
>
> 1 plant carried excellens and gaudens
>
> 1 plant carried punctulans and velans
>
> 1 plant carried punctulans and gaudens

Only excellens. velans or excellens. gaudens offspring were expected. The excess of the former may have been due to certation (see next paragraph). The two plants with punctulans are metaclines. Cytological observations on the metacline punctulans. velans showed a ⊙ 12 and one pair, the same complex-combination which characterizes this combination normally produced in the reciprocal cross. Metaclines from four different crosses showed similar agreement. One of these was tested in crosses and gave the typical breeding behavior expected from that complex-combination. If the exceptional offspring had resulted from loss of a lethal or a gametophyte factor from a complex by crossing over, then the subsequent breeding behavior should have differed. The occurrence of metaclines is evidence against the presence of a true gametophytic lethal in that particular cross.

2. Certation

Certation, the differential ability of two kinds of pollen to function, is found in some races of Oenothera. For example, the shulliana race produced in outcrosses using it as the ♂ parent 7 plants carrying the jugens and 39 carrying the maculans complex. The cross lamarckiana x muricata ♂ produces gaudens. curvans hybrids in which the filled pollen grains are of two kinds in equal numbers. One is larger and has the normal spindle-shaped starch grains, while the other is smaller and has starch grains which are sub-spherical. Pollen tubes from the latter are slower growing than those from the other class. The slower growing ones must be those of curvans, as shown by the following tests. When crossed on muricata with short styles, a few curvans gametes function, but when crossed on longer-styled lamarckiana, none of the curvans gametes function. Sparse and early bud pollination furnishes a greater opportunity for the slower-growing ones to accomplish fertilization. The ovules in the upper half of the ovary are fertilized largely by the faster-growing pollen tubes. A similar situation exists in the biennis and suaveolens races, except that only the pollen with spindle-shaped starch grains germinated in vitro or on the stigmas. These must carry the complex which is functional in the ♂ . The inactive pollen grains with the spherical starch grains must carry the other complex.

3. Megaspore competition (Renner effect).

Competition in megaspore quartets was discovered by Renner. In a study of O. hookeri, a homozygous form, he found that in the linear quartet of fully-formed megaspores the one nearest the micropylar end develops. In the majority of species outside the Onagraceae family which includes Oenothera, the megaspore nearest the chalazal end develops. In O. muricata (rigens. curvans) Renner (1921), found that in about half the linear quartets of megaspores the spore at the chalazal end developed faster than the one in the favored position at the micropylar end and ultimately was the one which formed the embryo-sac. Occasionally both developed embryo sacs. Since muricata rarely produced curvans eggs, the interpretation was that the rigens complex was sufficiently vigorous to compete successfully even when it was in the less favored position, as shown in Figure 40. This behavior as a result of megaspore competition, is known as the Renner effect.
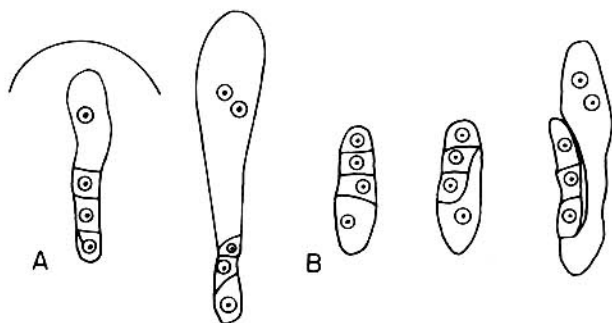
FIG. 40. Diagrams showing megaspore competition between the rigens and curvans complexes. The two megaspore quartets on the left are the ones with rigens in the favored position at the micropylar end. The two on the right are those with curvans at the micropylar end, showing that the basal megaspore with rigens is still the one that functions as the embryo-sac-mother-cell (after Renner 1921).

The relative "activities" or "strengths" of the two kinds of megaspores differ in different Oenothera races, and vary in different combinations. The rubens complex, for example (as stated by Sturtevant 1926a) "never succeeds in competition with velans, is usually unsuccessful against albicans, but never fails against curvans". Such behavior cannot be explained as due to lethals, but is explainable in terms of differences in competitive vigor of megaspore development. The frequencies of transmission of the two complexes in four races used as the female parent in outcrosses are shown in Table 42.

The data in Table 42 illustrate the effect of megaspore competition, since in each cross the ratio of the two complexes

Table 42. Frequencies of transmission of the two complexes in outcrosses using four races as the female parent.

♀ parent	number of offspring	
lamarckiana	12 velans	4 gaudens
nobska	51 pubens	3 aenescens
shulliana	66 jugens	4 maculans
muricata	mostly rigens	few curvans

deviates from a 1:1 ratio. Hence, the gaudens and velans complexes from lamarckiana, in addition to carrying a zygotic lethal, also show megaspore competition.

Does megaspore competition occur in other species outside of Oenothera? A test for its presence can be made only in situations where there is segregation for genes or deficiencies lethal to the embryo sac. In many plant species, only one of the two cells formed by the first meiotic division of the megaspore mother cells completes the second division; or if both go through division II, two of the resulting nuclei begin to degenerate immediately. In maize, evidence comes from two sources in which the linear quartet of megaspores includes deficient as well as normal megaspores, that is, in tertiary trisomics and in interchange heterozygotes with high frequencies of crossing over in the interstitial segments. In both of these the degrees of pollen and ovule abortion are similar (Burnham 1956). Had megaspore competition been frequent, ovule abortion should have been much lower than pollen abortion.

4. Self-incompatibility.

Self-incompatibility is another possible reason for the unusual breeding behavior in certain Oenothera, as shown by Steiner (1956) in a study of 17 different collections of O. biennis. The presence of a self-incompatibility factor in a given complex can be tested only if that complex is capable of functioning in ♂ and ♀ gametes and if the homozygote for that complex is viable.

The biennis races studied by Steiner have a ⊙ 14 and two complexes, one, designated α (alpha), that is usually transmitted through the egg; and the other designated β

(beta) that is usually transmitted through the pollen. On selfing they breed true; but intercrosses may give other results. For some of them the α complex is occasionally transmitted through the σ , the β sometimes through the egg.

It is thus possible to get $\alpha\alpha$ or $\beta\beta$ combinations in intercrosses between different races. Whenever $\alpha_1 \alpha_2$ offspring are not obtained, although it is known that the α_2 complex is occasionally transmitted through the male, it can be concluded that both carry the same zygotic lethal. Gametophytic lethals would preclude their transmission through a particular gametophyte, either σ or $\female$; but variation in the degree of certation or embryo sac competition for a non-lethal factor might permit an occasional one of the unexpected type to function. Seven of the 17 collections did transmit α occasionally through the pollen, 10 did not. Of the 75 crosses$_\wedge$in which $\alpha_1 \alpha_2$ might have been expected, 61 produced one or more; while 19 produced the $\beta_1 \beta_2$ combination. Surprisingly, the 33 $\alpha_1 \alpha_2$ combinations that were tested by controlled self-pollination were self-incompatible while the 19 $\beta_1 \beta_2$ combinations were self-compatible. By assuming a series of alleles similar to those in <u>Nicotiana</u>, using + for the self-compatible allele, the cross of two races might be $\alpha_1^{S_1} \beta_1^+ \times \alpha_2^{S_2} \beta_1^+$. The occasional $\alpha_1 S_1 \alpha_2 S_2$ would be self-incompatible, and $\beta_1^+ \beta_1^+$ would be self-compatible. Steiner goes on to suggest that such a system, associated with megaspore competition, could account for the origin of the true-breeding complex heterozygote in chance hybrids that occurred, and that the lethals might accumulate later.

made

Thus there are in <u>Oenothera</u> several different methods of establishing true-breeding heterozygotes. It is not surprising that <u>Oenothera</u> with all these special behaviors has offered so many problems which at first seemed unexplainable. Two additional examples will illustrate this.

Examples of true-breeding races

In the earlier stocks of <u>O. lamarckiana</u> both complexes were transmitted through both ovules and pollen. It was true-breeding because each complex carried a zygotic lethal. In 1934, a change took place such that in the new r-<u>lamarckiana</u> strain; <u>velans</u> was transmitted through the eggs, rarely through the sperm; <u>gaudens</u> was transmitted through the sperm, rarely through the eggs (Cleland and Hammond 1950). There were segmental changes in addition to the change in lethals. This strain of <u>lamarckiana</u>, when selfed, bred nearly true as it did before, but the outcrosses produced nearly all of one type in a given cross, but a different one in the reciprocal cross. This is also the breeding behavior for the <u>biennis</u> races used by Steiner for his studies of self-incompatibility just described.

<u>O. suaveolens</u> when selfed produces plants that carry the <u>albicans</u> and <u>flavens</u> complexes and an occasional weak plant with <u>flavens. flavens</u>. Here, the <u>albicans</u> and <u>flavens</u> complexes are transmitted through the $\female$, but only <u>flavens</u> is transmitted through the pollen. The <u>flavens</u> complex carries a recessive factor which is sublethal to the zygote.

The preceding discussions illustrate the fact that the various races may show very different breeding behaviors in selfs and crosses, depending on the number of independent chromosome groups, and what kinds of lethals and other factors which affect transmission are present.