Chromosomally Aberrant Progeny of Selfed Trisomic *Eleocharis erythropoda* Steud.¹

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Eleocharis erythropoda is a common sedge in wetland areas of the northern half of the United States and southern Canada. In the eastern half of the range, the second longest chromosome is trisomic and the populations are almost exclusively $2n+1=19$. In the western segment of the range the populations are normal with $2n=18$ (Harms 1968, unpubl.).

The meiotic behavior of the trisomic has been described in a previous paper (Harms 1968). Briefly, the chain trivalent of the three homologs separate equationally in the first meiotic division (i.e. sister chromatid separation) such that no bridge is formed. In the second division a two-one segregation invariably occurs so that the pollen grains formed possess either a nine-chromosome normal complement or ten-chromosome complement with an extra chromosome. With random recombination of these gametes and assuming a similar meiotic behavior in megasporogenesis one would expect to recover 25% 18-chromosome normal, 50% 19-chromosome reconstituted trisomics, and 25% tetrasomics in a selfed trisomic. However, included in the actual progeny recovered in a controlled selfed trisomic were four plants which were none of these chromosome classes. This paper is concerned with the analysis of these four progeny.

Materials and methods

The plant used in this study was collected in the field and transplanted to the greenhouse (Voucher Specimen: KANSAS: Allen Co.: n. edge of Chanute on US 69, roadside ditch, L. J. Harms 3726, 20 May, 1968. CONN). The plant was isolated so as to exclude foreign pollen and allowed to set seed. The achenes were harvested, treated for 30 minutes in H_2SO_4 , washed, and soaked in 100 ppm gibberellic acid for seven days. The treated achenes were then placed in vials and covered with water where germination occurred in five to ninety days. In some cases seedlings were grown to maturity (about three months), cold treated for six weeks, and placed in 14-hour illumination where flowering generally occurred in one month. In other cases, plants were allowed to flower naturally in the early Spring.

Meiotic stages were prepared by excising young anthers from spikelets which

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had been fixed for 24 hours in 3:1, alcohol: propionic acid. Anthers were squashed in propionocarmine, examined and photographed under phase. Somatic preparations were made from root tips treated for one hour in 0.25% colchicine, fixed in 2:1, alcohol: propionic acid, hydrolyzed in 1 N HCl for 10 minutes, and squashed in propionocarmine. Stainability of pollen with lactophenol-cotton blue was used as an index of pollen fertility.

In the ensuing discussion each plant is distinguished by a number (i.e. 3726-5) which refers to a number assigned to each plant in my crossing data book as it was analyzed.

Results

The parental trisomic karyotype is shown in Fig. 1 with 19 chromosomes. Since the chromosomes of *Eleocharis* are diffusecentric, they lack any visible constriction regions. The only means of karyotypically characterizing them is by differential length. The longest chromosome of the set ranges from 4.7-10 μ (avg. 7.9 μ). The second longest chromosome ranges from 0.6-3 μ smaller than the longest in the same cell and averages 6 μ in length. The third longest chromosome ranges from 3.3 to 6.0 μ (avg. 4.4 μ) and is 1.3-4.0 μ smaller than the second longest chromosome in the same cell. The smallest chromosomes in the complement are almost invariably 3 μ in length irregardless of the contraction. Rarely, a fifth size class is present which are very short fragment-types of chromosomes 1-1.5 μ in length. For the sake of comparison, the chromosomes will be referred to as L (long), ML (medium long), M (medium), S (short), or F (fragment) in accordance to the size classes outlined above. Correspondingly, the trisomic has a karyotype of 2L, 3ML, 4M, 10S.

In 3726-5 the karyotype appeared indistinguishable from that of the parent. However, in meiosis seven bivalents and a heteromorphic chain of five chromosomes was present at maximum pairing (Fig. 2). In prophase II the heteromorphic nature of the multivalent was more clearly discernable with two short chromosomes paired terminally with three longer chromosomes (Fig. 3). In some cases one of the small chromosomes was unpaired (Fig. 4) or the two small chromosomes were paired separately from the chain of three longer chromosomes (Fig. 5). In anaphase II the chromosomes of the multivalent showed frequent alternate segregation (Fig. 6). Several chromosome-combinations were seen in pollen mitosis as a result of multivalent segregation. Fig. 7 shows 10 chromosomes of 2L, ML, 2M, 5S and Fig. 8 shows 2L, 2M, and 6S chromosomes. In all cases 1L and 4S chromosomes are present and must therefore represent the bivalent elements in meiosis, whereas the multivalent must be composed of a S-S-L-ML-ML combination. Pollen grains showed 98.5% stainability.

The karyotype of 3726-14 was 2L, 2ML, 5M, and 10S chromosomes and differed from the parent in its lack of an extra ML chromosome and presence of an additional M-chromosome (Fig. 9). In meiosis eight bivalents and a chain of three chromosomes was generally present (Fig. 10) although more rarely one of the chromosomes of the trivalent was unpaired. Anaphase II segregation of the

Figs. 1-7. *Eleocharis erythropoda* (Magnification 1500 $\times$). 1, parental somatic chromosomes with 3 ML-chromosomes; $2n=19$, 2-7, progeny #3726-5. 2, diakinesis with 7 II+chain V. 3-5, propahse II. 3, 7 prs.+5 chromosome chain of 3 long-2 short (arrows). 4, 7 prs.+4 chromosome chain of 3 long-1 short+unpaired chromosome at each pole (arrows). 5, 8 prs.+long chromosome chain of 3 (arrows). 6, anaphase II showing alternate chromosome segregation of chain of 5 (arrow). 7, pollen mitosis with 2L, 1ML, 2M, 5S.

Figs. 8-13. *Eleocharis erythropoda* (Magnification 1500 $\times$). 8, progeny #3726-5 pollen mitosis with 2L, 2M, 6S. 9-13, progeny #3726-14. 9, somatic chromosomes with 5 M-chromosomes (arrows); $2n=19$. 10, metaphase I with 8 II+chain III. 11, anaphase II (one pole) showing tri-valent segregation (arrow). 12, pollen mitosis with 2 M-chromosomes. 13, pollen mitosis with 3 M-chromosomes.

Figs. 14–20. *Eleocharis erythropoda* # 3726–17 (Magnification 1500 $\times$). 14, somatic chromosomes with 2L, 3ML, 3M, 11S, fragment (arrow); $2n=19$ +fragment. 15, prophase II with 7 prs.+long chromosome chain of 3 and heteromorphic chain of 3 at each pole (arrows). 16–20, pollen mitosis. 16, L, ML, 2M, 5S. 17, L, ML, 5S. 18, L, ML, M, 5S. 19, L, ML, M, 6S, fragment (arrow) 20, L, ML, 2M, 6S, fragment (arrow).

trivalent is shown in Fig. 11 with each of the two terminal chromosomes passing to opposite poles and the central chromosome segregating randomly to either of the poles. The pollen formed has chromosome complements of either L, ML, 2M, 5S (Fig. 12) or L, ML, 3M, 5S (Fig. 13) in approximately equal proportions. One of the two M-chromosomes must therefore from the trivalent in meiosis. Pollen stainability was 95.6%.

Figs. 21-25. *Eleocharis erythropoda* #3726-19 (Magnification 1500 $\times$). 21, somatic chromosomes with 2L, 3ML, 4M, 11S; $2n=20$. 22, prophase II with 7 prs. + long and short chromosome chains of 3 at each pole (arrows). 23-25, pollen mitosis. 23, L, 2M, 4S. 24, L, ML, 2M, 6S. 25, L, 2ML, 2M, 6S.

The karyotype of 3726-17 was 2L, 3ML, 3M, 11S, 1F (Fig. 14). It differs from the parental trisomic in its lack of an M-chromosome and presence of extra S and F-chromosomes. In metaphase of meiosis the pairing appeared much like that of the typical trisomic with eight bivalents and a chain trivalent; however, in prophase II it became apparent that one of the bivalents was actually a heteromor-

phic trivalent with an F-chromosome paired terminally with two larger elements (Fig. 15). As a result of the segregation of the two trivalents, many combinations of chromosomes were found in pollen mitosis. Figure 17 with L, M, 5S must represent a pollen grain which received only the bivalent elements of meiosis. In Fig. 18 with L, ML, M, 5S the pollen grain has received one of the ML-trivalent elements but none of the smaller trivalent chromosomes, and the pollen grain in Fig. 16 with L, ML, 2M, 5S has in addition to one of the ML-trivalent chromosomes an M chromosome which must represent one of the chromosomes of the smaller heteromorphic trivalent. The pollen grain in Fig. 19 with L, ML, M, 6S, F has received one of the ML-trivalent elements and an S and F-chromosome of the shorter trivalent, whereas the pollen grain in Fig. 20 with L, ML, 2M, 6S, F has received all of the shorter trivalent elements (i.e. S-M-F) in addition to one of the ML-trivalent chromosomes. Pollen stainability was 70.25%.

The karyotype of 3726-19 differs from the trisomic parental in its possession of an extra S-chromosome (Fig. 21). In metaphase of meiosis the pairing appears similar to the parental trisomic but in prophase II one can see a short chromosome trivalent in addition to the long chromosome trivalent (Fig. 22). As in the previous example, numerous chromosome combinations were found in the pollen grains. The grain shown in Fig. 23 with L, 2M, 4S must represent a grain which received only the bivalent chromosomes. The grain shown in Fig. 24 with L, ML, 2M, 6S has received a single ML-chromosome from the large trivalent and two S-chromosomes from the smaller trivalent, whereas the grain in Fig. 25 has received two ML and two S-chromosomes from each of the two trivalents. The two trivalents must be composed of ML-ML-ML and S-S-S chromosome associations. Pollen stainability was 95.6%.

Discussion

Of the four progeny analyzed only 3726-14 lacks the trisomic ML-chromosome of the parent, however one of the M-chromosomes is now trisomic. The presence of an extra M-chromosome in somatic cells, trivalent pairing in meiosis, and pollen grains which have either a normal complement of nine chromosomes or ten chromosomes with an extra M-chromosome offer irrefutable evidence of this.

In the remaining three cases the trisomic ML-chromosome of the parent is present although in 3726-5 it has been somewhat altered. The first evidence of this is seen in meiosis where a heteromorphic chain of five chromosomes is formed rather than the typical chain of whree. In the prophase II stage it is possible to see that two small chromosomes are terminally paired to the three longer chromosomes. The only means by which such a pairing configuration could be achieved is through an interchange between one of the three ML-chromosomes and a non-homologous S-chromosome. The interchange segment is apparently of such a size that the interchange ML-chromosome appears longer than its two homologs. I was unable to detect this size difference in somatic preparations but it was detectable in pollen mitosis. The plant is correctly termed a tertiary trisomic. Burnham (1962) states that tertiary trisomics occur regularly, perhaps exclusively, among the progeny

of interchange heterozygotes although Blakeslee (1929, 1934) has found them in the progeny of lines of *Datura* which had no ring formation. The high fertility of the plant would indicate that both of the S-chromosomes are normal and the S-chromosome involved in the interchange has been lost.

Both 3726-19 and 3726-17 bear the reconstituted parental trisomic ML-chromosome. However in 3726-19 one of the S-chromosomes is also trisomic as evinced by the karyotype, meiotic pairing, and chromosome complement of the pollen grains. In 3726-17 an additional heteromorphic trivalent is formed in meiosis. Since the plant lacks one of the M-chromosomes but bears an additional S and F-chromosome, it would seem that one of the M-chromosomes has unequally fragmented to form the extra S and F-chromosomes. Analysis of the chromosome complement of the pollen grains gives conclusive evidence that the smaller trivalent is formed from a S-M-F combination which must have been derived by such a fragmentation. In most plant and animal groups chromosomal fragmentation would have lethal effects owing to their loss in subsequent divisions since many would be acentric. In *Eleocharis*, and perhaps all genera in the family, the centric activity is spread along the entire length of the chromosome and fragments retain activity. Löve, Löve, and Raymond (1957) have reviewed the occurrence of diffusecentric chromosomes in the plant kingdom.

The transverse fragmentation of chromosomes and the resultant increase in chromosome number has been termed agmatoploidy (Schrader and Hughes-Schrader 1956). Agmatoploidy has been studied on great detail in the genus *Luzula* (Juncaceae). In the unaltered karyotype the basic number is six with all of the chromosomes equal in length. Certain aneuploid races were found in which two or more of the chromosomes were shorter than the others in the set. In crosses between the unaltered chromosome types and the aneuploids, heteromorphic multivalents were formed with two or four shorter chromosomes pairing with a single large chromosome (Nordenskiöld 1961).

Agmatoploids artificially produced by X-irradiation exhibited a similar behavior in heterozygotes (Nordenskiöld, 1963). Harms (1968) has found a naturally occurring agmatoploid heterozygote in the 18-chromosome race of *Eleocharis erythropoda* and Strandhede (1965) reports agmatoploids in related European species.

The greatly lowered fertility reflected in the pollen stainability (70.25%) would be expected, since those gametes which receive only an S or an F chromosome from the trivalent would be deficient. Those gametes which receive the combinations M, MS, MF, or SF would be fertile. Thus, the expected sterility should be about 33.3% which is in adequate agreement with the observed sterility (29.75%). The high fertility of the three plants previously discussed indicates that the presence of extra chromosomes has no effect on viability.

Summary

Four chromosomally aberrant progeny of a selfed trisomic *E. erythropoda* were analyzed. Of these, two were reconstituted trisomics for the parental medium-long chromosome; however, one bore an additional trisomic short chromosome

and the other a fragmented medium chromosome. In the third plant one of the medium-long trisomic chromosomes bore an interchange segment of a non-homologous short chromosome and was thus a tertiary trisomic. In the last case the plant was trisomic for a medium chromosome only.

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