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The genetics of meiotic chromosome pairing in *Lolium temulentum* × *Lolium perenne* tetraploids

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Summary. The degree of preferential pairing of homologous chromosomes was estimated in a series of tetraploid hybrids of *Lolium temulentum* × *Lolium perenne* by means of cytological and genetic analyses. The correlations between the frequency of bivalents at first metaphase of meiosis in the hybrid tetraploids and the degree of preferential pairing calculated from the segregation pattern of isozyme alleles in a test cross was extremely high. The results showed clearly that suppression of heterogenetic pairing in these *Lolium* tetraploids is achieved by a genetic system involving the A chromosomes as well as the B chromosome system which has been known for some time. Certain similarities with the genetic system controlling pairing in polyploid wheats are discussed.

Key words: *Lolium* hybrids – Interspecific teraploids – Preferential pairing – Isoenzymes

Introduction

The use by plant breeders of tetraploid interspecific hybrids rather than their diploid counterparts can be attributed to their superiority on three important counts. Firstly, there is the advantage from the so called "Gigas effect" the consequences of increased cell size and its related pleiotropic effects giving larger plant parts. Secondly, tetraploidy serves to restore a degree of fertility to interspecific hybrids which are sterile as diploids. Finally, there is the undoubted benefit in terms of population stability of the preservation of hybrid gene combinations over sexual generations (Breese et al. 1981). Although tetraploidy per se serves to conserve some hybridity this is enhanced dramatically by preferential pairing of homologous chromosomes (Breese and Thomas 1977). This is seen in its extreme form in amphidiploids where intergenomic hybridity is completely preserved over successive generations of seed propagation. It is highly likely that this was as important in the evolution of natural allopolyploids as was the benefit resulting from increased fertility.

Complete preferential pairing of homologous chromosomes with no homoeologous association (amphidiploidy) has rarely if ever been achieved in artificial interspecific polyploids based on diploid species. It is quite common to find heteromorphic bivalents as well as multivalents at metaphase one of meiosis in hybrids. The presence of such configurations at metaphase implies both pairing of homoeologous chromosomes or chromosome segments and crossing over between them. It also implies a degree of recombination of genes from the two genomes with the inevitable consequence of a break up of some desirable heterozygous (intergenome) gene combinations. Thus, not only will the average phenotypic performance of the population be somewhat reduced in subsequent generations but the population variance will also be increased. The presence of multivalents and univalents at meiosis will also increase the chances of irregular disjunction of chromosomes at anaphase one leading in turn either to a loss of fertility or to the production of aneuploid progeny.

All these undesirable consequences of homoeologous chromosome association in induced interspecific hybrids must to some extent at least detract from their value for agricultural or horticultural use. This is especially so where synthetic cultivars have to be multiplied over four or five seed generations to obtain adequate amount of seed for commercial use.

The rate of deterioration of synthetic populations of tetraploid hybrids will therefore depend on the degree of homoeologous pairing, or stated conversely, on the degree of preferential pairing of homologous chromosomes. The theoretical considerations of Breese et al. (1981) and Breese (1983)

demonstrates this clearly. Not only is the rate of dissipation of heterozygous gene combinations affected but also the degree of heterozygosity at equilibrium. Moreover, the model also serves to demonstrate the striking superiority of tetraploidy over diploidy in conserving hybrid gene combinations.

The increasing range of potentially valuable tetraploid hybrid material becoming available in the *Lolium/Festuca* complex has emphasised the importance of obtaining accurate information on genetic systems which regulate the pairing of chromosomes at meiosis in such hybrids. Previous reports by Evans and Macefield (1972, 1973); Bowman and Thomas (1973); Clarke and Thomas (1976); Ahloowalia (1977); Breese and Thomas (1977); Lewis (1980) and Thomas et al. (1983) have showed that considerable variation in the degree of homoeologous pairing is evident in interspecific hybrids within this group. Taylor and Evans (1977) working mainly with diploid hybrids of *Lolium temulentum* and *Lolium perenne* were able to demonstrate that genetic factors controlling the frequency of homoeologous bivalents could be attributed to the A chromosomes as well as those previously known to be present on the B's of *L. perenne* (Evans and Macefield 1972, 1973). A preliminary assessment of the pattern of chromosome pairing in a few hybrid tetraploids indicated that these A chromosome effects resulted in fewer multivalents at metaphase 1 of meiosis (Taylor and Evans 1977). The present work was designed to examine this in more detail using both a conventional cytological analysis of metaphase 1 of meiosis in tetraploid hybrids and a genetic assay of the segregation of marker genes in a test cross of tetraploid hybrids to appropriate tester plants.

The rationale of this type of genetic assay was described by Breese and Thomas (1977). Basically it demands that the homoeologous chromosome arms of a tetraploid hybrid are 'marked' with contrasting alleles while homologous chromosomes have the same allele. Codominant alleles coding for enzyme variants (isoenzymes) which are capable of being separated by gel electrophoresis have been found to be particularly suitable for this purpose. Since many of these loci are multiallelic it is possible in many cases to use a tester genotype homozygous for yet another allele. The pattern of segregation of the marker genes in the test cross progeny is taken as a reflection of the way that the chromosomes concerned associated and separated at the first division of meiosis in the parental hybrids. The major constraint on the use of this method is the availability of enzyme systems showing the necessary genetic variation and of suitable methods of separating these isoenzymes. In the present study we were able to use three unlinked loci namely those coding for Phosphoglucose isomerase (PGI), Acid phosphatase (APH) and peroxidase (PER).

Materials and methods

Stocks

The two genotypes of both *L. temulentum* and *L. perenne* described previously by Taylor and Evans (1977) were again used for this experiment. Briefly their pedigress were as follows:

1. *L. perenne* Lp19 and Lp10 were half sib plants having originally been obtained from the three way cross [(Ba8973 × S23) × S24] and maintained clonally for several years. Both have a chromosome number of $2n = 2x = 14 + 1B$. Genetically they can be considered as being highly heterozygous.
2. *L. temulentum.* The two lines Ba3081 and Ba6902 used in this programme were completely unrelated having originated from the UK and France, respectively. Both were considered to be completely homozygous as they had been maintained by natural self-pollination of single plant selections over many generations. Both genotypes have a chromosome number of $2n = 2x = 14$.

Crossing and propagation of progeny

Four separate crosses were made with the two *L. temulentum* lines used as seed parents in separate crosses with the two *L. perenne* genotypes. Mature inflorescences of both Ba3081 and Ba6902 were emasculated prior to anthesis and pollinated later with pollen from either Lp19 or Lp10, suitable precautions against contamination by 'foreign' pollen being taken throughout.

L. temulentum		*L. perenne*
Ba3081	×	Lp19
Ba3081	×	Lp10
Ba6902	×	Lp19
Ba6902	×	Lp10

Eighteen to 21 days-old embryos were dissected out and cultivated on a simple agar medium containing sucrose and the major elements necessary for plant growth.

Induction of tetraploidy

Young seedlings having no more than one leaf emerging from the coleoptile were removed from the culture tubes and immersed in 0.2% aqueous colchicine prepared with sterile water for a period of 3 h. Following three rinses with sterile water the treated seedlings were replaced on fresh agar slopes and a suitable 'recovery' period allowed before transplanting into a suitable compost in pots.

Cytological analysis

Emerging inflorescences were fixed in Carnoy's fixative and squash preparations of pollen mother cells (pmc's) at first metaphase/anaphase of meiosis were made in aceto-carmine stain. Tetraploid plants or sectors were first identified and detailed analysis of meiosis subsequently performed using suitable slides. The frequency of quadrivalents, trivalents, bivalents and univalents together with the number of chiasmata were scored for 20 pmc's in each plant.

Genetic analysis

The Plan. As indicated earlier, the basic idea was to 'mark' homoeologous chromosomes of the *L. temulentum* × *L. perenne* tetraploid hybrids with contrasting isoenzyme alleles whose products could be separated by starch gel electrophoresis and to monitor the segregation of these alleles by means of a test cross. The overall plan is best illustrated by reference to one specific enzyme system such as PGI2. Both diploid *L. perenne* genotypes Lp19 and Lp10 contained the co-dominant *a* and *b* alleles while the *L. temulentum* genotype Ba3081 was homozygous *b*. The diploid interspecific hybrids would have been either *ab* or *bb* genotype and their cor-

responding tetraploids *aabb* (*ab* phenotype) or *bbbb* (*b* phenotype) since they were 'doubled' versions of the diploids. Clearly only the *aabb* genotypes would have been suitable for genetic analysis. In these hybrids a pair of chromosomes from the *perenne* genome would have carried the *a* alleles while their homoeologues on the *temulentum* genome would have carried the *b* alleles.

The segregation of the *a* and *b* alleles to the gametes is a direct reflection of the pairing affinities (and subsequent chiasma position) of the relevant chromosomes at meiosis. On the one extreme a random association of both homologous and homoeologous chromosomes, i.e. no preferential pairing of homologous chromosomes would result in a gametic output of 1 *aa:* 4 *ab:* 1 *bb*. Random chromatid assortment would alter this to 3 *aa:* 8 *ab:* 3 *bb*. The other extreme of complete preferential pairing of homologous chromosomes would result in all the gametes being of the *ab* type. The possiblities are summarized in tabular form in Table 1.

Since we have no information on the location of the marker locus relative to the centromere there is no way of knowing whether any random chromatid assortment would take place even when homologous and homoeologous chromosomes associate to form a quadrivalent. Consequently the expected gametic output following either random chromosome assortment or of random chromatid assortment can be taken as the base. Any decrease from the expected in the proportion of the *aa* and *bb* (dissociate) gametes would be an indication of preferential pairing of homologous chromosomes. This can be quantified according to the formula given in Table 1.

The gametic output of the tetraploid hybrids was assayed by means of a test cross to an autotetraploid *L. multiflorum* tester plant homozygous for a third codominant allele (*d*). The frequency of the three segregational classes amongst the test cross progeny gave a direct measure of the gametic output of each hybrid tetraploid parent. For this purpose it was assumed that all the gametic classes were of equal fitness.

Electrophoresis

The three enzyme systems, PGI, PER and APH, were analysed by horizontal starch gel electrophoresis. The enzymes were isolated from crude extracts obtained by crushing sections of young leaves in a few drops of cold 0.1 M Tris mercaptoethanol buffer pH 7.2. Each extract was absorbed onto small paper wicks which were inserted at intervals into a transverse cut in the gel. Electrophoresis was conducted at 4 °C at 300 Volts. Gels were developed (stained) in appropriate solutions of the following composition.

PGI – 12 mg Fructose-6-phosphate, 8 mg NADP, 15 mg MTT, 5 mg Phenazine methosulphate (PMS), 20 units Glucose-6-dehydrogenase, 5 ml 0.1 M $MgCl_2$, 95 ml 0.1 M Tris-HCl pH 8.0. Incubated in dark at 37 °C.

APH – 150 mg Sodium naphthyl acid phosphate, 75 mg Fast Garnet GBC, 2 ml 0.1 M $MgSO_4$, 98 ml 0.2 M acetate buffer, pH 5.5. Incubated in dark at 37 °C.

PER – 65 mg 3-amino-9-ethyl carbozole dissolved in 2.5 ml of dimethyl formamide, 2 ml of 0.1 M Calcium chloride, 1.5 ml of 30% hydrogen peroxide, 90 ml 0.2 M sodium acetate buffer pH 5.0. Incubated in dark.

Gels were fixed in either 50% glycerol (PGI and PER) or in 50% ethanol (APH) and the relative positions of each isozyme band noted for each extract (plant).

Table 1. The segregational pattern expected at the PGI 2 locus in *L. temulentum* × *L. perenne* hybrids. Hybrid genotype $T^bT^bP^aP^a$ (*L. temulentum*/*L. perenne*). Tester genotype $M^dM^dM^dM^d$ (*L. multiflorum*)

	Associate types	Dissociate types	
Genotype of gametes	T^bP^a	T^bT^b	P^aP^a
Isoenzyme classes	ba	bb	aa
Expected frequencies			
Random chromosome assortment	4	1	1
Random chromatid assortment	8	3	3
100% preferential pairing	1	–	–

% preferential pairing =

$$\left(1 - \frac{\text{Observed frequency of dissociate types}}{\text{Expected frequency of dissociate types}}\right) \times 100$$

Results

Cytological analysis

A total of 73 progeny plants which were either entirely tetraploid or had tetraploid sectors were analysed cytologically. These could be separated into eight classes based on the four different crosses indicated earlier plus the presence or absence of B chromosomes. It is worth noting that since these tetraploids are doubled versions of the diploid progeny of crosses involving a 1B pollen parent they would either contain no B's or four B chromosomes in addition to the normal complement of twenty eight A chromosomes.

The mean frequency of quadrivalents, trivalents, univalents and chiasmata were calculated from the analysis of 20 p.m.c.'s per plant. The data for each of the eight classes are summarized in Table 2. It is clearly evident that considerable differences existed between the various genotypic classes in terms of their meiotic pairing pattern. The progeny of Ba6902 × Lp19 in particular seemed to show a marked reduction in multivalent frequency and a corresponding increase in bivalent frequency.

A high frequency of multivalents is of course a clear reflection of a considerable level of homoeologous association in that quadrivalents and trivalents can only arise from such association. On the other hand although a high frequency of bivalents is indicative of a considerable degree of homologous association that some of these bivalents could have arisen from homoeologous association cannot be ruled out completely.

Statistical analysis of the results enable an assessment to be made of the effect of different parental genotypes and of B chromosomes on the chromosome pairing pattern of the progeny. The crossing design was such that the data can be analysed as a 2×2×2 factorial with one way classification. However as with many

Table 2. Frequencies of quadrivalents, trivalents, multivalents (quadrivalents + trivalents), bivalents, univalents and chiasmata in the 8 classes of hybrids

Hybrid class	No. of plants	Frequency of					
		IV	III	(IV+III)	II	I	Chiasmata
Ba3081 × Lp10 OB	6	1.22	0.90	2.11	9.60	1.24	21.98
Ba3081 × Lp10 +B	6	0.77	0.30	1.07	11.56	0.87	23.35
Ba3081 × Lp19 OB	14	0.55	0.67	1.23	11.37	1.23	21.42
Ba3081 × Lp19 +B	16	0.20	0.17	0.37	13.00	0.67	21.79
Ba6902 × Lp10 OB	10	0.74	0.51	1.25	11.33	1.01	22.15
Ba6902 × Lp10 +B	8	0.19	0.50	0.69	12.47	0.70	22.39
Ba6902 × Lp19 OB	7	0.16	0.11	0.27	12.42	2.30	17.54
Ba6902 × Lp19 +B	6	0.04	0.02	0.06	12.92	1.87	18.32

Table 3. Analysis of variance of the frequencies of multivalents, bivalents and chiasma frequencies in the eight classes of hybrids

Item	N	Multivalents M.S.	Bivalents M.S.	Chiasmata M.S.
Total	72			
Classes	7	3.388***	10.040***	29.730***
Error	65	0.432	1.729	3.706
Analysis of means				
OB v 4B	1	0.891***	3.367***	0.795
Lp19 v Lp10(perenne)	1	1.255***	2.892***	15.252***
Ba3081 v Ba6902(temulentum)	1	0.826***	1.665***	7.805***
Perenne/temulentum	1	0.001	0.340	5.824***
Perenne/B chromosome	1	0.040	0.133	0.005
Temulentum/B chromosomes	1	0.166	0.495	0.118
Perenne/temulentum/B chrom.	1	0.006	0.017	0.399
Error (adjusted)	65	0.054	0.216	0.464

*** $P=0.001$; ** $P=0.01$

cytological assessments each class does not comprise the same number of entries. Consequently the analysis was performed on class means with the error variance being corrected by use of the harmonic mean. A summary of the analysis is given in Table 3.

It is quite clear that the two levels of all three factors being analysed namely difference between *L. perenne* genotypes, difference between *L. temulentum* genotypes and difference between OB and +B plants are significant ($P=0.001$) for both the frequency of multivalents and of bivalents at metaphase 1 of meiosis. Furthermore the effects appear largely additive there being no significant interaction between the main factors. Taken in conjunction with the data presented in Table 2 the results show that *L. perenne* genotype Lp19, *L. temulentum* gentoype Ba6902 and B chromosomes contribute genetic effects which significantly lower the frequency of multivalents per p.m.c. and a corresponding increase in the frequency of bivalents. The number of unpaired chromosomes (univalents) did not appear to be influenced in any regular manner. The comparisons were all non-significant and have consequently been omitted from Table 3.

It is also evident from the analyses in Table 3 that there is significant variation in chiasma frequency between the two groups of *L. perenne* hybrids and also between the two groups of *L. temulentum* hybrids. Reference to Table 2 shows that this is mainly due to the low frequency of chiasmata in *L. temulentum* Ba6902 × *L. perenne* Lp19 hybrids. This is picked up as a significant *perenne/temulentum* interaction in the analysis in Table 3.

The effect of chiasma frequency on the frequency of the various configurations can sometimes be removed by examining the mean number of the multivalents, bivalents and univalents in cells of specific chiasma frequency and then comparing the different classes of progeny using Joing Regression and Covariance analyses.

However the range of p.m.c. chiasma frequency was considerably different in the different classes of hybrids in this experiment thus making comparisons between groups of classes e.g. Ba6902, Ba3081 rather questionable statistically.

Genetic analysis

At least three separate F_1 tetraploid hybrids from each of the eight genotype classes referred to earlier were used for test crossing. A total of 1939 test cross progeny were analysed for PGI phenotype, 1780 for PER phenotype and 1779 for APH phenotype. These were subsequently classified into "Associate" and "Dissociate" types according to the nomenclature of Breese and Thomas (1976). The term associate phenotype was used to denote the presence in the progeny of both marker alleles from the original tetraploid hybrid parent while dissociate phenotype was used to describe the progeny containing only one of the marker alleles for a particular enzyme system i.e. aa or bb in the model described earlier in Table 1. Since the phenotype of these enzymes can be translated directly as the genotype and since the tester contained a contrasting allele the frequency of associate and dissociate type phenotypes represent the frequencies of associate and dissociate type gametes produced by the original tetraploid hybrid plants under test.

Table 4 gives the frequency of associate and dissociate types gametes for each of the three marker genes produced by each of the 8 classes of hybrid tetraploids tested. Also given is the Dissociation Index (D.I.) which is merely the percentage of gametes which are of the dissociate type. This is done so as to give some indication of the nature of chromosome pairing in these hybrids. Complete association of homoeologous and homologous chromosomes with random chromosome assortment would be expected to give a dissociation index of 33.3% while with random chromatid assortment this would increase to 42.9%. On the other hand if chromosome pairing in the tetraploid hybrids was limited to homologues only, i.e. 100% preferential pairing, no dissociate type gametes would arise and the dissociation index would therefore be 0.

Several points are worth noting. It appears that some association of homoeologous chromosomes must have occurred in all genotypes as the dissociation index is greater than 0 in all classes. However it is also clear that the magnitude of dissociation index is different for the different genotypes and although there are minor

Table 4. a Frequencies of associate and dissociate type gametes together with the dissociation index (D.I.) for each of the three genetic markers; **b** X^2 analyses for deviation from 2:1 and 4:3 ratios; **c** contingency X^2 analyses of the data

a	PGI			PER			APH		
	Frequencies of			Frequencies of			Frequencies of		
Genotype	Ass.	Diss.	D.I.	Ass.	Diss.	D.I.	Ass.	Diss.	D.I.
Ba3081×Lp10 OB	86	85	49.7	107	64	37.4	104	67	39.2
Ba3081×Lp10 +B	143	70	32.9	140	73	34.3	139	74	34.7
Ba3081×Lp19 OB	160	46	22.3	137	57	29.4	166	40	19.4
Ba3081×Lp19 +B	220	16	6.8	180	20	10.0	164	36	18.0
Ba6902×Lp10 OB	140	128	47.8	152	81	34.8	142	91	39.1
Ba6902×Lp10 +B	347	44	11.3	251	90	26.4	269	55	18.0
Ba6902×Lp19 OB	202	28	12.2	142	63	30.7	160	45	22.0
Ba6902×Lp19 +B	215	9	4.0	209	14	6.3	214	9	4.0
Total	1,513	426		1,318	462		1,358	421	
b									
Overall X^2 [1]									
(2:1 Exp)	112.67***			43.6***			74.8***		
Overall X^2 [1]									
(4:3 Exp)	345.42***			207.6***			267.6***		
c									
Contingency X^2									
Overall X^2 [7]	308.4***			103.9***			127.2***		
OB/+B X^2 [1]	109.1***			37.8***			31.5***		
Ba3081/Ba6902 X^2 [1]	15.5***			1.7			11.4***		
Lp10/Lp19 X^2 [1]	115.9***			41.4***			56.7***		

*** $P=0.001$

Table 5. Estimates of % preferential pairing based on (a) random chromosome assortment and (b) random chromatid assortment using each of the three marker genes

Parental genotype	% Preferential pairing – Random chromosome assortment				% Preferential pairing – Random chromatid assortment			
	PGI	PER	APH	Mean	PGI	PER	APH	Mean
Ba3081 × Lp10 OB	0	0	0	0	0	12.7	8.5	7.1
Ba3081 × Lp10 + B	1.4	0	0	0.5	23.3	20.0	18.9	20.7
Ba3081 × Lp19 OB	33.0	11.9	41.7	28.9	47.9	31.4	54.7	44.7
Ba3081 × Lp19 + B	79.7	70.0	46.0	65.2	84.2	76.7	58.0	73.0
Ba6902 × Lp10 OB	0	0	0	0	0	18.9	8.9	9.3
Ba6902 × Lp10 + B	66.2	20.8	49.7	45.6	73.7	38.4	60.4	57.5
Ba6902 × Lp19 OB	63.5	7.8	34.1	35.1	71.6	28.3	48.8	49.6
Ba6902 × Lp19 + B	87.9	81.2	87.9	85.7	90.6	85.4	90.6	88.9

differences the overall pattern of variation is similar for the three marker genes. The correlation coefficient of dissociation index values of PGI and PER is 0.83, of PGI and APH is 0.91 and of PER and APH is 0.87. All these are significant at the 1% level. This is taken as an indication that the pairing pattern of three chromosome arms labelled is similar.

It is of some interest to examine the segregation pattern in more detail. This was done using the X^2 test (see Table 4b). Clearly the overall segregation of all three genes did not conform to either a 2:1 (random chromosome assortment) or 4:3 (random chromatid assortment) ratio. The contingency X^2 was then used to test for heterogeneity between families and to partition this heterogeneity (Table 4c). The test shows that the variation in segregation pattern for all three genes over the 8 families is highly significant. Furthermore the difference in segregation of all three genes between OB and +B hybrids and between *L. perenne* Lp10 and Lp19 is highly significant. This is due to a much lower frequency of dissociate gametes in both +B hybrids and in *L. perenne* Lp19 hybrids. This is interpreted as confirmation that both the presence of B's and of the LP19 genotye reduced the degree of homoeologous association of chromosomes. The test between *L. temulentum* Ba3081 and Ba6902 is not as conclusive. The segregation of both the PGI and the APH genes is significantly different in the two groups of hybrids. However the magnitude of X^2 are markedly less than those in previous tests. Furthermore the segregation of the PER gene does not differ in the two goups. This evidence is taken to indicate that while the genotype of Ba6902 lowers the degree of homoeologous association it does so to lesser extent than B chromosomes and *L. perenne* Lp19. It is also possible that different chromosomes are affected differently in this case.

The results are finally summarized in Table 5 as % preferential pairing based on the formula given earlier in Table 1. Two separate base lines were used in calculating these values. Firstly that expected assuming random chromosome assortment and secondly that expected assuming random chromatid assortment. There is no way of estimating which is the most appropriate. Indeed it is highly likely that a base line somewhere intermediate of the two extremes would be the more appropriate and therefore the correct values of preferential pairing are intermediate of the two values presented in Table 5. It is also likely that the base line that should be used would be different for the three genes considered here since random chromatid assortment is only possible on the occurrence of a single cross-over between the centromere and the locus in a quadrivalent association. We have no information as to the distance between any of these genes and its appropriate centromere.

Despite the inability to specify the % preferential pairing as a single figure the values given in Table 5 are nonetheless a realistic estimate of the magnitude of preferential pairing in the different genotypes. Not surprisingly since they are derived from the data in Table 4 they reflect the general pattern referred to above, i.e. hybrids containing B chromosomes, *L. perenne* Lp19 and *L. temulentum* Ba6902 showing substantially higher levels of preferential pairing than their counterparts with OB, *L. perenne* Lp10 and *L. temulentum* Ba3081. Of some significance however is that the degree of preferential pairing ranges from no detectable level in Ba3081 × Lp10 OB to between 80% and 90% in the Ba6902 × Lp19 + B hybrids.

Comparison of the cytological and genetical analyses

Although the cytological analysis given earlier provided no absolute values of preferential pairing the frequency of multivalents relative to bivalents in each of the eight F_1 families give an indication of the level of homoeologous chromosome association at meiosis. Multivalents can only arise through homoeologous pairing. Corre-

lation of multivalent frequencies (Table 2) with % preferential pairing (Table 5) gives therefore a meaningful comparison of the cytological and genetical analysis. The correlation coefficient values of multivalents with mean % preferential pairing is −0.81 when the values based on random chromosome assortment are used and −0.85 when those based on deviation from random chromatid assortment are used. Both these values are significant. Clearly the results from the two analyses broadly agree with each other in that a decrease in the frequency of multivalents (increase in bivalents) is associated with an increase in preferential pairing.

Discussion

The present work with artificially created interspecific tetraploids of *Lolium* shows quite clearly that genetic elements which modify pairing affinities of homoeologous chromosome exist in diploid populations of both *Lolium perenne* and *Lolium temulentum.* Previous work had shown that B chromosomes of *L. perenne* had the capacity to decrease the frequency of quadrivalents in tetraploid *L. temulentum* × *L. perenne* (Evans and Macefield 1972, 1973; Taylor and Evans 1977). Similarly there was an indication that genes on the A chromosomes could likewise reduce the frequency of quadrivalents at first metaphase of meiosis in such hybrids (Taylor and Evans 1977). The present report confirms these cytological observations and furthermore establishes beyond doubt that increased frequencies of bivalents and decreased frequencies of quadrivalents are indeed due to increased levels of preferential pairing.

Both the cytological and genetic analyses point to the presence in *L. perenne* Lp19, in *L. temulentum* Ba6902 and in B chromosomes of genetic factors which reduce the amount of preferential pairing in tetraploid hybrids of *L. temulentum* × *L. perenne.* Furthermore the effect of Lp19 and B chromosomes appear to be largely additive, with the effect of *L. temulentum* Ba6902 being more difficult to interpret since it has a marked effect on chiasma frequency. The cumulative effect of all three factors results in preferential pairing in the order of 80–90%. This approaches the 100% value of natural allopolyploids. That this is genetically controlled rather than due to structural divergence of homoeologous chromosomes is evident from the fact that no appreciable preferential pairing could be detected in similar crosses e.g. *L. temulentum* Ba3081 × *L. perenne* Lp10 without B chromosomes.

Although the evidence of a genotypic control of chromosome pairing is clearly established there is no information as to the nature and number of loci involved. It is highly likely that it involes more than one locus bearing in mind the additive nature of the variation in preferential pairing and the correlated effect of lower chiasma frequency due to *L. temulentum.*

Detailed knowledge of the genetic system or systems involved in the control of synapsis and subsequent crossing over is extremely limited for most polyploids. The main exceptions are *Triticum* and *Avena.* We know of course that in most if not all natural polyploid chromosome association at metaphase one of meiosis is in the form of bivalents and it is generally assumed that these are bivalents of homologous chromosomes. It is also known that in tetraploid and hexaploid *Triticum* species, this process is mediated mainly by the *Ph* gene on the long arm of chromosome 5B (Riley and Chapman 1958; Okamoto 1958). Similarly there is evidence that the three genomes of hexaploid *Avena sativa* are prevented from association at meiosis by a distinct genetic system (Rajhathy and Thomas 1972). It is highly likely that the diploid like behaviour of most if not all allopolyploids is achieved in this manner. The question of how such control systems arose independently in many separate genera is however difficult to answer. Indeed the precise origin of the *Ph* system in *Triticum* is not yet resolved satisfactorily. Riley et al. (1973) suggested three possibilities, two of which are of some interest in relation to the present investigation. Firstly they noted that in view of the capacity of B chromosomes from *Aegilops mutica* to compensate for the absence of the *Ph* gene it was considered possible that a segment of a B chromosome could have been incorporated into tetraploid *Triticum* at an early stage in its evolution. Secondly, it was suggested that a mutation at the time of, or immediately subsequent to, the origin of tetraploidy could account for the origin of the *Ph* allele. Finally they considered the possibility that an allele corresponding to *Ph* may have been present in the original B genome donor and that this would have been incorporated in the first tetraploid *Triticum.* The present results show that it is possible to have genetic variation for the control of homoeologous pairing present in diploid populations. The effect of this control only becomes evident in an interspecific cross both at diploid and polyploid level. Furthermore in addition to the B chromosome effect there is also a genotypic effect of the A chromosomes. There is no evidence at present whether these two are related in any way, i.e. whether the A chromosome effect is derived from a spontaneous incorporation of a B chromosome segment at some stage or whether the B chromosome in this population is derived from an A chromosome which happened to carry the pairing control genes. Unfortunately both *L. perenne* Lp19 and Lp10 are of a complex origin. They are half sibs derived from crosses between a *L. perenne* ecotype collected in North Africa and both cvr S.23 and cvr S.24.

There is also no way at present of knowing whether either part of the apparent dual control system of *L. perenne* has every been incorporated in natural polyploids. Although there are no such polyploids in the genus *Lolium* there are tetraploids, hexaploids and even octoploids and decaploids in the closely related *Festuca* genus. Comparison with the genetic system controlling pairing within the *Triticum* complex is inevitable in view of the extensive work done on that genus. B chromosomes of *Ae. mutica* (*Triticum mutica*) and *Ae. speltoides* (*T. speltoides*) like those of *L. perenne* have the capacity to suppress homoeologous pairing (Dover and Riley 1972). The *Ph* allele could even have originated from a diploid ancestor. That no such allele has ever been recovered from putative diploid progenitors of wheat might not in itself be all that surprising as its frequency within a diploid population would not be expected to be very high since an allele of this type would confer no adaptive superiority on individuals carrying it. There is every indication in *Loliums* that major control systems of the type described here are not widespread although a much smaller degree of preferential pairing has been noted in *L. multiflorum* × *L. perenne* tetraploids (Breese and Thomas 1977; Lewis 1980).

References

Ahloowalia BS (1977) Hybrids between tetraploid Italian and perennial ryegrass. Theor Appl Genet 49:229–235

Bowman JG, Thomas H (1973) B chromosomes and chromosome pairing in *Lolium perenne* × *Festuca arundinacea* hybrid. Nature (London) New Biol 245:80–81

Breese EL (1983) Exploitation of the genetic resources through breeding; *Lolium* species. In: McIvor JG, Bray RA (eds) Proc Symp Genet Resources Forage Plants. CSIRO, Melbourne, pp 275–288

Breese EL, Thomas AC (1977) Uniformity and stability of *Lolium multiflorum* × *Lolium perenne* allotetraploids. In: Sanchez-Monge E, Garcia-Olmedo F (eds) Interspecific hybridization in plant breeding. Proc 8th Eucarpia Congr, Madrid, pp 155–160

Breese EL, Lewis EJ, Evans GM (1981) Interspecies hybrids and polyploidy. Philos Trans R Soc London, Ser B 292:487–498

Clarke J, Thomas H (1976) Chromosome pairing in *Lolium perenne* × *Lolium multiflorum* amphiploids. In: Rep Welsh Plant Breed Stn for 1975, p 59

Dover GA, Riley R (1972) Prevention of pairing of homoeologous meiotic chromosomes of wheat by an activity of supernumerary chromosomes of *Aegilops*. Nature 240:159–161

Evans GM, Macefield AJ (1972) Suppression of homoeologous pairing by B chromosomes in a *Lolium* species hybrid. Nature (London) New Biol 236:110–111

Evans GM, Macefield AJ (1973) The effect of B chromosomes on homoeologous pairing in species hybrids. 1. *Lolium temulentum* × *Lolium perenne*. Chromosoma 41:63–73

Lewis EJ (1980) Chromosome pairing in tetraploid hybrids between *Lolium perenne* and *L. multiflorum*. Theor Appl Genet 58:137–143

Okamoto M (1958) Asynaptic effect of chromosome. 5. Wheat Inf Serv 5:6

Rajhathy T, Thomas H (1972) Genetic control of chromosome pairing in hexaploid oats. Nature (London) New Biol 239:218–219

Riley R, Chapman V (1958) Genetic control of the cytologically diploid behaviour of hexaploid wheat. Nature 182:713–715

Riley R, Chapman V, Miller TE (1973) The determination of meiotic chromosome pairing. In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp University of Missouri, Colombia MO, pp 731–738

Taylor IB, Evans GM (1977) The genetic control of homoeologous chromosome association in *Lolium temulentum* × *L. perenne* hybrids. Chromosoma 62:57–67

Thomas H, Morgan WG, Borrill M, Evans M (1983) Meiotic behaviour in polyploid species of *Festuca*. In: Brandham PE, Bennett MD (eds) Kew Chromosome Conf, vol II. Allen & Unwin, London Boston Sydney, pp 133–138