THE EFFECT OF RYE POLLEN AND TEMPERATURE UPON THE HOMOEOLOGOUS PAIRING IN *TRITICUM AESTIVUM* L. X *SECALE CEREALE* L. F_1 HYBRIDS

BY

MARCO ANTONIO QUINONES

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ABSTRACT

A study of meiotic chromosome behavior as influenced by temperature was conducted on wheat-rye hybrids produced from the cross T. aestivum L. c.v. Chinese Spring x S. Cereale L. c.v. Prolific. Two genotypic classes of hybrids were used, viz., those containing a complete haploid complement from each parent (i.e. 2n = 28) and those deficient for chromosome 5B of wheat (2n = 27).

Three different levels of temperature were employed, viz., 15°C, 21°C and 32°C and hybrid plants of each constitution (2n = 28 and 2n = 27) were allowed to undergo development during meiosis at each temperature level.

It was found that temperature of 32°C affected chromosome behavior by reducing homoeologous synapsis which in turn suppressed chiasma frequency.

A continuous variation of the frequency of homoeologous chromosome pairing was found to exist in both genotypes notwithstanding temperature regime. An hypothesis was advanced that such a distribution frequency was due to the effect of a polygenic system contributed by the heterozygous rye parent and which controlled chromosome behavior. The significance of such a system from both a theoretical and a practical standpoint is discussed.

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INTRODUCTION

Hexaploid wheat Triticum aestivum L. (2n = 6x = 42) is made up of three different genomes A, B and D. The three genomes were derived from closely related 14 chromosome diploid species. Each pair of homologous chromosomes has a corresponding homoeologous pair in each of the other two genomes.

In spite of the very close genetic relationships between homoeologous chromosomes in hexaploid wheats, normal meiotic behavior precludes pairing between homoeologues. This lack of pairing was explained by postulating gene mutation which suppressed homoeologous synapsis. Sears and Okamoto, (1958); and Riley and Chapman, (1958), showed that a gene (s) on the long arm of chromosome 5B ($5B^L$), was responsible for the suppression of homoeologous pairing at meiosis and that in the absence of this arm, homoeologous synapsis occurs as it does in the absence of the complete chromosome.

Since the discovery of the $5B^L$ gene (s) in wheat, many attempts have been made to elucidate its true method of action in the control of meiotic pairing in wheat and its hybrids.

Early reports in most hybrids between T. aestivum and 14 chromosome diploid species in the genera Secale, Agropyron, and the abandoned genera Aegilops, (now included in Triticum), showed that there is little chromosome pairing at meiosis presumably because homoeologous synapsis is precluded by the unmodified effect of chromosome 5B. Nevertheless, Nakajima, (1952); and Nakajima and Zennyozi,

(1966), have shown that depending upon the specific combination, wheat-rye F_1 hybrids can exhibit varying degrees of chromosome pairing even though chromosome 5B is present. Furthermore, the findings of Feldman, (1966), clearly illustrated that $5B^L$ activity affects the premeiotic spatial distribution and relative orientation of homologous and homoeologous chromosomes and that chromosomes 5A and 5D have an effect on premeiotic association opposite to that of their homoeologue 5B. His suggestion that every diploid species in the wheat group may have a gene similar to the one present on chromosome 5D, encouraged us to undertake a review on the meiotic behavior of wheat-rye hybrids.

REVIEW OF LITERATURE

The behavior of chromosomes, especially during the first meiotic metaphase in wheat-rye hybrids has been studied in detail for many years. Early reports from Kihara, (1924); Thompson, (1926); Muntzing, (1935); Liljefors, (1936); Nakajima, (1956); and others, have stressed the low frequency of bivalents at first metaphase of meiosis and showed the distant relationship of Secale with members of the wheat group. Some exceptions to this general condition have been reported. For example, Nakajima, (1952), described a wheat-rye hybrid with an unusually high frequency of bivalents. Later Nakajima and Zennyozi, (1966), showed that in wheat-rye hybrids there is a continuous variation in the mean number of bivalents among plants, ranging from complete asynapsis to 7.9 bivalents per plant and a range from 0 - 11 pairs per cell. They concluded that the variation in the number of bivalents observed was influenced by the genetic background of the wheat used in the cross..

Prior to the discovery of the $5B^L$ gene effect on the control of homoeologous pairing in hexaploid wheats by Sears and Okamoto, (1958); and Riley and Chapman, (1958); only one class of wheat-rye hybrids was reported, viz, 28-chromosome hybrids. These hybrids showed very little pairing at metaphase I of meiosis. With the knowledge of the $5B^L$ gene, Riley and Chapman, suggested that the genetic mechanism which restricts pairing between homoeologous chromosomes of

wheat, might also be responsible for the failure of wheat chromosomes to pair with those of certain species in related genera. Riley, Chapman and Kimber, (1959), tested this hypothesis by comparing 28-chromosome (5B present) wheat-rye and wheat-Aegilops longissima hybrids with 27-chromosome (5B deficient) hybrids and found an increased number of bivalents in the 27 chromosome hybrids along with trivalents and quadrivalents. To explain the presence of quadrivalents they suggested that homoeologous chromosomes from each of the three genomes of wheat and a rye chromosome might be associated. Nevertheless, the wheat-rye hybrid deficient for chromosome 5B, showed a lower frequency of bivalents per cell as compared with the level of wheat-Aegilops longissima pairing and they suggested that some interference from the rye chromosomes during meiosis may exist.

In a further examination of hybrids displaying homoeologous pairing, Muramatsu, (1959); Riley, (1960-1963); Cauderon, (1963); Upadhya and Swaminathan, (1963); and Lacadena, (1967) reported hybrids involving hexaploid wheat and most of the diploid species of Aegilops, Secale and Agropyron. In all but a few hybrids examined to date, the suppression of homoeologous pairing was effective in the presence of 5B, but not in its absence. Where exceptions occurred, they included hybrids between T. aestivum and the diploid species Triticum speltoides (= Aegilops speltoides) or T. tripsacoides (= Ae. mutica), in which considerable meiotic pairing occurred and which Riley, (1960), proposed to be due to the suppression of the $5B^{L}$ gene by the genotype of those species. Riley (1963); also pointed out the anomalous behavior of rye which when crossed with wheat, gave rise to hybrids in

which the absence of $5B^L$ cause only a relatively small increase in pairing. This finding led him to propose that the rye genotype inhibits pairing in a way very similar to that of chromosome 5B. However, Melnyk and Unrau, (1959); contrary to the established opinion, demonstrated that the chromosomes of rye are capable of intergeneric pairing at least with those of T. tauschii (Ae. squarrosa), donor of the D genome in T. aestivum. They found that up to 25% of the bivalents observed were formed by Secale-Aegilops conjugations.

Okamoto, (1962, 1963); and Riley, Chapman and Belfield, (1966); studied the effect of mutagens on the action of the 5B chromosome in wheat-rye hybrids. A mutated 5B might possibly be detected by an increased incidence of homoeologous chromosome pairing (i.e. suppression of diploidization). These investigations led to the finding that in at least some hybrids an unusually high frequency of homoeologous conjugations occurred. Nevertheless, it was not possible to recover or fix the "mutated" material in later generations, possibly because the high frequency of homoeologous pairing as exhibited by the few hybrids was due solely to the effect of the rye pollen per se as distinct from a mutational effect involving the $5B^L$ "locus".

One of the most important studies in the understanding of the effect of the 5B gene (s) was provided by Feldman, (1966), using plants of T. aestivum with different dosages of the long arm of 5B as an isochromosome. Feldman concluded that the $5B^L$ gene (s) operates by regulating the premeiotic spatial distribution of both homologous and homoeologous chromosomes. He also reported that to a lesser

extent, chromosomes 5D and 5A have an effect on premeiotic association which is opposite to that of their homoeologue $5B^L$, and that high temperature relaxes somatic association in plants with extra dosages of $5B^L$, presumably by affecting the action of the promoters.

In a later report, Feldman, Mello-Sampayo and Sears, (1966), concluded that the phenomenon of premeiotic association of homologous, and to a lesser extent homoeologous chromosomes (personal communication) in T. aestivum, is not exclusive of the meiotic cells, but rather a common situation in all cells throughout the life of the plant. These workers also presented a list of references which supported their concept that somatic association occurs normally in many animal and plant genera.

Very recently Feldman and Mello-Sampayo, (1967), presented evidence to show that the phenomenon of somatic association in T. aestivum is a very complex one. In addition to promoters 5D and 5A, the short arm of 5B ($5B^S$) also may act in the same manner as suggested by Riley, Chapman, Young and Belfield, (1966). Feldman (personal communication); also has evidence which suggest that the short arm of 5A and 5D are implicated in the same phenomenon. These data show clearly that all the homoeologous chromosomes of the group 5 in T. aestivum are implicated in the same phenomena and renders plausible Feldman's assumption that the $5B^L$ gene (s) initially promoted premeiotic association but subsequently mutated to a form with the opposite effect.

Feldman and Mello-Sampayo, (1967); studied the meiotic behavior of wheat-T. speltoides hybrids and the effect of temperature upon them. Their results led them to suggest that the T. speltoides genotype, has

a gene (s) which, in combination with those present in 5A, $5B^S$ and 5D can induce homoeologous pairing notwithstanding the presence of $5B^L$ in the same genotype.

In a very recent report by Driscol, Darvey and Barber, (1967), on the effect of colchicine on meiosis of T. aestivum, evidence was found to support early findings that premeiotic association in hexaploid wheat does in fact exist. Furthermore, when the drug was applied before the onset of meiosis it acts in a way very similar to $5B^L$, possibly enhancing its effect in the suppression of homologous pairing.

MATERIAL AND METHODS

All wheat-rye hybrids used in the present study were derivatives of T. aestivum L. c.v. Chinese Spring which genotypically were either euploid, 2n = 6x = 42 or monosomic for the 5B chromosome, 2n = 6x-1 = 41. The rye parental genotype was a strain of Secale cereale L. c.v. Prolific, 2n = 14. Both species were maintained by the Department of Plant Science; University of Manitoba.

Synthesis of Hybrids

The parental species were grown in a greenhouse in six-inch pots and crosses were made during late spring of 1967. With the aid of embryo culture method in the way described by Melnyk, (1957), 52, 28-chromosome hybrids (21 chromosome from wheat and 7 from rye), and 18, 27-chromosome hybrids (deficient for 5B), were established.

The hybrids were first grown in a growth chamber in which day and night temperatures averaged approximately 18°C and 10°C (± 1) respectively with a 16-hour photoperiod. These growing conditions were effective in initiating good tiller development during early growth of the hybrid plants.

Upon the development of the third tiller, 10 of the 52, 28-chromosome hybrids and 6, of the 18, 27-chromosome hybrids were cloned in such a way that three clonal units were sectioned from each parent plant. Both populations were then allowed to develop uniformily at 10°C for approximately 4 weeks. Prior to the onset of meiosis, each of the three clonal units derived from each parent plant were transferred to one of three different temperature regimes maintained during

meiotic development, viz., 15°C, 21°C and 32°C (± 1). The remainder of the population of uncloned hybrids were distributed equally among the three treatments resulting in a total of 24, 28-chromosome hybrids and 10, 27-chromosome hybrids tested under each temperature regime. A photoperiod of 16-hour duration was maintained throughout the study.

Cytological Studies

At meiosis, spikes from hybrids in each of the three temperature regimes were fixed in Carnoy's solution and after 48 hours were transferred to 70% alcohol until examination. Preparations of pollen mother cells at first meiotic metaphase were made using the aceto-carmine method.

In the analysis of F_1 hybrids, records were kept of the number of univalents, open and closed bivalents, trivalents and higher multivalents that occurred per pollen mother cell at metaphase I in individual plants in each treatment. In scoring the number of chiasmata per cell, open bivalents were scored as one chiasma, closed bivalents as well as trivalents as two chiasmata, and quadrivalents as three chiasmata. In order to facilitate comparisons of results between the different hybrids and to make possible a clearer evaluation of the pairing frequencies of individual hybrids, the complexities of pairing were reduced to a common denominator of a unit termed a "bivalent association" as suggested by Kihara, (1929). Thus, either a closed or open bivalent as well as a trivalent configuration was interpreted as one bivalent association, while a quadrivalent was considered equivalent to two bivalent associations. Approximately

100 pollen mother cells were examined and scored for each hybrid.

Cytological examination was conducted at a magnification of 1250 diameters, while photomicrographs were recorded at 1000 magnifications.

RESULTS

The variety Chinese Spring was chosen for this study as the wheat parent because it is known to possess the primitive genomic structure of the early wheats, also because of the relative ease with which it can be hybridized with rye. On the other hand, Secale cereale c.v. Prolific is of annual spring growth and used extensively in the Triticale breeding program in the Plant Science Department.

Cytological data of the F_1 hybrids at the three different temperature treatment are summarized in Tables I to VI inclusive. In Plates I and II, typical metaphase I figures of some of the hybrids are presented.

Meiosis of 28-chromosome F_1 Hybrids at 15°C, 21°C and 32°C

Seventeen 28-chromosome wheat-rye hybrids which were grown at the 15°C treatment were analysed at first meiotic metaphase. The average bivalent association per cell showed continuous variation among plants from 0.05 to 5.63 (Table I). Most of the bivalents were rod-shaped; trivalents and or more complex associations were not observed in the more asynaptic hybrids (Plate I. Fig. A). However, as the degree of homoeologous pairing increased, as for example, in hybrids H-36 and H-15 (Table I), rod and ring shaped bivalents were observed as well as trivalents and quadrivalents (Plate I. Figs. B and C). In general a wide variation in the pairing relationship occurred at the 15°C temperature, ranging from cells exhibiting complete asynapsis to some which included up to eighteen chromosomes

Table I. - Mean pairing frequency per cell in 28-chromosome (5B present) wheat-rye hybrids grown at 15°C

Hybrid	Cells Scored	Bivalents Univ. (Range)	Bivalents Open (Range)	Bivalents Closed (Range)	Triv. (Range)	Quad. (Range)	Aver. Biv. Ass.	Chias-mata (Range)
H-25	100	27.90 (26-28)	0.05 (0-1)				0.05	0.05 (0-1)
H-8	100	27.42 (22-28)	0.29 (0-3)				0.29	0.29 (0-3)
H-51	100	27.34 (24-28)	0.32 (0-2)	0.01 (0-1)			0.33	0.34 (0-3)
H-63	100	26.52 (20-28)	0.74 (0-4)				0.74	0.74 (0-4)
H-11	100	26.52 (16-28)	0.72 (0-6)	0.02 (0-1)			0.74	0.76 (0-6)
H-62	100	25.92 (22-28)	1.02 (0-3)	0.02 (0-1)			1.04	1.06 (0-3)
H-14	100	25.52 (18-28)	1.22 (0-5)	0.02 (0-1)			1.24	1.26 (0-5)
H-68	200	24.33 (18-28)	1.77 (0-5)	0.05 (0-1)	0.01 (0-1)		1.83	1.89 (0-5)
H-66	100	24.28 (20-28)	1.79 (0-4)	0.04 (0-4)	0.02 (0-1)		1.85	1.91 (0-4)
H-104	100	23.84 (16-28)	2.00 (0-5)	0.08 (0-1)			2.08	2.16 (0-7)
H-9	100	23.50 (18-28)	2.16 (0-5)	0.06 (0-1)	0.02 (0-1)		2.24	2.32 (0-5)
H-5	100	23.34 (20-28)	2.10 (0-4)	0.20 (0-2)	0.02 (0-1)		2.32	2.54 (0-6)
H-61	100	22.64 (17-28)	2.58 (0-5)	0.04 (0-1)	0.06 (0-1)		2.68	2.78 (0-5)
H-64	100	22.25 (16-28)	2.68 (0-6)	0.18 (0-2)	0.01 (0-1)		2.87	3.06 (0-6)
H-67	100	21.88 (18-28)	2.63 (0-5)	0.16 (0-2)	0.18 (0-1)		2.97	3.31 (0-5)
H-36	100	17.77 (13-28)	3.81 (0-6)	0.54 (0-3)	0.15 (0-2)	0.02 (0-1)	4.54	5.27 (0-10)
H-15	100	15.91 (9-28)	3.31 (0-7)	1.37 (0-3)	0.83 (0-3)	0.06 (0-1)	5.63	7.89 (0-13)

Plate I - First meiotic metaphase in hybrids of Triticum aestivum L. var. Chinese Spring x Secale cereale L. var. Prolific.

Fig. A - An asynaptic 28-chromosome hybrid grown at 15°C showing one bivalent and 26 univalents.

Figs. B and C - Hybrids with higher homoeologous pairing grown at 15°C.

B. two trivalents, two bivalents and 18 univalents.

C. One quadrivalent, four bivalents and 16 univalents.

Fig. D - Asynaptic hybrid grown at 21°C showing one bivalent and 26 univalents.

Figs. E and F - Hybrids with higher homoeologous pairing grown at 21°C.

E. three closed and one open bivalents and 20 univalents

F. one trivalent, five bivalents and 15 univalents.