An Example of Achiasmatic Meiosis from Tetraploid *Artemisia douglasiana* Besser (Compositae)¹

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Received October 2, 1969

Artemisia douglasiana Besser is known to include three chromosomal races at the diploid, tetraploid, and hexaploid levels, base number $x=9$ (Keck 1946, Estes 1969). Among the polyploids metaphase I configurations normally include multivalents and bivalents. However, Estes (1969) noted a single tetraploid plant in a population from Satus Creek, Washington, which appeared to be desynaptic; it possessed an average of 0.42 bivalents and 35.12 univalents per cell at metaphase I, and multivalents were completely lacking. Though the plant set only 29.9% good pollen, one hybrid, an octoploid, was formed from an interspecific cross with tetraploid *A. lindleyana*, the latter species serving as carpellate parent. This paper describes the meiotic characteristics of the octoploid hybrid and the chromosome numbers of a series of off-spring produced by open-pollination.

Materials and methods

The Satus Creek population occupies some 100 m² of sand and gravel floodplain where US Highway 97 crosses the creek immediately north of Satus Pass, north of Goldendale, Klickitat County, Washington, U.S.A. Living material of the meiotically atypical plant (Accession 11I), along with six other individuals, was transferred to greenhouse cultivation for one year and then to an irrigated tilled garden. Floral buds of the plant—from nature, the greenhouse, and the garden—and of the hybrids were killed and fixed in chloroform-alcohol-acetic acid (6:3:1). Meiotic preparations were then stained in alcohol-carmines-hydrochloric acid and squashed in Hoyer's medium.

Observations and results

Meiotic analysis of the desynaptic plant: Synapsis of the chromosomes was evident at pachytene, the earliest stage in which they were clearly distinct (Fig. 1A). Short unpaired regions were observed, but whether these non-

¹ The original collections, determinations, and hybridizations were completed while the author held a NSF Cooperative Graduate Fellowship in the Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon, U.S.A. Financial assistance was provided at the University of Oklahoma by the Faculty Research Committee and through NSF Grant GB-8115.

synapsed sites were the result of incomplete pairing or of the first chromosome separation following pachytene could not be ascertained from these preparations.

The bivalents remained closely associated through late-diplotene and occasionally early-diakinesis. However, chiasmata were lacking in most bivalents (Fig. 1B), and by mid-diakinesis the pairs had physically dissociated so that, as previously reported (Estes 1969), only 0.42 bivalents per cell remained. The prior association was still apparent, however, as the previously synapsed chromosomes were in close proximity, lying approximately parallel one to another (Fig. 1C).

The few remaining bivalents aligned normally at the equatorial plate (Fig. 1D). The univalents, on the other hand, were not associated with the spindle apparatus at any stage of meiosis I. Rather at what seemed to be the stage immediately succeeding diakinesis, the univalents tended to be clustered in the polar regions of the cell (Fig. 1D). The resulting distributions of chromosomes in meiocytes with all 36 chromosomes positioned at one pole or the other, did not fit the distribution predicted by the binomial distribution (Table 1). Therefore, either their movement to the poles was

Table 1. Apparent anaphase I disjunctional ratios in the *A. douglasiana* desynaptic tetraploid

Ratio	$\frac{18}{18}$	$\frac{19}{17}$	$\frac{20}{16}$	$\frac{21}{15}$	$\frac{22}{14}$	$\frac{23}{13}$	$\frac{24}{12}$	$\frac{25}{11}$	$\frac{26}{10}$	$\frac{27}{9}$	$\frac{28}{8}$	$\frac{29}{7}$	$\frac{30}{6}$	$\frac{31}{5}$	$\frac{32}{4}$	Total
Obs.	2	2	8	4	5	3	1	1	1	2	1	1	1	0	1	33
Exp.	7.7	7.3	6.2	4.7	3.2	2.0	1.9									33.0

The observed ratios fail to fit the binomial distribution. $\chi^2=36.73$.

not random, or the figures were artifacts of the squash technique. Because transitional stages, between diakinesis and the polar arrangement, were not observed, the latter case seems most likely. Perhaps with pressure on the meiocytes the unattached univalents were displaced from the relatively dense equator to the poles, with a slight tendency to move as a unit and hence to depart from distributional ratios such as 18:18 or 19:17 that would predominate following random segregation.

Eighty percent of the 130 observed telophasic cells formed restitution nuclei (Fig. 1E), which supports the suggestion, above, that the simulated patterns of anaphase-like disjunctions may have been partially an artifact. Often one of the two nuclei of a dyad, or a restituted nucleus, was misshapen, indicating its possible origin from the coalescence of extra-nuclear chromosomes with the nucleus of the microspore. It was obvious that some chromatin did remain in the cytoplasm of many of the restituted cells (Fig. 1E), but counts of the number of chromosomes involved usually could not be made.

From telophase I, the remaining stages of division were normal, except

Fig. 1. Meiosis in the desynaptic *Artemisia douglasiana*. A, pachytene. B, early-diakinesis. C, mid-diakinesis. D, metaphase I with 1 bivalent at the metaphase plate, note the apparent anaphase I disjunctional ratio-23:11 with one bivalent (arrow). E, restitution nucleus with extra nuclear chromatin (arrow). F, sporads of microspores, one tetrad and three dyads, with micronuclei (arrows) in one dyad.

that chromosomes excluded from the daughter or restitution nuclei of the first division were also excluded from the nuclei of the microspores (Fig. 1F). Of the 319 sporads analyzed, 84.6% contained one or more micronuclei and about 42% of these micronuclei eventually became enclosed within a separate wall. The exclusion of these chromosomes no doubt partially accounts for the low pollen fertility reported above.

Chromosome counts obtained from mitotic division in microspores of plant 11I were, as expected, highly variable; the observed gametophytic numbers ranged from 22 to 36.

Meiotic analysis of the open-pollinated progeny: Seven progeny obtained from open-pollinated heads attained maturity. These formed an aneuploid series: $2n=32, 39, 40, 42, 43, 45$, and 48 . At the time plant 11I was receptive to pollen, diploid, tetraploid, and hexaploid plants of other accessions were actively producing pollen and were available as possible pollen parents. If megasporogenesis had been normal in plant 11I, chromosome numbers of $2n=27, 36$, and 45 could have been expected among the progeny, or if its megaspores were non-reduced, progeny having $2n=45, 54$, and 63 might be formed. It is immediately obvious, therefore, that megasporogenesis in 11I was also aberrant. Because the pollen of tetraploid plants was overwhelmingly predominant in the garden (out of about 100 plants in flower, only 3 were $6x$ and no more than 6 were $2x$), the egg cells participating in these crosses, with the possible exception of one, likely had chromosome numbers ranging between 21 and 30. None of the progeny proved to be totally desynaptic, though univalents were occasionally produced.

*Meiotic analysis of the octoploid hybrid, *A. lindleyana* × *A. douglasiana*:* The seed parent of the octoploid hybrid was collected from the shore of the Columbia River near the village of Blalock, Gilliam County, Oregon, U.S.A. Although

Fig. 2. Meiosis in *A. lindleyana* × *A. douglasiana* octoploid hybrid. a, metaphase I, $33II\ 6I$ (see Table 2). b, anaphase I in the $5x$ sub-division of the panicle, disjunctional ratio-23:22.

relatively few of the chromosome configurations of meiocytes from the octoploid hybrid could be interpreted unambiguously, a number of significant observations were recorded:

1) In the five meiocytes observed at metaphase I, bivalents overwhelmingly predominated (Table 2 and Fig. 2A). The maximum number of multivalents per cell was only two.

Table 2. Chromosomal configurations at metaphase I in the *A. lindleyana* × *A. douglasiana* octoploid hybrid

Cell	IV	III	II ¹	I	Chiasmata
1	—	—	33	6	41
2	—	—	33	6	39
3	1	—	34	—	46
4	1	1	32	1	47
5	2	—	31	2	46

¹ includes single chromosomes that likely had been previously associated.

2) The average chiasma frequency for these five cells was 43.8 chiasmata per cell, or 1.2 chiasmata per pair of chromosomes.

3) 71 of 83 (86%) examined sporads included four microspores; however, 37 of these sporads had micronuclei. Only five dyads of microspores were found.

4) Fertility, as measured by the stainability of mature pollen and the presence of three nuclei in the grain immediately prior to another dehiscence, was 90%; however, self-pollination failed to produce any akenes among the florets of 130 heads.

5) Floral buds from one sub-division of the panicle were found to have an anomalous chromosome number and floral morphology. The somatic chromosome number was 45 (Fig. 2B) rather than the normal 72, and there were 4 rather than 5 anthers per flower. Pairing relationships could not be examined, because the cells that could be analyzed were in anaphase I. This segment of the inflorescence set 65% good pollen, but no akenes were produced in any of heads that were self-pollinated.

Discussion

In *Artemisia douglasiana* (Accession 11I) the high frequency of univalents, in lieu of bivalents, at metaphase I is considered to result from desynapsis (i.e., the paired units at pachytene separated prior to the cessation of prophase). Two causative factors have been proposed for desynaptic behavior: premature terminalization and achiasmata (Thomas and Rajhathy 1966). Observations of the meiocytes at early diplotene suggest that in *A. douglasiana* (11I) desynapsis resulted from a failure of chiasma formation.

The meiotic characteristics of this exceptional plant also corresponded closely with the composite description of strongly asynaptic organisms presented by Prakken (1943), the salient features of which are listed below, with the characteristics of 11I deviating from this norm listed in italics:

- 1) Defective spindle—the bivalents appeared to disjoin normally and therefore the spindle apparatus was considered to be functional.
- 2) No movement of the univalents to the equator.
- 3) Rare splitting of the univalents at anaphase I—none were observed to divide at anaphase I.
- 4) Interphase often with other than two nuclei (one, three, or four)—one, via restitution, but frequently with extra-nuclear chromosomes.
- 5) Regular second division.

An asynaptic triploid of *Allium amplexans* has been shown to vary from the typical condition in a similar manner, and a pentaploid hybrid was produced from a cross of this plant with a normal tetraploid (Prakken 1943). The desynaptic *Artemisia douglasiana* also produced at least one eupolyploid seedling (8x) from the cross-fertilization of a non-reduced gamete (the 5x progeny possibly resulted from the union of an $n=27$ gamete of the exceptional plant with a reduced gamete of a meiotically typical tetraploid or from the union of reduced gametes of 11I and a hexaploid). Apparently the results of meiosis and of consequent fertilization of both asynaptic and desynaptic plants are similar, if univalent formation is complete. The sporadic occurrence of desynaptic and asynaptic plants through recurrent mutation, recombination, or environmental modification could therefore allow for recurring cycles of polyploidization within a species complex. The changes in chromosome number would presumably result either by cross-fertilization between the plant and the members of the original population or by self-fertilization of the desynaptic or asynaptic organism.

The similarity of meiotic consequences may, under certain circumstances, extend to organisms in which univalent formation is a function of the non-homology of the inclusive genomes (i.e. interspecific hybrids). Wagenaar (1968) found, in hybrids between *Triticum crassum* and *Triticum turgidum* which produced only univalents in the PMC's, that non-reduction was a regular feature and was correlated with a delayed metaphase I and lagging chromosomes at anaphase I. The presence of micronuclei was noted in sporads of the hybrid so presumably aneuploid pollen grains were produced as well. However, the chromosome number of the vast majority of the seedlings was double that of the parent. An artificial hybrid produced from the intergeneric cross, *Viguiera porteri* ($n=17$) $\times$ *Helianthus angustifolius* ($n=17$), formed from 6–26 univalents at meiosis I with the higher frequencies predominating (Heiser 1963). Pollen-set in the hybrid was about 20%, and on the basis of tetracolpate rather than the normal tricolpate condition and the size and number of nuclei per sporad, Heiser concluded that most of the fertile grains were polyploid. The plant did not set seed, however.

The evolutionary potentialities of newly derived polyploids have been discussed in depth elsewhere (Ehrendorfer 1959, Lewis 1967, Jones 1961 and Stebbins 1959). As has been pointed out, the success of newly formed poly-

polyploid individuals would depend upon the comparative adaptive properties of the original and derived chromosomal races, the availability of habitats suitable for the expansion of the polyploids, and the breeding mechanisms in force within the complex. The evolutionary alternatives would be three-fold: coexistence, occupancy of new sites by the polyploids, or replacement of one race by the other. In a population in which desynaptic or asynaptic organisms recurred regularly, polyploid genotypes would be produced over a number of generations. Competition between progenitor and derived populations would therefore continue unless the capacity for desynapsis were lost.

In plant 11I, restitution was frequently incomplete, so that an aneuploid series was produced within the gamete population. The evolutionary effect of this phenomenon would again depend upon the genetic system of the complex and selection by the environment. If the genotype and the environment were tolerant of aneuploidy, the resulting polyploid population would most likely be polymorphic with respect to chromosome number. Selection, however, probably acts against aneuploidy in the *Artemisia ludoviciana* complex, as only three naturally occurring individuals have been reported to deviate from the euploid condition; even though non-disjunction at anaphase I does occur in all taxa investigated (Estes 1969). These three were members of *A. douglasiana*, two were trisomic hexaploids and one a monosomic tetraploid from Satus Creek. Experimental evidence of selection against genotypes with specific numbers of chromosomes has been reported in *Holcus mollis* by Jones (1954), who found that in artificially grown progeny there were seven chromosome levels that were not present in nature. A polyploid complex derived from desynaptic plants in which selection acts against aneuploidy should thus give rise to derived races that are strictly euploid. Therefore, the only effect of deviant gamete production would be to decrease the rate with which new polyploids are formed.

Two characteristics present in *A. douglasiana* in general and in plant 11I in particular could serve to enhance the effectiveness of this hypothetical scheme for polyploid production:

- 1) This species and its relatives include only herbaceous perennials. A desynaptic individual therefore may exist over an extended period of time, and a larger total number of unreduced gametes and hence polyploid offspring might be produced from one individual than would be possible if the plant were an annual.

- 2) The Satus Creek population is tetraploid. An increase in chromosome number via desynapsis could occur more easily within a previously established polyploid population than within a diploid one. A desynaptic tetraploid, crossed with its 4x sister plants, could yield directly the balanced hexaploid level. In diploids, however, desynapsis would most often give rise to the imbalance of triploidy.

These observations demonstrate the feasibility of the origin of polyploid

and aneuploid types through desynaptic and asynaptic intermediaries. However, even if polyploid types do originate in nature via this method, it is certainly not the sole, or perhaps even the prime method in the *Artemisia ludoviciana* complex. Genotypes occur that produce unreduced microspores to a limited extent ($<10\%$ in all cases) in all the taxa examined within the complex, including *A. douglassiana* (Estes 1969). In fact, the octoploid F_1 individual from the cross of *A. lindleyana* $\times$ *A. douglasiana* (11I) very likely arose from non-reduced gametes produced by both its parents. The origin of the $2n$ gametes from the normally synaptic members of the complex is most likely the result of meiotic spindle malfunction and failure of anaphase migration (Estes unpubl.); although sporadically occurring desynaptic cells within these individuals cannot be completely discounted, none were observed. The relative importance of these two modes of origin of polyploids within the *Artemisia ludoviciana* complex cannot yet be assessed, because the products of each should be very similar. However, differences may exist in the cytological characteristics of the two types of derived populations, primarily with respect to chiasma frequency. If desynapsis is a result of achiasmata, as in this instance, and if it results from genetic control rather than environmental modification, then polyploids produced in this manner may demonstrate a lower chiasma frequency. Also the production of polyploids through a low frequency of unreduced gametes by normally synaptic plants would be a continuous process; that by rare desynaptic plants with abundant unreduced gametes would be more sporadic.

Summary

Meiosis in *Artemisia douglasiana* (Satus Creek 11I) is achiasmatic, resulting in the production of univalents at metaphase I. Restitution nuclei are a major effect of this desynapsis, and the gametes were found to have the unreduced or close to the unreduced chromosome number. The chromosome numbers of open-pollinated progeny formed an aneuploid series intermediate between the parental chromosome level ($2n=4x=36$) and the hexaploid level ($2n=6x=54$). However, one of the off-spring from an interspecific cross proved to be euploid.

Desynapsis is proposed as one possible mechanism for the production of natural polyploids.

Acknowledgments

I am most grateful to Drs. Kenton Chambers, Warren Kronstad, and Ronald Tyrl for their critical reading of this manuscript and for their many helpful suggestions. Also fiscal assistance was provided by the National Science Foundation in the form of NSF Grant GB-8115. The drawing apparatus utilized in this study was purchased with funds made available

through the Faculty Research Committee, the University of Oklahoma.

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