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Cytogenetic Studies on the F_1 Hybrid of *Lolium multiflorum* × *L. rigidum* and the Species Relationship in the Genus *Lolium*

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Summary. Successful crosses have been made between *Lolium multiflorum* × *L. rigidum* and the F_1 hybrids cytologically compared with the two parents with respect to karyomorphology and chromosome pairing. The hybrid showed considerable homologies between the parental chromosomes though some amount of differentiation had also occurred. The extent of differentiation suggests that phylogenetically the species are related and possibly separated quite recently. A model is presented to account for the differentiation of sections *Craepalia* and *Eulolium* from a common stock. Presumably the ancestral form existed in the Mediterranean and was, briefly, an outbreeder with symmetrical karyotype.

Introduction

The genus *Lolium* comprises of 8 morphologically distinct species which have been artificially intercrossed with a view to study their breeding affinities. However sufficient investigations have not been carried out on the cytogenetical aspects of the hybrids (TERRELL 1966). In a previous paper MALIK and THOMAS (1966) described the karyomorphology of several *Festuca* and *Lolium* species including *L. multiflorum* and *L. rigidum*, in detail. The characteristics of the karyotypes in these two species indicated that chromosomes were apparently similar. The mean length of the chromosomes, however, differed between the two species: chromosomes in *L. rigidum* being smaller.

It is generally accepted that the cytogenetical information relative to chromosome pairing of hybrids is helpful in assessing species relationships. To interpret successfully the genomic relationship the two species were hybridized and a detailed meiotic study in the hybrids was undertaken to elucidate the extent of chromosome pairing. The differences in chromosome size between the two species could be of some help in determining the mode and extent of the differentiation between the parental species. Taking the present data in conjunction with the past an attempt is also made to consider the general problem of speciation in the genus *Lolium*.

Material and Methods

During 1964—65 a large number of controlled reciprocal crosses were made among several genotypes of *Lolium multiflorum* and *L. rigidum* using emasculation and hand pollination techniques. For cytological analysis whole heads of parents and their F_1 hybrids were fixed in Carnoy's (6:3:1) to which had been added a few drops of a saturated solution of ferric chloride. Smear preparations of anthers were made in acetocarmine.

Results

The reciprocal crosses between the two species were quite successful and yielded about 65—69% seeds. Majority of these seeds germinated (90%) and gave rise to F_1's. Most of the seedlings died out at an early stage of growth and only 15% survived; of these only 6 flowered in late February. The results indicated good amount of intercompatibility.

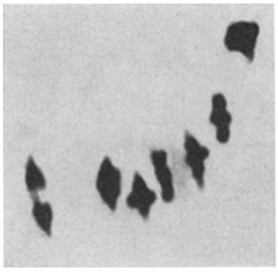


Fig. 1. First metaphase plate (7_{II}) in *L. multiflorum*.

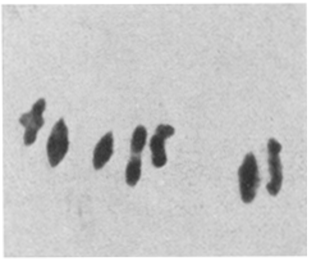


Fig. 2. First metaphase plate (7_{II}) in *L. rigidum*.

Data on chromosome pairing at Metaphase-1 in the two parental populations and their F_1 hybrids are given in Table 1. It will be seen that both the parents had predominant bivalent formation. Figures 1 and 2 show 7_{II} at M-1 in *L. multiflorum* and *L. rigidum* respectively. In *L. multiflorum* majority of the bivalents had 1—4 chiasmata per bivalent. The mean chiasma frequency per cell was 14.69 and the chiasmata ranged from 11—16. In *L. rigidum* the chiasmata ranged from 10—14 with a mean value of 13.66 per cell. In general the disjunction of the chromosomes at Anaphase-1 was regular followed by a normal second division. The pollen fertility was 93.8% in *L. multiflorum* and 90.6% in *L. rigidum*.

Table 1. Chromosome associations at Metaphase-I in *Lolium multiflorum*, *L. rigidum* and their F_1 hybrid.

	Univalents /cell	Bivalents with Xta				Multivalents /cell	Xta/ chromosome
		1	2	3	4		
L. rigidum	0.00	0.93	5.43	0.62	—	— —	0.97
L. multiflorum	— — —	0.25	5.85	0.83	0.06	— —	1.05
Hybrid (F_1)	0.38	2.48	4.07	0.16	—	0.031	0.80

Fig. 3. 7II with some of the bivalents being heteromorphic.

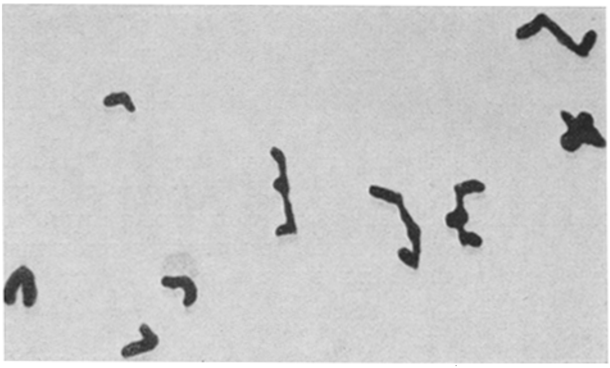


Fig. 4. Metaphase-I with 5II + 4I — note the unequal univalents.

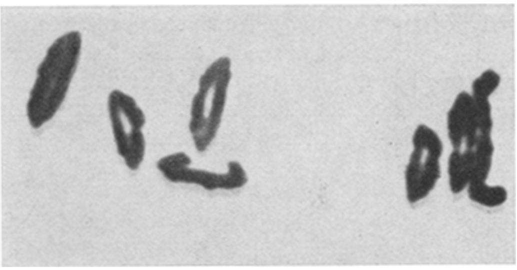


Fig. 5. One bivalent with mis-orientation.

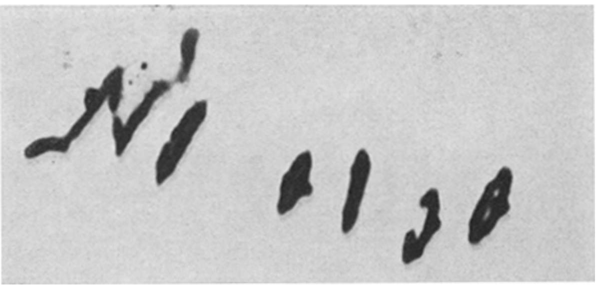


Fig. 6. P.M.C. with 5II + 1IV.

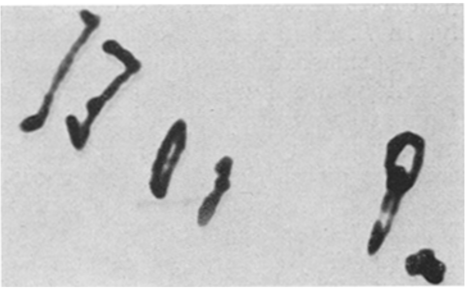


Fig. 7. Cell plate with unequal armed bivalents, some bivalents show interlocking.

Figs. 3—7. First metaphase plates in the F_1 hybrid.

Figures 3—7 depict the various types of P.M.C's at M-1. Though there was a high amount of pairing between the chromosomes of two species yet meiosis could not be classified as completely regular since univalents and multivalents were also observed. The majority of the cells had 7 bivalents (Figs. 3, 5 and 7). The average number of univalents per cell was 0.38 and they ranged from 0—4. Figure 4 shows 5II + 4I; univalents within the cell varied in size at M-1. On the whole 62% bivalents had two, 30% one and 8% had three chiasmata. Sometimes heteromorphic bivalents with unequal arms, 1—4, were also observed (Figs. 3, 4 and 7). Bivalents with misorientation (Fig. 5) and interlocking were also noticed (Fig. 7).

Of the 200 cells studied 9 contained multivalents; of these 7 cells had one quadrivalent each and two had one trivalent. The multivalents were of the disjunctional type. The mean chiasma frequency per cell was 11.27 and they ranged from 7—14. Anaphase-1 was generally normal, but more often it showed lagging chromosomes, some of these even divided at this stage. In many cells irregular number of chromosomes was present at each pole. In the second division misdivided chromosomes could be seen rather frequently. As a result of irregular meiosis, abnormal tetrads were commonly found. The pollen fertility was 5.8% and the grains varied in diameter from 14—18 μ.

Besides, certain other abnormalities were also observed. In some hybrids anthers with aneuploid chromosome numbers ranging from 10—16 per cell were found. Such cells could have arisen as a result of abnormal premeiotic divisions. Polyploid cells with 3—18 nuclei were noticed in some plants. Most of these cells exhibited synchronized divisions. These cells probably owe their origin to the failure of cytokinesis during premeiotic divisions. Spiralisation upsets were observed in some bivalents. Sometimes bivalents had centromeric upsets and were either mono-syntelic or bi-amphitelic (vide HENDERSON 1962). Isolated cells with suppression of wall formation were encountered. This failure of wall formation probably occurred subsequent to first or second division.

Discussion

The genus *Lolium* comprises of 8 species which fall into allogamous (*L. perenne* group species) and autogamous group (*L. temulentum* group species). The species belonging to the two sections have been extensively hybridized and the interspecific hybrids frequently reported (see TERRELL 1966). It is generally demonstrated that intragroup crosses are strongly interfertile compared to intergroup crosses.

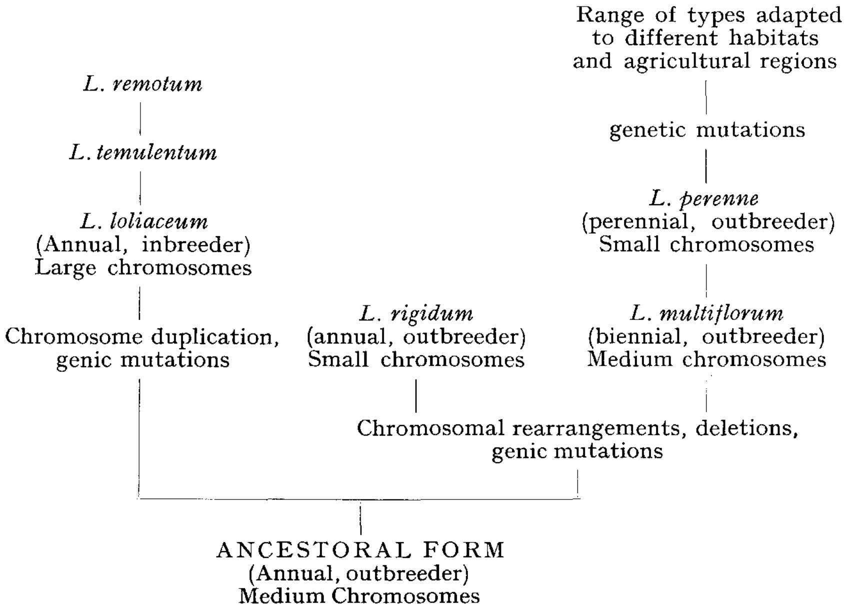
Lolium multiflorum has been crossed with other species but successfully hybridized with authentic *L. rigidum* for the first time in the present studies. The conclusions drawn from the studies are in accord with the general findings reported by TERRELL (1966) that intragroup species crosses in the genus *Lolium* yield high fertility.

A detailed meiotic study of the hybrids between the two species provides an opportunity to elucidate parental genome homologies and their phylogenetic relationship. Majority of the cells in the F_1, like the two parents, had seven bivalents. The mean chiasma frequency per cell of the F_1 was also comparable to the parents, showing no effective reduction in pairing in the hybrids. As pointed out earlier the basic karyotype of the two species is also quite similar. That some differentiation, however, exists with respect to the complement of two species is evident from the disparity in the size of the chromosomes complements of two species since *L. rigidum* had smaller chromosomes compared to *L. multiflorum*. This is further supported by the occurrence of non-pairing of some of the chromosomes, heteromorphic bivalents, multivalents and univalents of unequal sizes in the F_1. These features clearly indicate some amount of structural differences between the chromosomes of the two species. The differences between the parental species are not, however, of that magnitude to upset the associations of chromosomes at meiosis to a marked degree. In fact it appears that there is some amount of duplication in the segments of *L. multiflorum* with respect to *L. rigidum* complement. Consequently the hybrids between the two species could not be expected to show extensive failure of pairing at meiosis. From the phylogenetic point of view the apparent similarity of the basic karyotype and the ease with which species could be interbred points towards their relationship and the two species might have separated only fairly recently.

According to JENKIN (1955) *L. perenne* and *L. multiflorum* are two closely related species. Sometimes it is preferred to regard these two forms as subspecies (NAYLOR 1960). On the basis of divergent results in crossing these two species with those of the genus *Festuca*, some people question their closeness. The crosses between *L. multiflorum* × *L. rigidum* are also fully fertile and in this way it is comparable to the said situation. Indeed the results of hybridization among the three species (*L. multiflorum*, *L. rigidum* and *L. perenne*) indicate good intercompatibility. In addition they overlap in their geographical distribution and have an apparently similar basic karyotype. These features point towards insufficient isolation between these forms and one may be tempted to rank them as subspecies. Despite all this three forms can be distinguished morphologically and there are indications of some chromosome differentiation as well (MALIK and THOMAS 1966). Thus there seems to be sufficient justification in ranking each one of these forms as separate species. One may suggest that the genomes of the three species are partially homologous and the species had had a monophyletic origin. This suggestion would, thus, justify a general consideration of speciation in the genus *Lolium*. As stated earlier seven of its species are included in two species groups apparently classifiable as allogamous (*Lolium*) and autogamous (*Craepalia*). Whereas species in the section *Lolium* are outbreeders and range from annual (*L. rigidum*), biennial (*L. multiflorum*) to perennial (*L. perenne*), species in the section *Craepalia* are all annuals and inbreeders. It is generally accepted that in evolution inbreeding forms evolve from outbreeders (DARLINGTON and MATHER 1949). This would mean that species of the section *Craepalia* are derived from those of *Lolium*. Indeed NAYLOR and REES (1958) found that *L. temulentum* (*Craepalia*) had chromosomes one third larger than those of *L. perenne* (*Lolium*) and pointed out that in *L. temulentum* duplication of parts of chromosomes had occurred relative to *L. perenne* and the former had a secondary origin.

According to ESSAD (1962) *L. perenne* was the original species of the genus *Lolium* and through progressive evolution gave rise to forms like *L. rigidum* and the autogamous group of species represented maximum evolution of the group. On the basis of karyomorphology, however, this is not tenable. The karyotype of *L. perenne* belongs to 2a symmetry which involves shifts in the centromere position and *L. rigidum* has 1a type of symmetry (see MALIK and THOMAS 1966). Furthermore, it is interesting to observe that *L. rigidum* (belonging to *Lolium*) is allogamous but strict annual. Thus it resembles the section *Craepalia* in its annual habit and section *Lolium* in its breeding behaviour. This would perhaps tempt one to suggest that this species forms a connecting link between the two sections. This situation is, however, not well supported on the cytological ground since in the section *Lolium* karyotype consists of satellited chromosome pair of J type. In the *Craepalia* section of species karyotype has J and E types (MALIK and THOMAS 1966). The J type has proximal secondary constriction and the E type has distal. This difference between the J and E types could be explained on the basis of chromosomal rearrangement involving pericentric inversion. Above all the chromosomes in the section *Craepalia* are larger than those of the section *Lolium*. It will be recalled that NAYLOR and REES (1958) suggested chromatin duplication in *L. temulentum* relative to *L. perenne* and its secondary origin. If the above assumption is correct, obviously, one should expect larger chromosomes in *L. rigidum* compared to the other outbreeders. This is not the case as has been pointed out earlier. Clearly these view points regarding species differentiation in the genus *Lolium* are not well satisfactory and for this reason a model is presented.

According to this both the sections *Lolium* and *Craepalia* were derived from a common ancestoral form.



It is suggested that the original form was outbreeder and annual with basic number of x = 7. Its karyotype consisted of chromosomes of nearly equal size, all or almost all, with median to submedian centromeres. In one line of evolution (leading to inbreeders) fundamental changes sufficient to give rise to taxonomic species involved chromatin duplication as well as genetic mutations. The karyotype also underwent some chromosomal rearrangements. The karyotype however, remained symmetrical or more or less so. Perhaps no polyploids were produced. The chromosomal rearrangements involved translocations as well as inversions and this is supported by the studies of MALIK and THOMAS (1966) and NAYLOR and REES (1958). In another line of evolution karyotype remained symmetrical (leading to outbreeder) and involved deletions, rarely duplications, and large number of genic mutations. It is believed that the genus *Lolium* had a Mediterranean origin. Based on this presumption it is suggested that the ancestoral form existed in the Mediterranean region and perhaps resembled the present *L. rigidum* form. If it was so, obviously, the origin of form like *L. perenne* from an annual one could only occur through drastic changes. It is quite conceivable since we know life span (annual, biennial and perennial) and ecological adaptabilities are subject to genetical control. Thus, perennial habit in *L. perenne* might have been evolved during the course of migration towards north. This involved in all probability chromosomal rearrangements or repatterning as well as several genic mutations in different directions. Presumably only those favouring movement towards northward survived. Within the genus *Lolium* since all the species are diploids apparently polyploidy has played no role (or insignificant) in the evolution of the various species. The crosses between the species within the section *Craepalia* and within the section *Lolium* are quite successful compared to intersectional crosses (JENKIN 1955). This suggests diverse course of evolution and also that species within each of the two sections have separated quite recently.

Zusammenfassung

Lolium multiflorum × *L. rigidum* wurden erfolgreich gekreuzt und die Chromosomen in F_1-Hybriden bezüglich ihrer Struktur und Paarung mit denen der Eltern verglichen. Die Chromosomen der Eltern ließen eine weitgehende Homologie erkennen, aber in gewissem Ausmaß deutete sich auch eine Differenzierung an. Diese Beobachtung läßt den Schluß zu, daß die beiden Arten relativ eng verwandt sind und sich phylogenetisch nahestehen. Das vorgelegte Modell weist darauf hin, daß die Differenzierung der Sektionen *Craepalia* und *Eulolium* höchstwahrscheinlich von einem gemeinsamen Vorfahr ausging. Dieser existierte vermutlich im Mittelmeergebiet als einjähriger Fremdbefruchter mit symmetrischem Karyotyp.

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Kritische Betrachtungen zur Nomenklatur argentinischer Wildkartoffeln

IX. *Solanum famatinae* Bitt. & Wittm., und die damit verwechselten *S. chacoense*, *S. kurtzianum*, *S. leptophyes*, *S. puberulofructum*, *S. setulosistylum*, *S. sleumeri*, *S. spegazzinii*, *S. velascanum* und *S. vidaurrei*

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Critical Considerations on the Nomenclature of Argentinian Wild Potatoes

IX. *Solanum famatinae* Bitt. & Wittm.,
and those Species confused with it:
S. chacoense, *S. kurtzianum*, *S.leptophyes*,
S. puberulofructum, *S. setulosistylum*,
S. sleumeri, *S. spegazzinii*,
S. velascanum and *S. vidaurrei*

Summary. The most important species known to be resistant to nematodes, *Solanum famatinae* Bitt. & Wittm. is found chiefly in western, semi-arid parts of South America. Its spread extends from South Bolivia to the Argentinian province of La Rioja. The original material came from the Famatina mountains, but was almost completely lost in European museums during the war. Fifty years after its discovery the author succeeded in finding *S. famatinae* again at the typical sites in the Famatina mountains. A critical comparison of the original type with the recently described new species *S. puberulofructum* Correll and *S. sleumeri* Correll showed that the latter are identical with *S. famatinae*. Using even a narrow definition of species it is not possible to maintain that *S. puberulofructum* and *S. sleumeri* are separate species, especially since the place of their original discovery (and until now their only sites) are only separated by 3 kilometers in the valley of Yacutula-Pozo de Piedra (prov. Catamarca). Finally *S. famatinae* became confused in the literature of the last ten years with a number of Argentinian wild potatoes, whose differences are explained.

Solanum famatinae Bitt. & Wittm. hat im Laufe des letzten Jahrzehnts in der praktischen Kartoffel-