Colchicine-Induced Tetraploidy in Oenothera

Adolph Hecht, Indiana University

During the past few years following the discovery by Blakeslee (1937) of the use of the drug colchicine for inducing polyploidy in plants many species have been subjected to colchicine treatment, and the great majority of these have responded by giving rise to polyploid cells, tissues and plants. The genus *Oenothera*, owing to the peculiar cytogenetics of most of its species, should provide a fertile field for a study of the genetic and cytologic behavior of tetraploids, yet very little work of this kind has thus far been reported. Other than for the preliminary report (Hecht, 1940) of this present paper, the writer is aware of only one other colchicine study with *Oenothera*, viz. the report by Stomps (1939) of the induction of *O. "gigas"* following colchicine treatment of *O. lamarckiana*. This present study was undertaken in order to study the general effects of polyploidy in *Oenothera*, and to test further some of the existing theories and hypotheses which have been developed by analysis of the cytogenetic behavior of the diploid Oenotheras.

Methods

This investigation is part of a general study which the writer is conducting on the subgenus *Raimannia* of Oenothera, and for this reason almost all of the species treated belong to the Raimannias. The taxonomic treatment of this group by Munz (1935) has been followed throughout this work. In addition to the binomials given by Munz, each collection has been assigned a race name, which follows the specific name; in most instances this race name is the name of the locality where the seed was originally obtained. The cytogenetic characteristics of these races are discussed by the writer in a paper now in preparation.

Seeds of thirty species, races and hybrids were placed upon moist filter paper in petri dishes to germinate, and as soon as evidence of germination was visible the excess water was drained off and replaced with 5 to 7 cc. of a 0.2% solution of colchicine in tap water. In the first series of experiments (1940) all germinated seeds were removed and planted two days after treatment, and on successive days thereafter up to the sixteenth day after treatment, by which time most of the viable seeds had germinated. In the second group of experiments (1941) the germinated seeds were removed and planted one day after treatment, and for several successive days thereafter all newly germinated seeds were similarly treated. All of the seedlings grew very slowly at first, and a number of them were characterized by monstrous growths. Most of the latter soon died, but a few of them survived to maturity, usually by the growth of a lateral bud. As soon as all danger of frost was over selected seedlings from each of the cultures were transplanted

(all were transplanted in 1941) into the field where they remained throughout the season.

Observations and Discussion

Table I lists the cultures which were treated with colchicine, and records the occurrence of tetraploidy following the treatment. All of the hybrids which died following colchicine treatment (except no. 19) were ones which also died before reaching maturity as untreated diploids.

Some of the plants were chimaeras, having some branches which were tetraploid and others diploid. In some instances only one branch was tetraploid and the rest of the plant was diploid, and vice-versa.

TABLE 1. **Cultures Treated with Colchicine:** A "plus" indicates that tetraploid plants were obtained, and a "minus" where all remained diploid. Cultures which died before reaching maturity are so indicated.

	Culture	Effect of Treatment
1.	*O. affinis Buenos Aires*	+
2.	*O. affinis Argentina*	+
3.	*O. affinis Florida*	+
4.	*O. laciniata Rockport*	+
5.	*O. laciniata Van Buren*	—
6.	*O. longiflora Buenos Aires*	+
7.	*O. macrosceles Saltillo*	+
8.	*O. odorata Quequen*	—
9.	*O. parodiana Florida*	+
10.	*O. parodiana Buenos Aires*	+
11.	*O. rhombipetala Bridgeport*	+
12.	*O. stricta Buenos Aires*	+
13.	*O. (Onagra) hookeri*	+
14.	*O. affinis Buenos Aires* x *O. macrosceles Saltillo*	died
15.	*O. affinis Buenos Aires* x *O. (Onagra) hookeri*	died
16.	*O. laciniata Rockport* x *O. heterophylla Kaicaster*	died
17.	*O. laciniata Jacksonville* x *O. mollissima Buenos Aires*	died
18.	*O. longiflora Buenos Aires* x *O. affinis Buenos Aires*	—
19.	*O. longiflora Buenos Aires* x *O. drummondii Corpus Christi*	died
20.	*O. longiflora Buenos Aires* x *O. rhombipetala Bridgeport*	died
21.	*O. longiflora Buenos Aires* x *O. (Onagra) hookeri*	died
22.	*O. mollissima Canelones* x *O. indecora Bañado Medina*	+
23.	*O. mollissima Canelones* x *O. rhombipetala Bridgeport*	—
24.	*O. parodiana Florida* x *O. affinis Florida*	—
25.	*O. parodiana Florida* x *O. affinis Buenos Aires*	+
26.	*O. parodiana Florida* x *O. heterophylla Kaicaster*	tentatively +
27.	*O. parodiana Florida* x *O. longiflora Buenos Aires*	—
28.	*O. parodiana Florida* x *O. (Onagra) lamarckiana*	—
29.	*O. Parodiana Buenos Aires* x *O. (Onagra) hookeri*	died
30.	*O. rhombipetala Bridgeport* x *O. laciniata Rockport*	died

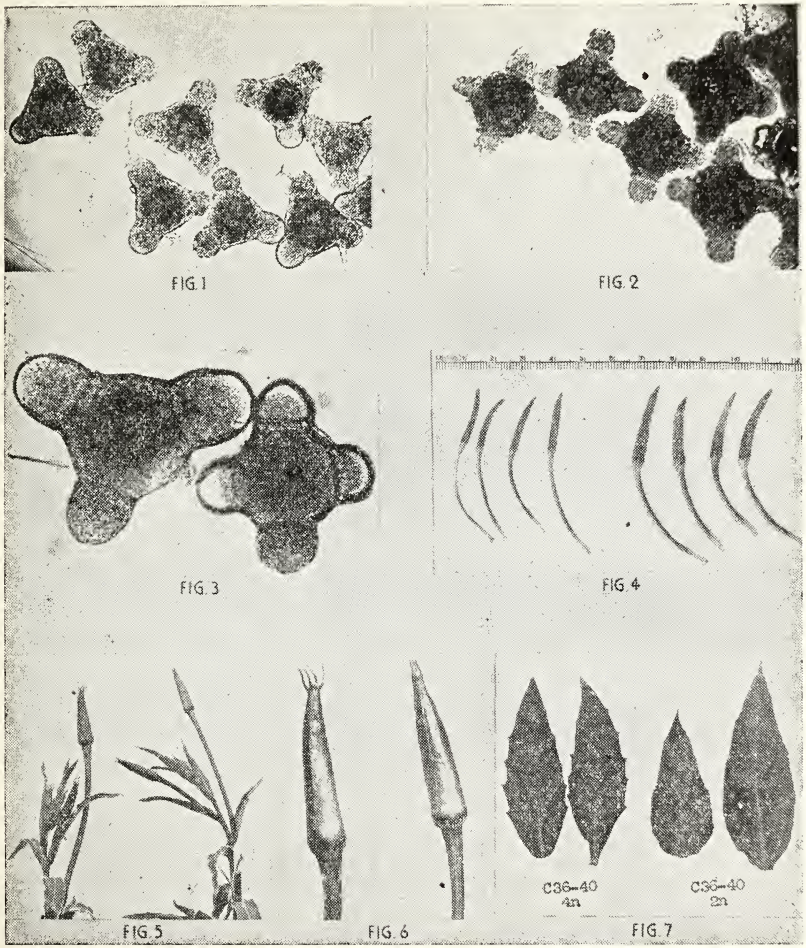


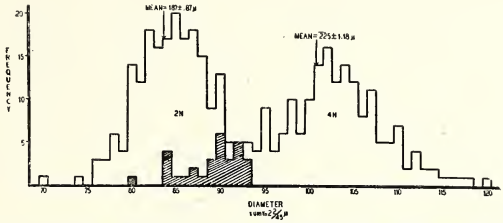
PLATE I. *Explanation of figures:* Fig. 1. Photomicrograph of pollen from diploid *O. rhombipetala Bridgeport.* Fig. 2. Photomicrograph of pollen from tetraploid *O. rhombipetala Bridgeport.* Fig. 3. Photomicrograph of pollen from tetraploid *O. laciniata Rockport.* Fig. 4. To the left, buds of diploid, and to the right buds of tetraploid *O. rhombipetala Bridgeport.* Fig. 5. On the left a shoot from the tetraploid, and on the right a shoot from the diploid of *O. affinis Argentina.* Fig. 6. On the left a bud from the tetraploid, and on the right a bud from the diploid of *O. affinis Argentina.* Fig. 7. To the left, leaves from tetraploid, and to the right leaves from diploid *O. rhombipetala Bridgeport.*

In other instances the division of the two chromosome types appeared to be more equal, such that one side of the plant was tetraploid and the other diploid.

The tetraploids were distinguished from the diploids on a basis of several general morphological differences which will be discussed later, and of course by chromosome count at meiosis (n=7), but a rapid and apparently reliable indicator of tetraploidy was found in the character of the pollen. Pollen from diploid Oenotheras is characterized by having three prominent lobes (Fig. 1), on which the germinal pores are located, and only quite rarely are grains with four or more lobes found. Lutz (1909) showed that four lobed grains such as are shown in Figure 2 are the rule in *O. "gigas"*, and occasionally grains with even a greater number of lobes (as many as eight) may be formed. This phenomenon has been studied and further correlated with chromosome number by Boedyn (1924, 1928). This pollen character of tetraploids is not peculiar to *Oenothera*, but owing to the fact that the germinal pores are located on prominent lobes the character is more obvious in this genus than in most others. Warmke and Blakeslee (1939) figure pollen grains from colchicine-induced tetraploids of *Nicotiana* with four germinal pores as compared with only three for the diploids, but other than for their being larger in size these tetraploid grains exhibit no surface features not present on the diploid grains. Four and more lobed grains are frequently found in *Oenothera* hybrids which show some degree of non-disjunction, but in such instances these are accompanied by one and two lobed grains. The latter likely represent the lesser chromosome-number cells following non-disjunction, and the former, those cells which contain more than the haploid complement. The four lobed pollen from some of the tetraploids, such as those of *O. rhombipetala Bridgeport*, have their lobes of equal size, and arranged in the shape of a Maltese cross (Fig. 2), but others, such as those of *O. laciniata Rockport*, have at least some of their pollen in the shape of the ordinary triangular haploid pollen, but with one or more smaller lobes emerging from some other surface of the grain (Fig. 3). As may readily be seen by comparing Figures 1 and 2, which are at the same magnification, the pollen from the tetraploids is appreciably larger than that from the diploids. Measurements of the four lobed grains from the tetraploids and of the three lobed grains from the diploids of *O. rhombipetala Bridgeport*, *O. laciniata Rockport* and *O. affinis Argentina* show distinct means for these two types of grains.

Some of the pollen from the tetraploids is three lobed, but the grains are larger than those produced by the diploids. A series of four-hundred measurements were made to compare the diameters of the three lobed grains from diploid and tetraploid *O. affinis Argentina*. Diameters were measured from the tip of a lobe perpendicular to the opposite base. Two hundred grains of each type were measured, and the results, plotted according to the micrometer units used—each of which equals 2 and 2/45 microns, are shown graphically in text Figure 1. The shaded area represents the overlap of the grains from the tetraploids on those from

the diploids. Since the mean diameter of the latter is thus 187± a standard error of 0.27 microns, and that of the former is 225±1.18 microns, it is apparent that the three lobed grains from the tetraploids are different from similarly-shaped haploid grains from the diploids, and the former likely contain the diploid chromosome number as do the four lobed grains. While such evidence is only presumptive it is supported by the fact that the pollen of long established tetraploid *Oenothera* species, such as *O. (Hartmannia) speciosa* and *O. (Kneiffia) glauca*, and the octaploid *O. (Kneiffia) fruticosa* have mostly three lobed pollen. *O. glauca* has previously been reported as tetraploid by Schwemmle (1924), and the present writer has been able to confirm this condition by chromo-



Text-figure 1. Block diagram comparing the distribution in size of three-lobed pollen grains from diploid and tetraploid *O. affinis Argentina*. The shaded area represents the overlap of the grains from the tetraploids on those from the diploids.

some counts. Dr. J. M. Beal of the University of Chicago has studied *O. speciosa* and found it to be tetraploid but has not yet published his findings; the writer has obtained plants of *O. speciosa* from Beal, and chromosome counts made from them confirm the latter's observations. *O. fruticosa* likely represents the first case of naturally occurring octaploidy to be reported for *Oenothera*. A cytological study of this octaploid is projected.

The tetraploids can be distinguished fairly well from the diploids on a basis of certain gross morphological differences, but each species differs to some extent in the expression of these tetraploid characteristics. In general, however, the tetraploids tend to be slightly taller, have slightly larger flowers, thicker leaves, stems, hypanthia, etc., and somewhat larger (especially broader) leaves. Preliminary measurements of leaf thickness of *O. rhombipetala Bridgeport* show the leaves of the tetraploids to measure 40 micrometer units to 30 for the diploid leaves. Similarly for *O. affinis Argentina* the tetraploid measurement is 30 units to 20 for the diploids. This difference seems to be due to larger cells rather than to more cells. A tetraploid character shown in most of the species, but especially well in *O. affinis* consists in the spreading of the calyx tips. In Figure 5, which shows a diploid shoot on the right and a tetraploid shoot on the left, the greater spread of the calyx tips of the tetraploid and the greater diameter of the tetraploid hypanthium is clearly evident. Figure 6 is a close-up of one bud from each. The calyx tips of tetraploid *O. rhombipetala Bridgeport* are as

closely appressed as are those of its diploid buds (Fig. 4), but the serrations of the leaves well serve to distinguish the tetraploids from the diploids. Figure 7 shows two leaves from a tetraploid plant on the left, and two from a diploid plant on the right; as may clearly be seen the margins of the diploid leaves are almost entire, whereas those of the tetraploid leaves are distinctly serrate.

A more detailed cytological analysis of these induced tetraploids is now in progress, but thus far a careful study has been made of the following two races: *O. rhombipetala Bridgeport* and *O. laciniata Rockport*. Since both of these as diploids show a circle of four and five pairs of chromosomes at meiosis, and segregates to seven pairs, the maximum configuration to be expected in the tetraploids would be a circle of eight and five circles of four. The circle of eight was seen in a number of cells (in at least twelve of those carefully examined), but no more than four circles of four were found in the material available. Several circles of six were observed, and of course all chromosomes not in circles were associated as bivalents.

The fertility of the tetraploids appears to depend upon the genetic situation already present in the species as a diploid. *O. laciniata Rockport* and *O. rhombipetala Bridgeport* showed almost as high fertility (except for self-sterility in the latter) in the tetraploid as in the diploid state, but tetraploids of *O. affinis Argentina* showed very appreciably reduced fertility. Seeds of all three were obtained, however, and the progeny which were grown the following year (1941) were uniformly tetraploid in appearance; cytological studies of these are now in progress.

A tetraploid of *O. rhombipetala Bridgeport* was pollinated with pollen from a diploid plant of this race, and one mature plant has been obtained from the seed produced. As would be expected it is a triploid, twenty-one being the somatic chromosome number. Preliminary cytological observations seem to show the presence of trivalent association of the chromosomes, and second meiotic division plates with ten and eleven chromosomes, respectively, though in most instances a few of the chromosomes appear to lag.

Spontaneous tetraploids have previously been reported for *Raimannia* by Schwemmle (1928). One plant from the F_2 of *O. affinis* x *O. odorata* was found to have several tetraploid branches; their bud and pollen characteristics as described by Schwemmle agree in all respects with the colchicine induced tetraploid *affinis* described here. *O. "gigas"*, the spontaneous tetraploid of *O. (Onagra) lamarckiana*, which is a classic in genetic literature, has recently been induced following colchicine treatment, by Stomps (1939). He finds that it is entirely similar to the spontaneously evolved *"gigas"*. At present little can be predicted about any future evolutionary significance of polyploidy in either *Onagra* or *Raimannia*. At least two of the *Raimannia* tetraploids seem to be rather highly fertile and true breeding, and do show somewhat increased vigor as compared to their diploid progenitors, but whether higher polyploids can be obtained from these yet remains to be seen. As has been mentioned, natural polyploid species of *Oenothera* do occur in the subgenera

Kneiffia and *Hartmannia*, but from an evolutionary standpoint neither of these has been as successful as either *Onagra* or *Raimannia*.

Summary

1. Tetraploid plants were obtained in about fourteen races and hybrids of *Oenothera* following treatment of the germinating seeds with colchicine.

2. The tetraploids differ from the diploids in general enlargement of all parts, and in several other characters peculiar to each of the species involved.

3. Whereas pollen grains from diploid Oenotheras are three lobed, many of those from the corresponding tetraploids are at least four lobed.

4. If the diploid configuration is a circle of four chromosomes, that of the tetraploid tends to be a circle of eight, but not all cells form circles this large. The bivalents tend to become tetravalents, but some of the associations of four fail also.

5. The evolutionary significance of polyploidy in *Oenothera* is briefly discussed.

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