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Cytogenetics of the Trigeneric Hybrid, (*Hordeum vulgare* x *Triticum aestivum*) x *Secale cereale**

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Summary. Chromosome pairing was studied in hybrids of (*Hordeum vulgare* × *Triticum aestivum*) × *Secale cereale*. Chiasma frequency per cell varied from 1.94 to 3.16 between the different hybrids. This variation was attributed to genetic variability in rye parents which affected homoeologous pairing. The pairing of rye chromosomes as revealed by Giemsa C-banding was a combination of nonhomologous association between rye chromosomes and associations with chromosomes of wheat and barley.

Key words: Intergeneric hybrids – Giemsa banding – Homoeologous chromosome pairing

Introduction

Chromosome pairing in hexaploid *Triticum aestivum* reflects a balance between genes that promote and those that supress homoeologous chromosome pairing. The *Ph* gene located on chromosome $5B^L$ has a major effect in restricting chromosome pairing to homologues and may activate other genes such as that on 3D. Genes are present in diploid species and genera such as *Secale* which suppress the 5B effect and permit pairing of wheat homoeologues. Nakajima (1952) observed variability in rye for effects on chromosome pairing in wheat-rye hybrids. Feldman (1966) and Dvorak (1977) observed a polygenic system in rye that promoted homoeologous chromosome pairing in wheat while Lelley (1976) suggested a system consisting of more than two alleles that probably act additively.

Chromosome pairing in barley-wheat hybrids has been variable. Negligible amounts of chromosome pairing have been reported in hybrids from *Hordeum vulgare* cv. 'Manker' × *T. turgidum* cv. 'Cocorit', 'Manker' × *T. aestivum* cv. 'Tobari 66' (Mujeeb et al. 1978) and the related species *T. timopheevi* × *H. bogdanii* (Kimber and Sallee 1978). A high chiasma frequency was observed in other *Hordeum-Triticum* hybrids such as *H. vulgare* cv. 'Betzes' × *T. aestivum* cv. 'Chinese Spring' (Cauderon et al. 1978; Fedak 1980) and in the reciprocal cross (Fedak 1980). Similarly, a high chiasma frequency of 2.23 was observed in a hybrid of *H. chilense* × 'Chinese Spring' (Martin and Chapman 1977). It has since been demonstrated that the chiasma frequency in different barley-wheat hybrids can vary by a factor of six depending on the parental cultivars employed (Fedak, manuscript in preparation).

Barley-rye hybrids have been produced at very low frequencies and chromosome pairing in such hybrids has been very low (Fedak 1979; Kruse 1967, 1969; Pickering and Thomas 1979). Trigeneric hybrids were obtained by pollinating the *T. timopheevi* × *H. bogdanii* hybrid with Imperial rye (Kimber and Sallee 1979). Chromosome pairing in this trigeneric hybrid was very low, not unlike that in the original *Triticum* × *Hordeum* hybrid (Kimber and Sallee 1978). Trigeneric hybrids were also produced by crossing 'Prolific' rye onto hybrids between 'Betzes' and 'Chinese Spring' (Fedak and Armstrong 1980) and their meiotic behavior will be the subject of this report.

Materials and Methods

The hybrid between *H. vulgare* cv. 'Betzes' and *T. aestivum* cv. 'Chinese Spring' was synthesized as described previously (Fedak 1980). This hybrid was pollinated by *S. cereale* cv. 'Prolific' to produce the trigeneric hybrid as described previously (Fedak and Armstrong 1980). The trigeneric hybrids were grown in a controlled environment cabinet maintained at a 16 hr day, a day/night temperature regime of 20/15°C and light intensity of 600 microeinsteins m^{-2} sec^{-1} provided by a combination of fluorescent and incandescent lamps. Two chromosome staining methods were used for study of meiotic chromosome pairing. Whole spikes were fixed in Carnoy's solution and the chromosome staining intensified by Snow's alcoholic-carmine prior to squashing in acetocarmine. The technique of Jones (1978) and as modified by Fedak and Armstrong (1980) was employed for the Giemsa C-banding procedure. The expected frequencies of chromosome configurations in the hybrids were calculated according to the formulae of Driscoll et al. (1979).

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Results

Meiotic behavior in the three trigeneric hybrids studied by the standard acetocarmine technique revealed the chiasma frequency to be 2.14, 3.14 and 3.64 for hybrids 4, 7 and 6, respectively (Table 1). The rod bivalent frequency ranged from 1.20 to 2.54 and ring bivalent frequency from 0.42 to 0.52 and the level of each in any particular hybrid paralleled the chiasma frequency. Within individual cells up to 5 bivalents were occasionally observed. Trivalent frequency among the hybrids ranged from 0.04 to 0.19. Sporocytes with high pairing such as 4^{II} and 1^{III} or 2^{II} and 1^{III} occurred in hybrid 6. Heteromorphic bivalents were observed at low frequencies and were assumed to be associations of rye with either wheat or barley chromosomes. Bivalents consisting of large chromosomes were assumed to consist of rye chromosomes but the chromosomes were not differentially stained and these assumptions could not be verified.

By means of Giemsa C-banding, rye chromosomes, including bivalents could be positively identified. They occurred exclusively as rod bivalents (Fig. 1) and their frequency varied from 0.02 to 0.12 per cell (Table 2). Configurations involving pairing of rye with non-rye chromosomes could be positively identified (Fig. 2) and their frequency ranged from a low of 0.01 in hybrid 7 to 0.04

Table 1. Observed (acetocarmine and Giemsa stained) chromosome pairing in trigeneric hybrids between (*Hordeum vulgare* × *Triticum aestivum*) × *Secale cereale*

Hybrid number		I	II			III	Other	Xta/ cell	No. of cells
			rod	ring	total				
4	acetocarmine	32.60	1.20	0.42	1.64	0.04	–	2.14	110
	Giemsa	32.04	1.17	0.31	1.48	0.09	–	1.94	52
6	acetocarmine	28.40	2.54	0.52	3.06		0.03	3.64	96
	Giemsa	29.63	2.08	0.23	2.31	0.25	–	3.16	108
7	acetocarmine	29.39	1.88	0.44	2.52	0.19	–	3.14	142
	Giemsa	30.91	1.65	0.26	1.91	0.09	–	2.40	80

Table 2. Observed and expected (based on 3, 4, and 5 homoeologues pairing at random[a]) chromosome pairing in the Giemsa-stained sporocytes

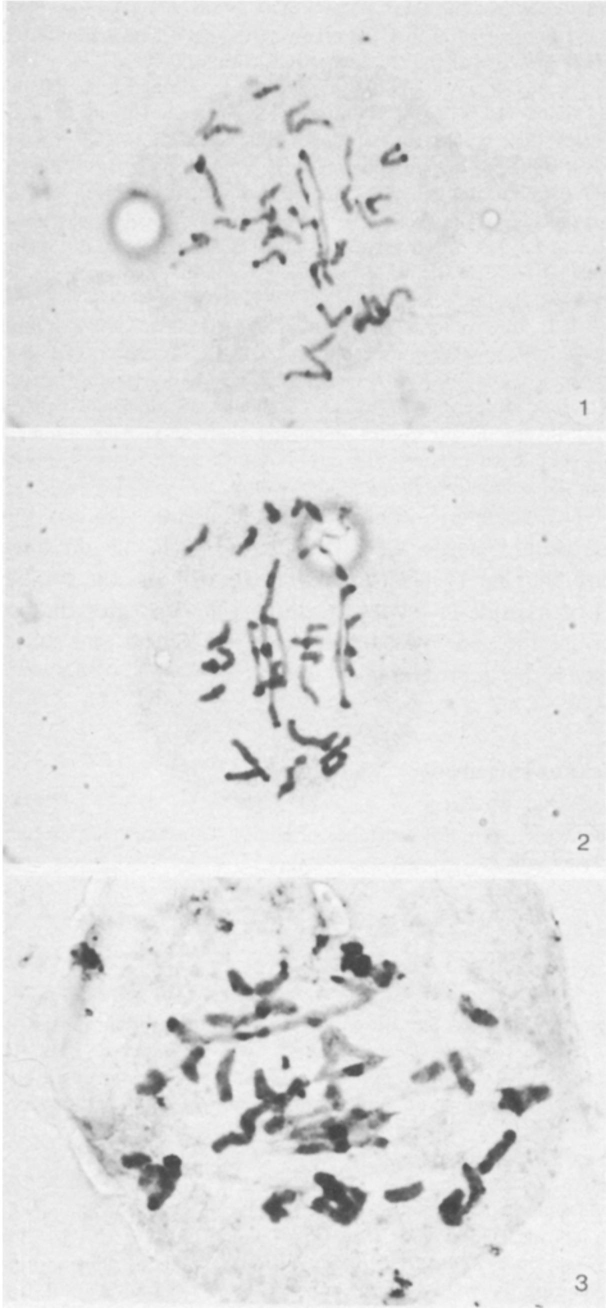
Hybrid number	I	II				III	chain	ring	V	C[a] value
		rod	ring	rye	rye non-rye					
4 obs.	32.04	1.17	0.31	0.12	0.04	–	–	–	–	
exp-5	31.18	1.75	0.02	0	0.59[b]	0.09	0.004	0	0	0.07
exp-4	31.23	1.69	0.03			0.10	0.006	0	–	0.07
exp-3	31.30	1.67	0.04			0.09	–	–	–	0.14
6 obs.	29.63	2.08	0.23	0.07	0.03	0.25	–	–	–	
exp-5	29.00	2.62	0.04	0	0.92[b]	0.21	0.014	0	0	0.11
exp-4	29.12	2.46	0.07			0.24	0.02	0	–	0.11
exp-3	29.15	2.45	0.12			0.24	–	–	–	0.22
7 obs.	30.91	1.65	0.26	0.02	0.01	0.09				
exp-5	30.38	2.08	0.02	0	0.76[b]	0.12	0.006	0	0	0.09
exp-4	30.45	1.99	0.04			0.15	0.01	0	–	0.09
exp-3	30.47	1.99	0.07			0.14	–	–	–	0.17

[a] Driscoll et al. 1979

[b] Under random chromosome pairing and chiasma formation among homoeologues 2/5 of the total bivalents would involve a rye chromosome

in hybrid 4. Several hyperploid cells were detected in the trigeneric hybrids such as cells with 41 chromosomes, 9 of which were rye chromosomes (Fig. 3). In this particular cell the sum of paired configurations was $6^{II} + 1^{III}$ of which $2^{II} + 1^{III}$ were rye chromosomes.

The trigeneric hybrids consisted of five genomes: A, B, D of wheat, the R from rye plus the barley genome. In other words, seven groups of chromosomes each with at least three homoeologues (A, B, D of wheat) and possibly five if the rye and barley genomes are homoeologous with the wheat genomes. To evaluate the extent of the homoeology between the five genomes, the observed chiasma frequency for each hybrid was used to calculate expected chromosome configurations by means of formulae provided by Driscoll et al. (1979). Three models were used to calculate the expected frequency of chromosome configurations; assuming the association at random of five, four and three homoeologous chromosomes, respectively. The best fit between expected and observed data would indicate the extent of homoeology between chromosomes of the five genomes from the three genera.



Figs. 1-3. Giemsa stained sporocytes in trigeneric hybrids: **1)** 35 chromosomes: $31^{I} + 2^{II}$ (with one rye-non-rye bivalent); **2)** 35 chromosomes. $23^{I} + 6^{II}$ (with one rye bivalent and one rye-non-rye bivalent); **3)** 41 chromosomes: (9 rye chromosomes): $26^{I} + 6^{II} + 1^{III}$ (with two rye bivalents and one rye trivalent)

The expected frequencies of configurations calculated from the three models were fairly similar (Table 2) with respect to univalents, bivalents (ring and rod) and trivalents. The observed frequencies showed a closer fit to the three or four homoeologue model compared to the five homoeologue model indicating that chromosome pairing and chiasma formation was more frequent between three rather than five homoeologues. This probably means preferential pairing between homoeologues from the three wheat genomes. There were major differences between the three models for expected frequencies of pentavalents and quadrivalents. However, in material of low chiasma frequency the expected values are extremely low and extremely large populations would be required to distinguish between the three hypotheses. A complicating factor was also encountered in that associations between nonhomologous chromosomes of the same haploid set can occur. The frequency of association of nonhomologous rye chromosomes ranged from 0.02 to 0.12. Presumably nonhomologous association of the barley and wheat chromosomes may also occur at some frequency. Nonhomologous associations may be involved in the formation of quadrivalents and pentavalents.

The greatest deviation from the expected values was with respect to the frequency of ring bivalents. The frequency of ring bivalents was higher than expected even for model 3. Such a frequency may suggest that pairing and/or chiasma formation was not completely random even among the three homoeologues of wheat.

Discussion

There was some discrepancy between the total amount of pairing observed by the two staining methods with lower values realized for the Giemsa technique. There are several alternative explanations for this discrepancy. It is possible that configurations not involving rye chromosomes may have been overlooked by the Giemsa technique because very little staining of the barley and wheat chromosomes occurred in most cells. Alternatively the Giemsa technique may cause disruption of some configurations. Higher scores were obtained for end-to-end associations for chromosomes of *Allium* (Fussel 1975, 1977) and *Ornithogalum* (Ashley 1979) when stained with Feulgen or 3H autoradiography than with Giemsa C-banding. This same phenomenon may have occurred in our studies but the discrepancy was not significant enough to alter final conclusions.

Chromosome pairing in the trigeneric hybrids reported here was quite high compared to trigeneric hybrids between other Triticinae species (Kimber and Sallee 1979). Observed chromosome pairing was assumed to be a sum of nonhomologous and homoeologous configurations. A low level of nonhomologous pairing has been reported for the haploids of each of the parental species. A chiasma frequency of 1.05 per cell has been reported for haploids of 'Chinese Spring' (Riley and Law 1965); 0.04 for 'Betzes' (unpublished) and 0.33 for rye haploids (Levan 1943) resulting in a sum of haploid chiasma frequencies for the three parental species of 1.42 which is somewhat lower than the observed chiasma frequency in either of the three hybrids studied. This implies that a major portion of observed pairing was of the homoeologous type.

Nordenskiold (1939) reported a chiasma frequency of 0.03 in a rye haploid while a range of 0.08 to 0.83 was reported by Levan (1943) for a number of rye haploids. These frequencies were in close approximation to paired configurations of rye chromosomes observed in this study. In addition to this nonhomologous pairing, rye chromosomes synapsed at a low frequency with either wheat or barley chromosomes. Whether the non-rye chromosomes were wheat or barley, could not be verified but probably revealed homoeologous associations. Associations between rye and barley chromosomes in barley × rye hybrids were negligible (Kruse 1969; Fedak 1979; Pickering and Thomas 1979) so that alien chromosomes paired with those of rye must be assumed to be wheat chromosomes. A summation of these results indicate chromosome pairing in trigeneric hybrids was a combination of homoeologous and nonhomologous associations.

There were differences between the three hybrids in their overall chiasma frequency. The barley-wheat hybrids, one of the parents of the trigenerics, were not necessarily the same plant but were of the same ancestry. The rye pollen was not obtained from the same plant so the variation in overall chromosome pairing was probably due to genetic variability between rye parents. The effect of the rye genotype on homoeologous chromosome pairing in wheat-rye hybrids has been amply demonstrated (Feldman 1966; Lelley 1976; Dvorak 1977).

The observed values of chromosome pairing agree rather well with the expected values assuming random pairing and chiasma formation among the homoeologues of the five genomes (Driscoll et al. 1979). However, the expected values assuming random pairing and chiasma formation among four or three homoeologues are not much different and the observed values perhaps fit the assumption that there is random pairing and chiasma formation among three homoeologues. It is probable that the reality lies somewhere between these three models for calculating expected frequencies. However, there would appear to be justification for assuming that the observed pairing is mainly between the homoeologues of wheat. Similar hybrids with significantly higher chiasma frequencies may help to differentiate among the three models. Therefore, the increased level of pairing observed in hybrids of barley × wheat and (barley × wheat) × rye as compared to haploid wheat then suggests that the effect of the $5B^L$ gene is suppressed by the presence of the barley and rye genomes.

Trigeneric hybrids in the Triticeae were obtained by Kimber and Sallee (1979) from the same three genera but from different species than used in our studies. Their hybrid involved crossing *S. cereale* onto an amphiploid of *T. timopheevi* × *H. bogdanii*. The chiasma frequency calculated from their observed pairing frequencies in the trigeneric hybrid was 1.80 which was lower than in any of our trigeneric hybrids. There are several possible explanations for the differences. The chiasma frequency in the *Triticum* × *Hordeum* hybrid was 0.99 and somewhat lower than the 1.81 observed in our 'Betzes' and 'Chinese Spring' hybrid (Fedak 1977). They used the rye cultivar 'Imperial' (Kimber, personal communication), which may have had a lesser effect on induction of pairing of wheat homoeologues than the cultivar 'Prolific' used in our studies. In general, there may be less homoeology expressed between the chromosomes of their three species than those employed in our studies.

The trigeneric hybrids are vegetatively vigorous but completely sterile and attempts at colchicine doubling have thus far been unsuccessful. Nevertheless the synthesis of hybrids involving the three genera creates the potential for gene transfer between the genera and subsequent crop improvements.

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