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Chromosome Relationships between *Lolium* and *Festuca* (Gramineae)

Prem P. Jauhar

Welsh Plant Breeding Station, Plas Gogerddan near Aberystwyth, SY23 3EB, Wales, U.K.

Abstract. With a view to eclucidating chromosome relationships between *Lolium perenne* (Lp), *L. multiflorum* (Lm) and *Festuca pratensis* (Fp), chromosome pairing in different diploid (2n=14), auto-allotriploid (2n=3x=21), trispecific (2n=3x=21), amphidiploid (2n=4x=28) and auto-allohexaploid (2n=6x=42) hybrids between them was analysed. At all these levels of ploidy there was very good chiasmate pairing between the chromosomes of the three species and, on the whole, there was little evidence of preferential pairing of the chromosomes of a particular species in the triploid, tetraploid and hexaploid hybrids. A critical test for this also came from the synaptic ability of the chromosomes of the single genome with those of the duplicated genome in the auto-allotriploids which formed predominantly trivalents with 2, 3 or even 4 chiasmata. Moreover, the homology between the Lp and Lm chromosomes seems strong enough to pass the discrimination limits of the B-chromosomes which do not suppress homoeologous pairing in the Lp LmLm triploid and LpLm diploid hybrids. — The triploids having two genomes of a *Lolium* species and one of *F. pratensis* had some male and female fertility which suggested genetic compatibility of the parental chromosomes resulting, presumably, in compensation at the gametic level. Also, the occurrence of comparable chiasma frequencies in the auto-allotriploids and trispecific hybrids showed that they were not markedly affected whether two doses of one genome and one of the other or all the three different genomes from the three species were present. From the trend of chromosome pairing in all these hybrids it is concluded that there is little structural differentiation between the chromosomes of the three species, no effective isolation barrier to gene-flow between them, and that they are closely related phylogenetically, having possibly evolved from a common progenitor. Taxonomic revision of the two *Lolium* species is suggested.

Introduction

Lolium and *Festuca* are highly valuable temperate genera of the grass family. There is a close proximity between these genera, and several hybrids between them, both natural and artificial, have been reported upon. Perennial ryegrass (*L. perenne* L.), Italian ryegrass (*L. multiflorum* Lam.) and meadow fescue (*F. pratensis* Huds.) are the most important diploid (2n=14) species of temperate grassland. Combining the desirable attributes of these species has obviously a great potential from the breeding point of view. A full understanding of the cytogenetic relationships between these species, however, would be of importance for effectively developing the breeding programmes already in progress at this Station and elsewhere.

Although the regular formation of seven bivalents in diploid (2n=14) hybrids between these species shows their phyletic relatedness, yet the two intergeneric diploid hybrids between *F. pratensis* and the two *Lolium* species are reported to be completely pollen sterile. This poses a challenging problem from the phylogenetic standpoint. In order to assess precisely the nature of chromosome differenti-

ation, if any, between these species, Jauhar (1975b) synthesized different auto-allotriploids incorporating two genomes of one species and one of the other. Chromosome pairing in such hybrids should be a critical test of affinities because the internal homologies of the chromosomes of different genomes can be best revealed by their synaptic competition in the auto-allotriploid condition. The same approach has therefore been continued and all the six auto-allotriploid hybrids between these species, together with some amphidiploids and auto-allohexaploids, were meiotically analysed. The nature of chromosome pairing in these hybrids combining different genomes in various proportions is reported and its phylogenetic significance discussed in this paper.

Materials and Methods

A wide range of material of perennial and Italian ryegrasses and meadow fescue available at this Station was used to make diploid (2n=14), auto-allotriploid (2n=3x=21) and trispecific (2n=3x=21) hybrids. Amphidiploids (2n=4x=28) and auto-allohexaploids (2n=6x=42) were synthesized by doubling the chromosomes of diploid and auto-allotriploid hybrids respectively. An Algerian collection of *Lolium perenne* (Ba 8973) which had 2n=14+1-3 B-chromosomes was used for making diploid or triploid hybrids with B-chromosomes. Several diploid collections of *Festuca pratensis*, Bf 55 (3), (11), (36), Bf 77 (6), and Bf 924, had different numbers of B-chromosomes[1].

Different diploid, triploid and trispecific hybrids were produced by hand emasculations and pollinations in the glasshouse. Although several hybrids could be obtained by using *Lolium* both as pollen and pistillate parent, better success was achieved using the embryo-culture technique (see Jauhar, 1975b) when *F. pratensis* was used as the female parent. Auto-allotriploids were synthesized by hybridizing the colchicine-induced autotetraploid of one species with the diploid of the other. Similarly, trispecific hybrids were made by crossing an amphidiploid between two species with the third diploid species.

All the auto-allotriploid and trispecific hybrids meiotically analysed in this study are listed in Table 2. Two of the triploid (Lp FpFp and Lm FpFp), and the trispecific hybrids were made by Mr. E. J. Lewis of the Station's Herbage Breeding Department, using the method described in Lewis and Jauhar (1974). Chromosome doubling by colchicine treatment of some diploid and triploid hybrids was done using dimethyl sulphoxide as a carrier which enhanced the efficacy of the treatment, as was also observed by Sanders and Hull (1970) and Kaul and Zutshi (1971). Mr. W. G. Morgan of the Station's Cytology Department helped with the colchicine treatments and subsequent chromosome counting.

For meiotic analyses panicles emerging from the flag leaf were fixed in 6:3:1 Carnoy's fluid to which a few drops of ferric chloride had been added. The anthers were stained with 1.5% acetocarmine or Snow's carmine (Snow, 1963). A combined staining with Snow's carmine and 1.5 aceto-orcein also gave very good differentiation between chromosomes and cytoplasm.

Pollen fertility was calculated by their stainability with one per cent cotton blue in lactophenol.

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1 *Abbreviations.* The following abbreviations are used in this paper: *Lolium perenne*—Lp; *Lolium multiflorum*—Lm; *Festuca pratensis*—Fp. The auto-allotriploids are abbreviated as Lp LmLm, LpLp Lm, Fp LpLp, Fp LmLm, Lp FpFp, and Lm FpFp, and the trispecific hybrids are designated as LpFp Lm, LmFp Lp, and Lm LpFp. The gap between two genomic designations indicates the direction in which the cross was made, the genome(s) contributed by the female parent appearing first and separated by a gap from the genomic contribution of the male parent. For example, Fp LpLp shows that Fp genome comes from the female parent, and in LpFp Lm, the Lp and Fp genomes were contributed by the maternal amphidiploid (LpLp FpFp) parent.

Results

A. Diploid Hybrids

Chromosome pairing and chiasma frequencies in the parental species and their diploid hybrids are summarized in Table 1. All the three different types of hybrids showed very good chiasmate pairing, but there were some differences between hybrids even from the same parents.

(i) L. perenne × *L. multiflorum.* These two species are easily crossable and are inter-fertile. The diploid hybrids had almost as good chromosome pairing as

Table 1. Chromosome pairing in some diploid species of *Lolium-Festuca* and their hybrids

Parents and hybrids	No. of plants analysed	Mean and range of configurations				B Chromosomes	Chiasmata		No. of cells scored
		IV	III	II	I		per cell ± S.E.	per paired chromosome	
L. perenne	3	—	—	6.93 (4–7)	0.15 (0–2)	—	11.32±0.12	0.817	50
L. multiflorum	3	—	—	7.00	—	—	12.24±0.08	0.874	75
F. pratensis	3	—	—	6.97 (6–7)	0.07 (0–2)	—	11.83±0.12	0.849	60
L. perenne × *L. multiflorum*									
Cross (i)	3	—	—	6.84 (6–7)	0.32 (0–2)	—	10.84±0.18	0.792	50
Cross (ii)	1	—	—	6.94 (6–7)	0.12 (0–2)	—	12.44±0.11	0.896	50
Cross (iii)[a]	2	—	—	6.87 (5–7)	0.33 (0–4)	2	11.33±0.10	0.823	30
L. perenne × *F. pratensis*									
Cross (i)	2	—	—	6.88 (6–7)	0.25 (0–2)	—	10.80±0.18	0.785	50
Cross (ii)	2	—	0.04 (0–1)	6.86 (6–7)	0.18 (0–2)	1	10.84±0.10	0.783	50
Cross (iii)	1	—	—	7.00	—	—	11.13±0.16	0.795	24
F. pratensis × *L. multiflorum*									
Cross (i)	2	0.02 (0–1)	0.08 (0–1)	6.12 (5–7)	1.45 (0–4)	—	10.02±0.19	0.798	60
Cross (ii)	2	—	—	6.84 (6–7)	0.32 (0–2)	—	10.86±0.16	0.794	50
Cross (iii)	2	—	—	6.82 (5–7)	0.36 (0–4)	1	10.80±0.24	0.792	25

[a] One desynaptic hybrid scored in this cross is not given here.

the parental species (Table 1). One hybrid had slightly more chiasmata per cell (12.44) and per paired chromosome (0.896) than either of its parents, suggesting some heterotic effect for chiasma formation. The number of univalents per cell, however, was marginally higher compared to that in the parents. Two of the hybrids included here had 2 B-chromosomes derived from the Algerian *perenne* (Ba 8973) parent, but they did not seem to affect pairing or chiasma formation (Table 1, Fig. 1). Fig. 1, for example, shows a metaphase cell with $7_{II}+2B$ having 12 chiasmata. The B's also formed a ring- or rod-bivalent in several cells.

A desynaptic hybrid (2n=14+2B) was also meiotically analysed but is not included in Table 1. In the desynaptic, the paired chromosomes fell apart precociously so that at diakinesis and metaphase mostly univalents were observed (Figs. 2 and 3). Fig. 2 shows a metaphase cell with $3_{II}+8_{I}+B_{II}$ and Fig. 3 shows all $14_{I}+2B_{I}$ at metaphase I. Meta-anaphase and subsequent stages, likewise, were irregular.

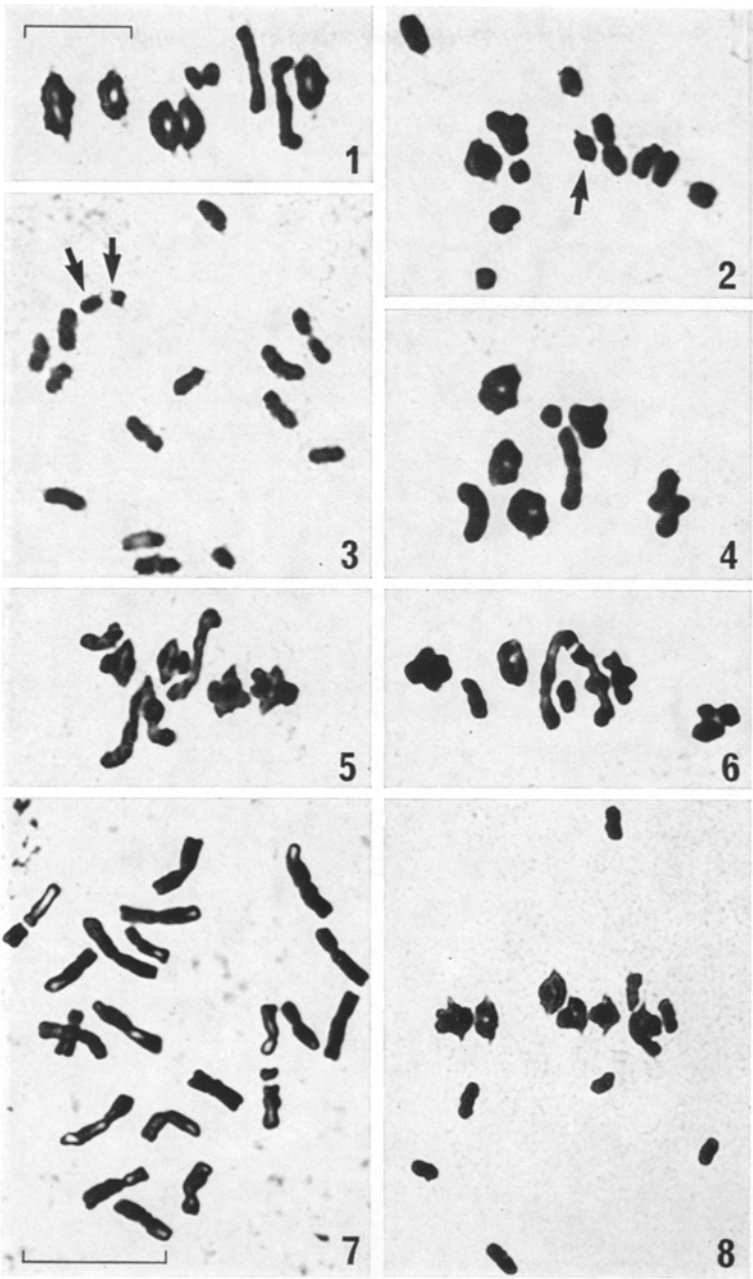
Very good seed set was obtained from these hybrids on open pollination and under selfing. F_2 progeny (selfed and open-pollinated) have been raised for meiotic analyses. There was a marked variation in morphology and vigour in the F_2 progeny. Even after one cycle of selfing there was appreciable loss of vigour. Although exactly *albina* type plants were extremely rare, *xanthas* and some other chlorophyll-deficient plants were observed.

(ii) L. perenne×*F. pratensis.* Several hybrids, some of which had B-chromosomes, were made in this combination, but only three crosses, one having a B-chromosome derived from the male *pratensis* parent Bf 77 (6), are reported here Meiosis was characterized by bivalent formation (Table 1), but cells with a trivalent+univalent and cells with two univalents were also observed occasionally. There was some variation in chiasma frequency between the three hybrid families and, since they involved the same parents, the results are comparable. A B-chromosome derived from the *Festuca* parent did not affect chromosome pairing or chiasma frequency in two hybrids (Fig. 4).

(iii) F. pratensis×*L. multiflorum.* Meiosis in two of the three crosses was characterized by bivalent formation. In one cross, however, a quadrivalent (Fig. 6) and some trivalents (Fig. 5) were observed (Table 1). In the 60 cells analysed in two hybrids, a total of one quadrivalent, five trivalents and 87 univalents were recorded in addition to bivalents. The chiasma frequency per paired chromosome was 0.798 which is comparable with other hybrids, two of which had a B-chromosome from the *Festuca* [Bf 55 (11)] parent.

(iv) Male and Female Fertility in the Intergeneric Hybrids. Although the six hybrid families between *F. pratensis* and the two *Lolium* species showed fairly good meiotic pairing with high chiasma formation (Table 1), there was high pollen sterility as judged by the non-dehiscence of anthers. However, the pollen fertility as judged by their stainability with cotton blue in lactophenol was not estimated.

Some female fertility was evident because some of the seeds set on these plants under open pollination were viable and F_2 plants have been established from them. Whether they resulted from backcrossing to one of the parents has yet to be decided from their morphology. Their chromosomal status is also not known so far.



Figs. 1—8. Cytology of diploid (2n=14) and auto-allotriploid (2n=21) hybrids of *Lolium-Festuca*. Figs. 1–6 and 8 have the same magnification. The bars represent 10 μm. Fig. 1. Metaphase in LpLm hybrid (2n=14+2B) showing $7_{II}+2$ Bs with 12 xta. Figs. 2 and 3. Metaphase cells in a desynaptic LpLm hybrid (2n=14+2B) showing $3_{II}+8_{I}+1\ B_{II}$ (arrowed), and $14_{I}+2$ Bs (arrowed), respectively. Fig. 4. $7_{II}+1$ B in LpFp hybrid with 11 xta. Figs. 5 and 6. $1_{III}+5_{II}+1_{I}$, and $1_{IV}+4_{II}+2_{I}$ in FpLm hybrids. Fig. 7. Somatic chromosomes of Fp LpLp triploid hybrids (under phase contrast). Fig. 8. Metaphase with $7_{II}+7_{I}$ with 16 xta in LpFpFp triploid

B. Auto-allotriploids

Meiosis in all the six different types of auto-allotriploids which had two genomes of one species and one of the other, *viz.* Lp LmLm, LpLp Lm, Fp LpLp, Fp LmLm, Lp FpFp and Lm FpFp, was analysed, several hybrids being studied in each genomic constitution. Most of the hybrids had 21 chromosomes. Hybrids with 20 or 22 chromosomes were observed occasionally but are not included in this report. Fig. 7 shows somatic chromosomes of an Fp LpLp hybrid. The data on chromosome pairing and chiasma frequency are listed in Table 2. Features such as lagging and dividing univalents at anaphase, micronuclei at telophase and tetrad stages and occasional formation of restitution nuclei were common to all the triploids. Mosaicism for chromosome number was also occasionally observed in some hybrids.

The most remarkable meiotic feature of all the auto-allotriploids was the formation of a large number of trivalents. In all of them, except Lm FpFp, a maximum of 7_{III} (the highest theoretically possible) was observed in several cells. The mean trivalent frequency per cell was also high (Table 2). In two Lp LmLm triploids which had a B-chromosome derived from the *perenne* parent (Ba 8973), 7_{III} were observed in several cells and the mean trivalent frequency was as good or even slightly higher than that in the three 0B-triploids of this genomic constitution. The data were therefore combined. Fig. 9, for example, shows a cell with $5_{III}+2_{II}$ ($+2_{I}$ not in the photograph)$+1$B having 18 chiasmata. Similarly, in one Fp LmLm triploid a B-chromosome from the *Festuca* parent (Bf 55–36) did not affect trivalent or chiasma frequencies. Thus, all these auto-allotriploids behaved meiotically like autotriploids. All the different types of trivalents like frying pan-, chain-, V-, Y- and rarely ϒ-, ϟ- or T-shaped were observed (Figs. 9–13). On the basis of mean trivalent frequency, however, they can be divided into two categories:

(i) High Trivalent Frequency Triploids. Fifteen triploids of four genomic constitutions, Lp LmLm, LpLp Lm, Fp LpLp and Fp LmLm, having either the three genomes from the two *Lolium* species, or two *Lolium* genomes plus one of *F. pratensis*, had a mean of 3.61 to 3.95_{III} per cell, and a maximum of 7_{III} (Fig. 12) was realized in all of them. Out of the total of 500 cells analysed in these auto-allotriploids, as many as 29 (5.8% of the cells) had 7_{III}, and 67 (13.4% of the cells) had $6_{III}+1_{II}+1_{I}$ (Fig. 10). Thus, there was little evidence of preferential pairing among the chromosomes of the duplicated genome to the exclusion of those of the single genome. A few multivalents higher than trivalents were also observed (Table 2). Chiasma frequency per cell and per paired chromosome varied from 14.84 to 15.72, and 0.790 to 0.867 respectively.

The mean percentage of pollen fertility as judged by their stainability with cotton blue in three types of triploids was: Lp LmLm=48.5; Fp LpLp=47.4; Fp LmLm=43.8. There was a marked variation in the size of stainable and non-stainable pollen grains, the small ones (Fig. 14) presumably having low number of chromosomes. In some hybrids of Lp LmLm and Fp LmLm genomic constitutions high pollen fertility (Fig. 14) was clearly reflected in anther dehiscence. The presence of female fertility was evident from the production of as many as 14 seedlings from about 800 apparently normal seeds set on them under open pollination. One

Table 2. Chromosome pairing in auto-allotriploid and trispecific hybrids in *Lolium-Festuca*

Triploid hybrids and genomic constitutions[a]	Number of hybrids analysed	Total no. of cells analysed	Mean and range of chromosome configurations						B chromosomes	Chiasmata	
			VI	V	IV	III ± S.E.	II	I		per cell ± S.E.	per paired chromosome
Auto-allotriploids											
(i) Lp LmLm[b]	5	150	—	—	0.03 (0–1)	3.80 ± 0.18 (0–7)	3.60 (0–7)	2.36 (0–6)	1	15.04 ± 0.14	0.809
(ii) LpLp Lm	3	100	—	—	0.02 (0–1)	3.76 ± 0.22 (0–7)	3.72 (0–8)	2.21 (0–5)	—	14.84 ± 0.17	0.790
(iii) Fp LpLp	4	160	0.03 (0–1)	—	0.05 (0–1)	3.95 ± 0.16 (0–7)	3.04 (0–6)	2.67 (0–6)	—	15.72 ± 0.14	0.858
(iv) Fp LmLm	3	90	—	—	0.01 (0–1)	3.61 ± 0.16 (0–7)	3.56 (0–7)	3.01 (0–5)	1	15.60 ± 0.13	0.867
(v) Lp FpFp	5	250	0.01 (0–1)	0.01 (0–1)	0.02 (0–1)	1.96 ± 0.10 (0–7)	5.03 (0–9)	4.87 (0–9)	—	16.32 ± 0.11	1.012
(vi) Lm FpFp	3	120	0.01 (0–1)	—	—	2.18 ± 0.15 (0–6)	4.84 (1–7)	4.71 (1–8)	—	17.20 ± 0.12	1.056
Trispecific hybrids											
(i) LpFp Lm	5	225	—	—	0.05 (0–1)	4.18 ± 0.12 (0–7)	2.88 (0–9)	2.48 (0–6)	—	15.16 ± 0.10	0.819
(ii) LmFp Lp	4	300	0.02 (0–1)	0.01 (0–1)	0.04 (0–1)	4.28 ± 0.11 (0–7)	2.80 (0–8)	2.24 (0–8)	—	14.45 ± 0.09	0.770
(iii) Lm LpFp	5	150	—	—	0.08 (0–1)	4.08 ± 0.12 (1–7)	2.76 (0–8)	2.96 (0–7)	—	15.00 ± 0.14	0.831

[a] Genomic contributions from the female and male parents are separated by a gap.
[b] Lp = *Lolium perenne*, Lm = *Lolium multiflorum*, Fp = *Festuca pratensis*. All the hybrids had 3x = 21 chromosomes.
Two Lp LmLm triploids had one B-chromosome from the Lp parent; one Fp LmLm triploid had a B from the Fp parent.

of these seedlings had 27 chromosomes, but the chromosomal status of the rest is not yet known.

(ii) Low Trivalent Forming Triploids. Eight triploids with the genomic constitution Lp FpFp and Lm FpFp, having one *Lolium* and two *Festuca* genomes, showed relatively lower trivalent frequency per cell, though a maximum of 7_{III} was realized in the Lp FpFp auto-allotriploids (Table 2). The mean trivalent frequency varied from 1.56 to 2.80_{III} per cell in the eight triploids studied. Therefore these auto-allotriploids also behaved meiotically more or less like autotriploids although there was some evidence of preferential pairing of the *Festuca* chromosomes as reflected in a higher bivalent frequency. Several cells with $7_{II}+7_{I}$ were observed (Fig. 8); the *Festuca* chromosomes probably paired as bivalents and the *Lolium* chromosomes remained as univalents in such cells. The Lp chromosomes, being smaller than Fp chromosomes, were discernible as relatively smaller univalents than the Fp univalents in the Fp LpLp or Fp LmLm auto-allotriploids. Pairing between *Festuca* and *Lolium* chromosomes was also evident in the formation of trivalents, some of them being heteromorphic (Fig. 11). Of the total of 370 cells analysed only 7 (1.9% of cells) had 7_{III} and 17 (4.6% of cells) had $6_{III}+1_{II}+1_{I}$ (Fig. 11). Frying-pan trivalents with 3 or 4 chiasmata were quite common, though allother shapes were also observed.

Another notable feature of these auto-allotriploids was the formation of a significantly ($P<0.001$) higher number of chiasmata per cell (16.32 and 17.20) and per paired chromosome (1.012 and 1.056) compared with the other triploids (Table 2). This was perhaps a reflection of more 'recognition sites' on the larger *Festuca* chromosomes which formed a high proportion of ring bivalents (Fig. 8) or frying pan trivalents with 3 or 4 chiasmata. Some higher multivalents were also recorded.

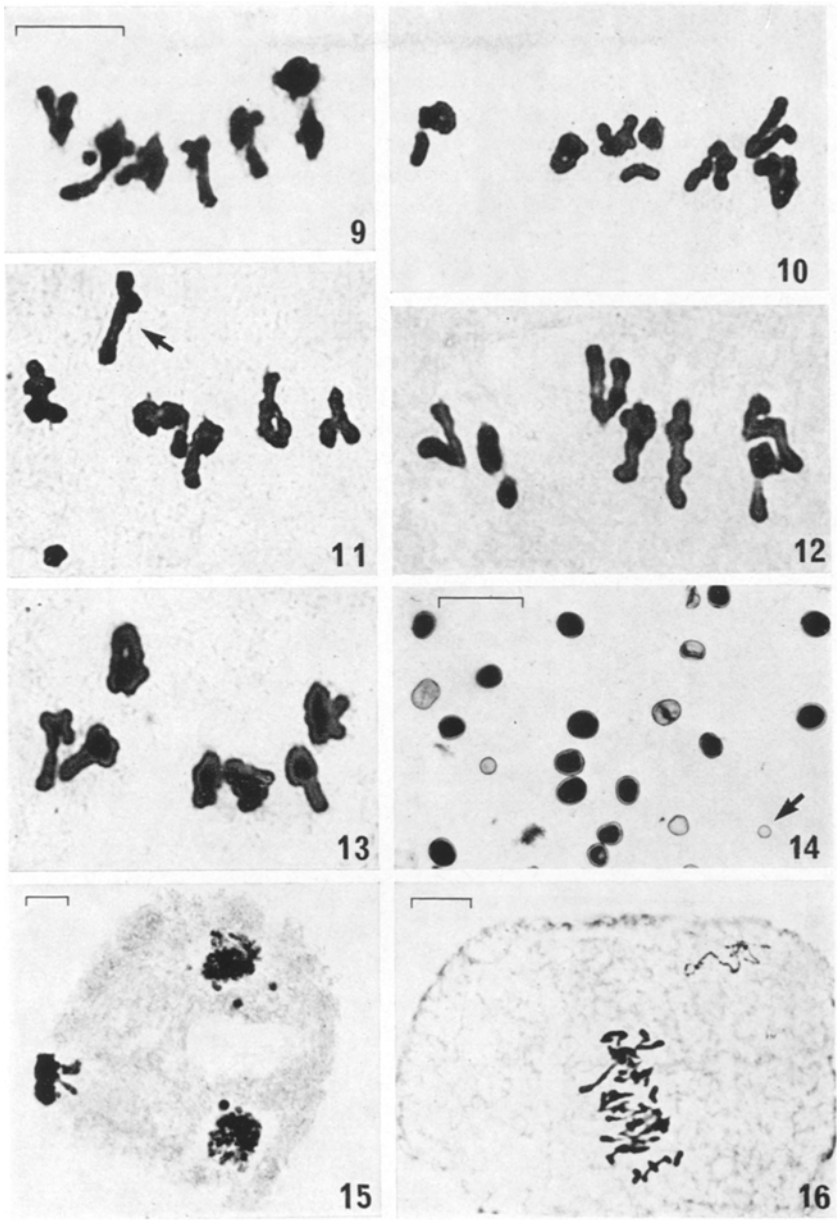
Pollen fertility, based on stainability, was not calculated, but lack of anther dehiscence clearly showed that the pollen fertility of these triploids was low.

C. Trispecific Hybrids

Fourteen trispecific hybrids of three genomic constitutions (LpFp Lm, LmFp Lp and Lm LpFp) having the three genomes in a common background were analysed. Meiosis in all of them was typical of that of an autotriploid, forming up to 7_{III} (Fig. 13) in numerous cells with mean trivalent frequency ranging from 4.08 to 4.28 (Table 2). Of the 675 cells scored, as many as 50 cells (7.4%) had 7_{III} and 98 cells (14.5%) had $6_{III}+1_{II}+1_{I}$. The mean chiasma frequency ranged from 14.45 to 15.16 per cell, and 0.770 to 0.831 per paired chromosome, a reflection of good pairing of chromosomes. Configurations higher than trivalents were recorded in some cells.

D. The Nature of Chromosome Pairing in the Auto-allotriploid and Trispecific Hybrids

(i) Inter-genomal Pairing. Intergenomal pairing was a predominant feature of all the six types of auto-allotriploids. The two homologous sets of chromosomes paired with the third related or homoeologous set to form trivalents (Fig. 12). This type of synapsis, restricted to two homologous and the third homoeologous set,



Figs. 9—16. Meiotic stages in some auto-allotriploid, trispecific and auto-allohexaploid hybrids. Figs. 9–13 have the same magnification. The bars represent 10 μm, except for Fig. 14. Fig. 9. Metaphase I in Lp LmLm triploid showing $5_{III}+2_{II}$ ($+2_{I}$ not in the photograph)$+1$ B with 18 xta. Note 4 frying-pan trivalents. Figs. 10 and 11. $6_{III}+1_{II}+1_{I}$ in Fp LpLp and Lm FpFp triploids, respectively. Note a heteromorphic trivalent in Fig. 11 (arrowed). Figs. 12 and 13. 7_{III} in Fp LmLm auto-allotriploid and LmFpLp trispecific hybrids, respectively. Fig. 14. Pollen grains of Fp LmLm triploid showing marked variation in size of stained and unstained pollen grains. Small pollen grains (arrowed) have, presumably, a small proportion of the chromosome complement. The bars represent 100 μm. Fig. 15. Tripolar spindle in LmLm FpFpFpFp auto-allohexaploid. Fig. 16. Metaphase in LpLp FpFpFpFp hybrid showing non-synchronised condensation of the chromosome complement (under phase contrast). While the condensed chromosomes are well congressed on the metaphase plate, uncondensed chromosomes (appearing like pachytene chromosomes) are placed towards the periphery

resulted in balanced pairing which formed equal numbers of bivalents and univalents. For instance, in the cells having $5_{III}+2_{II}+2_{I}$, the 5_{III} can be attributed to synapsis of five homologous pairs and the five homoeologous partners from the third genome, 2_{II} could have resulted from the homologous pairs, and the two remaining chromosomes of the single genome were left as univalents.

Additionally in the trispecific hybrids, pairing was restricted mostly to the homoeologous sets resulting predominantly in trivalents. Thus, when all the seven homoeologous sets paired, 7_{III} were formed (Fig. 13). A lesser number of trivalents, depending upon the complete homoeologous sets involved in pairing, was accompanied by the appropriate number of bivalents and univalents. Such balanced pairing, *e.g.* $6_{III}+1_{II}+1_{I}$, or $4_{III}+3_{II}+3_{I}$, was the dominant feature of all the 14 trispecifics.

(ii) Intra-genomal Pairing. Apart from balanced pairing restricted only to homologous and/or homoeologous sets of chromosomes, there was some evidence of intra-genomal, or inter-homoeologous group pairing in the auto-allotriploid and allotriploid hybrids. Chromosome configurations higher than trivalents (Table 2) could have formed only if such a pairing occurred. The formation of $3_{III}+5_{II}+2_{I}$, or $8_{II}+5_{I}$, or $9_{II}+3_{I}$ were also clear cases of unbalanced pairing which was present in about 6.5% of the cells in auto-allotriploids and 11.6% of the cells in the trispecifics.

E. Amphidiploids

The diploid hybrids reported above (Table 1) were treated with colchicine to produce amphidiploids. Only two amphidiploids of *L. perenne* × *L. multiflorum* and two of *F. pratensis* × *L. multiflorum* have been analysed for this report. Meiosis in all these amphidiploids was characterized by the formation of high frequency of quadrivalents (Table 3). In the two LpLp LmLm amphidiploids as many as 6_{IV} were recorded in some cells, the mean (IV+III) frequency per cell being 3.08, which is typical of that of an autotetraploid. Similarly, in the other two apparently intergeneric amphidiploids FpFp LmLm, a maximum of 5_{IV} was recorded in some cells, the mean (IV+III) frequency per cell being 2.10. Chiasma frequency per cell and per paired chromosome was also quite high in these amphidiploids (Table 3).

F. Auto-allohexaploids

The two auto-allohexaploids (LpLp FpFpFpFp and LmLm FpFpFpFp) derived by chromosome doubling of the auto-allotriploids (Lp FpFp and Lm FpFp) showed, rather surprisingly, a high frequency of hexavalents (Table 3). Even at that level of ploidy the chromosomes of *Lolium* and *Festuca* did not pair preferentially to form only bivalents and quadrivalents, but as many as 3_{VI} were recorded, the mean being 1.60_{VI+V} for the LpLp FpFpFpFp hexaploid and 1.16_{IV+V} for LmLm FpFpFpFp. Chiasmata per cell and per paired chromosome were also quite high (Table 3).

Meiosis in general was highly irregular resulting in high pollen sterility. An interesting feature was the non-synchronization of condensation of bivalents and the stage of development of meiosis in the complement. In some cells at diplotene

Table 3. Chromosome pairing analysed in some amphidiploids and auto-allohexaploids of *Lolium-Restuca*

Amphiploids and genomic constitutions[a]	No. of hybrids studied	Total no. of cells analysed	Mean and range of chromosome configurations						Xta per cell	Xta/ per paired chromosome
			VI	V	IV ± S.E.	III	II	I		
Amphidiploids (2n = 4x = 28)										
(i) LpLp LmLm[b]	2	50	—	—	2.26 ± 0.24 (0–6)	0.82 (0–3)	7.62 (2–14)	1.24 (0–4)	21.36	0.798
(ii) FpFp LmLm	2	60	—	—	1.65 ± 0.20 (0–5)	0.45 (0–2)	9.48 (4–14)	1.10 (0–4)	22.25	0.827
Auto-allohexaploids (2n = 6x = 42)										
(i) LpLp FpFpFpFp	1	20	1.15 (0–3)	0.45 (0–2)	3.45 ± 0.40 (2–6)	1.65 (0–5)	5.35 (2–10)	3.35 (1–6)	33.30	0.862
(ii) LmLm FpFpFpFp	1	25	0.92 (0–2)	0.24 (0–1)	4.44 ± 0.37 (2–7)	1.36 (0–5)	5.28 (2–9)	2.84 (0–6)	32.80	0.838

[a] Genomic contributions from the female and male parents are separated by a gap.
[b] Lp = *L. perenne*, Lm = *L. multiflorum*, Fp = *F. pratensis*.

or diakinesis, for example, while the majority of the bivalents showed good condensation and appeared as characteristically expected at diplotene or diakinesis, some chromosomes had the appearance of single threads typical of leptotene stage. Similarly, at metaphase I some bivalents had only progressed up to the stage characteristic of pachytene bivalents (Fig. 16). Such bivalents which lagged behind in development never seemed to catch up with the rest of the complement. It is difficult to determine whether they came from the neighbouring cells as a result of "cytomixis"—a phenomenon reported in some *Festuca* species and several other grasses of hybrid origin (Church, 1929a, b). The fact that the relatively uncondensed chromosomes were sometimes additional to the complement and were always placed near the periphery would, however suggest their being, "cytomixed" from the adjoining cells which were presumably at an earlier stage of meiosis than the recipient cells. Several disjunctional abnormalities like tripolar (Fig. 15) and quadri-polar spindles were also observed.

Discussion

Both natural and synthetic hybrids between *Lolium* and *Festuca* species have long fascinated evolutionists and plant breeders, and some of these hybrids are among the most widely studied in the grass family. However, phylogenetic relationships between these two extremely important temperate grass genera are far from clear. Diploid hybrids between some *Lolium* and *Festuca* species show regular meiosis with mostly 7_{II}, and chiasma frequency per cell and per paired chromosome in the hybrids is quite comparable to that in the parental species (Table 1). Although such good pairing is a reflection of the phylogenetic proximity between *F. pratensis* and the two most important species of *Lolium*, *L. perenne*

and *L. multiflorum*, the reported high, in some cases complete, pollen sterility of the two intergeneric hybrids merits further cytogenetic investigation. Moreover, it has been argued that chromosome pairing in hybrids cannot be taken as a true index of phylogenetic relationship. Darlington (1937) was among the first to question the validity of basing such conclusions on counts of bivalents and univalents in the hybrids, and it has been suggested that chromosome pairing in the amphiploids is a more realistic test of homology (Darlington, 1963; Stebbins, 1971).

As well as analysing chromosome pairing in diploid hybrids (2n=14) and their amphidiploids (2n=28), Jauhar (1975b) adopted an additional approach to studying chromosome differentiation in these species by analysing chromosome pairing in the triploid hybrids where one genome was present in a double dose and another in its haploid state. Analysis of synaptic competition in these auto-allotriploids should be a critical test of affinity of the chromosomes of the constituent genomes. Continuing the same approach, all the six auto-allotriploids and some of the derived auto-allohexaploids have been analysed in the present investigation.

I. Chromosome Pairing and Fertility of the Diploid Hybrids

(i) L. perenne×*L. multiflorum (2n=14).* Regular meiosis in the three diploid (2n=14) hybrid families with chiasma frequency per cell and per paired chromosome being as good as in the parents (Table 1) shows their close relationship, which is further borne out by their complete inter-fertility. There was some indication of heterotic effect for chiasma frequency in one of the hybrids. Thus, there does not seem to be any barrier to free gene-flow between the two species, and in this respect they do not satisfy the condition of being separate, valid biological species (see Jauhar and Joshi, 1970). However, meiotic analyses of the F_2 progeny of these hybrids are awaited before commenting further on their biosystematic status. The loss of vigour and the appearance of chlorotic plants in the F_2 population could be attributed to inbreeding depression in the progeny of these naturally out-breeding species.

Desynapsis in one of the F_1 hybrids (2n=14+2B) which showed high falling-apart of the paired chromosomes at diakinesis and metaphase I (Figs. 2, 3) cannot be attributed to B-chromosomes, because pairing in another two hybrids from the same cross, which also had 2 B-chromosomes, showed normal meiosis (Table 1; Fig. 1), chromosome pairing and chiasma formation being as good as in the other 0 B-chromosome hybrids (Table 1). It appears therefore that B-chromosomes from the Algerian *perenne* (Ba 8973) parent did not affect pairing or chiasma frequency in these diploid hybrids.

Some similar diploid *Lolium* species hybrids lacking pairing, described as asynapsis (Naylor, 1960), have been reported. This could be a reflection of recessive desynaptic gene(s) floating in the parental populations which express themselves in the hybrids and, consequently, result in desynapsis. The possibility of the occurrence of a spontaneous gene mutation which brings about this condition cannot be ruled out. Numerous cases of desynapsis are known in grasses and other species (see Jauhar and Singh, 1969), but desynapsis in the present hybrid was partial of the type reported in *Pennisetum typhoides* (Jauhar, 1969).

(ii) L. perenne×*F. pratensis (2n=14).* Similar frequencies of chromosome configurations and of chiasmata per cell and per paired chromosome in the three families, one of which had a B-chromosome derived from the *Festuca* parent (Fig. 4, Table 1), shows that the *Festuca* B, like the Algerian *perenne* B in the LpLm hybrids, did not affect pairing or chiasma formation. A B-chromosome derived from the Fp parent did not suppress homoeologous pairing in an auto-allotriploid Fp LmLm also (Jauhar, 1975b). Some differences in chiasma frequency per cell (Table 1) could be attributed to genotypic differences because these hybrids were derived from the same cross and grown in the same conditions and fixations were made on the same day. Similar genetically-controlled differences in chiasma frequencies in different populations and inter-population hybrids of *F. pratensis* were observed by Rees and Dale (1974).

(iii) F. pratensis×*L. multiflorum (2n=14).* Of the three hybrid families, some trivalents (Fig. 5) and a quadrivalent (Fig. 6) in one (Table 1) could either be attributed to the presence of a small translocation in one of the parental complements which on homoeologous pairing produces the observed multivalents, or to residual intra-genomal homology. If there is a segmental interchange, so many univalents (mean=1.45 per cell) would not be expected in this hybrid unless there is also a partial failure of homoeologous pairing. A detailed karyotypic analysis could provide an answer to this problem. It is likely that small structural rearrangements have occurred in some populations of these species.

A B-chromosome derived from the *Festuca* parent did not suppress homoeologous pairing as reflected in bivalent formation or chiasma frequency in two hybrids (Table 1).

Pollen sterility, as judged by the non-dehiscent anthers, in these intergeneric hybrids needs critical investigation. Although the chromosomal nature of their sterility was inferred from the fact that it is largely removed by chromosome doubling (see Lewis and Jauhar, 1974), this problem deserves reinvestigation, particularly in view of the fact that appreciable pollen stainability and some female fertility (see section II) is found in the Fp LpLp and Fp LmLm auto-allotriploids. If male sterility in these diploid intergeneric hybrids (LpFp and FpLm) is entirely chromosomal in nature, they would be expected to be female sterile also. However, a small F_2 population from hybrids grown under open pollination was established, clearly showing some female fertility. The role of unfavourable gene interactions in bringing about sterility in the intergeneric diploid hybrids cannot be completely dismissed. This inference receives support from the work of Reusch (1959) who found marked differences in the rate of endosperm growth and of viable seed production between different genotypic combinations of *L. perenne* and *F. pratensis*.

II. Chromosome Pairing and Fertility in Auto-allotriploids

(i) High Trivalent Forming Triploids. In four auto-allotriploids, Lp LmLm, LpLp Lm, Fp LpLp and Fp LmLm, the two homologous sets of chromosomes paired freely with the third related or homoeologous set to form predominantly trivalents. In all of them a maximum of seven trivalents was realized and the mean trivalent frequency was also very high (Table 2). Thus, all these genomically auto-allotriploids behaved meiotically as autotriploids and there was hardly any

evidence for preferential pairing of the homologues to the exclusion of their homoeologues. Even in the two intergeneric triploids, Fp LpLp and Fp LmLm, the presence of two sets of *Lolium* homologues did not put the chromosomes of single *Festuca* genome at a synaptic disadvantage because they had a mean of 3.95_{III} and 3.61_{III} per cell, respectively. These observations are in accord with those reported earlier for some auto-allotriploids (Jauhar, 1975b). Since even a small amount of differentiation can lead to preferential pairing (see Stebbins, 1950), it would appear that the chromosomal differentiation between *F. pratensis* and the two *Lolium* species is much too low to permit any noticeable preferential pairing. Furthermore, the little difference between trivalent and chiasma frequencies in the Fp LpLp and Fp LmLm triploids shows that there is hardly any noticeable differential affinity between homoeologues. This conclusion is further substantiated by the formation of up to 14_{II} in the 28-chromosome hybrids of *F. pratensis* (2n=14), *L. perenne* (2n=14) and *L. multiflorum* (2n=14) with *F. arundinacea* (2n=42) (Crowder, 1953).

There was no evidence of the suppression of homoeologous pairing as reflected in trivalent formation or chiasma frequency in the two Lp LmLm triploids which had a B-chromosome derived from the Lp (Ba 8973) parent or in an Fp LmLm triploid which had a B-chromosome from the Fp (Bf 55-36) parent. B-chromosomes have been reported to suppress homoeologous pairing in some hybrids betweeen *L. multiflorum* and *L. perenne* (Evans and Macefield, 1974), but no such effect of Bs was noticeable in the diploid or auto-allotriploid hybrids reported in this paper or in an Fp LmLm triploid reported earlier (Jauhar, 1975b). It is possible that either the Bs from a specific source suppress homoeologous pairing and/or the interaction of the Bs with a particular genotype produces this effect. The genotypes used by Evans and Macefield were different from the present hybrids, but even they noticed a rather small effect of Bs in the diploid hybrids and none in the derived amphidiploids. Since Bs from the same Algerian *perenne* parent markedly suppress homoeologous pairing in *L. temulentum* × *L. perenne* hybrids (Evans and Macefield, 1973), it seems that the homology between the *L. perenne* and *L. multiflorum* chromosomes is strong enough to pass the discrimination limits of the B-chromosomes.

(ii) Low Trivalent Forming Triploids. The other two apparently intergeneric auto-allotriploids (Lp FpFp and Lm FpFp), where the *Festuca* genome was present in duplicate, the trivalent frequency was, however, noticeably lower compared to the other triploids (Table 2). It may be mentioned here that the Fp chromosomes are somewhat larger than the Lp or Lm chromosomes as observed earlier also by Essad (1962, p. 43) and Malik and Thomas (1966, p. 172). Although a maximum of 7_{III} was realized in the Lp FpFp triploids also, low mean trivalent frequency and the formation of $7_{II}+7_{I}$ (Fig. 8) in many cells showed that the preferential pairing among the *Festuca* chromosomes was comparatively stronger, resulting in a greater exclusion of somewhat smaller *Lolium* chromosomes as univalents.

This could be explained on the basis of presence of more "recognition sites" or "zygomeres" (Sybenga, 1966) in the larger *F. pratensis* than in the *L. perenne* or *L. multiflorum* chromosomes. The presence of two doses of Fp chromosomes, therefore, puts the *Lolium* chromosomes at a synaptic disadvantage. This hypothesis of more "recognition sites" is borne out by the higher proportion of ring

bivalents, well reflected in significantly higher chiasma frequencies per cell ($P < 0.001$) and per paired chromosome in the Lp FpFp and Lm FpFp than in the Fp LpLp or Fp LmLm triploids (Table 2).

(iii) Male and Female Fertility. Despite the preponderance of trivalents in the auto-allotriploids, the percentage of pollen fertility, as judged by their stainability in cotton blue, was remarkably high in some of them (Lp LmLm=48.5; Fp LpLp =47.4; Fp LmLm=43.8). It is surprising to note that although LpFp and FpLm diploid hybrids are largely pollen sterile, yet when two doses of Lp or Lm genomes are present with one of Fp, fairly high pollen stainability and some female fertility is obtained in the auto-allotriploids. This could be due, at least partly, to the formation of balanced gametes with all or mostly *Lolium* chromosomes, but as the probability of the formation of such balanced gametes would be low, it is reasonable to assume that the pollen grains containing mixtures of apparently different, but presumably genetically compatible, *Lolium-Festuca* chromosomes may be stainable and seemingly functional. This perhaps could be interpreted as compensation at the gametic level, so that the absence of some *Lolium* chromosomes is made good by the presence of the corresponding chromosomes of *Festuca*. Although pollen viability was not tested by artificially culturing them, some amount of female fertility in these auto-allotriploids is confirmed by the production of F_2 plants from the seed set on them under open-pollination.

Apparently low pollen fertility as judged by lack of anther dehiscence in the Lp FpFp and Lm FpFp triploids is difficult to explain. It was caused, perhaps, by genomic imbalance. From the data available it appears that one genome of *Festuca* with two of *Lolium* confers more pollen fertility than when two genomes of *Festuca* are combined with one of *Lolium*.

III. Chromosome Pairing in the Trispecific Hybrids

(i) Inter-genomal Pairing. From the study of chromosome pairing in 14 trispecific hybrids (genomically LpFp Lm, LmFp Lp and Lm LpFp) incorporating the genomes of *L. perenne*, *L. multiflorum* and *F. pratensis* in a common background, it is clear that the chromosomes of these three species show very good pairing as reflected in high trivalent frequency (Table 2). A maximum of 7_{III} is found in 50 cells and $6_{III}+1_{II}+1_{I}$ in 98 cells of the 675 scored (mean of over 4_{III} per cell). Thus these trispecifics, like the auto-allotriploids mentioned above, behave meiotically as autotriploids. Moreover, chiasma frequency per cell and per paired chromosome is also high and quite comparable to those in the four auto-allotriploids Lp LmLm, LpLp Lm, Fp LpLp and Fp LmLm, and demonstrates that the genomic constitution of the triploids having two genomes of one species and one of the other or all the three different genomes from the three species, do not markedly affect chiasma frequency (Table 2). Also chromosome pairing and chiasma formation are not affected whether a particular genome(s) is contributed by the maternal or paternal parent, that is, no cytolpasmic effects are evident. These observations further show conclusively that the genomes of the three species are little differentiated, at least in terms of their pairing behaviour.

(ii) Intra-genomal Pairing. While in the majority of the cells there was inter-genomal and balanced pairing restricted only to homoeologous sets forming

either all trivalents or trivalents plus an appropriate number of bivalents and univalents, some intragenomal and/or inter-homoeologue pairing must have occurred to form multivalents (Table 2). Hexavalents, for example, could result from both homologous as well as intra-genomal pairing, indicating structural hybridity for segmental interchange in some chromosomes. Such segmental interchange could easily have occurred as a result of crossing over between the *L. perenne* and *L. multiflorum* chromosomes, or *Lolium* and *F. pratensis* chromosomes which had paired before the formation of gametes in the maternal amphidiploid parents used in this study. There is thus some evidence of small structural rearrangements between some of the species.

IV. Chromosome Pairing in the Amphidiploids and Auto-allohexaploids

That the *Lolium* and *F. pratensis* chromosomes are little differentiated is further borne out by the formation of quadrivalents in the LmLm FpFp amphidiploids and the formation of pentavalents and hexavalents in the LpLp FpFp FpFp and LmLm FpFpFpFp auto-allohexaploids (Table 3).

The formation of up to 6_{IV} (mean$=3.08_{IV+III}$) in the LpLp LmLm amphidiploids, shows that they behave meiotically as autotetraploids. Crowley and Rees (1968) observed almost similar quadrivalent and trivalent frequencies in the raw, unselected autotetraploids of *L. perenne*. However, in the advanced generation tetraploids selected for high fertility they found an increase in quadrivalent frequency. Thus, presumably, there was a natural selection for genes which ensure regular disjunction of quadrivalents, resulting in higher fertility of the tetraploids. On the other hand, in the LpLp LmLm tetraploids analysed by Mr J. Clarke of the Station's Cytology Department (see data in Thomas and Thomas, 1972, p. 118), there is a considerable "cytological diploidization" in the successive generations, presumably due to the natural selection of gene(s) which condition regular meiosis with bivalent formation as observed in synthetic tetraploids of *Pennisetum typhoides* (Jauhar, 1970).

The formation of as many as 5_{IV} (mean$=2.10_{(IV+III)}$ per cell) in the FpFp LmLm amphidiploids shows that they also behave meiotically more like autotetraploids than as allo-tetraploids. These FpFp LmLm and LpLp LmLm amphidiploids could be more appropriately considered as intervarietal autotetraploids (Stebbins, 1957) or *intra-specific autotetraploids* (Jauhar and Joshi, 1969), which means that they are autoploids having some hybridity though of the infraspecific level. Essad (1956) also found a mean of $2.89_{(IV+III)}$ per cell in the LpLp FpFp amphidiploids as compared to the marginally higher mean of $3_{(IV+III)}$ in the autotetraploid LpLpLpLp produced by chromosome doubling of the same parent as used in the production of amphiploids. Since the same genotype of *perenne* was doubled to produce autotetraploids, Essad's data provide a critical test that the LpLp FpFp amphidiploids behave meiotically as autotetraploids and that there is little differentiation between the chromosomes of *L. perenne* and *F. pratensis*. The present results support Essad's (1962) conclusion that *L. perenne* is a modified form of *F. pratensis*.

It is remarkable that Nitzsche (1974) has obtained some plants like *L. perenne* in the progeny of diploid hybrids between *F. pratensis* and *L. multiflorum*, a

situation similar to the reconstruction of *Triticum vulgare* and *T. spelta* from crosses between *T. compactum* and *T. dicoccum* (MacKey, 1963). From these results it appears that the dividing lines between the three species are conditioned by a single or few recombinational units. If these species indeed differ by a few genes, it may be possible to transmutate one species into another by induced mutagenesis as has been done in the hexaploid species of *Triticum* (Swaminathan, 1963). Single gene (or recombinational unit) differences between the hexaploid *Triticum* species led MacKey (1954) to group *T. vulgare*, *T. compactum*, *T. spelta* and *T. sphaerococcum* as subspecies of *T. aestivum* L., a system with which the wheat breeders and taxonomists now widely agree (see Sears, 1959). A similar taxonomic revision of the species in the *Lolium-Festuca* complex could perhaps be conveniently found, based on their cytogenetic relationships.

Formation of hexavalents and pentavalents resulted in highly irregular meiosis and pollen sterility, as judged by completely non-dehiscent anthers, in the auto-allohexaploids (Table 3). It is interesting that even at that level of ploidy when the Fp genome is present in quadruplicate with an Lp or Lm genome in duplicate, the chromosomes of the two genera should pair to form up to 3_{VI} (a mean of 1.60_{VI+V} in LpLp FpFpFpFp, or 1.16_{VI+V} in LmLm FpFpFpFp). These could also therefore be termed according to Jauhar and Joshi's terminology as *intraspecific autoploids*. Carnahan and Hill (1955) also produced some auto-allohexaploids by chromosome doubling the *L. perenne* (2n=14)×*F. elatior* L. (*F. pratensis* Huds., 2n=28), but they could not be studied meiotically. It was suggested, however, by these workers that there was a possibility of combining desirable characters of these hexaploids with those of the widely adapted hexaploid tall fescue. The present studies do not support this suggestion of Carnahan and Hill because of the highly irregular meiosis and high pollen sterility of these auto-allohexaploids. Lewis (1966) has already made some progress in combining desirable characters of *Lolium* species with those of tall fescue, and this programme could well be more effectively carried out as the genetic control of chromosome pairing in the latter has been demonstrated (Jauhar, 1975 a, c).

Conclusion

On the basis of chromosome pairing in the different diploid (2n=14) auto-allotriploid (2n=3x=21), trispecific (2n=3x=21), amphidiploid (2n=4x=28) and autoallohexaploid (2n=6x=42) hybrids between the three species *Lolium perenne*, *L. multiflorum* and *Festuca pratensis*, it is concluded that there is little differentiation between the chromosomes of these species, and that they are closely related phylogenetically, having possibly evolved from a common progenitor.

The synaptic ability of the chromosomes of the three species, despite the presence of their own homologous partners at different levels of ploidy, coupled with the genetic compatibility of their chromosomes, showing, presumably, compensation at the gametic level which results in some male and female fertility in the auto-allotriploids, and their easy crossability in nature and by man, all provide conclusive evidence that there is no effective isolation barrier to free gene-flow between these species. Inter-specific gene transfer may be possible from the standpoint of breeding. In the light of these findings there is ample justification

for these three species being in the same genus *Festuca*, an old, diverse and well adapted genus of the Festuceae, and that the two *Lolium* species *L. perenne* and *L. multiflorum* could, in fact, be varieties of the same species.

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