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Cytogenetics of *Lolium perenne*

Part 2. Chiasma Distribution in Inbred Lines

A. Karp and R. N. Jones
Department of Agricultural Botany, University College of Wales, Aberystwyth (Wales)

Summary. An analysis of the patterns of chiasma distribution within bivalents, in a number of inbred lines of *Lolium perenne,* reveals a striking potential for variation in this character, as well as a polygenic genetic basis for its control. In parent plants the chiasmata have a regular pattern of localisation which restricts their formation to the ends of the chromosome arms: this strict localisation is progressively lost with inbreeding and novel patterns of distribution are found where chiasmata occur in more interstitial and proximal regions. The variation is continuous and originates from genotypic differences between the different parent plants, rather than from segregation among families deriving from individual parents.

Key words: *Lolium perenne* – Inbred lines – Meiosis – Chiasma distribution

Introduction

In *Lolium perenne* chiasmata are characteristically formed in a terminal location at the ends of the chromosome arms, and have a highly regular pattern of distribution both among the bivalents within pollen mother cells (pmcs) as well as between the different pmcs within a plant. An analysis of the patterns of variation in a number of inbred lines, and families of inbred plants, derived from selfing four different parent plants of variety S23, revealed a polygenic system of genetic regulation over the components of chiasma variation which was postulated to operate at two levels of control (Karp and Jones 1982). The components studied were (i) the mean pmc chiasma frequency, (ii) the cell variance, which quantifies the variation between pmcs, and (iii) the bivalent variance, which measures variation between bivalents within cells. All three components showed a correlated response to inbreeding: mean pmc chiasma frequency was reduced, and the cell and bivalent variances were increased, but the relationships between them were complicated by a certain level of independent segregation of the controlling genes which manifested itself in the advanced S4 and S5 inbred generations. It was in order to account for these complications that the two-level model of control was proposed.

The present work is concerned with a fourth level of chiasma variation, namely that involving the distribution pattern of chiasmata within bivalents. The same pedigrees and inbred lines of plants are involved, as described in the previous paper, and evidence will be presented to show that polygenically controlled variation exists for differences in chiasma localisation, and that this polygenic system also operates at two levels of control. The data are supplemented by additional analyses on some inbred lines of German origin that are genetically distinct, and unrelated to the S23 material which forms the main part of the study.

Materials and Methods

The material consists of four inbred pedigrees of *L. perenne* var. S23, obtained from four different parent plants. The work described here is mainly concerned with variation between the most advanced S5 generation lines. The relationships between the lines, and their ancestry in terms of the pedigree structures, have already been described in detail (Jones and Jenabzadeh 1981; Karp and Jones 1982). Basically there are three S5 lines deriving from each of the P3, P15 and P30 parents, and two from the P6 parent, and the relationships between the lines, within pedigrees, is determined by the generation of inbreeding at which they diverged into separate lines of descent. Some additional inbred lines of *L. perenne* of German ancestry (the "Deutsches Weidelgras" or WD lines), were also available for study. The nine lines of this material, which were at the S9 generation when studied, originated from the German ryegrass varieties Odenwälder (lines 003, 038, 064, 089, 109), Odengrün (206, 271) and Semperweide (375, 388) as described by Utz and Oettler (1978), and they are quite unrelated and

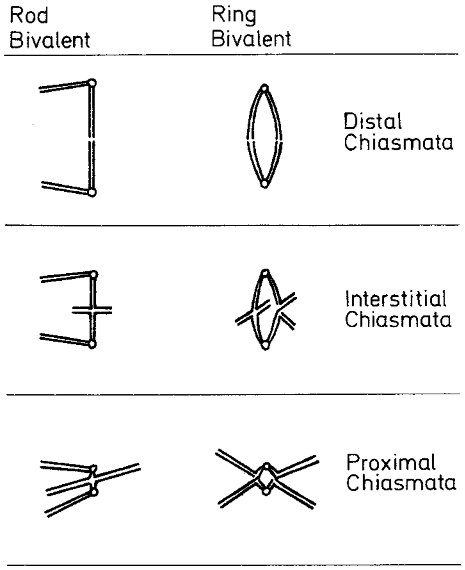

Fig. 1. Diagrammatic representations of rod and ring bivalents to show the scheme of classification of chiasmata according to their distal, interstitial and proximal locations

developmentally different to S23. Seed of this material was kindly supplied by Dr. U. Posselt of the Hohenheim University of Stuttgart.

Analyses on the patterns of variation in chiasma distribution were made on bivalents at metaphase I (MI) of meiosis, from inflorescences fixed in Carnoy's fluid. Samples of 20 MI pmcs were used for each plant and chiasmata classified into three categories depending upon their position within the bivalents. Chiasmata at the ends of chromosome arms, with no side-arms visible beyond the point of exchange, were classified as distal. Those in the regions intermediate between the centromere and the end of the arms, such that side-arms were equal to or less than the length of arm between the centromere and the chiasma, were classified as interstitial; and those adjacent to the centromere, with side-arms greater in length than the distance between centromere and chiasma, as proximal.

The basic system of classification is illustrated in Fig. 1. In addition to these simple patterns there are the more infrequent cases of rod bivalents with two chiasmata in one of the arms, and ring bivalents with two chiasmata in either one or both of the arms, but essentially the position of the chiasmata can be readily classified within the prescribed scheme. The number of chiasmata found in the three classes were expressed as percentages of the total number of chiasmata for each sample of 20 pmcs in each plant. The numbers of plants involved in each of the S5 families are given in Fig. 2. All data in the from of percentages were transformed to angles for the purpose of statistical analyses.

Results

The Parent Characteristics

The pattern of chiasma distribution in the parent plants, based on the overall means of the four parents, is given in the form of a histogram in Fig. 2, together with the data for the S5 generation. As the histogram clearly shows the parents are characterised by having predominantly distal chiasmata (mean ca. 90%), and their bivalents have a regular appearance in the form of dichiasmate rings and monochiasmate rods (Fig. 3). Proximal chiasmata, at a mean of 0.8% (0.7, 2.1, 0.0, 0.2 in P3, P6, P15 and P30 respectively), were hardly ever seen and interstitial ones occur in a low mean frequency of about 10%. This pattern of distribution is typical of outbred population plants of the variety S23 from which the parents were originally taken.

Patterns of Variation Among Families in the S5 Generation

In the S5 generation, in contrast, there is a marked difference in the distribution, and a much higher proportion of chiasmata are found in the interstitial and proximal locations (Fig. 2). Bivalents have a far less regular appearance (Fig. 3) and are quite unlike those normally found in outbred population material of

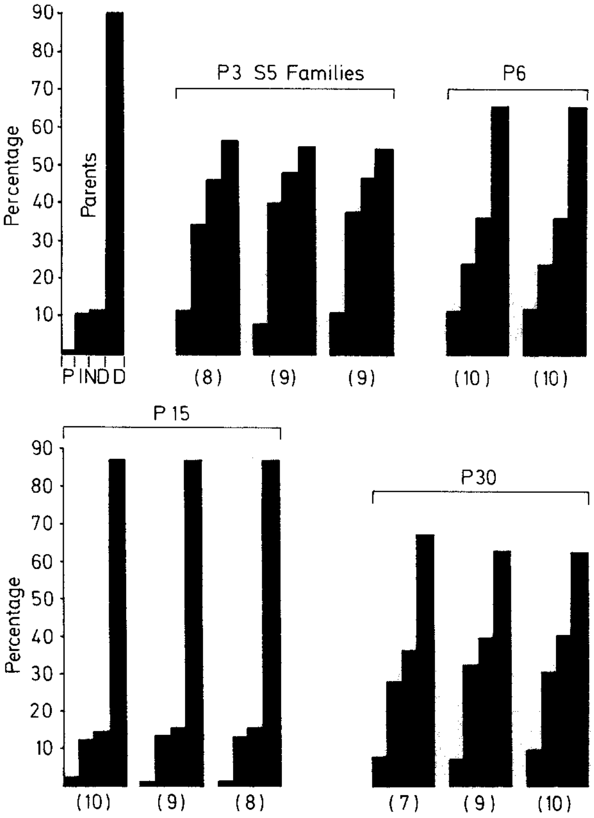

Fig. 2. Histograms showing the patterns of chiasma distribution within bivalents in the Parents and the four pedigrees of S5 families analysed in 1980. Columns within each block, from left to right, represent the proportion of proximal (P), interstitial (I), non-distal (ND = P + I) and distal (D) chiasmata. Numbers of plants comprising each of the S5 families are given beneath their respective histograms

perennial ryegrass. In relation to the structure of the breeding scheme it will be observed that the bulk of the variation lies between pedigrees, and that there are virtually no differences segregating out between the families within pedigrees. Analysis of variance confirms these observations. There are highly significant differences between S5 families overall, for all three categories of chiasma localisation ($P < 0.001$), and this variation resides at the between pedigrees ($P < 0.001$) rather than the between families within pedigrees level (NS). When the data for families within pedigrees are pooled the analysis shows that for proximal chiasmata the P3 and P6 pedigrees have identical patterns of variation and that all the other combinations are different at the 1.0% level. For interstitial chiasmata all combinations are different ($P < 0.01$) and for nondistals the paired combinations all differ also at the 0.1% level except for the P6/P30 pair which is non-significant. As far as individual pedigrees are concerned it will be seen that the highest percentage of non-distal chiasmata is to be found within P3 (46.0%) and the lowest within P15 (14.5%), and that the range between these two pedigrees is quite considerable. Although the P15 pedigree resembles the mean of the parents very closely for this character, it does have a slight excess of non-distals (14.5 cf. 11.42%), and a larger excess when compared with the P15 parent itself (14.5 cf. 7.6%).

The contrasting patterns of chiasma distribution found between the pedigrees of S5 families are under genotypic control. This is established by further analyses designed to remove the effect of possible correlated responses due to chiasma frequency variation. In the first place the mean pmc chiasma frequencies for the P15 and P30 pedigrees are virtually identical at 8.43 and 8.19 respectively, but the percentages of non-distal chiasmata are widely different at 14.5 and 37.4%. Secondly the analysis of variance has been repeated after recalculating for all S5 individuals the percentages of proximal, interstitial and non-distal chiasmata for the dichiasmate ring bivalent class only, and then for the monochiasmate rod bivalents only, thus removing any effect due to chiasma frequency variation at the bivalent level. In both cases the same levels of significance ($P < 0.001$) were found for variation between pedigrees, and there were no significant differences between families within pedigrees. A further analysis was also carried out, at the cell level, based on the mean percentage of non-distal chiasmata for each of the four pedigrees, taking only those pmcs with a fre-

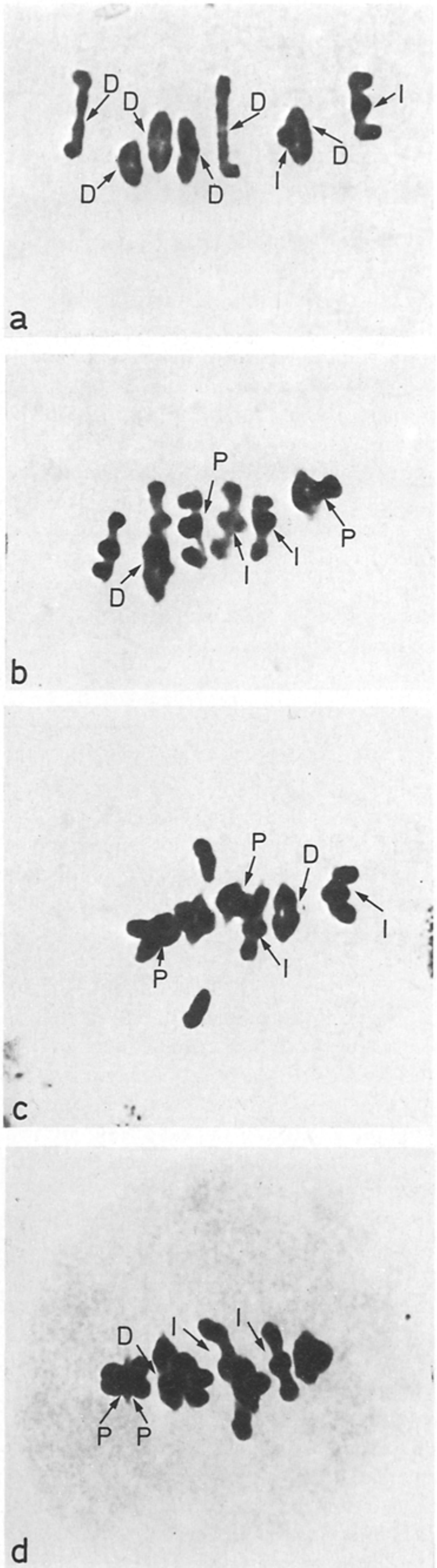

Fig. 3a–d. Metaphase I bivalent configurations in a typical Parent cell (**a**), and in three pmcs from the P30 S5 (**b**, **c**, **d**). Note the examples of distal chiasmata in the Parent (arrowed) and the examples of interstitial and proximal chiasmata in the S5

quency of nine chiasmata per cell: the means for the P3, P6, P15 and P30 pedigrees were 3.75, 3.43, 1.15 and 3.46 respectively, and P15 was significantly different ($P < 0.001$) from the other three.

The results for the earlier generations of inbreeding are not given in detail other than to say that segregating differences among families were found in all generations from S1–S4, and that the variation between families within pedigrees diminished, relative to the variation between pedigrees, as the inbreeding advanced. At the S4 the patterns of variation and the levels of significance were virtually identical to those of the S5, except for within pedigree differences for proximal chiasmata in P15 (barely significant, $P < 0.05$), and for interstitials in P3 (barely significant, $P < 0.05$). The major part of the variation for chiasma distribution therefore segregated out in the early S1–S3 generations of selfing, and in this respect the character behaves differently to those for chiasma frequency, and cell and bivalent variances, which were still segregating at S5 (Karp and Jones 1982). Evidently the parent plants have less heterozygosity for this character or else there are fewer genes involved in its control.

Deutsches Weidelgras (WD) Lines

Nine additional WD lines derived from German *L. perenne* populations were also included in the study. These lines were at S9 when fixed, and each one was derived from a different parent plant by the method of single-seed descent (Utz and Oettler 1978); and they therefore represent nine different pedigrees on the same basis as the S23 scheme. They have not previously been studied cytologically and were considered potentially useful in gaining a better evaluation of the range of variation in chiasma distribution, as well as providing a contrasting type of experimental material.

Four of the lines were each represented by five replicate plants (viz. 038, 109, 206, 375), one line by three replicate plants (388) and the other four by two each (003, 064, 089, 221); in all other respects they were handled in the same way as the S23 material.

The patterns of variation in chaisma distribution for the different WD lines, in terms of their percentages of proximal and interstitial chiasmata, are displayed in the two histograms in Fig. 4. The results have been combined with the corresponding data for the S23 families in order to demonstrate the continuous nature of the variation for chiasma distribution within bivalents. Mean values for the four parents of the S23 lines are also included for the purposes of comparison. As far as the percentage of proximal chiasma is concerned (Fig. 4 a) the extremes of the variation are found between the WD lines 388 (0.0%) and 038 (15.7%), and the S23 fall into related groupings with the P15 families having the lowest values and P6 the highest. Differences between WD lines are significant at the 0.1 percent level, and as for S23 lines the variation is still significant when the effect of chiasma frequency is removed by repeating the analysis on percentages of proximal chiasmata in the dichiasmate rings ($P < 0.01$) and monochiasmate rod bivalents ($P < 0.01$) only. For the percentage of interstitial chiasmata (Fig. 4 b) the WD lines differ from S23 in that the differences between them do not show any significance, although when the proximal and interstitial chiasmata classes are added together, as non-distals, the variation between them is significant ($P < 0.01$). It is also interesting to note in Fig. 4 b that for interstitial chiasmata in S23 the families belonging to the four pedigrees show quite

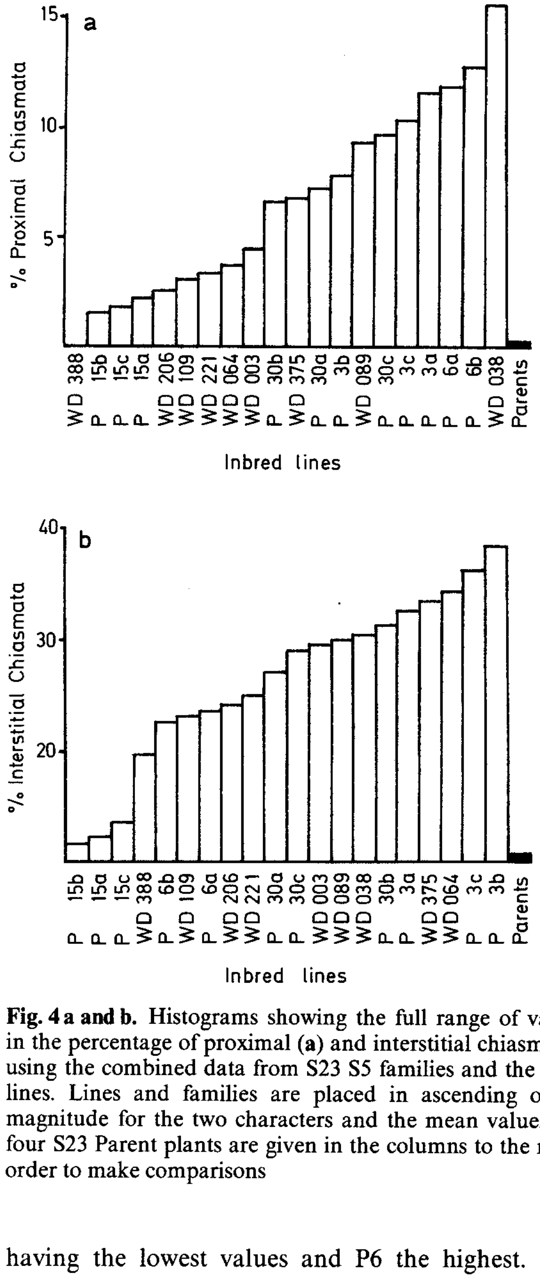

Fig. 4 a and b. Histograms showing the full range of variation in the percentage of proximal (**a**) and interstitial chiasmata (**b**), using the combined data from S23 S5 families and the WD S9 lines. Lines and families are placed in ascending order of magnitude for the two characters and the mean values of the four S23 Parent plants are given in the columns to the right, in order to make comparisons a distinct separation from one another, from the lowest values in P15 to the highest in P3, without any overlap. Aside from these detailed considerations the main point to emerge from the distribution patterns of the 20 families (lines) of plants shown in Fig. 4, is that the variation in chiasma distribution and localisation is continuous, and under polygenic control.

Inbreeding Effects in S23

The results described above for S23 refer mainly to the pattern of chiasma distribution within the most advanced inbred generation available, namely the S5, and only brief reference has been made to the release of variation among the earlier S1–S4 segregating generations. In this section we consider how the distribution patterns change in response to increasing homozygosity in the different inbred pedigrees, using regression analyses on the mean values for proximal and interstitial chiasmata over the P-S5 generations. In the analysis the data for the parent plants are the means of the two seasons, 1979/80; that of the S1–S4 was obtained in 1979, and that of the S5 in 1980. As there was no significant difference in parent values over the two seasons we feel justified in combining the 1979 S1–S4 and the 1980 S5 results together.

The relationship between chiasma distribution characteristics and inbreeding is shown in the graph in Fig. 5. As expected there is an increase in the proportion of both proximal and interstitial chiasmata with advancing inbred generations, and the interstitial values are always greater than those of the proximal. Levels of significance are assigned to the individual regressions in Fig. 5. The joint regression is highly significant ($P < 0.001$), and so too are the heterogeneity of means items for the overall regressions ($P < 0.001$), for pedigrees ($P < 0.001$) and for chiasma classes (P/I, $P < 0.001$). There are no interactions, and no heterogeneity of slopes for the overall regression lines, for pedigrees or for chiasma classes. The rate of change is the same for both proximal (mean $b = 2.24$) and interstitial chiasmata (mean $b = 2.26$) and there is an average percentage increase in both classes of 2% per generation of inbreeding. The change is a straight inbreeding effect and does not involve any deliberate element of selection for cytological characters at any stage in the breeding programme. When joint regression analyses are carried out for the two chiasma classes separately, with fewer degrees of freedom, essentially the same outcome is obtained but with some heterogeneity of slopes for the proximal chiasmata class ($P < 0.05$). Non-distal chiasmata are not included in the joint regression, but on the basis of analyses within the individual pedigrees the regressions are all significant ($P < 0.01$) except for P15. The differences in means among the regressions confirm the earlier findings from the analysis of variance. There are different mean levels of chiasma distribution classes running down the four inbred pedigrees, which relate back to genotypic differences at the parental generation.

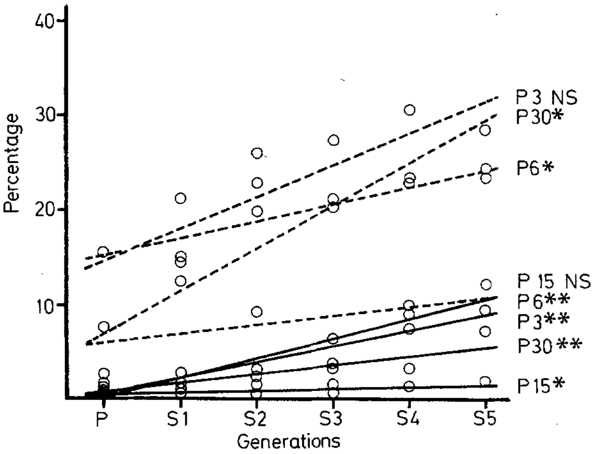

Fig. 5. Graphs showing the change in the mean proportions of proximal and interstitial chiasmata in the four S23 pedigrees over generations of inbreeding. The continuous lines represent proximal chiasmata and the discontinuous ones the interstitials

Relationships Between the Components of Chiasma Variation

In the first paper in this series (Karp and Jones 1982) a two-level model of control was proposed in order to accommodate the patterns of variation in the components of chiasma variation (viz. (i) pmc chiasma frequency, (ii) cell variance, (iii) bivalent variance), and as an aid to interpreting and understanding the interrelationships between them. The model proposed a level I control to account for correlated effects arising as a consequence of inbreeding, and level II regulation to explain some of the anomalies of inbreeding as well as the independent segregation of some of the variables within generations.

Chiasma distribution within bivalents is the fourth variable contained within the model (Fig. 5, paper 1), and we now assess and extend the analysis presented in this paper in order to explore the relationships between this fourth variable – for which we will use the single character of percentage non-distal chiasmata – and the other three. Particular attention has been paid to the relationship between chiasma distribution and chiasma frequency.

(i) Chiasma Frequency

At a general level of analysis, plotting overall means for all individuals across all pedigrees, within each genera-

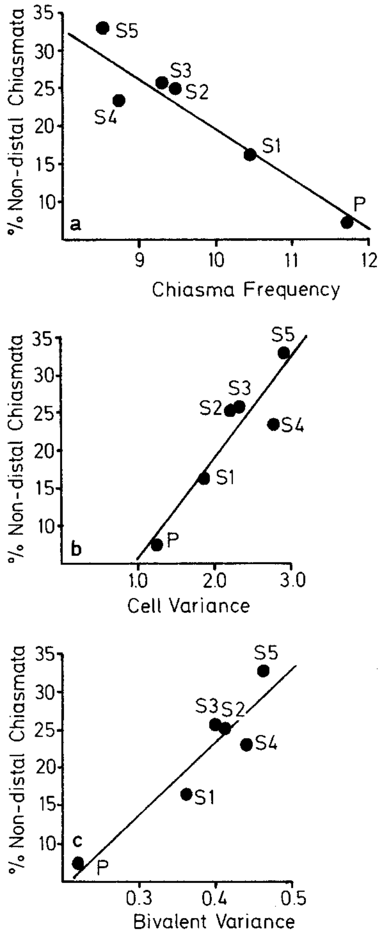

Fig. 6a–c. Graphs showing the interrelationships between the percentage non-distal chiasmata and the other three components of chiasma variation, using mean values for the generations

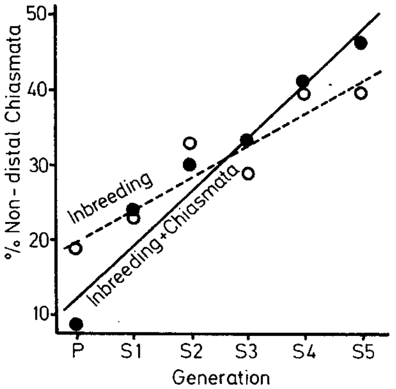

Fig. 7. Percentage non-distal chiasmata plotted against generations. The broken line (○ - - - - ○) is based on mean percentage non-distal chiasmata calculated from the pmc chiasma class range 9–11 only, and shows an effect due to inbreeding which is independent of chiasma frequency. The continuous line (●—●) is a plot using percent non-distals calculated from unselected data and shows a higher rate of increase and an effect due to inbreeding which is dependent upon chiasma frequency

tion, there is a strong negative correlation between percent non-distal chiasmata and chiasma frequency ($P < 0.01$, Fig. 6 a). When the regressions are calculated separately for individual pedigrees the same trends (in terms of slope) are still found, but only those of P3 and P30 are significant (Table 1). The strongest correlation is within the P3 pedigree, which is the one with the highest level of non-distal chiasmata in all of the inbred generations. It may also be recalled at this juncture that the P3 and P30 pedigrees were also the only ones to show a significant level of reduction in chiasma frequency with inbreeding (paper 1); and that in P15 there is no significant increase in percentage non-distal chiasmata with inbreeding either.

The difficulty in trying to elucidate the relationship between chiasma frequency and distribution is that both of these characters are affected (albeit in opposite directions) by inbreeding, and there is the problem of trying to separate out the inbreeding effects on distribution which are independent of chiasma frequency from those which are not. In attempting to do this we have restricted our detailed analysis to the P3 pedigree only, which is the one with the strongest expression for these characters, and we will make only brief reference to the other pedigrees where appropriate. The analysis is based in the first instance on the graph of non-distal chiasmata against generations shown in Fig. 7. When the mean percentage of non-distal chiasmata, using all the data from each plant within each of the generations, is plotted against generations, the regression line is positive and significant at the 1.0% level; and there is a rate of increase in non-distal chiasmata of 6.9% per generation. This increase is due to both the inbreeding effect as well as to the influence of the progressive reduction in chiasma frequency which is dependent upon inbreeding. In order to remove this chiasma effect a second line has been plotted, based on a selected range of pmc chiasma frequency classes (and therefore the same mean pmc chiasma frequency) within each of the six generations. This has been done by calculating the percentage of non-distal chiasmata within pmc classes of 9, 10 and 11 only, which are widely represented among the plants within each generation; and then taking the mean of the three classes. The regression line is again positive and significant ($P < 0.01$), but it now represents an effect due to inbreeding which is independent of chiasma frequency, and the rate of increase in non-distal chiasmata is now lower at 4.2% per generation. The difference in slope between the two regression lines ($P < 0.05$) gives us an indication that variation in mean pmc chiasma frequency itself may have a small effect upon the percentage of non-distal chiasmata, over and above that which is due directly to inbreeding. We are mindful of the statistical shortcomings of this argument, however, and have sought confirmation of the effect by extending the analysis to a more testing situation, namely that within the P3 S5 generation, where inbreeding is not a complicating issue. A regression analysis was carried out for percentage non-distal chiasmata against chiasma frequency, using non-distals calculated from each of the eight pmc chiasma frequency classes over the range 4–11. Each of these classes of pollen mother cell is again widely represented among the twenty-six plants comprising the P3 S5. The regression is negative and significant at the 1.0% level, showing that at the cell level the non-distal chiasmata are highest in pmcs with the lowest chiasma frequencies, and vice versa. This is a more sensitive and valid test than the one described above because the confounding effect due to inbreeding has been removed and we are dealing with the maximum level of non-distal chiasmata (S5) over a wide range of prescribed chiasma frequencies. From this analysis we can estimate that the level of change in non-distal chiasmata per unit of chiasma frequency variation within the P3 S5 is small, and of the order of 1.7%. The same relationship was found to hold for the P30 S5 generation as well ($P < 0.05$), and over the same pmc chiasma frequency range; but in P6 and P15 where the range of available pmc classes was smaller the regressions were non-significant, although the slopes were again negative. In the P30 S5 the rate of change of non-distal chiasmata was 1.2% per unit of chiasma frequency. The direct effect of chiasma frequency upon distribution is therefore quite small, but additional evidence for its existence is forthcoming from yet a further analysis within the P3 pedigree, this time at the level of the bivalent. In the P3 S5 dichiasmate ring bivalents the mean frequency of non-distal chiasmata is 43.3%; in the monochiasmate rods it is higher at 48.9%, a difference of five percent. There is no significant variation between the three S5 families for this character in either rings or rods, and when the data from families are pooled the difference in mean between the two forms of bivalent is significant ($P < 0.05$, t-test). In the P6 S5 the difference is even greater, 41.4 against 31.6, and the significance correspondingly increased ($P < 0.001$); in P15 and P30 the differences are small, but in the same direction. The effect is also confirmed for a sample of the WD lines, with values of 34.0 and 41.4% for rings and rods respectively ($P < 0.05$).

It would appear that chiasmata occurring in dichiasmate ring bivalents have a higher probability of forming in distal locations than do those occurring in monochiasmate rods, apparently as a result of an 'interference' effect which can act across the centromere. A reduction in chiasma frequency, in circumstances where pmcs contain mainly dichiasmate rings and monochiasmate rod bivalents as in P3 S5, leads to an increase in the proximal and interstitial localisation of chiasmata, and from what has been said above it would seem that a likely basis for this effect is a change in the ratio of rods to rings. The more rods the more non-distal chiasmata. In the P3 S5 generation the frequency of rings plus rods with more than one chiasma in the same arm is negligible (< 3.0%), and there is no possibility of these types influencing the relation between frequency and distribution. To test the theory that the change in the proportion of rods to rings forms the basis of the frequency/distribution relationship we have calculated the ratio of rod to ring bivalents over the same range of pmc chiasma classes (4–11) within the S5, and then plotted this ratio against non-distal chiasmata for each of the eight pmc classes involved. The regression is positive, and significant at the 1.0% level. When the number of rings in the different pmcs is plotted against the same range of classes there is also a strong positive regression ($P < 0.001$). Conversely the number of rings per cell against non-distal chiasmata gives a negative regression ($P < 0.01$). Essentially the same trends are found when the analysis is repeated using the variation over generations of inbreeding, rather than within the S5.

In summary it may be stated that there is strong evidence for a negative correlation between the proportion of non-distal chiasmata and pmc chiasma frequency, independent of any effect due to inbreeding, and that a basis for this relationship can be found in the changing ratio of rods to rings that is associated with variation in the number of chiasmata per cell. The relationship is best revealed in the most advanced S5 inbred generation with the maximum level of non-distal chiasma formation, and within an individual genotypic class showing high expression for the character. It must be stressed however that this effect is a relatively minor one, and that the bulk of the change in distribution pattern is due to the inbreeding effect which is independent of chiasma frequency.

In relating chiasma distribution to the model for control we see the level I response in the correlated behaviour of chiasma frequency and non-distal chiasmata to inbreeding. Evidence for level II control, which may affect either of the two variables independently, is argued along the following lines, albeit briefly. In the first place we have seen that chiasma frequency and non-distal chiasmata can vary independently of one another over generations of inbreeding. Secondly there is a difference in the pattern of segregation for the two variables within the S5. Non-distal chiasmata differ only between pedigrees whereas chiasma frequency variation can be found between families within pedigrees as well (paper 1). Thirdly there are segregating differences at the S5 for non-distal chiasmata within uniform ring and rod bivalent classes.

Table 1. Summary of regression analyses of variance for relationships between non-distal chiasmata and the other three components of chiasma variation at the level of individual pedigrees, over generations of inbreeding (ND = percentage non-distal chiasmata; Xta = pmc chiasma frequency; cv = cell variance; bv = bivalent variance)

Components	P3	P6	P15	P30
ND/Xta	<0.001	NS	NS	<0.05
ND/cv	<0.05	<0.001	NS	<0.05
ND/bv	<0.001	NS/<0.05	NS	NS

(ii) Cell Variance

Cell variance is positively correlated with the non-distal location of chiasmata when the two components are plotted as means of all individuals over generations of inbreeding ($P < 0.05$, Fig. 6 b). Regressions for the individual pedigrees are given in Table 1, and the strongest correlation is found within P6. The P15 pedigree, which is not significant, did not show any significance for cell variance against inbreeding either (paper 1). Independent behaviour of the variables, at level II control, is suggested by differences in segregation patterns: for cell variance, in contrast to non-distal chiasmata, there are no differences between S5 pedigrees, but there is segregation between two families ($P < 0.05$) within the P30 pedigree.

(iii) Bivalent Variance

As for cell variance, bivalent variance is positively correlated with non-distal chiasmata over generations of inbreeding ($P < 0.01$, Fig. 6 c). At the level of individual pedigrees the strongest correlation is within P3 (Table 1). Level II independent control is implied both by the absence of segregation for this character among S5 pedigrees, and its presence between two families within the P6 pedigree ($t = 2.5$, $P < 0.05$ for bivalent variance, and $t = 0.03$, NS for non-distal chiasmata).

Discussion

The components of chiasma frequency variation in *L. perenne* are under genotypic control, as indeed they are for the many other species that have previously been investigated (Rees 1961; John and Lewis 1965). For *L. perenne* a two-level model of control for the three components of chiasma variation (viz. chiasma frequency, cell variance and bivalent variance) was fully discussed in paper 1 (Karp and Jones 1982). Suffice it to say here that the component of distribution within bivalents has a similar quantitative basis to its variation to the other three and can readily be accommodated within the same scheme (Fig. 5, paper 1). The evidence for genotypic control comes from a breakdown of the normal pattern of localisation due to inbreeding, coupled with segregation among the S5 pedigrees of plants. The loss of control arises by regular increments over generations of selfing and the patterns of segregation can be traced back to genetic variation between individual parent plants. It is taken that the breakdown in control may be explained in the same way as that in rye (Rees 1955), namely genetic imbalance following the forcible change from a heterozygous outbreeding system to one of selfing and progressive homozygosity.

In some lines the changes are of large magnitude and there is indirect evidence from the correspondence between the patterns of chiasma distribution and U-type exchanges that the effects represent a real shift in the sites of chiasma formation rather than artefacts due to differential rates of terminalisation. This evidence will be presented separately in a subsequent paper in this series.

As far as the mechanism which determines chiasma distribution is concerned we can say little with reference to perennial ryegrass other than that it has a genetic basis, and that an element of chiasma interference may be involved as well. The question of interference is a long standing one and originates from a series of papers by Mather (Mather 1940) concerning the chiasma frequency/chromosome length relationship. According to Mather's scheme chiasma formation within a bivalent is sequential with the first formed one occurring at a differential distance *d*, with respect to the point of origin (centromere), and successive ones then spaced out at intervals determined by the interference parameter *i*. Support for this theory has been forthcoming from analyses of male meiosis in grasshoppers, where the point of origin appears to be at the telomere rather than the centromere as envisaged by Mather (Henderson 1963; Southern 1967; Fox 1973). The mechanism suggests a physical basis to the control and requires chiasmata to be formed in sequence one after another. Jones (1974), on the basis of studies with a rye distributional mutant, has taken issue with this sequential model and has argued that interference phenomena are manifestations or consequences of control rather than control mechanisms in their own right. Certainly in *L. perenne* it has been possible to induce large shifts in the distribution of chiasmata by breaking down the normal system of regulation, and there is every reason to believe that these changes result from genotypic effects rather than from any physical basis of chromosome reorganisation. We have found too that within this genotypic framework of change there is an interference constraint, which allows for a greater redistribution within the monochiasmata rods, than it does within the dichiasmate ring bivalents. This constraint holds across a range of genotypes within both the S23 and the WD lines; it is a small effect, as already mentioned, and does not appear to have the consistency of expression expected simply on the basis of a physical process.

A final point of discussion which presents itself from the *L. perenne* data given here is the question of why there should be such a strict control over chiasma localisation in the parent material anyway? The question is part of a much wider one and concerns the general issue of the role of chiasma frequency and distribution in relation to recombination. The evidence that change in an established pattern of distribution in a heterozygous outbreeder does result in novel recombinants and a release of variability is not particularly compelling, but what there is is summarised in Rees and Jones (1977). In relation to *L. perenne,* and to *L. multiflorum* and *Festuca pratensis* as well, Rees and Dale (1974) have described differences in chiasma frequency and distribution between natural populations, which are genetically determined, and which they believe to be adaptive. Short lived populations were found to have higher chiasma frequencies and a greater proportion of non-distal chiasmata than the longer lived more perennial ones; furthermore the short lived types displayed less variation, and less response to selection, for a number of quantitative characters. The argument is that in the short lived populations the high frequency, together with the higher proportion of non-distal chiasmata which is associated with it, has disrupted the super gene linkage groups in the mid regions of the chromosomes and released the variability. In the longer lived perennial forms with lower frequencies, and a more strict distal localisation of chiasmata, the super gene complexes have tended to remain intact and to preserve the potential variability. In relation to this model the S23 variety of *L. perenne* with which we are concerned can well be classified as a long lived perennial type of ryegrass. It is highly persistent, highly polymorphic and has a relatively low chiasma frequency with distal localisation. The pattern of distal localisation in the parent plants may therefore be seen as an adaptive device, along the lines proposed by Rees and Dale, which functions to preserve and to regulate genetic variation. With forced inbreeding the system of regulation has been broken down and chiasmata redistributed within the super gene complexes in the interstitial and proximal regions of the chromosomes. The extent to which this disruption and redistribution of chiasmata will lead to an extended range of variability, and the extent to which it may be of potential use for manipulation by the breeder, remains to be evaluated.

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Dr. R. N. Jones
Department of Agricultural Botany
The University College of Wales
Penglais, Aberystwyth
Dyfed, SY23 3DD (Wales)

Dr. A. Karp
Department of Biochemistry
Rothamsted Experimental Station
Harpenden, Herts. AL5 2JQ (England)