Theor. Appl. Genet. 59, 139-143 (1981)



# Genetic Segregation in Relation to Chromosome Pairing in Tetraploid Hybrids Between *Lolium perenne* and *L. multiflorum*

E.J. Lewis
Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth (UK)

**Summary.** Segregation at one of the loci controlling tiller-base pigmentation was studied to determine the mode of inheritance in tetraploid hybrids between *Lolium perenne* and *L. multiflorum*. The results could be explained by tetrasomic inheritance and thus did not support previous reports of a degree of preferential chromosome pairing in this material. However, double reduction and aneuploidy may to some extent have masked any tendency to disomic segregation brought about by preferential pairing. Moreover, there was significant heterogeneity between families in the segregation ratios which may indicate genetically controlled differences in pairing behaviour. The results are related to previous cytological and genetic studies.

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

## Introduction

In an earlier publication (Lewis 1980), cytological evidence was presented to support the view that tetraploid hybrids between *Lolium perenne* and *L. multiflorum* show a moderate but significant degree of preferential meiotic chromosome pairing. This was based on a comparison of bivalent, multivalent and chiasma frequencies, and their inter-relationships, between the hybrids and autotetraploid *L. perenne*. The evidence was of necessity circumstantial in nature since there are at present no cytological means of distinguishing between the constituent parental chromosomes forming the different metaphase configurations in the hybrids.

The way in which the parental chromosomes associate at meiosis in the hybrids will of course be reflected in the mode of inheritance and the extent to which they pair preferentially can thus be ascertained by genetical means. The restriction of pairing to true homologues would result in strict disomic segregation while any homoeologous pairing would be reflected in a corresponding amount of tetrasomic segregation.

The degree of preferential pairing calculated from the cytological data was of the order of 33% (Lewis 1980) and this agrees very closely with the value obtained by Breese and Thomas (1977) from genetical analyses in their material. It is evident from these results that a considerable amount of homoeologous chromosome pairing, and thus tetrasomic segregation, must take place in such hybrids.

The present paper attempts to assess the relative extent of disomic and tetrasomic segregation at one of the loci controlling anthocyanin pigmentation in these hybrids and to relate this to the data already presented on chromosome pairing in the same plants (Lewis 1980). The possible danger of relating the pairing behaviour of the full chromosome complement to a genetic situation which is restricted to the particular set of four chromosomes carrying this locus is appreciated and has been taken into consideration. Some of the more important factors that can complicate studies involving tetrasomic segregation, such as the occurrence of double reduction and aneuploidy, which have been discussed in detail by Doyle (1973), have also been taken into consideration in the interpretation of the results.

## Material and Methods

Details of the plant material used were given by Lewis (1980). Anthocyanin pigmentation of the tiller-base in this material is controlled by two complementary dominant factors C and R (Jenkin 1930). The initial *L. perenne* female parents were of the constitution ccccRRRR, and therefore devoid of anthocyanin pigmentation ('non-red'), while the *L. multiflorum* pollinators were 'red' based (CCCCRRRR). The $F_1$ hybrids, identified by their 'red' base colour, were then duplex for the C factor (CCccRRRR). $F_1$

0040-5752/81/0059/0139/$ 1.00

plants with 2n = 28 chromosomes were selfed and also backcrossed to the 'non-red' parental material. Insufficient numbers of selfed progeny were however obtained for further study. The seedlings obtained from backcrossing were screened for base colour and the ratio of 'red':'non-red' recorded for each family. Screening was repeated several times over a period of several months to avoid mis-classification, since the intensity of pigmentation was found to vary considerably, particularly in the very young seedlings.

## Results

If pairing amongst the four chromosomes carrying this locus was strictly confined to true homologues, all gametes produced by the hybrids would have the constitution Cc and no 'non-red' seedlings would appear in the backcross progeny. That is to say, inheritance would be strictly disomic.

If, on the other hand, pairing between the four chromosomes was completely at random, tetrasomic inheritance would ensue. A certain proportion of the gametes would carry the recessive allele only, and a corresponding proportion of 'non-red' seedlings would thus be produced. The actual gametic output, and hence the proportion of such seedlings, would depend both on the frequency of quadrivalent formation and the randomness of disjunction of chromatids at the locus when a quadrivalent is formed. The latter depends primarily on whether crossing-over can take place between the locus and the centromere. When random pairing results in bivalents, or when a quadrivalent is formed and the locus in question is completely linked to the centromere, the resulting segregation is the same. In the present material the expected ratio of 'red':'non-red' seedlings would then be 5:1.

When a quadrivalent is formed and the locus is sufficiently far from the centromere to allow the formation of a chiasma between them, sister chromatids at the locus can become attached to different centromeres. Consequently, the sister alleles may be included in the same or in different gametes, depending on the distribution of the chromatids at the two meiotic anaphases. With equational separation at first anaphase and appropriate orientation at the second, sister chromatids at the locus can arrive in the same gamete (double reduction). With 50% cross-over between the locus and centromere, and random separation at second anaphase the maximum frequency for this event would be 1/7. At this level the ratio expected from the present test cross would be 3.67 'red':1 'non-red'.

As already intimated, aneuploidy could also affect the segregation ratio in these hybrids. Aneuploid gametes arise as a result of incomplete pairing, or of numerical non-disjunction (3:1 separation) of quadrivalents. Since unpaired chromosomes are very frequently excluded from the spore nuclei, monosomic gametes tend to be more common than trisomic gametes. Assuming that gametes which are deficient for one of the chromosomes carrying the locus in question are functional, random loss amongst these four chromosomes will have a non-random influence on segregation frequency (Bernardo 1965). Thus, in a gamete which would normally have the constitution Cc, the loss of the dominant allele would be manifest in the backcross progeny ratio whereas the loss of the recessive allele would not.

The formulae derived by Doyle (1973) for the calculation of expected segregation frequencies take each of the above factors into account. They are based on the formulae of Mather (1936), Fisher and Mather (1943) and Catcheside (1956) and the notation is similar to that used by these workers. The frequency of double reduction is given as $\alpha$ and that of gametes, which are monosomic, disomic and trisomic for the chromosome in question, as m, d and t respectively. For the duplex $F_1$ hybrids under consideration, the expected frequencies of the various kinds of gametes are as shown in Table 1. The expected ratio of 'red':'non-red' seedlings then becomes $t(6-\alpha) + d(5-2\alpha) + 3\,m$: $t\,\alpha + d(1+2\,\alpha) + 3\,m$.

Since the chromosome carrying the C locus has not yet been identified, the values of m, d and t have to be derived from the proportions of 2n = 27, 2n = 28 and 2n = 29 plants obtained. The large numbers involved precluded the chromosome counting of the actual backcross progeny and the proportions amongst the $F_1$ hybrids themselves have been used instead. These were 5/19, 12/19 and 2/19 respectively. Assuming that aneuploidy occurs at random amongst the seven chromosome sets, the values of m, d and t are then 5/133, 126/133 and 2/133. The values for m and t should probably be slightly higher since some of the 2n = 28 plants may not have been true euploids but compensated aneuploids of the type $2n = 4x - 1 + 1$, which cannot be identified as such.

However, using the values calculated for m, d and t in the above formula, the ratio of 'red':'non-red' seedlings becomes 4.66:1 when $\alpha$ takes its minumum value of 0,

**Table 1.** Relative proportions of the various gametes formed as a result of double reduction, incomplete pairing and numerical non-disjunction (after Doyle 1973)

| Gametic genotype | Relative proportion |
|---|---|
| CCC | $t\alpha/6$ |
| CCc | $t(3-\alpha)/6$ |
| Ccc | $t(3-\alpha)/6$ |
| ccc | $t\alpha/6$ |
| CC | $d(1+2\alpha)/6$ |
| Cc | $d(2-2\alpha)/3$ |
| cc | $d(1+2\alpha)/6$ |
| C | m/2 |
| c | m/2 |

**Table 2.** Numbers of 'red' and 'non-red' seedlings, and their ratios in backcross families

| Family number | Number of seedlings 'red' | 'non-red' | Ratio 'red': 'non-red' |
|---|---|---|---|
| 1 | 112 | 22 | 5.09:1 |
| 2 | 105 | 29 | 3.62:1 |
| 3 | 105 | 20 | 5.25:1 |
| 4 | 128 | 38 | 3.37:1 |
| 5 | 159 | 47 | 3.38:1 |
| 6 | 239 | 73 | 3.27:1 |
| 7 | 134 | 42 | 3.19:1 |
| 8 | 187 | 28 | 6.68:1 |
| 9 | 194 | 37 | 5.24:1 |
| 10 | 156 | 32 | 4.88:1 |
| 11 | 191 | 42 | 4.55:1 |
| 12 | 182 | 33 | 5.52:1 |
| Pooled | 1892 | 443 | 4.27:1 |

**Table 3.** $\chi^2$ analyses of ratios of 'red': 'non-red' seedlings

| | | $\chi^2$ | d.f. | Probability |
|---|---|---|---|---|
| Departure from | 5:1 | 8.94 | 1 | 0.01 – 0.001 |
| " " | 3.67:1 | 8.27 | 1 | 0.01 – 0.001 |
| " " | 4.66:1 | 2.73 | 1 | NS |
| " " | 3.50:1 | 14.27 | 1 | < 0.001 |
| Heterogeneity[a] | | 20.97 | 11 | 0.05 – 0.02 |

[a] Calculated from contingency values

and 3.50:1 when $\alpha$ is at its maximum of 1/7. There are, thus, four ratios to be considered in the interpretation of the segregation data obtained i.e. 5:1, 3.67:1, 4.66:1 and 3.50:1.

Table 2 shows the numbers and ratios of 'red':'non-red' seedlings in the different backcross families and the results of testing these against the four ratios are summarised in Table 3.

Considering first the analyses in the absence of aneuploidy, i.e. the comparison with the 5:1 and 3.67:1 ratios, in only one backcross family (family 8) is there any positive suggestion that segregation might at least be partially disomic at this locus. Indeed the overall ratio, 4.27:1, is significantly less than 5:1 indicating chromatid segregation rather than chromosome segregation. The overall ratio is at the same time significantly greater than 3.67:1 and so indicates an $\alpha$ value less than its theoretical maximum of 1/7. However the heterogeneity $\chi^2$ is significant at the 5% level suggesting that the families are not consistent in this respect and that they may thus exhibit genetic control in this segregation. This of course is in keeping with the cytological observations in this material (Lewis 1980) which show marked variation between $F_1$ plants in quadrivalent frequency. By the same token, the relatively low level of quadrivalent frequency reported would lead one to expect an $\alpha$ value less than its maximum 1/7 and so a ratio greater than 3.67:1, even if the frequency of recombination between the C locus and the centromere is high.

When the effect of aneuploidy is taken into consideration it is found that the overall ratio of 4.27 'red': 1 'non-red' seedlings does not differ significantly from the higher of the two theoretical ratios, 4.66:1. This suggests on the one hand that the results may be satisfactorily explained on the basis of chromosome segregation together with the assumed level of aneuploidy. However, as already noted, the significant heterogeneity $\chi^2$ indicates that the families are not consistent in their segregation behaviour. Several of the ratios are significantly lower than 4.66:1 indicating once more that there is probably some degree of chromatid segregation. There is again very little evidence of disomic segregation. Of the several ratios which are higher than 4.66:1, that found in family 8 (6.68:1) provides the only positive suggestion of this but the difference still does not reach statistical significance.

Taking the analysis as a whole, there is therefore little or no direct evidence of disomic segregation at this locus. There is on the other hand, fairly good evidence of some degree of chromatid segregation. Its effect, and that of aneuploidy when present, would of course be to counteract and reduce the effect of preferential chromosome pairing as estimated from the segregation ratios. It is nevertheless still difficult to reconcile these results with the level of preferential pairing estimated from the cytological data (Lewis 1980).

It will be recalled however that the latter represented the level for the whole chromosome complement and it is conceivable that some homoeologous groups tend to form preferential bivalents more frequently than others. Neither individual homoeologous groups, nor parental chromosomes within them, can however be identified at meiosis. The only cytological evidence as to uniformity of behaviour between and within the seven homoeologous groups is that which can be obtained from frequency distribution analyses of the different types of chromosome associations. These can be carried out by the method described by Hall (1955), using large samples of pollen mother cells (PMCs). Thus, if the probability of quadrivalent formation, for example, is the same for each set of chromosomes the number of PMCs with 0, 1, 2 ... 7 quadrivalents should follow a binomial distribution.

Figure 1 shows the observed and theoretical distributions of quadrivalents for the hybrids, together with those for the autotetraploid *L. perenne* for comparison. The deviation from the theoretical binomial distribution is non-significant in both groups (P = 0.30 – 0.20 in the hybrids and P = 0.50 – 0.30 in the autotetraploids). There is, thus, no evidence in either case that certain sets of four

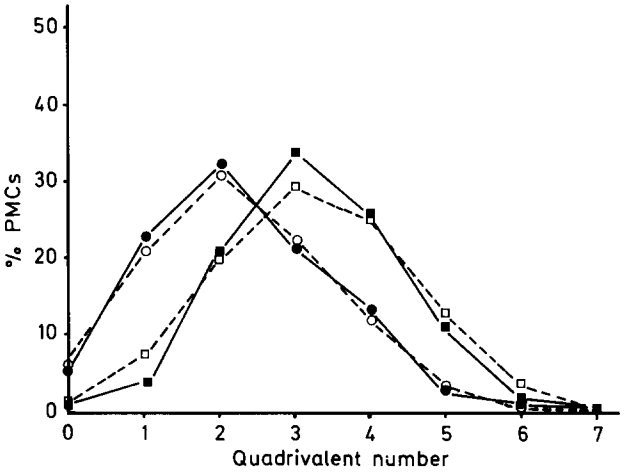


Fig. 1. Observed (continuous line) and theoretical (broken line) frequency distributions of PMCs with 0-7 quadrivalents in hybrids (circle) and autotetraploid *L. perenne* (square)

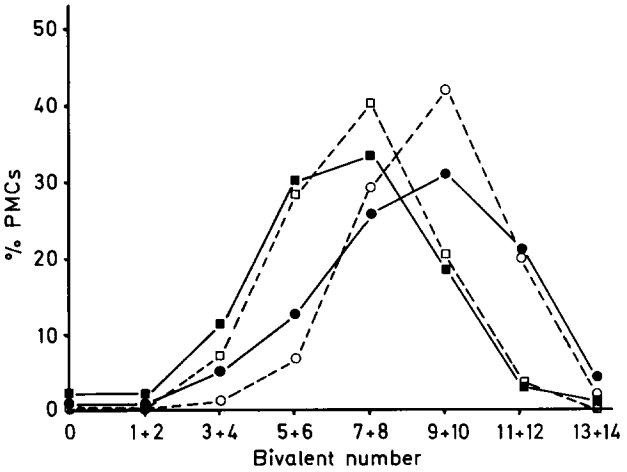


Fig. 2. Observed (continuous line) and theoretical (broken line) frequency distributions of PMCs with 0-14 bivalents in hybrids (circle) and autotetraploid *L. perenne* (square)

chromosomes tend to form quadrivalents more frequently than others.

Some grouping of the data was necessary before the bivalent distributions could be tested in the same way. As reported by Lewis (1980), very few PMCs with odd numbers of bivalents were recorded, and Hall's analysis could not therefore be validly applied to the data as it stood. Consequently the bivalent frequency classes have been combined in pairs as shown in Figure 2. The theoretically expected cell frequencies were first calculated for the fifteen classes, and then combined in pairs in the same way as the observed data.

It is clear from Fig. 2 that the observed bivalent distributions show greater deviation from theoretical expectation than do those for quadrivalents in Fig. 1. This is particularly so in the case of the hybrids where the deviation is highly significant ($P < 0.001$); the deviation just reaches significance at $P = 0.05$ in the autotetraploids. It would thus appear, in so far as the method described by Hall provides a sufficiently precise test of this, that the different homoeologous chromosome groups tend to vary in their frequency of bivalent formation, particularly in the hybrids. It is therefore possible that the group in question may show a lower tendency to form bivalents, and more especially preferential bivalents, than some of the others and this could account for the discrepancy between the segregation and cytological results.

## Discussion

The overall lack of clear cut evidence of preferential chromosome pairing from these results was somewhat unexpected in view of the cytological results obtained in the same material (Lewis 1980), and the segregation results at another locus in very similar material (Breese and Thomas 1977). Both of these studies had shown that the level of preferential pairing was of the order of 33% and the present results clearly fail to corroborate this. The backcross families did vary significantly in their segregation ratios, suggesting genetically controlled differences in pairing pattern in the hybrids, and there was a tendency in one or two of the families towards disomic segregation. Overall, however, there was very little evidence of any appreciable degree of preferential pairing, even when the possible effects of chromatid segregation and aneuploidy were taken into consideration.

The most probable explanation for the discrepancy between the present results and the previous results referred to is that the different homoeologous chromosome groups vary in their tendency to form preferential bivalents. It was argued by Lewis (1980) that preferential pairing occurs in these hybrids because of some structural differentiation between the parental genomes. There is, of course, no reason whatsoever why such differentiation should be uniform from one homoeologous group to another and it is therefore quite conceivable that the different groups vary in their tendency to form preferential bivalents. The binomial analyses did in fact give some indication of some non-randomness in bivalent formation.

The cytological results (Lewis 1980) were for the whole chromosome complement and thus relate to the average pairing behaviour over the seven homoeologous groups. The pairing behaviour of certain of these groups could well differ significantly from this overall value and it is suggested that the group carrying the C locus does show a below average tendency to pair preferentially.

Such a variation in level of preferential pairing between homoeologous groups could similarly account for the discrepancy with the results of Breese and Thomas (1977) if

it is assumed that the two loci concerned are located in different homoeologous groups. That is to say, the group carrying the C locus could show considerably less preferential pairing than that carrying PGI/2, the isozyme locus used by these workers. The PGI/2 locus has already been assigned to a particular chromosome (Lewis, Humphreys and Caton 1980) but the location of the C locus is not yet known.

The possibility that the different homoeologous groups may vary in their degree of preferential pairing has obvious and important practical breeding implications. The desirable combination of agronomic features displayed by some of these hybrids is based on several contrasting parental characteristics (Lewis 1980). If, as seems likely, the loci controlling these are widely distributed throughout the parental genomes their mode of inheritance might well be different, and this would have a bearing on aspects of selection and stability.

## 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 the work. I am indebted to those of my colleagues who provided advice and assistance, particularly Dr H. Thomas for valuable discussion and comments.

## Literature

Bernardo, F.A. (1965): Segregation in new allopolyploids of *Gossypium*. 4: The effect of chromosome elimination on segregation frequencies. Canad. J. Genet. Cytol. 7, 284-290

Breese, E.L.; Thomas, A.C. (1977): Uniformity and stability of *Lolium multiflorum* X *L. perenne* allotetraploids. Proc. 8th Congr. Eucarpia, Madrid, 155-160

Catcheside, D.G. (1956): Double reduction and numerical non-disjunction in maize. Heredity 10, 205-218

Doyle, G.G. (1973): Autotetraploid gene segregation. Theor. Appl. Genet. 43, 139-146

Fisher, R.A.; Mather, K. (1943): The inheritance of style length in *Lythrum salicaria*. Ann. Eugenics 12, 1-23

Hall, B.M. (1955): Binomial analysis of chromosome behaviour in hybrid plants. Proc. Pennsylvania Acad. Sci. 29, 121-126

Jenkin, T.J. (1930): Inheritance in *Lolium perenne* 3: Base-colour factors C and R. J. Genet. 22, 389-394

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

Lewis, E.J.; Humphreys, M.W.; Caton, M.P. (1980): Chromosome location of two isoenzyme loci in *Lolium perenne* using primary trisomics. Theor. Appl. Genet. 57, 237-239

Mather, K. (1936): Segregation and linkage in autotetraploids. J. Genet. 32, 287-314

Received October 15, 1980
Communicated by R. Riley

Mr. E.J. Lewis
Welsh Plant Breeding Station
Plas Gogerddan, Aberystwyth (Wales)