THE DISTRIBUTION OF QUANTITATIVE DNA CHANGES ASSOCIATED WITH THE EVOLUTION OF DIPLOID *FESTUCEAE*

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SUMMARY

Increase in nuclear DNA amount associated with the evolution of diploid species in the genus *Festuca* and the genus *Lolium* was achieved by an allocation of approximately equal amounts of DNA to each chromosome of the complement. Thus, each chromosome of the haploid complement of *Festuca drymeja* has approximately 0.17 pg more DNA than its homoeologous counterpart in *F. scariosa*. Each chromosome of *Lolium temulentum* has approximately 0.15 pg more DNA than its *L. perenne* homoeologue. Because small chromosomes within the complement acquire as much extra DNA as the larger chromosomes, it follows that the relative difference in size and DNA content is greater between the smaller than the larger homoeologous chromosomes of species with different DNA amounts. For this reason we should expect, and indeed have confirmed, that pairing at meiosis in interspecific hybrids is less effective for small than large bivalents. At the same time, however, observations at pachytene in F_1 species hybrids show that, despite large DNA differences, homoeologous chromosomes are capable of complete and effective pairing in a high proportion of pollen mother cells. In other words the effect of large differences in DNA amount upon the pairing of homoeologous chromosomes is surprisingly small.

A further consequence to be expected of the kind of DNA change which we have described is that the chromosomes of species with high DNA amounts will, in general, be more alike in size and DNA content than chromosomes within complements of species with low nuclear DNA amounts.

1. INTRODUCTION

LOLIUM and *Festuca* are genera of the tribe *Festuceae* in the family *Gramineae*. *Lolium* species are all diploids ($2n = 14$). The chromosome complements of inbreeding species are, however, very different from those of outbreeders in that they are comprised of larger chromosomes and contain about 50 per cent more DNA. *Festuca* species comprise both diploids and polyploids. The diploids, like the *Lolium* diploids, have 14 chromosomes. We find, as in *Lolium*, a very considerable variation between them, both in chromosome size and in nuclear DNA amount. This DNA variation, being independent of change in chromosome number, must of course derive from loss or gain of DNA within chromosomes. The present work is concerned with two problems. The first was to find out how the quantitative DNA changes associated with the evolution of these diploid *Festuceae* are distributed within complements. The second was to ascertain the consequences of such changes upon chromosome pairing at meiosis.

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2. MATERIALS AND METHODS

(i) *Nuclear DNA measurements*

Roots were fixed in 4 per cent neutral formaldehyde for two hours at room temperature ($23^{\circ}\text{C} \pm 2^{\circ}\text{C}$), washed in distilled water (several changes) for 24 hours, fixed in alcohol:acetic acid (3:1) and then stored at 4°C until required. Roots were then washed in distilled water, hydrolysed in 5 N HCl at room temperature for 60 minutes. After hydrolysis the roots were washed in distilled water, stained in Feulgen solution at pH 2.2 for one hour, passed through three changes of SO_2 water, again washed in distilled water and stored at 4°C overnight. Roots were squashed and mounted in 45 per cent acetic acid. DNA amount was estimated from absorption measurements at 565 nm in a Vickers M85 Scanning Microdensitometer. Ten 2C nuclei were measured in each of five to six replicates in each species. Values were made "absolute" by using preparations of *Allium cepa*, whose 2C nuclear DNA amount is taken to be 33.5 pg, as a standard.

(ii) *Preparations of meiosis and mitosis*

These were made after alcohol:acetic acid fixation by staining in aceto carmine and Feulgen stains respectively.

Seeds of plants of all the *Lolium* and *Festuca* species used were obtained from the Welsh Plant Breeding Station. We are especially grateful to Dr Martin Borrill and Dr Hugh Thomas of the Station not only for making available the material but for much advice and information about the species.

3. THE DISTRIBUTION OF DNA CHANGE BETWEEN CHROMOSOMES
FESTUCA(i) *DNA variation among diploids*

Table 1 shows the 2C nuclear DNA amounts in eight *Festuca* diploids. The differences between species are substantial and significant. *Festuca drymeja* has almost two and a half times more nuclear DNA than *F. alpina*. We shall deal with the taxonomic and phylogenetic implications of the DNA variation among these diploid and, as well, among polyploid *Festuca* species in detail elsewhere. However, it is worth noting that the distribution of DNA amounts corresponds closely with taxonomic status.

TABLE 1
The 2C nuclear DNA amounts (picograms) in diploid
Festuca species

Montanae	<i>F. drymeja</i>	7.17
	<i>F. donax</i>	6.36
	<i>F. altissima</i>	5.79
Scariosae	<i>F. scariosa</i>	4.73
Bovinae	<i>F. pratensis</i>	3.96
Ovinae	<i>F. polesica</i>	3.40
	<i>F. rupicaprina</i>	2.96
	<i>F. alpina</i>	2.88

(ii) *Festuca drymeja* and *F. scariosa*

Even though the 2C nuclear DNA amount in *F. drymeja* (7.17 pg) exceeds that in *F. scariosa* (4.73 pg) by 52 per cent the species produce viable hybrids. As would be expected the chromosomes of *F. drymeja* are considerably larger. The question is, where in the *F. drymeja* complement is the extra, supplementary DNA located? Is it found in all chromosomes of the complement? If so, how much in each chromosome and where?

(a) *The mitotic complements*

If we were to assume that rank in order of size corresponds with rank in respect of homology, namely that chromosomes 1, 2, 3 etc. in increasing order of size in *F. drymeja* were homologous or, more strictly, homoeologous with 1, 2, 3 etc. in *F. scariosa* we could easily answer the question as to how the extra DNA in *F. drymeja* chromosomes was distributed. Chromosomal DNA amount within a nucleus is correlated with chromosome size. Measurements of increase in size in each *F. drymeja* chromosome relative to its *F. scariosa* homoeologue would tell us how much of the extra DNA was located in each. Unfortunately we cannot make this assumption. We have no evidence that rank in order of size corresponds with rank in order of homoeology. It is for the same reason that comparisons of mitotic complements within the genus in general are of limited use in determining where the DNA changes are located. Because *F. drymeja* and *F. scariosa* may be hybridised, observations at meiosis in the F_1 hybrids enable us to recognise pairs of homoeologous chromosomes and, in consequence, to determine how the DNA differences are distributed among the chromosomes.

(b) *Meiotic configurations*

Fig. 1 shows a first metaphase of meiosis in the F_1 hybrid *F. drymeja* $\times$ *F. scariosa*. All seven bivalents are asymmetrical. The asymmetry is manifested mainly by a difference in length between homoeologues and to a lesser degree by a difference in chromatid contraction. The general asymmetry tells us that the extra DNA in *F. drymeja* is distributed among all seven chromosomes of the haploid complement.

FIG. 1.—First metaphase of meiosis in a pollen mother cell of the hybrid *F. drymeja* $\times$ *F. scariosa*. Horizontal bar ca. 10 microns.

(c) *Identifying the chromosomes*

To find out how much of the extra DNA is found in each of the *F. drymeja* chromosomes, we need to identify the *F. drymeja* and *F. scariosa* chromosome in each of the seven asymmetrical bivalents. In the absence of distinctive markers the problem can be resolved as follows.

The difference in nuclear DNA amount between *F. drymeja* and *F. scariosa* is 52 per cent. We could assume that the larger chromosome within each bivalent derived from *F. drymeja* and the smaller from *F. scariosa* given one condition, namely that the sum of the DNA amounts in the larger chromosomes exceeds the sum of the DNA amounts in the smaller by 52 per cent. Chromosome size, as we have stated, is proportional to DNA amount. By measuring the volumes of the large and small chromosomes in each bivalent within a cell we obtain estimates of their relative DNA amounts. (Volumes were estimated from measurements of chromatid length and width in photographs and assuming chromatids to be solid cylinders.)

(d) *The measurements*

The chiasma frequency at first metaphase in pollen mother cells of the F1 hybrid is very low. The mean frequency, based on scoring 20 pollen mother cells in each of five F1 hybrids, was 3.4 per cell, as compared with 9.6 in *F. drymeja* and 12.8 in *F. scariosa*. Even so we found three cells which contained not more than two univalents. Cells with more than two univalents are, of course, of no use for our purpose because it is not possible to say which of the univalent pairs are homeologues. From measurements in these three cells we show, in the upper row in table 2, the volumes of the smaller chromosomes in each pair in order of increasing size. Below are the volumes of their homoeologues. It will be observed that the difference between the volume totals of the small and large chromosomes is 42 per cent. That it is less than the DNA difference (52 per cent) permits us to conclude with confidence that the smaller members of each pair belong to *F. scariosa*, the larger to *F. drymeja*. One might well ask, however, why it is that the difference in volumes should be so much smaller than the 52 per cent DNA difference between species. The reason is made clear in fig. 2. Fig. 2(a) shows a bivalent with a terminal chiasma. The difference in volume between the homoeologues may be readily and precisely measured. In fig. 2(b) the chiasma is interstitial, but because the chromosome segments distal to the chiasma are of equal length there is no problem about estimating the volume of each of the two homoeologous. The difficulty

TABLE 2

The volumes of the small chromosomes in each of seven bivalents ranked in order of increasing size (top row) and, below, the volumes of their homoeologous partners. Volumes in arbitrary units. Festuca drymeja × F. scariosa

	98	113	124	136	157	175	197	1000
	150	176	186	205	222	220	265	1424
Difference	56	63	62	69	65	45	68	424
Percentage difference	53	56	50	51	41	26	35	42

FIG. 2.—Asymmetrical bivalents with a terminal chiasma (a), an interstitial chiasma with equal (b) and unequal (c) distal segments.

arises with bivalents of the kind shown in fig. 2(c). Here the chiasma is again interstitial but, in contrast to fig. 2(b), there is a difference in length not only between segments proximal to the chiasma but also between segments distal to the chiasma. It is, perhaps, more likely that the long segment distal to the chiasma “belongs” to the longer segment proximal to the chiasma. We cannot, however, be sure. We have in cases of this kind, therefore, restricted our measurements to the segments proximal to the chiasma. For this reason we should expect the difference between the volumes of large and small chromosomes to be underestimated.

(e) *The distribution of differences*

Before considering the results in table 2 in detail it is worthwhile considering what patterns of DNA change we should look for to account for the 52 per cent difference between complements. Two obvious possibilities are:

Each *F. drymeja* chromosome contains 52 per cent more DNA than its *F. scariosa* homoeologue. In this case the larger *F. drymeja* chromosomes would carry, in absolute terms, more extra DNA than the smaller *F. drymeja* chromosomes.

Each *F. drymeja* chromosome contains the same amount of extra DNA. Since the difference in total chromosomal DNA between the 1C complements is 1.22 pg it would mean that each *F. drymeja* chromosome carried 0.17 pg more DNA than its homoeologue. It would follow, also, that the percentage difference in DNA amount between *F. drymeja* and *F. scariosa* chromosomes would be greater for the smaller than for the larger homoeologous pairs.

Taking volumes as a reflection of DNA amounts we observe from table 2 that the difference in volumes, in absolute terms, is much the same for all chromosome pairs, whereas the percentage difference is greater for the smaller pairs. The indication is that the extra DNA in *F. drymeja* is distributed more or less equally throughout the complement *i.e.*, *ca.* 0.17 pg per chromosome. This conclusion is confirmed by the graph in fig. 3, where the volume of each of the larger, *F. drymeja* chromosomes is plotted against its smaller, *F. scariosa* homoeologue. The regression is highly significant, which means of course that the smaller *F. scariosa* chromosomes are, in general, homoeologous with the smaller *F. drymeja* chromosomes.

FIG. 3.—The volumes of the larger (*F. drymeja*) chromosomes in each bivalent at first metaphase of meiosis plotted against the volumes of the smaller homoeologous chromosomes of *F. scariosa*. Volumes in arbitrary units.

More important, the regression does not depart significantly from a slope of 1 ($b = 0.97$). A slope of 1 would be expected if the extra DNA were shared out equally among the *F. drymeja* chromosomes (as in (b) above). If the larger *F. drymeja* chromosomes had acquired more extra DNA than the smaller (as in (a) above) the slope of the regression would be greater than 1. If, alternatively, the slope were less than 1 it would mean that the smaller *F. drymeja* chromosomes had acquired more extra DNA than the larger.

To summarise, our evidence indicates that during the divergence and evolution of these species the chromosomes of *F. drymeja* acquired approximately equal amounts of supplementary DNA, *ca.* 0.17 pg. We appreciate that such a conclusion is valid only on the assumption that speciation was not accompanied by translocation of large chromosome segments between chromosomes within complements. The evidence is against such structural change. At meiosis in the *Festuca* hybrid, and in the *Lolium* hybrid described below, there is no multivalent formation such as would be expected as a result of translocation.

LOLIUM

(i) *DNA variation among diploids*

Table 3 shows the 2C nuclear DNA amounts in inbreeding and outbreeding *Lolium* species. The inbreeders have about 50 per cent more DNA than the outbreeders. *Lolium temulentum*, an inbreeder, has 6.23 pg of nuclear DNA. *L. perenne*, an outbreeder has 4.16 pg (2C values) and smaller chromosomes. As in *Festuca* the question is, where in the *L. temulentum* complement is the supplementary DNA located? As in *Festuca* the answer comes from observations at meiosis in the F1 hybrid, *L. temulentum* $\times$ *L. perenne*.

TABLE 3

The 2C nuclear DNA amounts (in picograms) in Lolium species (Data from Hutchinson, Rees and Seal, 1979)

Inbreeders	<i>L. persicum</i>	6.35
	<i>L. temulentum</i>	6.23
	<i>L. remotum</i>	6.04
	<i>L. loliaceum</i>	5.49
Outbreeders	<i>L. canariensis</i>	4.23
	<i>L. rigidum</i>	4.33
	<i>L. multiflorum</i>	4.31
	<i>L. perenne</i>	4.16

(ii) *Meiotic configurations in hybrids of L. temulentum and L. perenne: first metaphase*

Despite the 50 per cent difference in DNA amount, the chromosomes of *L. perenne* and *L. temulentum* pair effectively at meiosis in the hybrid (Rees and Jones, 1967). The bivalents, fig. 4, are all asymmetrical. As in the *Festuca* hybrid the DNA changes have affected all seven chromosomes. We have measured the volumes of the small and large chromosomes in each of the seven bivalents (table 4). On average the sum of the volumes of the large chromosomes exceeds the volumes of the smaller members of the seven bivalents by 39 per cent, well below 50 per cent. We can conclude therefore that the smaller chromosome in each bivalent derives from *L. perenne*, the larger from *L. temulentum*. That the percentage difference

FIG. 4.—First metaphase of meiosis in a pollen mother cell of the hybrid *L. temulentum* $\times$ *L. perenne*. Horizontal bar ca. 10 microns.

TABLE 4

The volumes of the small chromosomes in each of seven bivalents ranked in order of increasing size (top row) and, below, the volumes of their homoeologous partners. Volumes in arbitrary units from measurements in ten cells. *Lolium temulentum* × *L. perenne*.

	103	118	125	140	155	168	191	1000
	173	173	188	190	203	226	241	1394
Difference	70	55	63	50	48	58	50	394
Percentage difference	68	47	50	36	31	35	26	39

is that much lower than expected in the *Lolium* than in the *Festuca* hybrid is due, at least in large part, to the higher proportion of interstitial chiasmata which, as we have explained, makes for the underestimation of differences.

(a) *The distribution of differences*

Table 4 shows that the differences in volumes are at least as great within small as within large bivalents. If anything the differences are greater. This is confirmed in fig. 5 where we have plotted the volume of each of the *L. temulentum* chromosomes against the volume of its *L. perenne* homoeologue. The regression is significant. As in *Festuca* we may conclude that the smaller *L. perenne* chromosomes are in general homoeologous with smaller members of the *L. temulentum* complement. The slope of the regression, b , is 0.83 but not significantly different from 1 ($P = 0.10-0.20$).

FIG. 5.—The volumes of the larger (*L. temulentum*) chromosomes in each bivalent at first metaphase of meiosis plotted against the volumes of the smaller homoeologous chromosomes of *L. perenne*. Volumes in arbitrary units.

The extra DNA in the *L. temulentum* complement, therefore, is distributed roughly equally among the haploid set, about 0.15 pg per chromosome. This is much like the situation in *Festuca*, although there is a suggestion that the smaller chromosomes of *L. temulentum* carry slightly more extra DNA than the larger chromosomes (table 4).

4. THE DISTRIBUTION OF DNA CHANGE WITHIN CHROMOSOMES

(i) *Pachytene analysis*

On the face of it, the location of supplementary DNA *within* chromosomes should be readily determined and mapped by examining the pachytene associations formed by homoeologous pairs in hybrids between species differing in DNA amounts, such as those described above in *Festuca* and *Lolium*. The method is explained in fig. 6. The expectation is that chromosome segments rich in supplementary DNA material would be detectable as loops or overlaps in pachytene bivalents. Such loops and overlaps have been detected and described in *Allium* hybrids (Jones and Rees, 1968) and, indeed, in the hybrid *Lolium temulentum* $\times$ *L. perenne* (Rees and Jones, 1967). While loops and overlaps were detectable in these hybrids the pachytene chromosomes were too long and intertwined to make feasible a detailed comprehensive analysis. Pachytene in the *F. drymeja* $\times$ *F. scariosa* hybrid is very much more amenable to cytological analysis.

(ii) *The Festuca hybrid*

It will be recalled that there is widespread failure of pairing at metaphase of meiosis in pollen mother cells. The average chiasma frequency is between three and four per cell with generally not more than one chiasma per bivalent. The bivalents are formed by the larger homoeologous pairs; the smaller chromosomes provide the univalents. To a large degree the failure of pairing at metaphase is a reflection of failure of pairing at pachytene.

FIG. 6.—The formation of loops to be expected in pachytene bivalents involving homoeologous chromosomes of different lengths.

Most cells at pachytene contain chromosomes which are unpaired or partly paired, notably the smaller chromosomes of the complement. Even so a sufficient number of pachytene were found containing six or seven bivalents suitable for analysis.

Fig. 7 shows typical pachytene configurations in a pollen mother cell containing seven bivalents. The loops or buckles are clearly discernible in the four smaller bivalents. Even more interesting is the conspicuous absence of buckles in the larger chromosomes. The frequency with which loops appear in each of the seven bivalents are given in table 5 (data from seven pollen mother cells with seven bivalents at pachytene).

FIG. 7.—Pachytene configurations of each of the seven pairs of homoeologous chromosomes in the *Festuca* hybrid. Bivalents numbered in order of increasing length. Arrows denote loops in the smaller bivalents (1 to 4). Horizontal bar *ca.* 10 microns.

From these observations we conclude

(a) That despite large differences in length and DNA amount homoeologous chromosomes may pair side by side in intimate association throughout the length of the bivalent. This is especially true of the three larger bivalents. It will be recalled that these larger bivalents form chiasmata in most pollen mother cells. We conclude that the pairing not only looks effective but is so with respect to chiasma formation.

(b) Loops are frequent in the smaller bivalents. This should not surprise us. Our previous analysis showed that the smaller bivalents are more asymmetrical than the larger. Evidently the inordinate asymmetry impedes pairing in comparison with the larger homoeologues. Moreover the pairing is more often than not ineffective because these small chromosome pairs rarely form chiasmata. It is, even so, worth noticing that even these smaller pairs occasionally produce bivalents at pachytene with apparently "normal" pairing throughout.

TABLE 5

The frequency of loop formation in each of the seven bivalents of the Festuca hybrid. Bivalents in order of increasing size

Chromosomes	1	2	3	4	5	6	7
With loops	7	4	6	2	1	0	0
Without loops	0	3	1	5	6	7	7

The premise that supplementary DNA segments within chromosomes may be located by mapping the loops of unpaired chromatin at pachytene, as outlined in fig. 6, is clearly unsupportable (cf. Jones and Rees, 1968). While this, in one sense, may be unfortunate the demonstration that pairing at pachytene between homoeologous chromosomes of vastly different length and DNA content may be accomplished completely and effectively is, in itself, quite surprising and, indeed, of compelling interest. It poses problems which, on the one hand, bear upon the question of homology and, on the other, upon the mechanics of pachytene pairing. We shall consider these questions in detail in another paper. For the present we wish to emphasise the following points,

(a) That large differences in DNA amount, of the order of 20–40 per cent (table 2), do not prevent complete and effective pairing between homoeologous chromosomes at pachytene. This we conclude from the behaviour of the larger chromosomes in the *Festuca* hybrid at both pachytene and first metaphase of meiosis.

(b) That where the DNA differences become exaggerated, of the order of 50 per cent or more (table 2) as between the smaller homoeologues, the pairing at pachytene is more often than not incomplete and ineffective.

(b) is by no means surprising, (a) is very surprising indeed.

5. SUMMARY AND CONCLUSIONS

In both the *Lolium* and *Festuca* diploids investigated our evidence indicates that increase in nuclear DNA amount was achieved by roughly equal DNA increments to each chromosome of the complement. The distribution of extra DNA within the complement is not therefore at random, in other words it is not proportional to chromosome length. The smaller chromosomes of the complement acquire as much extra DNA as the larger. Indeed in *Lolium* they may acquire more. There is evidently a constraint upon the way in which the extra nuclear DNA is distributed. One of the consequences is that the smaller homoeologues in hybrids between the species differing in nuclear DNA amount are structurally more dissimilar than the larger homoeologues. As a result we may expect, as in the *Festuca* hybrid, that the smaller homoeologues will be more prone to failure of pairing at pachytene and to failure of chiasma formation. In this context it is, paradoxically, equally important to emphasise that large differences in DNA amount between homoeologous chromosomes do not necessarily prevent effective pairing at pachytene. This is made clear by the behaviour of the larger homoeologous chromosomes in the *Festuca* hybrid. In the *Lolium* hybrid the point is even more forcibly endorsed. Despite the large DNA differences between *all* homoeologous pairs in this hybrid the chiasma frequency averages well over one per bivalent. Indeed

the chiasma frequency in the *Lolium* hybrid (11.0 per pollen mother cell) is not significantly lower than that of the *L. perenne* parent, 11.6 (Hutchinson *et al.*, 1979).

Another consequence of the kind of DNA accretion which we have described is that an increase in the total nuclear DNA will lead to an increasing similarity in the size and DNA content of chromosomes within the complement. The smaller chromosomes "catch up" with the larger. The point is worth stressing. There is recent evidence to suggest that chromosome sizes and shapes may, in themselves, be of functional and adaptive importance (Mizuno and Macgregor, 1974; Rees *et al.*, 1978; Bennett, 1982). If so, the achievement of the appropriate shapes and sizes may be a not insignificant attribute of much of the repetitive, untranscribed DNA sequences so plentiful in the chromosomes of eukaryotes.

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