HIGH TRANSMISSION FREQUENCY OF A TRIPSACUM CHROMOSOME IN CORN[1]

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AMONG the progenies of advanced corn-backcross generations of hybrids of 2n *Zea mays* L. × 2n *Tripsacum dactyloides* L., several 21-chromosome stocks have been isolated which contain the 20 normal chromosomes of corn plus an extra chromosome from Tripsacum. In one of these, the extra chromosome has been transmitted through the egg in 91 percent of the 80 offspring which have been grown and checked cytologically for chromosome constitution. This is a surprisingly high frequency.

There are a number of conceivable explanations with related precedents for high transmission of this sort, in addition to the possibility of parthenogenetic development of unreduced eggs. The manner of development of the female gametophyte in corn from a single megaspore, normally the basal cell of a linear quartet, provides an obvious means if the extra chromosome can somehow be included in the functional megaspore at disproportionately high frequency. SINGLETON and MANGELSDORF (1940) and RHOADES (1942) found no deviation from basal spore functioning in studies of gametic lethals and nonrandom segregation. RHOADES (1952), however, showed that abnormal chromosome 10 may be included in the basal megaspore 70 percent of the time because of neocentric activity of the terminal knob. KAYANO (1957) working with Lilium and RUTISHAUSER (1960) working with Trillium have found that lagging supernumerary or fragment chromosomes are found most often near that end of the anaphase I spindle where the cell destined to produce the egg will be located. This behavior is apparently not typical of corn trisomes for if it were, EINSET (1943) should have found more frequent transmission of shorter chromosomes instead of the reverse, since frequency of lagging at anaphase I was thought to be inversely related to relative chromosome length.

AVERS (1954) reported a tendency for precocious division of univalents in aster microsporocytes, with evidence that these univalents regularly separate equationally twice during meiosis so that high transmission frequency results. In contrast, corn univalents tend to be retarded in division, and there has been no evidence that equational separation ever occurs more than once during meiosis.

Differential viability of spores, gametes or zygotes provides another basis for distorted transmission frequencies. Although ordinarily selection acts against the

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individual of aberrant constitution, exceptions have been reported in addition to the well known case of *kappa* in Paramecium. LUIG (1960) found distorted segregation ratios in wheat through both male and female gametes, and SEARS and LOEGERING (1961) reported a gene in wheat which causes the early abortion of pollen grains not carrying it, from heterozygous but not hemizygous plants. CAMERON and MOAV (1957) reported a gene in *Nicotiana plumbaginifolia* which seems to cause the degeneration of pollen not carrying it when the chromosome on which it occurs is added to the *Nicotiana tabacum* complement. In this case female transmission remains normal. Analogous to these latter two cases in plants, is the segregation distorter (*SD*) gene of Drosophila (SANDLER and HIRAIZUMI 1960) which somehow seems to bring about the destruction of many of the gametes from heterozygous males which carry its normal allele. It has been suggested that *SD* induces breakage of its homologue and that gametes which receive the breakage products are deficient and therefore inviable. However, the at least superficially similar action of the pollen-killer gene studied by SEARS could not readily be attributed to production of deficiencies by break-induction in the homologue, since pollen of hemizygous plants (deficient for the entire homologue) is normal.

Occurrences of unusual transmission even less well understood (because information is more fragmentary) are the seemingly advantageous male gametophyte factors of MANGELSDORF (1958) in corn-teosinte hybrid progeny, and the frequent recovery of a Drosophila sc^8 deficient chromosome from heterozygotes (attributed by MATHER [1939] to a probable maternal effect).

Corn trisomes have been found by EINSET (1943) to be transmitted through the egg with frequencies of *less* than the 50 percent expected from unbiased distribution to progeny. The frequency of loss of the extra chromosome in EINSET's studies was inversely related to chromosome length, and this loss was therefore thought to be due to the failure of lagging univalents (unassociated with their homologues at metaphase I because of lack of chiasma formation) to be included in spore nuclei.

Corn trisomes are transmitted only rarely through the pollen apparently because of slower pollen tube growth of those grains containing 11 chromosomes (MCCLINTOCK and HILL 1931; EINSET 1943).

As is commonly the case with alien addition races, most Tripsacum chromosomes seem to be rapidly lost from corn stocks containing them. Plants of the first corn backcross generation of corn-Tripsacum hybrids contain a complete Tripsacum genome as well as two corn genomes (MANGELSDORF and REEVES 1939). These plants have been completely pollen sterile, but when pollinated by corn they produce a few seeds in which a few Tripsacum chromosomes usually accompany the normal corn complement. By the fourth corn backcross generation often only a few plants are found to carry even a single Tripsacum chromosome, and so far only two addition races have been readily maintained. One of these is the high transmission stock described in this paper. In the other an interchange has occurred between a corn and a Tripsacum chromosome so that trivalent configurations are regularly formed, and the interchange Tripsacum

chromosome is transmitted through the egg with a frequency of about 50 percent (MAGUIRE 1957). No male transmission is possible by ordinary techniques in this case since in plants carrying this extra chromosome the anthers do not dehisce, and no pollen is shed. When anthers from mature flowers are dissected and and the contents examined, an occasional normal appearing pollen grain is found. Recently, anthers from such flowers have been chopped up and applied to the silks of a number of ears of normal corn plants. Since four seeds have been produced in this way, all of which contained the extra chromosome, further study of its transmission through pollen may prove interesting.

This paper is a report of information now available on the behavior of the Tripsacum chromosome which is transmitted at a high frequency when added to the corn complement.

MATERIALS AND METHODS

The Tripsacum derived chromosome of these experiments (Figure 1) has physical properties similar to those of chromosome 10 of corn. It is about 44 percent of the total length of corn chromosome 1, with an arm ratio of about 2.0

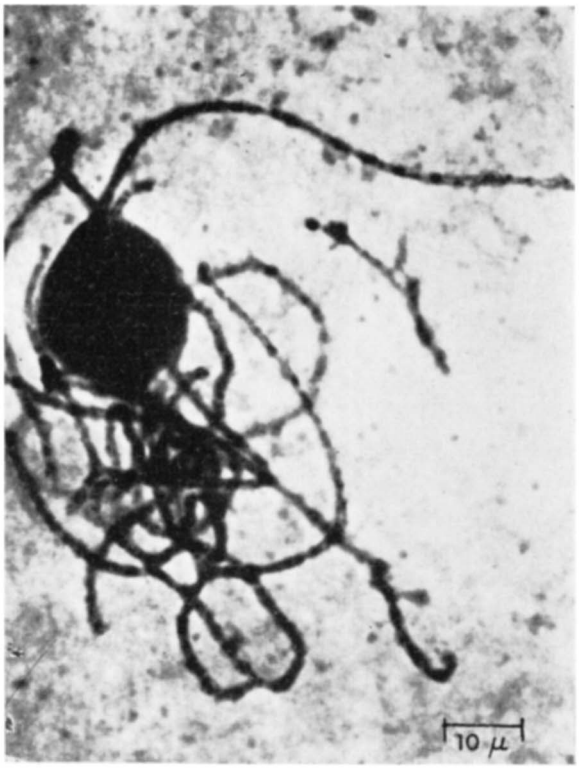

FIGURE 1.—Photomicrograph of microsporocyte from a 21-chromosome plant showing the Tripsacum chromosome at upper right as a univalent with several folds.

and a small terminal knob on the short arm. It does not synapse with any of the corn chromosomes at pachytene and has always been found to be univalent at diakinesis and metaphase I in 21-chromosome plants. Both ovule and pollen fertility are low in 21-chromosome plants but normal in their 20-chromosome sibs. Twenty- and 21-chromosome plants are indistinguishable on the basis of gross morphological characteristics.

Microsporocyte samples were fixed in 3:1 alcohol-acetic mixture and refrigerated until examined in acetocarmine smear preparations. Young ears were fixed in 3:1 alcohol-acetic mixture and refrigerated until embedded in tissuemat, or were fixed in Craf solution and refrigerated in 70 percent alcohol until embedded in tissuemat. Tissuemat blocks were sectioned at 11μ, stained in HEIDENHAIN's iron hematoxylin, destained in ferric ammonium sulfate and mounted in balsam. Chromosome number determinations of plants were made from microsporocyte smear preparations in all cases. Percentage of laggards and 11-chromosome microspores were estimated from smear preparations by use of a systematic scanning procedure.

RESULTS AND DISCUSSION

Genetic studies: The stocks carrying the extra Tripsacum chromosome were testcrossed as female parent to a multiple recessive tester which was bm_2 lg_1 a_1 su_1 pr y_1 gl_1 wx g_1 (bm_2 =brown midrib, lg_1 = liguleless leaf, a_1 = no anthocyanin in plant or kernel) su_1 = sugary endosperm, pr = red aleurone, Y_1 = yellow endosperm, gl_1 = glossy seedling, wx = waxy endosperm, g_1 = golden). In 156 progeny in which no segregation was expected or found for a_1, pr, and Y_1, segregation for bm_2, lg_1, su_1, gl_1, and wx did not deviate significantly from 1:1. This is evidence that the extra chromosome did not carry dominant alleles for these markers. There was difficulty of classification for g_1, but there seemed to be a deficiency of the recessive class, possibly because of the presence of a G_1 allele (a chromosome-10 marker in corn) on the Tripsacum chromosome. Testcrosses of this stock to chromosome-10 markers are currently underway to determine whether this is the case. The genetic tests have demonstrated, however, that parthenogenetic development of unreduced eggs is not the cause of the high transmission frequency of the extra chromosome.

Microsporogenesis and pollen sterility: In microsporocytes the extra chromosome was frequently found to lag at anaphase I. The average frequency of the lagging based on observations of at least 100 cells from each of six plants was about 80 percent (Table 1). When it did not lag it was found in progress toward one pole without having divided. The laggards frequently separated equationally at late anaphase I; they were often not included in a telophase I nucleus but remained in the cytoplasm and appeared as laggards again at anaphase II. The frequency of these laggards apparently decreased steadily as the stage of meiosis progressed until only about 34 percent of microspore quartets contained at least one cell with a laggard (Table 2). In about one tenth of these quartets two cells contained laggards, and no quartets were found with laggards in three or four cells.

Although microspore samples were collected from a number of plants, only

TABLE 1

Anaphase I laggard frequencies

Plant no.	Percent of at least 100 microsporocytes containing laggards
P6110–2	88%
P6252B–2	89%
P6111–1	85%
P6110–5	76%
P6112–6	66%
P6108B–4	73%
	average = 80%

TABLE 2

Quartet laggard frequencies

Plant no.	Number of quartets containing no laggard	Number of quartets with a laggard in one cell	Number of quartets with laggards in two cells	Percent quartets with at least one laggard
P6252B–3	85	36	22	41
P6112–4	81	25	8	29
P6110–2	83	32	14	36
P6112–1	70	30	11	37
P6108A–3	58	25	5	34
P6108A–2	71	29	0	29
			average	34%

one of these contained stages of microspore division suitable for chromosome counting. In this sample, 40 percent of 78 analyzable cells contained 11 chromosomes. In this case, the extra chromosome was apparently lost during microsporogenesis with a frequency of about ten percent. This frequency of loss agrees well with expectation from frequency of laggards found at the quartet stage. There is no evidence that the extra chromosome ever separates equationally twice during meiosis in microsporocytes.

In anaphase I when the extra chromosome was found on the spindle and not lagging, it was always mixed with the other chromosomes (and distinguishable from them only when the others could be matched). There was no evidence that it has unusual centric activity which would tend to locate it in such a position that it would be contained in the basal megaspore a large proportion of the time at the end of the second megasporocyte division.

Alien addition Tripsacum chromosomes are not constant in their effect upon pollen sterility. In a previous study (MAGUIRE 1957), the presence of a particular Tripsacum chromosome resulted in complete pollen sterility. In the present study, the percentage of abortive pollen was variable as indicated in Table 3. This variability is similar to that of disomic plants homozygous for substitution of a Tripsacum segment into the corn complement (MAGUIRE 1961). In two cases 21-chromosome plants having a high percentage of normal appearing pollen were selfed.

TABLE 3

Pollen sterility in 21-chromosome plants

	Percent normal appearing pollen					
	0	1–20	21–40	41–60	61–80	81–100
Number of plants	50	12	1	3	0	0

Among the 12 progeny of these two selfed plants were three 22-chromosome plants with the extra chromosome present in duplicate. Thus it seems to be transmitted readily through the pollen, and either there is no marked differential functioning or viability of 10 versus 11-chromosome pollen, or 10-chromosome pollen is less likely to survive but more likely to effect fertilization if it survives (presumably because of more rapid pollen tube growth). Unlike the case in tobacco reported by CAMERON and MOAV (1957), the extra chromosome does not appear to be somatically lost with a significant frequency. Twenty-chromosome sectors have never been found in tassels of 21-chromosome plants although many samples have been intensively studied. Thus both 10- and 11-chromosome functional pollen grains are apparently produced from 21-chromosome tissue, and any selection at the microspore, male gametophyte or gamete level can probably be at most only partial.

Megasporogenesis and ovule sterility: Although 210 ovules were examined in stages ranging from meiotic prophase to stages of embryo sac development, no anaphase I or anaphase II configurations and only one metaphase I were found, probably because these stages are of very short duration. It could not be determined therefore, whether the extra chromosome separates equationally in both meiotic divisions of megasporocytes or has extra centric activity at anaphase I of megasporogenesis. Nor could it be determined whether the Tripsacum chromosome tends to sink to the basal end of the meiotic spindle where it is often included in the basal megaspore. If this latter form of behavior actually occurs, it seems improbable that the mechanism is purely mechanical, since corn univalent and other Tripsacum chromosomes added to the corn complement do not behave this way. RHOADES (1942) showed that gravity did not influence the distribution of abnormal chromosome-10 in corn by inverting the plants.

Forty-four of the ovules examined were at stages of development appropriate for determining which of the four megaspores is functional. In all of these ovules the basal megaspore developed while the others disintegrated. Thus, it seems highly improbable that megaspore replacement occurs with a frequency which could affect transmission frequency substantially.

The percentages of normal seed on ears of 21-chromosome plants pollinated by normal pollen are listed in Table 4. Ovule fertility was consistently so low that selection could have occurred against those ovules in which the egg carried only the normal ten corn chromosomes. Since with random distribution the

TABLE 4

Ovule fertility of plants carrying the extra chromosome

Plant no.	Total number ovules	Number of defective seeds	Number of normal seeds	Percent seeds set
P5922–3	323	0	23	7.1
P5922–4	291	0	21	7.2
P5922–5	305	0	11	3.6
P5924–4	345	0	10	2.9
P6012–13	279	0	6	2.2
P6013–1	362	0	6	1.7
P6013–3	334	0	8	2.4
P6012–8	151	0	13	8.6
P6017C–2	182	4	21	11.5
P6012–4	143	0	2	1.4
P6012–9	211	1	4	1.9
P6013–8	195	0	12	6.2
P6013–7	214	1	12	5.6
P6013–1	362	0	6	1.7
P6013–2	313	0	2	0.6
P6017B–3	357	4	4	1.1
P6017B–4*	101	41	40	80.2
		average for 21-chromosome plants = 4.1%		

* 22-chromosome plant.

basal megaspore would carry the extra chromosome half the time, if it is lost from nuclei because of lagging as frequently as in microsporogenesis, it might be included in as many as 40 percent of eggs. When it is included in a nucleus after lagging, particularly when it divides equationally after the other chromosomes have proceeded toward the poles, it is possible that it would tend to be distributed to the two central spores most often. The data of MORGAN (1950) and RUSSELL and BURNHAM (1950) suggested selective eliminations from the basal megaspore of chromatids which lagged because they were involved in chromatid bridges, although RHOADES and DEMPSEY (1953) found evidence that some single exchange chromatids from a paracentric inversion were included in the basal megaspore. Since the level of ovule fertility is so low, and about nine percent of surviving ovules contained 20 chromosomes, even under these conditions, some of the eggs of the possibly favored constitution may not have developed. The set of seed on the ears of two plants (with half normal-appearing pollen) which were self-pollinated was eight percent and two percent. Among 12 progeny of these selfings there have been three 22-chromosome plants, eight 21-chromosome plants and one 20-chromosome plant. Identical expectations would follow such selfings from high production of 11-chromosome eggs with random mortality, and from random production of 11-chromosome eggs with high selection against 10-chromosome eggs before fertilization. Since slightly different expectations would follow post-fertilization selection, this type of occurrence might be distinguished from the other two possibilities with large selfing progenies.

In the case of a 22-chromosome plant (pollinated by normal pollen) the proportion of seeds formed (Table 2) was comparatively high although approximately half of these seeds were defective. In this 22-chromosome plant, eleven normal appearing bivalents were invariably found in microsporocytes, and meiosis was entirely regular. All of the eggs presumably carried eleven chromosomes, and the eight plants so far grown from this ear all had 21 chromosomes. So it appears that there could have been only comparatively little selection against seed formation from ovules with 11-chromosome embryo sac nuclei in a plant of 22-chromosome constitution when pollinated by normal 10-chromosome pollen. The defective seeds (not found on the other ears in substantial proportion) may have resulted from genetic segregation. MANGELSDORF (1958) reported defective endosperm mutants in corn-teosinte hybrid progenies.

With the exception of the differential transmission in wheat described by LUIG (1960), previously reported instances in higher organisms of apparent incompatibility effects of the sort possible here have been confined to male spores, gametes or gametophytes. In the case in tobacco, CAMERON and MOAV (1957) concluded that the effect of the pollen-killer gene was exerted only where nuclei both with and without it shared common cytoplasm. Such a situation would prevail only briefly, if at all, in corn megasporogenesis and wheat microsporogenesis at the end of anaphase I before the cell plate is formed.

It has been pointed out by CAMERON and MOAV (1957) that the common existence of genes similar to the pollen-killers would probably not be recognized since they would tend to become homozygous in cross fertilizing species, and reversion mutants to susceptible alleles would be classed as lethals. They have also pointed out that possibilities for introgression would be enhanced by the existence of such genes in divergent species.

CAMERON and MOAV have drawn attention to the similarities of the action of a pollen-killer gene to an antigen-antibody type reaction. Although it can be questioned whether antibodies are formed in plants, it is generally inferred that they are, from the accumulated evidence from self-sterility factors. It is also apparent that if many genes are capable of inducing fatal antibody production in cells which do *not* contain them, this action is usually inhibited in some way, for otherwise many gametes of heterozygous organisms would be killed.

The possibility of a subtler action of antigen-antibody type specificity was suggested to PETERSON (1960) from his studies of unstable loci in corn, and it is possible that investigation of immunological reactions in plants would prove fruitful.

SUMMARY AND CONCLUSIONS

An extra chromosome derived from Tripsacum in an otherwise apparently normal corn complement has been transmitted to a high proportion (91 percent) of the progeny through the egg. Limited data indicate that this chromosome may be transmitted through the pollen with an apparent frequency approximating that found in microspores, although differential pollen tube growth rate may have an undertermined influence. Both ovule and pollen sterility are high in 21-

chromosome plants, and normal in 20-chromosome sibs. The extra chromosome rarely if ever separates equationally in both meiotic divisions of microsporocytes, and it does not show extra centric activity in microsporocytes. Genetic data rule out the possibility of parthenogenetic development of unreduced eggs. Direct examination of ovules shows no evidence of megaspore replacement. Remaining possibilities for this high egg transmission frequency are equational separation of the Tripsacum chromosome in both megasporocyte divisions (unlike microsporocyte divisions), unusual centric activity of the Tripsacum chromosome at anaphase of megasporocytes (again unlike microsporocytes), sinking of the Tripsacum chromosome to a basal position near the meiotic spindle followed by its eventual frequent inclusion in the basal megaspore (unlike other corn and Tripsacum univalents), or unusual, strong selection against those ovules in which the basal megaspore does not contain the extra chromosome. It is suggested that this may result from incompatibility of megaspores, embryo sacs or embryos of the normal corn constitution with maternal background which contains the Tripsacum chromosome. Additional data from selfings of 21-chromosome plants may indicate whether such selection, if it occurs, does so after fertilization.

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