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A Possible Effect of Heterochromatin on Chromosome Pairing

(Giemsa banding/triticale/amphiploid/synthetic species)

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ABSTRACT **Rye chromosomes were selectively stained in the meiosis of triticale by means of heterochromatin banding techniques. Compared to wheat chromosomes, rye chromosomes showed reduced pairing at first meiotic metaphase. Within the rye genome this pairing failure was associated with the presence of large, terminal heterochromatic bands. Since these terminal bands of rye chromosomes are late replicating, the effect of heterochromatin could arise from an overlap between the processes of chromosome replication and chromosome pairing.**

Triticale (X *Triticosecale* Wittmack) is the amphiploid between species of tetraploid or hexaploid wheat (*Triticum* L.) and rye (*Secale* L.). In triticale a varying number of homologous chromosomes fail to pair at meiosis and are seen as univalents at first metaphase (1, 2). It has been suggested (3–7) that these univalents are mainly chromosomes of the rye genome. This conclusion was based on two general observations made on octaploid triticale. First, some triticales have a strong tendency to revert to hexaploids that resemble wheat (3). Second, individual chromosomes of rye contributed to aneuploidy much more often than wheat chromosomes (5). By contrast, in hexaploid triticales a substantial number of the aneuploids were contributed by the wheat genomes as well (8, 9). However, in order to extrapolate from aneuploidy to meiosis, we must assume that all deficiencies will be transmitted with approximately the same frequency.

Ideally what is needed is a method whereby the genomic origin of the univalents themselves can be ascertained. Such a method was suggested to us by the recent reports (10–12) that in somatic cells of triticale the wheat and rye chromosomes can be strained differentially with Giemsa. Rye chromosomes generally carry at least one large and terminal band, unlike wheat chromosomes, where terminal bands are both infrequent and small. It is generally accepted that chromosome regions that strain differentially with Giemsa are heterochromatic.

MATERIALS AND METHODS

Genotypes used were as follows:

(a) *AABBRR*—Stewart durum (*T. turgidum* L.) × Prolific rye (*S. cereale* L.) — 6A190, and a variety of triticale × triticale F_1 hybrids.
(b) *AABBR*—Stewart durum × Rosner triticale.
(c) *ABRR*—6TA204 triticale × UC90 rye.

All pollen mother cell material was fixed in Carnoy's II. Mean meiotic pairing was determined on aceto-carmine squashes. Pollen mother cells from the same florets were stained for heterochromatin as follows: anthers containing pollen mother cells at first metaphase were squashed on slides in a drop of acetic acid and the coverslips were removed. The slides were dipped in 45% acetic acid or Carnoy's I, air dried at room temperature, treated in saturated $Ba(OH)_2$ solution (pH about 13.2) for 5 min, and washed thoroughly in demineralized water (anions and cations) prior to incubation in 0.30 M NaCl–0.03 M sodium citrate, pH 7 (2× SSC) for 1 hr at 60–65°. The slides were washed again and stained with Leishman's or Giemsa stains in phosphate buffer at pH 6.8 for 3–5 min.

The staining procedure made it difficult to score entire cells, so only those chromosomes with no degree of overlap were recorded. Ring bivalents are more likely than univalents to overlap other chromosomes, while rod bivalents seem to be intermediate in this respect. Consequently, the ring bivalents, rod bivalents, and univalents recorded are not in proportion to one another (Table 1), but are regarded as a random sample of each configuration.

The distribution of only the most prominent terminal heterochromatic bands was recorded for each configuration (Fig. 1, Table 1). This was done for two reasons. First, one or two wheat chromosomes do have small terminal bands (e.g., chromosome 1B). Second, it was felt that only the largest terminal bands would be plainly seen in chiasmata (Fig. 1). Thus, we hoped to separate the wheat chromosomes from the rye chromosomes and to record the distribution of chiasmata relative to the rye heterochromatin.

TABLE 1. *The distribution of terminal heterochromatin in ring bivalents, rod bivalents, and univalents in hexaploid homozygous (6A190) and heterozygous (hybrid) triticale**

No. terminal bands	Ring bivalents		Rod bivalents		Univalents	
	6A190	Hybrids	6A190	Hybrids	6A190	Hybrids
2	0	6	7	27	17	143
1 Paired	15	12	2	6	—	—
1 Unpaired	—	—	25	61	12	133
0	65	103	15	40	7	60
Banded/ total (%)	18.75	14.89	69.39	70.15	86.56	82.14

* Illustrations of the various chromosome types can be seen in Fig. 1.

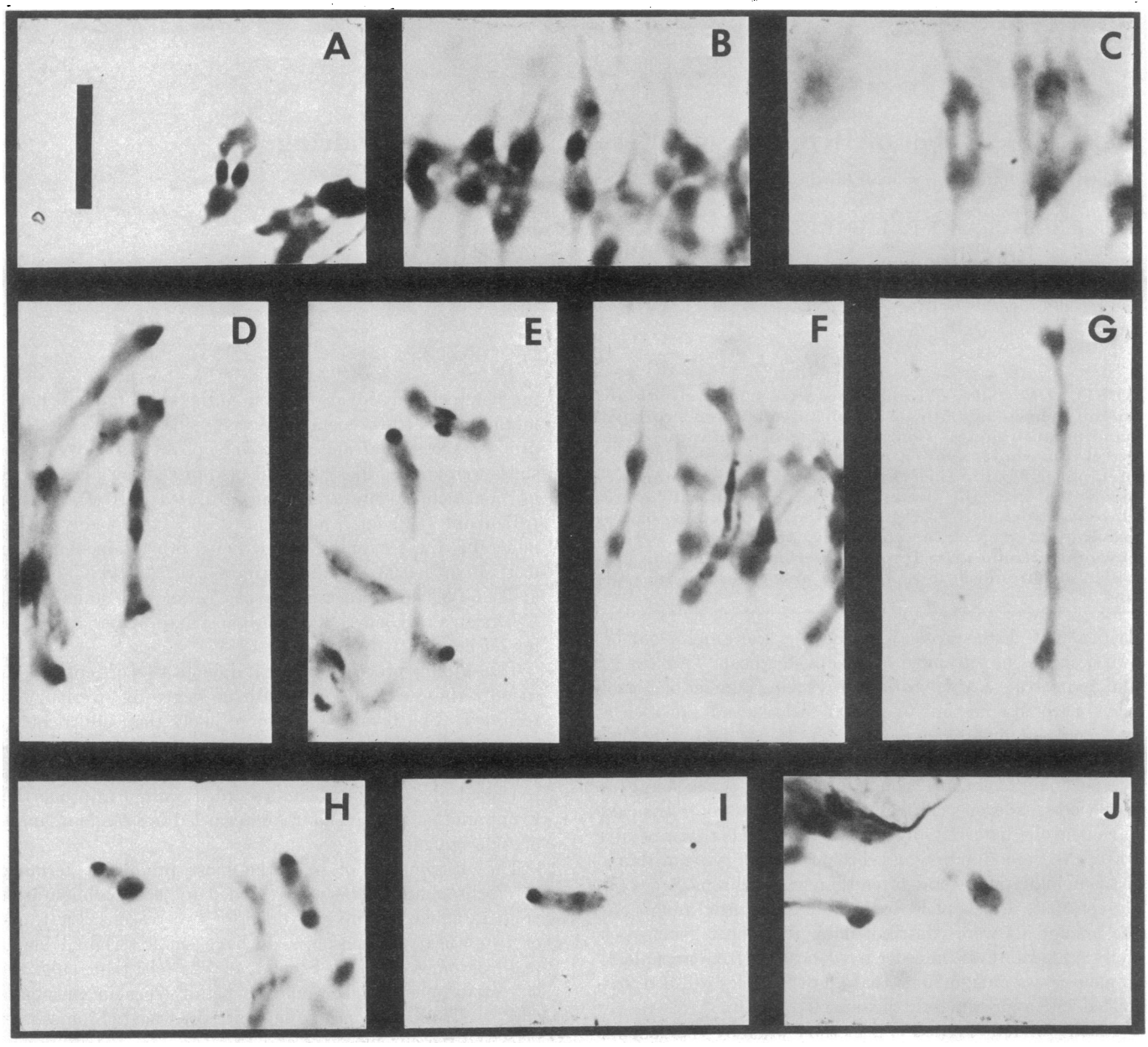

FIG. 1. Metaphase chromosomes of triticale stained for heterochromatin. (*A*, *B*, and *C*) Ring bivalents of chromosomes with 2, 1, and 0 terminal heterochromatic bands, respectively. (*D*) Rod bivalent of chromosomes with two terminal bands. (*E*) Two rod bivalents composed of chromosomes with 1 terminal band. Coorientated bivalent showing the heterochromatic arms unpaired. Short unstretched bivalent with paired heterochromatic arms. (*F*) Rod bivalent composed of chromosomes with one terminal band with paired heterochromatic arms. (*G*) Rod bivalent of chromosomes showing no heterochromatic bands. (*H*, *I*, and *J*) Univalents with 2, 1, and 0 terminal heterochromatic bands, respectively. (*A*) from tetra-Prelude (*T. aestivum* L. *AABB*) × Prolific rye. All others from 6A190. *Bar* represents 10 μm.

Data from the triticale × triticale F_1's were pooled.

RESULTS

Rye chromosomes were readily identified by the presence of one or two conspicuous terminal bands (Fig. 1); these chromosomes dominated the rod bivalent and univalent classes (Table 1). Unlike the rye chromosomes, the wheat chromosomes contributed little to the rod bivalent and univalent classes but were found in great excess among ring bivalents. Some chromosomes may have been misclassified as being either rye or wheat. To check this possibility, aceto-carmine squashes of pollen mother cells were made so as to record the mean pairing in 6A190 (Table 2). From Table 1, 18.75% of ring bivalents in 6A190 were stained for terminal heterochromatin. Of the 15.14 ring bivalents per cell in 6A190 (Table 2), 18.75% may then be considered to be rye chromosomes. In the same way 69.39% of all rod bivalents showed terminal heterochromatin (Table 1), and, therefore, this percentage of the 5.10 rod bivalents (Table 2) in 6A190 were rye chromosomes (Table 2). Finally, 80.56% (Table 1) of the 1.50 univalents in 6A190 (Table 2) would also be rye chromosomes. If we add up the estimated contribution of the rye genome to these three configurations, the total number of chromosomes should approximate 14—the number of rye

TABLE 2. *Mean observed and predicted chromosome pairing in AABBRR, AABBR, and ABRR genotypes*

Genotype and predicted pairing	Chromosome configuration			Chromosome number	No. cells examined
	Ring bivalents	Rod bivalents	Univalents		
*AABBRR**	15.14	5.10	1.50	42	28
Predicted wheat pairing	12.30	1.56	0.30	28.02	—
Predicted rye pairing	2.84	3.54	1.21	13.97	—
AABBR†	12.32	1.68	7.00	35	25
ABRR‡	4.07	2.93	14.00	28	14

* Mean meiotic pairing in 6A190 was determined from the same florets that provided pollen mother cells for heterochromatin staining.

† Rosner triticale and Stewart durum differ by one reciprocal translocation. Ring IV's were coded as two ring II's, and open IV's were coded as one ring II and one rod II.

‡ To avoid including homoeologously paired wheat chromosomes, only cells with 14 or more univalents were considered.

chromosomes in triticale. The value obtained was 13.97 (Table 2), which is very close. This suggests that during the scoring there was no gross bias introduced by using only the large terminal heterochromatic bands to identify rye chromosomes in meiosis. Furthermore, in three cases where the complete cell could be analyzed, only seven chromosome pairs with terminal heterochromatic bands could be seen.

An indication of the relative pairing of wheat and rye chromosomes in triticale can be obtained from the backcrosses to each of its parents. In triticale × rye (*ABRR*) the rye genome forms a considerable number of rod bivalents, while in wheat × triticale (*AABBR*) the wheat chromosomes form mainly ring bivalents (Table 2). These observations agree well with the genomic pairing in 6A190 *AABBRR* that was estimated from the heterochromatic staining (compare Table 2). Therefore, in triticale, rye chromosomes pair less often than wheat chromosomes.

The reason for this abnormal behavior of the rye chromosomes actually seems to be related to the presence of the terminal heterochromatin. Among rod bivalents with a single terminal band, most were paired in the nonheterochromatic arm (Table 1, Fig. 1).

Since several of the rye chromosomes carry only one large band located on the short arm, the association of heterochromatin with pairing failure could be fortuitous. On the other hand, reluctance of the heterochromatic arms to pair was also indicated by the relative behavior of rye chromosomes with two large terminal bands. Compared to chromosomes with a single terminal band (Table 1), these chromosomes were more likely to be found as univalents and least likely to pair as ring bivalents ($X^2 = 18.79$, 2 degrees of freedom, $P \leq 0.005$). In these cases, both the long and short arms were heterochromatic and both showed pairing failure. Therefore, the low pairing of heterochromatic arms cannot be simply explained by a low average arm length.

DISCUSSION

Why should the presence of terminal heterochromatin in the rye chromosomes interfere with their pairing in triticale? In the heterochromatic telomeres of rye, DNA starts and finishes its replication later than in the rest of the chromosomes (13–15). Meiosis in rye is relatively slow, however, compared to wheat and triticale, and this slow meiotic cycle may be necessary to accommodate the late-replicating ends of the rye chromosomes. In triticale, unless the rye telomeres replicate substantially earlier than in rye itself, they could be a possible cause of meiotic difficulties (16, 17).

In maize and barley, chromosome pairing is believed to begin at or near the telomeres (18–20). Perhaps the telomeres cannot engage in chromosome pairing until they are replicated. An overlap between the replication phase of rye telomeres and prophase pairing could explain why rye chromosomes have such difficulty pairing in hexaploid triticale.

Some triticales exhibit better pairing than others. Presumably in high-pairing lines either the heterochromatin content of the rye chromosomes has been reduced or the meiotic cycle has been adjusted to suit the requirements of both wheat and rye chromosomes. So far no highly asynaptic lines of triticale (with a mean of 5 or more univalents per cell) have been timed with respect to meiosis.

S. montanum Guss. has some chromosomes without large heterochromatic bands (N. L. Darvey, personal communication). This would explain why a triticale with *S. montanum* as the rye parent showed better pairing than 6A190 (21). Some of the triticale hybrids we examined showed rod bivalents with marked heteromorphism for the size of the terminal bands. Hence, it may be possible to select for low heterochromatic content in triticale. It might also be possible to artificially reduce the amount of heterochromatin present in a species by chemical (22) or other means (23, 24).

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