Theor, Appl. Genet. 58, 137-143 (1980)

THEORETICAL AND APPLIED GENETICS

Chromosome Pairing in Tetraploid Hybrids Between *Lolium perenne* and *L. multiflorum*

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Summary. Chromosome association at first meiotic metaphase in tetraploid hybrids between *Lolium perenne* and *L. multiflorum* was compared with that in autotetraploid *L. perenne*. The hybrids were found to have significantly higher levels of bivalent frequency, and lower levels of multivalent and chiasma frequency. A significant increase in multivalent frequency with increasing chiasma formation was found in both groups, but the increase was much less in the hybrids. These differences in chromosome associations between the two groups must therefore reflect differences in chiasma distribution and it is suggested that the results indicate a significant degree of preferential bivalent pairing in the hybrids.

Key words: *Lolium perenne* – *L. multiflorum* – Tetraploid hybrids – Preferential chromosome pairing

Introduction

In common with many other synthetic allopolyploids, tetraploid hybrids between the diploid (2n = 14) species *Lolium perenne* and *L. multiflorum* show some multivalent association at meiosis, indicating the occurrence of homoeologous as well as homologous chromosome pairing. Further homoeologous pairing must also occur in the form of heterogenetic bivalents, but there are no currently available cytological techniques that can be used to identify this in the present material. Since homoeologous pairing results in recombination between the *L. perenne* and *L. multiflorum* genomes, there must be a gradual dissipation of interspecific hybridity through consecutive seed generations. The extent to which this occurs is clearly of great significance in a breeding context since the very desirable combination of agronomic features obtainable in the initial hybrids (Breese and Davies 1975) is based on several contrasting parental characteristics. Restricting the amount of interspecific recombination, by maximising the degree of preferential or homogenetic chromosome pairing, must therefore be a primary objective in the breeding of this material.

There are contrasting reports of the levels of multivalent and bivalent pairing found in tetraploid *L. perenne* × *L. multiflorum* hybrids. Clarke and Thomas (1976) reported a lower level of multivalent pairing and a corresponding higher level of bivalent pairing than that found in autotetraploid forms of the parental species. Ahloowalia (1977) on the other hand found that the multivalent frequency in F_1 plants was high with most pollen mother cells (PMCs) containing three or four quadrivalents.

While a high frequency of bivalents may well be indicative of preferential pairing, bivalent pairing can be conclusively shown to be preferential only if it results in disomic segregation. Breese and Thomas (1977) were able to demonstrate partial disomic segregation in test crosses between certain of their F_1 hybrids. Using a isozyme genetic marker, which has recently been shown by Lewis, Humphreys and Caton (in press) to be located on chromosome 6, they calculated the level of preferential pairing to be 34% for this particular set of four chromosomes.

Ahloowalia (1977) however concluded, on the basis of his cytological results and segregation ratios obtained for two genetic markers (fluorescent-roots and awned-florets), that 'for all practical purposes, these allopolyploids resembled autotetraploids in their breeding and genetic behaviour'.

In view of these conflicting results, the present investigation was made to further examine chromosome pairing in relation to genetic segregation in this allotetraploid. This paper reports on a detailed comparison of pairing behaviour in a range of F_1 hybrids with that of an autotetraploid form of *L. perenne*, which formed the first part of this investigation.

0045-5752/80/0058/0137/$ 1.40

Materials and Methods

The crosses were made by placing six tetraploid genotypes of *L. perenne* amongst large numbers of tetraploid *L. multiflorum* plants for mass pollination. The anthocyanin-pigmented base marker, which was shown by Jenkin (1930) to be controlled by two complementary dominant factors C and R, was used to distinguish hybrids from selfs (and to study segregation in the second part of the investigation). The *L. perenne* plants were of the constitution ccccRRRR and therefore devoid of anthocyanin pigmentation ('non-red') while the *L. multiflorum* pollinators were 'red' based (CCCCRRRR). As expected, the majority of the progeny were 'red' based, and therefore hybrids, and 25 of these together with 10 *L. perenne* from the original 'non-red' population were randomly chosen for the study. Inflorescences were fixed in Carnoy's fluid to which a few drops of ferric chloride had been added, and 25 PMCs per plant were analysed after staining in alcoholic hydrochloric acid-carmine (Snow 1963).

Results

Pattern of Chromosome Association at First Metaphase

The 19 hybrids and 7 autotetraploids analysed included 7 and 3 aneuploids respectively. The results which are summarised in Table 1 are for all 26 plants since the exclusion of the aneuploids did not materially affect the overall results. Before comparing the data for the two groups it is desirable to consider the pairing behaviour of the autotetraploids in the light of previous reports and to determine whether it is typical for this kind of material.

Each set of 4 homologous chromosomes can associate as (a) 1 quadrivalent, (b) 1 trivalent and 1 univalent, (c) 2 bivalents, (d) 1 bivalent and 2 univalents or (e) remain unpaired as 4 univalents. Durrant (1960) has derived formulae which predict the frequencies of the various types of configurations, with varying chiasma frequencies, assuming random chiasma formation between the four homologues. The values obtained for mean quadrivalent frequency per PMC in this study (3.04-3.56) show reasonably close agreement with expectation, but the frequency of univalents and trivalents was much lower, and bivalent frequency higher than expected. According to the model proposed by John and Henderson (1962), where it is further assumed that pairing is usually obligatory for the 4 homologues, equal numbers of quadrivalents and bivalents are expected, with trivalents and univalents rare.

The present results, while showing a very low frequency of trivalents and univalents, again clearly deviate from the expectation of equal numbers of quadrivalents and bivalents. It would appear therefore that the pairing behaviour of this material does not conform to either of the models described. It does however show very close agreement with that reported several times previously in autotetraploid *L. perenne* (Myers 1945; Ahloowalia 1967; De Roo 1968; Crowley and Rees 1968; Simonsen 1973) and to this extent provides a valid basis with which to compare chromosome pairing in the hybrids.

This comparison (Table 1) very clearly shows that the hybrids as a group had a lower quadrivalent frequency and a corresponding higher bivalent frequency. (There was, as expected, a very high negative correlation between the mean frequency per plant of these two types of configuration; $r = -0.9688$ for the hybrids and $r = -0.9563$ for the autotetraploids). In so far as a mean frequency of 6.54 represents the level of bivalent pairing that can be expected from the association of 4 fully homologous chromosomes in this material, the increase over this found in the hybrids (2.44) suggests a degree of preferential pairing of approximately 33%, $\left(\frac{2.44 \times 100}{14.00 - 6.54}\right)$.

The mean trivalent frequency was of the same order in both groups. There was a slight increase in the frequency of unpaired chromosomes in the hybrids over that in the autotetraploids and a decrease in the overall mean chiasma frequency. Two of the aneuploid hybrid plants had 29 chromosomes but pentavalents were found in only one of these (1 V in each of 4 PMCs).

Detailed analyses of the between and within group variation in pairing behaviour are given in the next section. However, before considering these it is worth noting a marked feature of pairing behaviour which is common to the hybrids and autotetraploids, and which is apparent from Tables 2 and 3.

Table 2 shows the proportion of chromosomes involved in the various kinds of associations previously referred

Table 1. Chromosome association and chiasma frequency at first metaphase in 7 autotetraploids and 19 hybrids

		Chromosome association					
		V	IV	III	II	I	Chiasmata
L. perenne (4 x)	Mean	–	3.26	0.37	6.54	0.35	24.09
	Range		3.04-3.56	0.08-1.04	5.20- 7.56	0.08-0.80	22.80-25.04
Hybrids	Mean	0.01	2.11	0.30	8.98	0.51	22.13
	Range	0-0.16	1.16-3.60	0.04-1.00	6.56-10.88	0.04-1.52	19.84-23.88

Table 2. Proportion of chromosomes involved in the various kinds of associations at first metaphase

		Percentage of chromosomes associated as				
		(a) IV	(b) III + I	(c) II + II	(d) II + 2I	(e) 4I
L. perenne (4 x)	Mean	46.57	1.86	51.00	0.57	–
	Range	43.43-50.86	1.14-2.86	48.00-53.14	0-1.71	
Hybrids	Mean	32.57	2.13	64.26	1.04	–
	Range	18.86-51.43	0.57-4.00	46.29-76.57	0.-2.29	

Table 3. Frequency of PMCs with 0-14 bivalents

		Number of bivalents															
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Number of PMCs	*L. perenne* (4 x)	2	0	2	0	11	1	29	3	30	0	18	0	3	0	1	100
	Hybrids	2	0	2	0	14	3	31	3	67	6	78	4	54	4	7	275

Table 4. Analysis of variance in bivalent, multivalent and chiasma frequencies

		Bivalents[a]	Multivalents[b]	Chiasmata
Source	d.f.	MS	MS	MS
Hybrids vs *L. perenne* (4 x)	1	3216.954**	12.870***	491.734***
Between plants	24	293.216***	0.568***	32.472***
Between PMCs (within plants)	624	21.768	0.095	3.290
Hybrids				
Between plants	18	166.472***	0.717***	37.986***
Between PMCs (within plants)	456	21.424	0.097	3.353
L. perenne (4 x)				
Between plants	6	673.447***	0.207*	15.930***
Between PMCs (within plants)	168	22.699	0.090	3.106

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

[a] Data transformed to $x^{(1.25)}$;

[b] data transformed to $\sqrt{x+1}$

to, and Table 3 the frequency distribution of PMCs with the various numbers of bivalents from 0-14. (The aneuploids have been excluded from these results although their inclusion did not substantially affect the overall picture). There is a very marked preponderance of classes (a) and (c) in Table 2 and this is reflected in the very low frequency of PMCs with odd numbers of bivalents in Table 3. The clear implication is that there is some restriction of pachytene association to 'pairs' of chromosomes, similar in effect to that found by Timmis and Rees (1971) in autotetraploid rye.

Variation in Chromosome Association at First Metaphase

Analysis of the total variation in bivalent, multivalent and chiasma frequency is given in Table 4. The data for bivalent and multivalent (quadrivalent + trivalent) frequency have been transformed because there was a significant overall negative correlation between plant mean and mean square in the former and a significant positive one in the latter. These were effectively removed by the transformations $x^{1.25}$ and $\sqrt{x+1}$ respectively.

The analysis clearly shows that the hybrids as a group

differ significantly from the autotetraploid *L. perenne* in all three characters. It further shows that, despite large cell to cell variation in bivalent and multivalent frequencies, genotypic differences are significantly greater both among the hybrids and the *L. perenne* plants for these two characters. Similarly, the between-plant differences in chiasma frequencies are highly significant in both groups.

The magnitude of the cell to cell variation in bivalent and multivalent frequency can be deduced from the standard deviation values given in Table 5, in relation to the plant mean values given in Table 1. The within plant variation in chiasma frequency was small in comparison.

The relative magnitudes of the within-plant variation in the hybrids and *L. perenne* material are analysed for the three characters in Table 6. The plant mean squares calculated from the transformed data have been used for bivalent and multivalent frequency. The analysis shows that the differences between individual plants are greater than differences between the hybrids and autotetraploids as groups.

Further analysis of this plant to plant variation in mean square values showed that significant differences exist among the hybrids for within-plant variation in bivalent and multivalent frequency but not in chiasma frequency. This was also true in the *L. perenne* plants but this was due mainly to the deviation of a single plant.

Relation of Bivalent and Multivalent Pairing to Chiasma Number

Regression analyses based on plant mean values showed that there was no significant relationship between either bivalent or multivalent frequency and chiasma frequency. Analyses based on individual PMC values, however, showed the relationships illustrated in Figs. 1 and 2. For these analyses, mean frequencies of bivalents and multivalents were calculated for each PMC chiasma level, from all the PMCs analysed within the hybrid and autotetraploid groups regardless of genotype. According to Evans (1968) this method can separate the effect of chiasma frequency from any other factor which might affect the degree of chromosome association at metaphase. The means are of necessity based on varying numbers of cells and this has to be borne in mind in the interpretation of the data. Classes with fewer than 5 PMCs were however omitted.

The four regressions illustrated in Figs. 1 and 2 are all significant at $P < 0.001$. Apart from the marked differences between the hybrids and autotetraploids in levels of bivalent, multivalent and chiasma frequencies already shown to be significant, the figures illustrate two features of interest.

Firstly, for each comparable chiasma frequency, that is from 21-26, the hybrids have more bivalents and fewer multivalents. The higher bivalent and lower multivalent

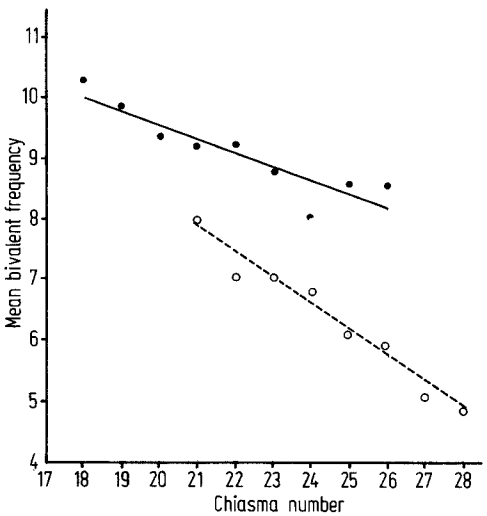

Fig. 1. Mean bivalent frequencies at specific PMC chiasma levels. Hybrids ——; *L. perenne* (4 x) – – – –

Table 5. Within plant variation (standard deviations) in bivalent, multivalent and chiasma frequencies

		Bivalents	Multivalents	Chiasmata
Hybrids	Mean	2.17	1.11	1.81
	Range	1.58-2.78	0.76-1.38	1.31-2.41
L. perenne (4 x)	Mean	2.40	1.23	1.74
	Range	1.26-2.90	0.62-1.47	1.34-2.06

Table 6. Analysis of variance of plant mean-squares for bivalent[a], multivalent[b] and chiasma frequencies

		Bivalents	Multivalents	Chiasmata
	d.f.	MS	MS x 100	MS
Hybrids vs *L. perenne* (4 x)	1	8.1503	0.0224	3.1052
Between plants	24	49.0686	0.1134	5.3942

[a, b] Plant mean squares calculated from the transformed data

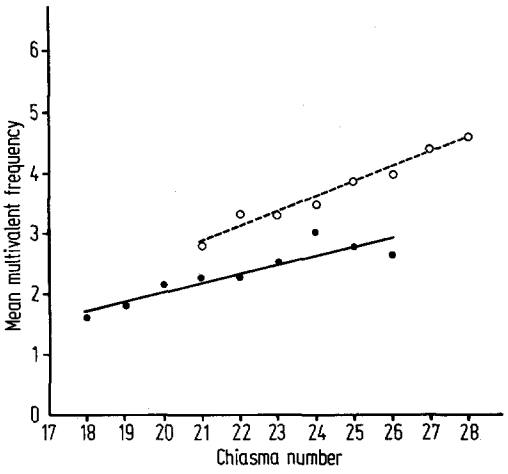

Fig. 2. Mean multivalent frequencies at specific PMC chiasma levels Hybrids ———; *L. perenne* (4 x) – – – –

frequency in the hybrids must therefore reflect a difference in the distribution of chiasmata as distinct from a difference in frequency.

Secondly, the slopes of the regression lines for the hybrids and autotetraploids differ significantly ($P < 0.01$) in both Figs. 1 and 2. Thus the rate of decrease in bivalent frequency with increasing chiasma number is lower in the hybrids. Likewise, the rate of increase in multivalent frequency with increasing chiasma number is also lower in the hybrids than in tetraploid *L. perenne*. This means that the magnitude of the difference in pattern of chiasma distribution between the two groups depends on the level of chiasma frequency.

There would therefore appear to be some quite fundamental differences in chromosome association between the hybrids and tetraploid *L. perenne*, underlying the more apparent differences.

Discussion

This investigation provides evidence that there was a marked shift towards diploid-like chromosome pairing behaviour in the tetraploid hybrids between *L. perenne* and *L. multiflorum* examined, in comparison with autotetraploid *L. perenne*. It also indicates that the increase in the level of bivalent pairing was likely to be the result of preferential chromosome association. The estimated level of preferential bivalent pairing was however relatively low (of the order of 33%) and on the evidence of multivalent frequency alone an appreciable degree of homoeologous pairing takes place. This of necessity results in the segregation of genes differentiating the parental species – termed secondary segregation by Darlington (1928). As mentioned earlier, the retention of maximum hybridity is highly desirable in the breeding of this material and seeking means of achieving this is a primary objective of this work.

The demonstration of significant genotypic variation in chromosome pairing behaviour in the present hybrids is encouraging from this viewpoint. The generally heritable nature of chromosome behaviour (Rees 1961) suggests that, to the extent that diploid-like pairing can be equated to preferential pairing, selection for increased levels of the latter may be effective. The marked within-plant variation in chromosome pairing behaviour, which is not uncharacteristic of synthesised polyploids in general, and even some natural polyploids (Morrison and Rajhathy 1960; McCollum 1958) must however be regarded as counteractive to effective genotypic selection in the present context. Nevertheless, the negative plant mean/mean square correlation found for bivalent pairing makes possible the selection of genotypes with a high mean value and a relatively low mean square. There were four such genotypes in the range of hybrids examined.

No instances appear to have been reported of successful selection for increased bivalent pairing in synthetic allotetraploids but there are several examples of natural tetraploids of putative hybrid origin, where it can be inferred that diploid-like pairing behaviour has evolved from a multivalent forming one (Tutin 1957; Hovin 1958; Ramanujam and Srinivasachar 1943; Skovsted 1937; Beasley 1942). These reports give no evidence, however, of how cytological diploidisation was achieved. Only in hexaploid *Triticum aestivum* (Riley and Chapman 1958) has the mechanism of genetic regulation of pairing, giving rise to disomic segregation, been precisely established.

Natural polyploids that form only bivalents at meiosis but nevertheless show polysomic inheritance have been reported (Nordenskiold 1945, 1953; Dawson 1941), and there are reports of a shift towards more diploid-like pairing in certain synthesised autotetraploids (Gilles and Randolph 1951; Swaminathan and Sulbka 1959; Jauhar 1970).

In seeking means of increasing preferential pairing it is important to bear in mind that the pattern of associations observed at metaphase is but the end result of a pairing process consisting of several important and vital steps. Differences in the relative frequencies of the various associations may be due to differences either in the processes of initiation of synapsis or in the frequency and distribution of chiasmata, or in both. As we have seen in Figs. 1 and 2, there is evidence that the higher bivalent and lower multivalent frequency in the hybrids reflect a difference in the distribution of chiasmata as distinct from a difference in their frequency, which was previously shown and is also apparent from these figures. Whether there are also differences in synaptic initiation cannot be directly ascertained from the present study, but there is some indication that this may be so.

Riley (1960) suggested that the genetic mechanism controlling diploid-like meiotic and genetic behaviour in *T. aestivum* (Riley and Chapman 1958) is not directly related to chiasma formation. He indicated that it probably operates by affecting the process which attracts chromosomes together and initiates pairing, reducing the attraction to such an extent that effective pairing can only take place between fully homologous chromosomes. Structural differentiation between homoeologues is implied and the effect of the mechanism is described as the genetic enhancement of the differential affinity resulting from it. Feldman et al. (1972) verified an earlier postulation (Feldman 1966) that the spatial distribution of *T. aestivum* chromosomes, in somatic and premeiotic cells, reflects this differential attraction between homologues and homoeologues. They showed that the chromosomes are not distributed at random within the cell; a close association exists between homologues and somewhat looser association between homoeologues.

That there is some structural differentiation between *L. perenne* and *L. multiflorum* chromosomes, and hence differential spatial association between homologues and homoeologues in the hybrids, may be implied by Figs. 1 and 2. They show that there is a smaller rate of increase in the number of multivalents (and a corresponding smaller rate of decrease in bivalent number), with increasing chiasma number, in the hybrids compared with the autotetraploids. This is to be expected if the chromosomes of the hybrids tend to be associated as 'pairs' rather than 'fours', since additional chiasmata would then less frequently convert pairs of potential bivalents into multivalents. This phenomenon thus provides a strong indication that preferential pairing occurs in the hybrids and further supports the earlier assertion that their higher bivalent frequency reflects this. In this, the results of the present investigation appear to agree well with the genetic results obtained by Breese and Thomas (1977) in similar material. The discrepancy between them and those of Ahloowalia (1977) may well reflect significant population differences in chromosome structure differentiation between the parental materials used.

Acknowledgement

I wish to thank Professor J.P. Cooper, Director of the Welsh Plant Breeding Station, for his interest and encouragement during the course of this work. I am indebted to those of my colleagues who provided advice and assistance, particularly Dr H. Thomas for valuable discussions and comments.

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Received April 29, 1980
Communicated by R. Riley

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