BRIDGE AND FRAGMENT ABERRATIONS IN *PODOPHYLLUM PELTATUM*

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McCLINTOCK (1933) observed bridges and fragments at meiotic anaphase in maize plants that also possessed paracentric inversion loops at pachytene. Crossing over within these loops was proposed as a mechanism for the formation of the bridge and fragments. Since then numerous organisms in which the pachytene stage has not been suitable for cytological investigation have been reported to be heterozygous for paracentric inversions on the basis of the circumstantial evidence of meiotic anaphase bridge and fragment configurations. However, the validity of using bridges and fragments as indicators of inversions was questioned by MATSUURA (1950), HAGA (1953), and WALTERS (1950), who proposed that chromatid breakage and fusion could account for them.

Podophyllum peltatum, a clonal, herbaceous woodland plant found in eastern and central North America, has permitted a further examination of this question. *Podophyllum* is of value in that pachytene and anaphase stages of meiosis are suitable for cytological examination, the chromosomes are large, few in number ($2n = 12$), and to some extent the chromosomes are identifiable as individuals (NEWMAN 1959). Data are presented which demonstrate bridges and fragments at anaphase which are not due to inversions identifiable at pachytene, suggesting that some mechanism other than crossing over in inversions is responsible.

MATERIALS AND METHODS

Material for the examination of microsporogenesis in pollen mother cells was collected in Ann Arbor, Michigan; Springfield, Illinois; St. Louis, Missouri; and Memphis, Tennessee. Young flower buds were fixed and stored in Newcomer's Fixative (1953) and temporary squash slides were prepared by SNOW's (1963) alcoholic carmine technique. For the critical pachytene stage, the anthers were stained with alcoholic carmine for one hour, washed in 45% propionic acid, and the contents of the locules squeezed out into a drop of 45% propionic acid. After placing a cover glass over the drop, the application of heat and gentle pressure flattened the cells and spread the chromosomes. Anthers with other stages of meiosis were stained for about 12 hours.

RESULTS

The genome of *Podophyllum peltatum* is characterized by six large chromosomes: two indistinguishable metacentrics designated by the letters A and B, two indistinguishable submetacentrics designated by the letters C and D, and two distinguishable acrocentrics designated by the letters E and F. Nucleolar organiz-

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FIGURE 1.—Photomicrograph and diagram of pachytene stage of meiosis demonstrating lack of inversion loops on chromosomes E and F. In the diagram the centromeres are represented by the small circles and the nucleolus by the letter N.

ing regions were found only on the E and F chromosomes. These two chromosomes may be differentiated in pachytene (FIGURE 1) by a difference in the length of the short arm and by the position of the secondary constriction on the

FIGURE 2.—Anaphase-II chromosome E first-division bridge and fragment configuration. Small fragment is indicated by the arrow.

long arm. The E chromosome has a longer short arm than the F chromosome, and the secondary constriction is located in the distal third of the long arm in E, whereas it is located at about the midpoint of the long arm in F. In the anaphase stages of both first and second meiotic divisions, the E and F chromosomes may be distinguished by the difference in the length of the short arms (Figure 2).

Anaphase I and II bridge and fragment aberrations were found in all clones in which these stages were available for examination. A general relationship between fragment size and thickness of bridge was observed; cells with long fragments have thin bridges and cells with short fragments have thick bridges. Bridges were found which joined the short arms of the submetacentric and E chromosomes as well as the long arms of the metacentric, submetacentric, E, and F chromosomes. Bridges were not observed between the short arms of the F chromosome. Fragments were generally rod-shaped but occasionally were U-shaped and rarely plus-shaped or circular.

Both free and attached fragments were observed in metaphase I. Bridges appear soon after the separation of homologous chromosomes during anaphase I and in some cases have an attenuated appearance in telophase I. Most if not all of the bridges seen in the first division continue to exist in the second division, as no cell wall is formed between the two interphase nuclei. From 1.2 to 21.7% (mean = 7.4%) of the prophase II cells examined contained a bridge and fragment aberration. Most of these cells contained a single bridge and a single fragment.

Measurements of fragment size made from photomicrographs of early and middle anaphase II chromosome-E and -F bridge and fragment configurations are presented in Table I. The fragments are grouped into three size categories: small (1–5 μ), medium (6–10 μ), and large (11–15 μ). The number of small fragments associated with chromosome E bridges is significantly higher ($P = .01 - .001$) than would have been expected if fragment size were random. Although there is no statistical difference ($P = 0.5 - 0.3$) between the three size categories for the F-chromosome fragments, there were more fragments in the

TABLE 1

Size of chromosome E and F associated fragments as determined from photomicrographs of early and middle anaphase-II cells

Clone number	Chromosome-E fragments				Chromosome-F fragments			
	Total fragments	Fragment size			Total fragments	Fragment size		
		1–5 μ	6–10 μ	10–15 μ		1–5 μ	6–10 μ	10–15 μ
Ann Arbor A8	17	10	3	4	13	3	5	5
Ann Arbor A11	6	5	1	0	2	0	2	0
Ann Arbor A15	6	1	3	2	10	5	2	3
St. Louis D62	3	3	0	0	11	1	7	3
Totals observed:	32	19	7	6	36	9	16	11
Totals expected:		10.67	10.67	10.67		12	12	12
		$\chi^2 = 9.8$				$\chi^2 = 2.16$		
		$P = 0.01-0.001$				$P = 0.5-0.3$		

medium-sized category. Accordingly, it appears that chromosome E tends to produce small fragments despite its similarity in length to Chromosome F.

The length and incomplete spreading of the pachytene chromosomes did not allow for analysis of all six bivalents in any one cell. Since the two metacentric bivalents could not be differentiated, the presence or absence of inversion loops in these chromosomes was determined only when the entire length of both bivalents could be analyzed in a single cell. This type of analysis also held for the submetacentrics. Chromosomes E and F (Figure 1) could easily be distinguished in pachytene on the basis of the position of both the centromere and secondary constriction.

Of the 15 clones for which pachytene figures were analyzed, two [one from Springfield, Illinois (Spr A1) and one from St. Louis, Missouri (SL D3)] were heterozygous for inversions. Inversion Spr A1 is located in a submetacentric chromosome and is pericentric, with the centromere approximately in the middle of the inverted segment. In clone SL D3 there is a paracentric inversion located in either a metacentric or submetacentric chromosome.

Since crossing over within paracentric inversion loops is a possible explanation for the bridge and fragment configurations described above, a comparison of chromosome-E and -F pachytene and anaphase-II chromosomes was made. These data are summarized in Table 2. Eight clones lacked chromosome-E and -F inversion loops in pachytene but demonstrated chromosome-E and -F bridge and

TABLE 2

Summary of data on the presence of pachytene inversion loops and chromosome-E and -F meiotic anaphase bridge and fragment configurations in clones of Podophyllum peltatum

State	Clone	Inversions (all chromosomes)	Chromosome-E and -F bridge and fragments
Michigan	AA A2	none	present
	A5	none	present
	A6	none	present
	A8	none	present
	A9	none	present
	A11	none	present
	A15	none	present
	A23	none	no data
	A24	none	no data
	AA C1	none	no data
	AA E2	none	no data
	AA F1	none	no data
Illinois	Spr A1	pericentric (metacentric chrom.)	present
Missouri	SL D3	paracentric (metacentric or sub- metacentric chrom.)	no data
Tennessee	Riv A12	none	no data

Eight clones do not have chromosome-E and -F inversions but demonstrate chromosome-E and -F bridge and fragment configurations.

fragment configurations. Thus, in these clones the bridges and fragments may occur in the absence of inversions. The comparison of pachytene and anaphase stages was made on a clonal basis, since flower buds containing both of these stages were rarely found. It is possible of course, that these data may be misleading in that some clones may contain at least two different chromosomal populations, some with and some without chromosome-E and -F inversions. However, there is no positive evidence for such clonal heterozygosity in any of the material examined.

DISCUSSION

The demonstration of inversion loops as proof of the presence of inversions has been limited mainly to work on maize pachytene and *Drosophila* salivary gland chromosomes. Pachytene loops have also been demonstrated in *Chorthippus* (DARLINGTON 1936), *Fritillaria* (FRANKEL 1937), and *Tulipa* (UPCOTT 1939). As pachytene chromosomes are technically difficult or impossible to analyze in most organisms, observations of bridge and fragment configurations have been used extensively as indirect evidence for the presence of paracentric inversions. Extensive lists of plants and animals which were reported to have inversions on the basis of this evidence are given by DARLINGTON (1937), STEBBINS (1950) and DOBZHANSKY (1951).

The breakage-fusion hypothesis: Another hypothesis to explain meiotic bridge and fragment configurations was proposed by MATSUURA (1950) working with *Trillium*, WALTERS (1950) working with *Bromus*, and HAGA (1953) working with *Paris*. It was proposed that breakage and fusion of chromatids in a four-strand stage could produce bridge and fragment configurations (see diagram in WALTERS 1950). A first-division bridge and fragment could arise from a break and fusion between two sister chromatids with a chiasma between the breakpoint and the centromere. A second-division bridge and fragment configuration could be produced by a break and fusion between two sister strands with no chiasma between the point of breakage and the centromere. The distance from the end of the chromosome to the point of the break should be equal to half the length of the fragment. Long fragments would thus indicate breaks proximal to the centromere; short fragments indicate breaks distal to the centromere.

Evidence in favor of the breakage and fusion hypothesis was based on cytological features of the bridge and fragment configuration which could not easily be explained by the inversion hypothesis. Since homologous pairing is necessary for inversion-caused bridges, univalent anaphase-I bridges and bridges between nonhomologous chromosomes could not have resulted from this cause. Univalents, composed of two sister chromatids joined by a centromere, may result from a failure in the pairing of homologous chromosomes, failure of chiasma formation between paired chromosomes, or the lack of a homologous chromosome. In some organisms, univalents which normally do not separate until the second division may come to the equatorial plate in metaphase I and undergo precocious division. Bridges occurring during the division of univalents in anaphase I were reported by HAGA and WALTERS. WALTERS estimated that between 20 and 30% of the

bridges observed in the first division of *Bromus* hybrids were between precociously separating univalents. HAGA illustrated a single case of a bridge and fragment configuration between two unpaired nonhomologous chromosomes in *Paris verticillata*. Univalent bridges were not observed in *Podophyllum*.

Cytological evidence from paired chromosomes also suggests that crossing over within an inversion is not responsible for all bridge-fragment configurations. This category includes fragments occurring in metaphase I, variation in fragment size, bridges without fragments, and a high frequency of second-division bridges.

Fragments produced from crossovers within inversions should remain in place in metaphase I and would not be expected to appear until the separation of chromatids in anaphase I. Attached metaphase-I fragments were observed by HAGA and WALTERS and were explained by the breakage-fusion hypothesis. Similar fragments were observed in *Podophyllum*.

Variation in fragment size is a striking feature in the cytology of *Paris quadrifolia* (GEITLER 1937) and *Paris verticillata* (HAGA 1953). Fragment size varies from very small to almost twice the length of the chromosome arm involved in the bridge. Similar variation in fragment size was also observed in *Podophyllum* (Table 1). Variation in fragment size in *Tradescantia* was explained by SWANSON (1940) as being the result of crossing over within inversion loops of very small inversions located along the length of most of the chromosomes. However, this does not seem likely in light of McCLINTOCK's (1933) finding that in pachytene small inversions tend to pair in a nonhomologous manner.

HAGA proposed that bridge-without-fragment configurations cannot readily be explained by the inversion hypothesis. Such aberrations would require an extremely small terminal inversion. Here again, small inversions are likely to pair nonhomologously. Bridge-without-fragment configurations have been reported by HAGA (1953) and JOHN *et al.* (1960). This aberration may be explained as the result of an almost terminal breakage-fusion forming a minute, microscopically invisible fragment.

The occurrence of a large number of second-division bridges (3.5 bridges per dyad) reported by WALTERS (1950) also suggests that inversions are not involved in all bridge-fragment configurations. Two chiasmata are required to produce a second-division bridge according to the inversion hypothesis. In *Bromus* hybrids very little chromosome pairing was observed at pachytene, the chiasma frequency was very low in metaphase I, and in no case were two chiasmata found in one arm of a bivalent.

In *Podophyllum* there is evidence that both inversion and breakage-fusion bridges may occur. Crossing over within the SL D3 inversion could produce a bridge-fragment configuration. Most of the bridge-fragment configurations, however, cannot be explained by the inversion hypothesis. The E and F chromosomes of eight of the clones listed in Table 2 exhibit the bridge-fragment configuration in meiotic anaphase stages but showed no evidence at pachytene for inversion heterozygosity. Other configurations were also found which suggest the breakage and fusion hypothesis, such as metaphase-I fragments, variation in fragment size, and bridges without fragments. Univalent bridges were not observed.

In Podophyllum, fragments associated with E and F chromosome bridges vary in size from twice the length of the chromosome arm involved in the bridge to small spherical bodies, suggesting that breakage and fusion may occur at various points along these chromosomes. A statistically greater number of small fragments is associated with the E chromosome and, while not statistically significant, a greater number of medium-sized fragments is associated with the F chromosome (Table 1). If the breakage-fusion mechanism occurs in Podophyllum, this suggests that the regions of high breakage frequency coincide with the heterochromatic regions of the secondary constrictions of the E chromosome and possibly of the F chromosome. HINTON, IVES and EVANS (1952) also proposed that regions of heterochromatin may be especially susceptible to breakage. In studies of the mutator gene *hi* in *Drosophila melanogaster*, they found that most of the breakage and reunion points of inversions and a transposition were in regions of heterochromatin. They suggested that mutator genes act primarily through heterochromatin. THERMAN-SUOMALAINEN (1949) reported chromosome breakage occurring exclusively at the secondary constrictions of the mitotic chromosomes of *Podophyllum emodi* after treatment with pyrogallol.

Examples of nonrandom distribution of breaks have also been reported by other authors. Excessive breakage in distal chromosome regions was reported by REES and THOMPSON (1955), WALTERS (1950), SWANSON (1942) and MARQUARDT (1952). Breakage in proximal regions was reported by SAX (1940) and HAGA (1953).

From an evolutionary standpoint, a consideration of the relationship of bridge and fragment configurations to paracentric inversions is the most interesting. Inversions have been found to play an important part in the evolution of the Diptera, especially in the genus *Drosophila* (WHITE 1965; DOBZHANSKY 1951). The major advantage to populations with inversions is that recombinations of adaptive gene orders generally do not occur within inverted segments. Crossing over within inversions leads to bridge and fragment configurations and thus to the production of inviable gametes.

Although inversions have been found to be numerous and important in the evolution of some *Drosophila* species, little work of this nature has been carried out in plants owing to the lack of suitable chromosome. Nevertheless, inversions have been reported to occur in natural populations of plants which exhibit the indirect evidence of bridge-fragment configurations. STEBBINS (1950) and MUNTZING (1961) state that inversions are a common feature of plant cytogenetics. STEBBINS notes that *Paris* and *Paeonia* are the two genera which exceed all others in the frequency of inversions. MUNTZING states that inversions are especially frequent in plants with a vegetative propagation, where they accumulate without the disadvantage of partial sterility. He cites *Paris quadrifolia* as a plant which seems to be heterozygous for numerous inversions.

The breakage and fusion hypothesis and the demonstration of noninversion bridge and fragments in the present work suggests the need for a reevaluation of the importance of inversions in the evolution of higher plants. The frequency of inversions in plant populations may have been overestimated by STEBBINS

and by MUNTZING. *Paris quadrifolia* which was used by both as an example of a plant with numerous inversions, has aberrations similar to those of *Paris verticillata*, which was used by HAGA in developing the breakage and fusion hypothesis.

Data on inversion pachytene loops of the present work indicate that inversions occur in natural populations of plants, but these data are insufficient to make any conclusions as to their importance. However, there is no reason to assume that inversions are an important aspect in the evolution of higher plants. Inversions may occur but the advantages of this system may not have been widely utilized. A similar hesitation to generalize on the inversion work of *Drosophila* was presented by WHITE and ANDREW (1962), who stated that the high frequency of inversion polymorphism in *Drosophila* may have led some geneticists to exaggerate the frequency of inversions in other organisms.

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SUMMARY

An analysis of specific pollen mother cell chromosomes in eight clones of the plant *Podophyllum peltatum* revealed the presence of anaphase bridge and fragment configurations but an absence of pachytene inversion loops. Spontaneous breakage and fusion may have been responsible for these anaphase aberrations rather than crossing over within paracentric inversions. A high rate of breakage and fusion in the nucleolar organizing region of one chromosome is indicated by a nonrandom distribution of fragment length. These data, coupled with a review of the literature on bridges and fragments, suggest that the frequency of paracentric inversions in plants, which has been inferred primarily from observations of anaphase bridge and fragments, may have been overestimated.

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