CYTOGENETIC STUDIES OF *LOLIUM MULTIFLORUM* LAM., *FESTUCA ARUNDINACEA*

SCHREB., THEIR HYBRIDS AND AMPHIDIPLOIDS

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# STELLINGEN

### I

Er is opvallend veel associatie van chromosomen in de hybriden tussen *Lolium multiflorum* en *Festuca arundinacea*.

Dit proefschrift.

### II

Een te nauwe verwantschap tussen de genomen van *L. multiflorum* en *F. arundinacea* en het slecht funktioneren van de genetische regulatie van de chromosoomparing zijn de oorzaken van de onregelmatige en onstabiele meiose van de amphidiploïden.

Dit proefschrift.

### III

Verdubbeling van het chromosoom aantal levert niet altijd een verbetering van de chromosoom-associaties in de amphidiploïde op.

Dit proefschrift.

### IV

De bepaling van het aantal micronuclei per tetrade is geen goede maatstaf voor de meiotische onregelmatigheid in de hybriden en amphidiploïden tussen *L. multiflorum* en *F. arundinacea*.

Dit proefschrift.

### V

De hypothese van JAUHAR om de genetische regulatie van de chromosoomparing in *F. arundinacea* te verklaren is te simplistisch.

Jauhar,P.P., 1975. Nature 254, 595-597.

## VI

De affiniteits berekeningen volgens KIMBER en ALONSO zijn van nut bij
het bepalen van de genoomsamenstelling van *F. arundinacea*.

Kimber,G. and L.C.Alonso. 1981. Can.J.Genet.Cytol. 23, 235-254.

## VII

Dat in tarwe geen volledige resistentie tegen *Septoria nodorum* gevonden
is, hoeft geen nadeel voor de plantenveredelaar te zijn.

## VIII

De verbetering van de opname en het verbruik van stikstof door de plante
verdient meer aandacht in de tarweveredeling.

## IX

Chemische verbindingen met een -SH groep onderdrukken de vorming van
sclerotiën bij *Sclerotium rolfsii* en de vorming van sporen bij *Phomopsis
viticola*.

Chet,I., Y.Henis and R.Mitchell. 1966. J.Gen.Microbiol. 45, 541-546.
Pezet,R. and V.Pont. 1980. Can.J.Microbiol. 26, 356-362.

## X

De investeringen van multinationale ondernemingen in de ontwikkelings-
landen hebben helaas meer ten doel de ondernemingen dan de ontwikke-
lingslanden te ontwikkelen.

## XI

Het veredelen van grassen heeft mede ten doel de koeien smakelijker ete
te geven.

G.Kleijer.
Cytogenetic studies of *Lolium multiflorum* Lam., *Festuca arundinacea*
Schreb., their hybrids and amphidiploids.

22 oktober 1982.

à Tammine
Annelore
Pascal

# PREFACE

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Finalement, je tiens à remercier mes parents qui m'ont permis de faire des études.

Contents

# 1. INTRODUCTION

Interspecific and intergeneric hybridization of plants has widely been used for genetic and evolutionary studies and for plant breeding. In the latter case, several different approaches can be distinguished. The following two are the most important:

1. Transfer of one or a few specific genes from one species to another. Many successful examples are known (LACADENA, 1978).

2. The creation of allopolyploids, combining the good characteristics of both parents. These may be entirely new forms, or the reconstruction of existing allopolyploids for the introduction of new genetic variation.

Although many important crop plants are allopolyploids (*Triticum aestivum* L., *Avena sativa* L., *Festuca arundinacea* Schreb., *Gossypium* spp., *Nicotiana tabacum* L.), newly made allopolyploids so far have had little success. A few have been tested on a large scale, but are still inferior to the parental species or the corresponding natural forms. Examples are resynthesized *Brassica napus* L., *Raphanobrassica* (the amphidiploid of *Raphanus sativus* and *Brassica oleracea*, MCNAUGHTON and ROSS, 1978) and the amphidiploid of *Lolium perenne* L. or *L. multiflorum* Lam. and *Festuca pratensis* Huds. (GRIFFITHS *et al.*, 1979; JOGGI, 1980, personal communication). The only newly created allopolyploid cultivated on an agronomically important scale is hexaploid Triticale, derived from the hybrid between *Triticum durum* Desf. (2n=28) and *Secale cereale* L. (2n=14) (ANONYMOUS, 1979).

Some of the difficulties encountered with newly established allopolyploids are due to a lack of meiotic stability. In natural allopolyploids chromosome pairing is restricted to homologous chromosomes which ensures disomic inheritance and intact transfer of the parental genomes to subsequent generations. In several established allopolyploids chromosome pairing has been shown to be controlled by genes suppressing homoeologous pairing. Such genes are found in a number of species of the Gramineae and have been studied in detail especially in wheat (*Triticum aestivum*, RILEY and CHAPMAN, 1958). Comparable systems may operate in

*Avena sativa* (GAUTHIER and MCGINNES, 1968; RAJHATHY and THOMAS, 1972)
and *Festuca arundinacea* (JAUHAR, 1975a). In new allopolyploids such
systems have not, or insufficiently been developed, and the resulting
allosyndetic, homoeologous pairing can have genetically and reproduct-
ively undesired consequences. In addition, in many cases the combina-
tion of relatively unrelated genomes may lead to unbalances resulting in
meiotic abnormalities not related to chromosome pairing. Very often,
however, such unbalanced combinations of genomes may also, or even stron-
ger, negatively affect other fertility components.

The species hybridized in the experiments reported here belong to the
genera Lolium and Festuca, agronomically valuable genera of the grass
family, of the latter especially the broad leaved species of the section
Bovinae. There is a close phylogenetic relationship between the two ge-
nera, and hybrids can be obtained between most of the species (JENKIN,
1959). Two cross combinations have been extensively reported in the li-
terature. The first is the cross between *L. perenne*, 2n=14 (perennial
rye-grass) or *L. multiflorum*, 2n=14 (Italian rye-grass) with *F. praten-
sis*, 2n=14 (meadow fescue), or the cross between the corresponding auto-
tetraploids. The second is the cross between *L. perenne* or *L. multiflo-
rum* and *F. arundinacea*, 2n=42 (tall fescue).

The first type of hybrid is completely male sterile in spite of good
meiotic chromosome pairing (PETO, 1933; WIT, 1959; ESSAD, 1962; GYMER
and WHITTINGTON, 1975). Colchicine treatment of the F1 hybrid partially
restores fertility (ESSAD, 1962). The amphidiploids produced directly
by crossing tetraploid rye-grass and tetraploid meadow fescue are nearly
completely male sterile (HERTZSCH, 1961). Selected allopolyploid strains
have some promise for practical use (GRIFFITS *et al.*, 1979; JOGGI, 1980,
personal communication). By backcrossing with *L. perenne* or *L. multiflo-
rum* as recurrent parent useful genes from *F. pratensis* may be introduced
into these Lolium species.

PETO (1933) was the first to make artificial crosses between *L. perenne*
and *F. arundinacea*. The allopolyploids derived from such hybrids, espe-
cially those involving *L. multiflorum* are of considerable interest to
the plant breeder, as the characteristics of the two species are comple-
mentary. *L. multiflorum* has a rapid establishment, good production in

the year of sowing and good palatability. *F. arundinacea* shows persistence, winter hardiness, drought resistance and an extended period of heading. BADOUX (1973) studied the relative influence of the parents on hybrid performance. For many morphological characters the hybrids were very similar to *F. arundinacea*. For other characters, such as date of heading, crude protein content and fibre content the hybrids were intermediate between the parents. Cytological observations of the hybrids showed pairing between Lolium and Festuca chromosomes (PETO, 1933). CROWDER (1953a) found up to 14 bivalents in hybrids between *L. multiflorum* and *F. arundinacea*. Most cells, however, contained unpaired chromosomes as well as multivalents in addition to bivalents. BUCKNER, HILL and BURRUS (1961) found a rather low number of univalents. All these hybrids were completely male sterile.

Meiosis of the amphidiploids of *L. multiflorum* and *F. arundinacea* was studied only on a rather limited scale. HILL and BUCKNER (1962) and WEBSTER and BUCKNER (1971) reported only the number of univalents, laggards and micronuclei in tetrads. Complete meiotic·analyses were carried out by LEWIS (1966) and JAUHAR (1975b). In both cases many univalents and multivalents were reported. WEBSTER and BUCKNER (1971) selected the amphidiploid for fertility and seed set during six generations. This resulted only in meiotically instable plants representing nearly the entire range from 42 to 56 chromosomes. The same observation was made by SPECKMANN (1980, personal communication) who even found that after three generations of selection for fertility no plants with 56 chromosomes were present. This lack of success can be explained by the meiotic irregularities with high levels of aneuploids in the progeny as a consequence. Too narrow a genetic basis of the parental material used until now may also have been an important factor.

Hybrids as such (PETO, 1933; HERTZSCH, 1961; SULINOWSKY, 1966) and amphidiploids (PETO, 1933; HILL and BUCKNER, 1962) of *L. multiflorum* and *F. arundinacea* have been extensively used in backcross programs to either the Lolium or the Festuca parent. The only success obtained until now is the variety 'Kenhy' which originates from a backcross of an amphidiploid with *F. arundinacea* (BUCKNER *et al.*, 1977). This variety has 42 chromosomes. This approach presently receives more attention from plant breeders than the production of allopolyploids.

In the study reported here, seven distinctly different *F. arundinacea* and three *L. multiflorum* genotypes were combined into hybrids and amphidiploids in order to analyse possible effects of the genotype on meiotic stability and other fertility components. Among the descendants of hybrids between amphidiploids, euploids were selected in order to study the possibility that recombination can lead to genotypes with a more regular and stable meiosis.

## 2. MATERIAL AND METHODS

### 2.1 Material

The species used in this study were *Festuca arundinacea* (2n=42) and *Lolium multiflorum* (2n=14 or 4n=28). The following varieties or populations of *F. arundinacea* have been included:

- Alpes          a population from the Swiss Alps
- Alta           a variety from the USA, early
- Ludion         a French variety, mid late (breeder: INRA)
- Manade        a variety from the South of France, very early (breeder: Vilmorin)
- Mayens        a Swiss ecotype from an elevation of 1800 m, very late
- NZ T544       received from Dr. Rumball, Grassland Division, DSIR, Palmerston North, New Zealand
- Portugal (EMP)    received from Estaçao Agronomica Nacional Oieras, Portugal

For the Lolium parent, 3 varieties were chosen:

- Lior or Turilo    a diploid Swiss variety. Turilo is closely related to Lior (breeder: FAP Reckenholz), 3 clones
- Manawa        a diploid hybrid rye-grass (*L. multiflorum* x *L. perenne*) Grassland Division, DSIR, Palmerston North, New Zealand, 2 clones
- Amenda        a tetraploid Dutch variety, mid-late (breeder: Zwaan and De Wiljes), 2 clones

### 2.2 Methods

#### 2.2.1 The crosses

The plants were sown or cloned in the autumn of 1973 and overwintered outside for vernalization. In the beginning of April 1974, the plants were taken into the greenhouse and planted in pots of 16 cm $\emptyset$. The temperature in the greenhouse was $15^{o}C$ during the day and $10^{o}C$ during the night for the first 4 weeks. Thereafter, these temperatures were $20^{o}C$ and $15^{o}C$ respectively.

Spikes of the rye-grass parent were emasculated approximately three days before anthesis. Only the lower two flowers of a spikelet were emasculated. The others were removed. The spikes were covered with an isolation bag in order to avoid pollination by other plants. Three days after emasculation, the spikes were pollinated with pollen of the tall fescue parent by opening the bag at the top and shaking the pollen on the spike. Three days later, the pollination was repeated as not all flowers flowered at the same time. The pollen of tall fescue was obtained by bagging the panicles of the plants and collecting the pollen once a day, mostly early in the afternoon after dehiscence of the anthers.

Reciprocal crosses were only made with the tall fescue variety Ludion and the population Portugal as female parents. The panicles were emasculated and covered with an isolation bag. Three days later, a flowering spike of the rye-grass variety Turilo was put in the bag. After three more days, the spike of Turilo was replaced by a freshly cut spike.

The hybrid seeds were harvested at maturity. After removal of the glumes, the seeds were sterilized with a 0.1% mercuric chloride solution during 4 minutes and washed six times in sterile distilled water. The seeds were put in tubes on an orchid agar medium (ROMMEL, 1958) in order to germinate. When the first leaf was approximately four cm long, the seedlings were transplanted to small jiffy pots and placed in the greenhouse at $20^{o}C$.

## 2.2.2 Chromosome doubling

Doubling of the chromosome number was carried out using the method of MORGAN (1976). When the plants had four to five tillers, the tillers were separated. A small triangular incision was made above the stem apex and the segment thus formed was removed. The leaves were trimmed and all roots removed. The tillers were then placed into water for 15 hours and afterwards into a 0.2% colchicine + 2% dimethylsulphoxide (DMSO) solution for 8 hours at $20^{o}C$. DMSO is added to the colchicine solution because it enhances the penetration of the colchicine into the cells (KAUL and ZUTSHI, 1971; SANDERS and HULL, 1970).

Following this treatment, the tillers were thoroughly washed under running water for two hours. The tillers were then placed into an appropriate soil mixture in the greenhouse.

2.2.3  Cytological observations

Chromosome counts of the amphidiploids and their descendants were made by
splitting up the tillers of a plant and after removal of the roots placing
them into a culture tank containing an aerated solution of 0.003 M $NaNO_3$,
0.0025 M $MgSO_4$, 0.015 M $KH_2PO_4$ and 0.0001 M $FeC_6H_6O_7 \cdot 5H_2O$ at $20^0C$.  After
three or four days, the newly formed roots were taken and treated with a
freshly prepared saturated solution of bromonaphthalene for four hours at
$20^0C$.  The roots were then fixed in a 3 : 1 alcohol acetic acid mixture
and stored in a refrigerator until used.  The root tips were hydrolysed
in 1N HCl at $60^0C$ for ten minutes and stained in a Feulgen solution.

After determinating the appropriate stage on one of the three anthers of
a floret, the two others were fixed in 3 : 1 alcohol acetic acid for
meiotic studies.  The anthers were stained according to the Feulgen pro-
cedure.  In some cases, when the anthers were not stained well enough,
they were additionally stained in 45% aceto-carmine.  Preparations were
made permanent using the evaporation technique of BRADLEY (1948), which
has the advantage of not requiring removal of the cover slip.

Pollen stainability was determined on 100 pollen grains from each of six
anthers just before dehiscence.  The pollen grains were stained in a mix-
ture of aceto-carmine stain and glycerine.  They were classified either
in two or three classes:

Two classes:
1)  completely stained, round pollen and
2)  other pollen

Three classes:
1)  completely stained round pollen
2)  partially stained pollen and
3)  empty shrivelled pollen

Germination of the pollen grains of the amphidiploids was tested in pe-
tri-dishes on a solid agar medium, as described by COLLET (1968).  The
pollen grains were collected between 14 and 15 p,m.  After 30 minutes,
pollen tube growth was examined for 400 pollen grains per plant.

## 3. RESULTS AND DISCUSSION

### 3.1 The crosses

The results of the crosses are presented in tables 1 and 2. In the fol-
lowing discussion *F. arundinacea* will be abbreviated to F.a. and *L.*
*multiflorum* to L.m. Most crosses with the diploid L.m. as female parent
gave good results. A statistical analysis showed that the differences
between the three clones of Lior, between the two of Manawa and between
the two of Amenda were not significant. For this reason, in table 1, the
crossing results of the different clones of a variety were pooled.
There were differences in percentage of seedset among the different
cross combinations varying between 28.6% for the cross Manawa x Mayens
and 63.4% for the cross Manawa x Alta. On an average, the seedset with
Manawa as female parent was better than with Lior but the difference was
not significant. Two tall fescues, Ludion and N.Z. gave very good re-
sults when crossed with Lior or Manawa. The population Portugal had a
low percentage of seedset in the two cross combinations. The results
with the other tall fescues were variable. Part of the seeds was very
small and has not been included in the results presented in table 1. Ten
hybrids of the combination Lior x Ludion were albinos.

A small percentage of the seeds of F.a. gave albino plants. In a special
trial, 28,000 seeds of the variety Manade and 10,000 of Ludion were ger-
minated in order to select twins. In both varieties, albinos were found,
0.06 and 0.11% respectively.

About 10% of the hybrid plants died within two weeks after germination,
probably due to the transfer from the nutrient agar medium to the jiffy
pots. The germination of the hybrid seeds of the cross combinations
with Manawa was much better (average 76.6%) than that of the cross combi-
nations with Lior (average 50%) and the differences were highly signifi-
cant (1% level). In both cases, the hybrids with Mayens as male parent
had the lowest percentage of germination, significant at the 5% level
after comparison using orthogonal coefficients.

Crossing F.a. with tetraploid L.m. proved to be much more difficult than
with the diploid L.m. The results are considerably worse, not only the

Table 1. Results of the crosses of diploid (2x) and tetraploid (4x) *L. multiflorum* (Lm) with *F. arundinacea* (Fa). Results of 1974. Manawa is 2x-(Lm x Lp)

| Cross | Number of flowers | Number of seeds | % Seedset | Number seeds germin. | % Germin- ation | Number of plants |
|---|---|---|---|---|---|---|
| **2x-(Lm x Fa)** | | | | | | |
| Lior x Alpes | 679 | 257 | 37.8 | 134 | 52.1 | 120 |
| Lior x Alta | 717 | 276 | 38.5 | 162 | 58.7 | 146 |
| Lior x Ludion | 633 | 379 | 59.9 | 242*) | 63.8 | 215 |
| Lior x Manade | 465 | 134 | 28.8 | 78 | 58.2 | 72 |
| Lior x Mayens | 363 | 212 | 58.4 | 86 | 40.6 | 77 |
| Lior x N.Z. | 537 | 284 | 52.9 | 155 | 54.6 | 142 |
| Lior x Portugal | 623 | 225 | 36.1 | 125 | 55.6 | 114 |
| Total | 4017 | 1767 | 44.0 | 982 | 50.0 | 886 |
| **2x-(Lm x Lp) x Fa** | | | | | | |
| Manawa x Alpes | 315 | 185 | 58.7 | 154 | 83.2 | 147 |
| Manawa x Alta | 354 | 226 | 63.4 | 161 | 71.2 | 147 |
| Manawa x Ludion | 396 | 243 | 61.4 | 184 | 75.7 | 172 |
| Manawa x Manade | 449 | 188 | 41.9 | 156 | 83.0 | 151 |
| Manawa x Mayens | 395 | 113 | 28.6 | 78 | 69.0 | 76 |
| Manawa x N.Z. | 384 | 231 | 60.2 | 171 | 74.0 | 144 |
| Manawa x Portugal | 175 | 69 | 39.4 | 57 | 82.6 | 54 |
| Total | 2468 | 1255 | 50.8 | 961 | 76.6 | 891 |
| **4x-(Lm x Fa)** | | | | | | |
| Amenda x Alpes | 154 | 50 | 32.5 | 16 | 32.0 | 12 |
| Amenda x Alta | 192 | 35 | 18.2 | 3 | 8.6 | 2 |
| Amenda x Ludion | 312 | 88 | 40.0 | 20 | 22.7 | 13 |
| Amenda x Manade | 192 | 24 | 12.5 | 1 | 4.2 | 0 |
| Amenda x Mayens | 214 | 27 | 12.6 | 0 | 0.0 | 0 |
| Amenda x N.Z. | 230 | 25 | 10.9 | 10 | 40.0 | 4 |
| Amenda x Portugal | 174 | 34 | 19.5 | 3 | 8.8 | 2 |
| Total | 1468 | 283 | 19.3 | 53 | 18.7 | 33 |

*) = 10 albinos

percentage of seedset, but also the percentage of germination. Only the combinations Amenda x Alpes and Amenda x Ludion gave relatively good results

The variety Ludion gave very good results in all three combinations (with Lior, Manawa and Amenda) and the differences between Ludion and the other varieties were significant at the 5 percent level. The highest percentage of small shrivelled seeds was found in the cross combinations with the tetraploid Amenda: 55% as compared to 6.6% and 9.9% in the crosses with Manawa and with Lior respectively. These small shrivelled seeds had about $\frac{1}{4}$ of the length of a normal seed and were much flatter.

The results of the reciprocal cross are presented in table 2. Here, both seedset and germination were very low. All the seeds were small and only one seed germinated.

Table 2. Results of the crosses between *F. arundinacea* and *L. multiflorum*, variety Turilo. Results of 1980

| Cross | Number of flowers | Number of seeds | % Seedset | Number seeds germin. | % Germin- ation | Number of plants |
|---|---|---|---|---|---|---|
| Fa x 2x-Lm | | | | | | |
| Ludion x Turilo | 1405 | 77 | 5.0 | 0 | 0.00 | 0 |
| Portugal x Turilo | 1597 | 56 | 3.5 | 1 | 1.80 | 1 |
| Total | 3002 | 133 | 4.4 | 1 | 0.75 | 1 |

The results of these crosses agree with those found by other authors. GRÖBER et al. (1974) obtained a seedset of 22.2% using diploid L.m. as female parent and 6.1% with the tetraploid L.m. HERTZSCH (1960) obtained 7.7% seedset with the diploid and 1.7% with the tetraploid L.m. as female parent. Both authors found that the crosses with diploid L.m. as pollen parent gave a very poor seedset and hardly any seed germination. Using tetraploid L.m. as pollen parent, the results were better than with the diploid as pollen parent. CROWDER (1953a) and LEWIS (1966) obtained a seedset of 38.1 and between 48 and 92% respectively, using the diploid rye-grass as female parent. The reciprocal crosses gave very poor results in both cases.

BEDDOWS (1965) also crossed largely self-incompatible L.m. with F.a., but without emasculation. His results varied from 16 to 56% seedset but he did not mention if these differences were significant. BUCKNER (1960) crossed *L. perenne* (L.p.) and L.m. with F.a. without emasculation. The range of viable hybrid seed production per plant from the L.m. crosses was 0 to 7 and from the L.p. crosses 0 to 1. BADOUX (1973) used for his crosses partially the same genotypes as used in this study. Yet he found a much lower seedset and germination of the hybrid seeds. Furthermore, he found the lowest percentage of seedset in the cross combinations with Manawa as female parent in contrast to our results (table 1). This seems to indicate that the success of hybridization is influenced also by other factors than the genotype of the parents.

In most studies, the crosses between L.p. and F.a. showed a lower percentage of seedset than L.m. x F.a. (BUCKNER, 1960; HERTZSCH, 1960; GRÖBER *et al.*, 1974). The results presented here showed that Manawa, which is a diploid hybrid between L.m. and L.p. had a slightly better crossability with F.a. than the L.m. variety Lior. A negative influence of L.p. on the cross compatibility with F.a. is not expressed in Manawa. Probably the results of the crosses depend mainly on the genotypes of the parents used. CROWDER (1953a) found an equal seedset for the crosses L.p. x F.a. and L.m. x F.a.

Germination of the hybrid seeds of L.m. x F.a. varied between 29.6% for one cross combination reported by BEDDOWS (1965) and 68.2% found by HERTZSCH (1960). The results presented in this study showed a better germination for the hybrids with Manawa as female parent (variation from 69.0 to 83.2%) than the germination reported by BEDDOWS (1965) and HERTZSCH (1960).

In general, hybrids between L.p. or L.m. with F.a. can be obtained rather easily with or without emasculation. Lolium should be taken as the female parent. With Lolium as the male parent, the crosses are much more difficult and from the low percentage of hybrid seeds obtained, only a few germinate. The cross compatibility as well as the germination rate of the hybrid seeds depend on the genotype of the parents.

## 3.2 Colchicine treatment

The seedlings were treated with colchicine when they had approximately
5 tillers. In total 256 plants (1350 tillers) were treated; 72% of the
tillers survived after the colchicine treatment. After several months,
chromosome counts were made on the newly formed tillers. In 75% of the
plants, sectors with 56 chromosomes were found. All these plants were
mixoploids. The plants flowered one year later and then only 40% of the
plants, which originally had a doubled sector, had panicles with dehiscent
anthers assumed to have 56 chromosomes. Thus the final percentage of
successful doubling is 30% of the 256 plants treated.

This method of chromosome doubling gave also good results in the exper-
iments of MORGAN (1976), who found a percentage of polyploids at the mi-
xoploid level of 70%. In the present study, even 75% of the tillers
showed doubled sectors, but when these tillers came into flower, a great
deal of the doubled sectors did not develop into a (part of) panicle
as was apparent from very limited appearence of dehiscent anthers. It
is possible that this 'loss' of a doubled sector can be explained as in-
herent to the normal growth of the plant and random loss of sectors from
the apex. It might also be that the tissues with 28 chromosomes are mo-
re vigourous than those with 56 chromosomes and that a selection takes
place against the sectors with 56 chromosomes. These could be reasons
why more than 50% of the doubled sectors detected in vegetative tissue
after colchicine treatment were 'lost' at flowering.

The percentage of successful doubling is nearly as high as the average
percentage (38%) found by DIJKSTRA and DE VOS (1975), who used the me-
thod of meiotic doubling. Meiotic doubling may be caused by fusion of
egg cells having the somatic number of chromosomes, with pollen of al-
ready doubled plants. The advantages of this method are the absence of
mixoploids and the possibly greater heterozygosity of the doubled plants
compared to plants doubled with colchicine in the vegetative stage. The
method of meiotic doubling seems to be very dependent on environmental
conditions because the authors found great differences between years.
The methods described by MORGAN (1976) and DIJKSTRA and DE VOS (1975)
are much more successful than other methods described before (DIJKSTRA
and DE VOS, 1975).

## 3.3  Cytological observations

### 3.3.1 The parents

#### 3.3.1.1  *F. arundinacea*

Panicles of two varieties of F.a. are shown in Fig. 1.  Small morphologi-
cal differences between the panicles of different varieties and popula-
tions were found (see Fig. 1, middle and right) but the basic structure
of the panicles was the same.



Fig. 1.  Left:  spike of *L. multiflorum* (L.m.) variety Lior;  middle:
panicle of *F. arundinacea* (F.a.) variety Ludion;  right:  pa-
nicle of *F. arundinacea* (F.a.) population Alpes.

Chromosome association at first metaphase of meiosis, pollen stainability
and fertility expressed as the number of seeds per spikelet are presented
in table 3. The data of the Festuca parents are mean figures of at least
five plants used as pollen parents. Most of the chromosomes paired as
bivalents (Fig. 2). The number of rod bivalents was rather high and va-
ried between 8.31 for Alta and 12.99 for Alpes. In all varieties or po-
pulations, a few univalents were found, the highest number in Portugal,
0.54 and the lowest number in Alpes, 0.09. This low number of univalents
in Alpes is surprising because Alpes has the highest number of rod biva-
lents. For the Festuca parents, there was no significant correlation bet-
ween the number of univalents and the number of rod bivalents ($r = 0.24$)
nor between univalents and number of arms bound ($r = -0.28$). The number
of arms bound was calculated as the sum of the rod bivalents plus twice
the number of ring bivalents and v-chaped trivalents plus three times the
number of chain quadrivalents plus four times the number of ring quadri-
valents. In Portugal and Alta, some tri- and quadrivalents were found.
An analysis of the number of arms bound for the first 20 cells of five
plants for each variety of F.a. did not only show significant differences
between varieties but also significant differences within varieties (bet-
ween plants of the same variety), although these differences were smaller
than the differences between varieties (table 4).



Fig. 2. Metaphase I of meiosis. Left: *L. multiflorum* Lior with 6 ring
bivalents and 1 rod bivalent; right: *F. arundinacea* Alpes with
10 ring bivalents and 11 rod bivalents. The bar represents 10 µm

Table 3. Chromosome associations, numbers of arms bound, pollen stainability and seed fertility of the parents (1975). Seed fertility was measured as number of seeds/spikelet after cross pollinisation

| Variety | No. plants | Number of cells | Uni valents | Bivalents ring | Bivalents rod | Bivalents total | Tri- valents | Quadri- valents | Number of arms bound | Pollen stainability, % | Seed fertility |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Alpes | 8 | 223 | 0.09 | 7.95 | 12.99 | 20.94 | | | 28.92 | 93.37 | 1.95 |
| Alta | 5 | 114 | 0.21 | 12.33 | 8.31 | 20.65 | | 0.12 | 33.38 | 87.16 | 1.72 |
| Ludion | 5 | 138 | 0.27 | 8.44 | 12.38 | 20.82 | | | 29.35 | 69.29 | 1.75 |
| Manade | 7 | 130 | 0.11 | 10.51 | 10.43 | 20.95 | | | 31.49 | 87.30 | 1.27 |
| Mayens | 5 | 125 | 0.42 | 10.72 | 10.05 | 20.77 | | | 32.07 | 96.16 | 1.53 |
| N.Z. | 5 | 130 | 0.12 | 11.25 | 9.69 | 20.94 | | | 32.18 | 92.17 | 2.12 |
| Portugal | 5 | 150 | 0.54 | 7.81 | 12.84 | 20.65 | 0.03 | 0.01 | 28.50 | 80.45 | 1.66 |
| Lior 1 | 2 | 60 | | 5.46 | 1.53 | 7.00 | | | 12.45 | 98.58 | 2.64 |
| Lior 2 | 4 | 120 | | 6.24 | 0.74 | 7.00 | | | 13.25 | 87.75 | 1.69 |
| Lior 3 | 4 | 120 | | 6.22 | 0.77 | 7.00 | | | 13.22 | 86.33 | 2.00 |
| Manawa 1 | 6 | 180 | | 5.86 | 1.13 | 7.00 | | | 12.86 | 87.47 | 0.86 |
| Manawa 2 | 4 | 120 | | 6.13 | 0.86 | 7.00 | | | 13.12 | 88.94 | 0.87 |
| Amenda 1 | 3 | 84 | 0.31 | 7.16 | 4.00 | 11.16 | 0.12 | 1.28 | 23.16 | 93.58 | 1.45 |
| Amenda 2 | 3 | 51 | 0.08 | 7.00 | 4.54 | 11.54 | 0.04 | 1.18 | 22.98 | 94.61 | 0.52 |

For ten plants meiotic analyses were carried out over two years (1974 and 1975). For all plants but one, the average number of arms bound was higher in 1974 than in 1975. A t-test was carried out with the number of arms bound for each plant. The differences between years were significant at the 1% level for eight of the ten plants. The differences between years of the other two plants were not significant. A number of plants was vegetatively propagated and for ten different clones meiotic analyses were carried out in the same year on two plants. A t-test with the number of arms bound showed no significant differences for four clones, between the two plants of a clone. For two clones, the difference was significant at the 5% level, and for four clones the difference was significant at the 1% level. The data of tables 3 and 4 only represent analyses carried out in 1975. When two plants of a clone were analysed, the plant with the highest number of cells analysed was taken to establish the average data presented in table 3 and the analysis of variance presented in table 4.

Table 4. Analysis of variance of the number of arms bound for the seven *F. arundinacea* parents

| Item | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Between varieties | 6 | 2386.4 | 397.7 | 189.4 | 0.001 |
| Within varieties | 34 | 5020.6 | 147.7 | 70.3 | 0.001 |
| Error | 659 | 1388.3 | 2.1 | | |
| Total | 699 | 8795.3 | | | |

These results of the meiotic analyses confirm the results of EVANS *et al.* (1973) and CROWDER (1953b), who also found in F.a. a low percentage of univalents, and some multivalents. EVANS *et al.* (1973) found the same percentage quadrivalents for the variety Alta as in this study. MALIK and THOMAS (1966a) also noted a low percentage of univalents. They observed that most of the bivalents were ring bivalents while in this study on an average more rod bivalents than ring bivalents were found. MALIK and THOMAS (1966a) did not find significant differences between and within varieties in their material. CROWDER (1953b) showed significant plant differences with respect to the number of cells with all the chromosomes paired as bivalents, but averages of selections did not differ.

Pollen stainability was high for all varieties and populations of F.a. and varied between 69.3% for Ludion and 96.2% for Mayens. The same range of pollen stainability has been found by MALIK and THOMAS (1966a). Seed fertility was the highest for the population of New Zealand (2.12 seeds/ spikelet), the lowest value (1.27) was found for the variety Manade. There was no significant correlation between the number of arms bound and seed fertility ($r = 0.11$), between the number of arms bound and pollen stainability ($r = 0.46$) and between pollen stainability and seed fertility ($r = 0.09$) calculated with the averages presented in table 3. When calculated with the individual plant data (31 plants) the correlation between pollen stainability and seed fertility was significant at the 5% level ($r = 0.44$). The other two correlations were not significant ($r = 0.20$ for both sets of data). This means that chromosome pairing did not influence pollen stainability and seed fertility; with some univalents and a high number of rod bivalents genetically balanced gametes are formed in a sufficient quantity to assure good pollen stainability and seed fertility. Owing to the rather allogamous character of tall fescue, the pollen of a plant will not be used for the fertilization of the ovule of the same plant but will serve for the fertilization of ovules of other plants. Since the correlation between pollen stainability and seed fertility is significant at the 5% level, this means that there must be also a correlation between pollen stainability and good ovules.

Conclusion:

Meiosis of F.a. shows some irregularities owing to the presence of univalents and multivalents. The number of rod bivalents is high and in several varieties more rod than ring bivalents were found. CROWDER (1953b), MALIK and THOMAS (1966a), and EVANS et al. (1973) also found a few univalents and a rather high number of rod bivalents. This indicates that in these varieties chromosomes pairing and chiasma formation is not optimal. No significant correlation ($r = 0.28$) was found between the number of univalents and the number of arms bound so that a reduction of the number of arms bound does not mean an increase of univalents. Meiosis of F.a. also showed some lack of stability. For the same plant, significant differences in number of arms bound were found over two years and a number of plants even showed significant differences within clones, analysed in the same year. Highly significant differences were observed in number of arms

bound between and within varieties (table 4). CROWDER (1953b) found dif-
ferences in the number of cells with 21 bivalents between the three
plants of a clone for some genotypes but he did not mention significant
differences within clones. The differences in numbers of arms bound bet-
ween plants of the same clone are difficult to explain. The plants grew
up together in the same greenhouse under the same environmental condi-
tions. Meioses were fixed approximately at the same time every day. It
could be possible that for those plants which showed significant differ-
ences meioses were fixed on different days so that small differences in
environmental conditions could occur but it seemed difficult to present
this as the only cause for the differences found. The differences in
numbers of arms bound of plants analysed over two years can be explained
by the influence of the environment. In 1974 it was systematically high-
er than in 1975 indicating better environmental conditions for chromosome
pairing and chiasma formation in 1974. The significant differences bet-
ween and within the Festuca varieties are thus partly due to other causes
than genetic differences between plants and varieties. F.a. is a poly-
morphic species with wide distribution. MALIK and THOMAS (1966b) showed
that in a number of geographical populations a considerable amount of
chromosomal differentiation had occurred. Genetical differences and en-
vironmental influences can thus be responsible for the differences found
between and within varieties. MALIK and THOMAS (1967) did not find si-
gnificant differences between or within the genotypes of various popula-
tions in their study. They made an analysis of variance with plant ave-
rages which is less exact than with individual cell data as used in this
study. Chromosome pairing seems to affect very little pollen stainabili-
ty and fertility only slightly as shown by the insignificant correlations.

### 3.3.1.2  L. multiflorum

A spike of L.m. is shown in Fig. 1. The diploid Lolium showed very regu-
lar pairing and no univalents were found (Fig. 2). Most of the chromo-
somes paired as ring bivalents with a range from 5.46 for the first clone
of Lior to 6.24 for the second (Table 3). An analysis of variance on the
average number of arms bound per plant showed that there were no signifi-
cant differences between the Loliums. Pollen stainability was high in
all cases, varying between 86.3% for Lior 3 and 98.6 for Lior 1, and seed

fertility was good. The clones of Manawa were less fertile than those of Lior and a t-test on the basis of individual plants showed that the difference was significant at the 1% level. The analysis of fertility was made in 1975 on two years old plants. At that moment, the plants were less vigourous than in the first year. There was no significant correlation between the number of bound arms and pollen stainability ($r = 0.05$), number of bound arms and seed fertility ($r = 0.14$) and pollen stainability and seed fertility ($r = 0.46$).

The tetraploid L.m. showed mainly bivalent pairing, 11.16 and 11.54 bivalents respectively for the two clones of Amenda. Some univalents and trivalents were found but their frequency was low. On an average, more than one quadrivalent per cell was found with a maximum of three per cell. Most of the quadrivalents were alternate chains or rings, which assures a regular distribution of the chromosomes among the gametes. The number of quadrivalents was rather low for an autotetraploid and was also low compared to the number of quadrivalents in autotetraploids of *L. perenne*. Here LEWIS (1980) found a range between plants from 3.04 to 3.56 quadrivalents and AHLOOWALIA (1967) 3.91 quadrivalents per cell with a maximum of 7. NITZSCHE (1974) found that in tetraploid L.p. 89.6% of the chromosomes formed quadrivalents and in L.m. 63.9%. A low number of quadrivalents as observed in Amenda has been reported earlier in certain synthesized autotetraploids (GILLES and RANDOLPH, 1951; SWAMMINATHAN and SULBHA, 1959; JAUHAR, 1970). Pollen stainability was high for both genotypes, 93.58 and 94.61 respectively. The seed fertility of Amenda 1 was high (1.45) as compared to that of Amenda 2. The difference was not significant probably owing to the fact that for Amenda 2, the fertility of only two plants could be analysed.

In comparison with diploids, a reduction of the number of arms bound per chromosome has been found for the tetraploids. The same phenomenon was found in *L. temulentum* and *L. persicum* (MARY et al., 1973) AHLOOWALIA (1967) observed that the tetraploids of *L. perenne* had almost twice the number of chiasmata per cell than the diploids, i.e. only a slight reduction per chromosome.

Conclusion:

Diploid Lolium showed very regular pairing and most of the chromosomes paired as ring bivalents. As in F.a. pollen stainability and seed fertility were not correlated with the number of arms bound and differences

in seed fertility are due to other causes than pollen stainability and number of arms bound. Tetraploid Lolium showed some irregularities by the formation of some uni- and trivalents. Quadrivalents were found in an astonishingly low number for a tetraploid.

### 3.3.2  The hybrids

#### 3.3.2.1  Introduction

All hybrids between diploid Lolium and F.a. examined had the expected 28 chromosomes. The inflorescences of the hybrids were mostly of the pani- cle type (Fig. 3). They were always simpler (less branched) than those of F.a. and resembled quite well the descriptions given by CROWDER (1953a) and BEDDOWS (1965). At flowering time, the anthers were not dehiscent and all the hybrids were male sterile. In some cases, degeneration had already started before meiosis, so that no meiotic analyses could be made.
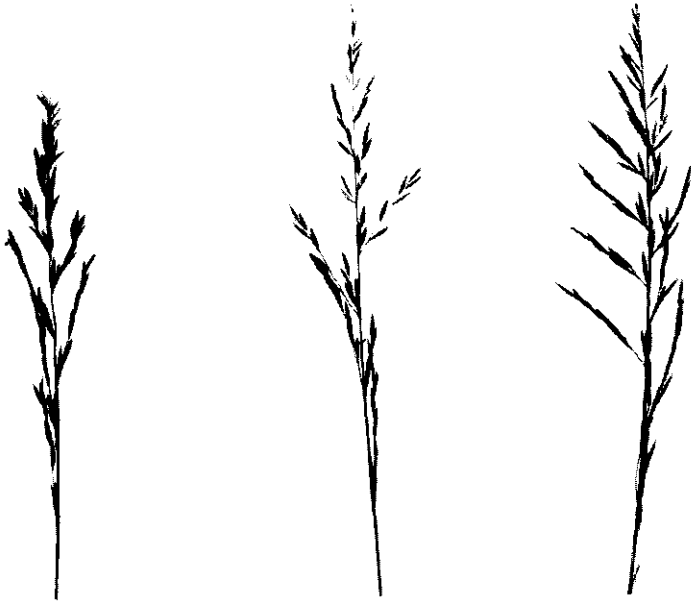


Fig. 3.  Panicles of the hybrids L.m. x F.a.  Left:  Lior x Alpes; middle:  Lior x Alta;  right:  Lior x N.Z.

The results of the metaphase I analyses of the different hybrids are pre-
sented in tables 5 and 6. The results of the meiotic analyses of the hy-
brids with Lior or Manawa as female parent will be discussed separately
later in this chapter (see sections 3.3.2.2 and 3.3.2.3). The results
from different plants of a cross combination and from the cross combina-
tions of the three clones of Lior and from the two clones of Manawa with
the same Festuca parent have been pooled. For ten plants, meiotic analy-
ses were carried out over two years (1975 and 1976) and ten cells or mo-
re could be analysed each year. A t-test for the number of arms bound
showed that for nine of the ten plants the difference between years was
not significant and for one plant the difference was significant at the
5% level. For the number of univalents, a t-test showed that for only
two plants the differences between years were significant (at the 5% le-
vel). The number of cells analysed is not identical for each plant. The
highest number of cells per plant was 39 but for most of the plants 20
cells or less were analysed. The reason for this low number of cells was
that the cells were difficult to analyse due to poor spreading of the
chromosomes and some stickiness in most of the cells.

In respect to the number of univalents and the number of arms bound,
three analyses of variance were carried out. The first was to test the
influence of the parents on univalent formation and number of arms bound
in the hybrids. The second was to detect significant differences between
the hybrids and the third was carried out to see whether the hybrids with-
in a cross combination showed significant differences.

### 3.3.2.2  *Hybrids of Lior x Festuca*

#### 3.3.2.2.1  Univalents

In these hybrids (table 5 and Fig. 4.3 and 4.4) the number of univalents
varied between 2.36 for the cross with Alpes and 4.05 for the cross with
Manade. Two classes of crosses could possibly be distinguished, one with
a relatively low number of univalents (Alpes, Ludion and Portugal), and
one with a high number of univalents (the other four F.a. parents).

Table 5. Chromosome associations of the hybrids Lior x *F. arundinacea* (range in parentheses)

| | No. plants | No. cells | Uni-valents | Bivalents ring | Bivalents rod | Bivalents total | Tri-valents | Quadri-valents | Penta-valents | Hexa-valents | No. arms bound | % cells without univalents |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lior x Alpes | 10 | 125 | 2.36 (0-10) | 6.06 (0-11) | 4.26 (0-10) | 10.32 (3-14) | 0.41 (0-3) | 0.94 (0-4) | | | 20.34 | 17.6 |
| Lior x Alta | 7 | 188 | 3.67 (0-18) | 5.65 (0-10) | 4.33 (1-10) | 9.98 (4-14) | 0.51 (0-4) | 0.69 (0-3) | 0.02 (0-1) | | 18.95 | 10.6 |
| Lior x Ludion | 10 | 169 | 2.94 (0-11) | 5.62 (1-11) | 5.08 (1-11) | 10.90 (6-14) | 0.44 (0-3) | 0.57 (0-3) | | 0.01 (0-1) | 19.15 | 13.0 |
| Lior x Manade | 7 | 75 | 4.05 (0-12) | 6.31 (2-12) | 3.83 (1-8) | 10.13 (6-14) | 0.48 (0-2) | 0.56 (0-2) | | | 19.32 | 4.0 |
| Lior x Mayens | 9 | 92 | 3.96 (0-12) | 5.93 (1-11) | 4.41 (1-9) | 10.35 (5-14) | 0.52 (0-3) | 0.45 (0-4) | | | 18.92 | 18.5 |
| Lior x N.Z. | 6 | 133 | 3.79 (0-16) | 5.88 (2-11) | 4.40 (0-9) | 10.28 (5-14) | 0.44 (0-3) | 0.59 (0-3) | | | 19.01 | 12.0 |
| Lior x Portugal | 7 | 116 | 2.57 (0-8) | 6.30 (2-12) | 4.10 (0-11) | 10.41 (5-14) | 0.43 (0-3) | 0.82 (0-4) | | 0.01 (0-1) | 20.43 | 16.4 |
| Total and means | 56 | 898 | 3.29 | 5.91 | 4.41 | 10.31 | 0.46 | 0.67 | 0.002 | 0.002 | 19.41 | 13.2 |
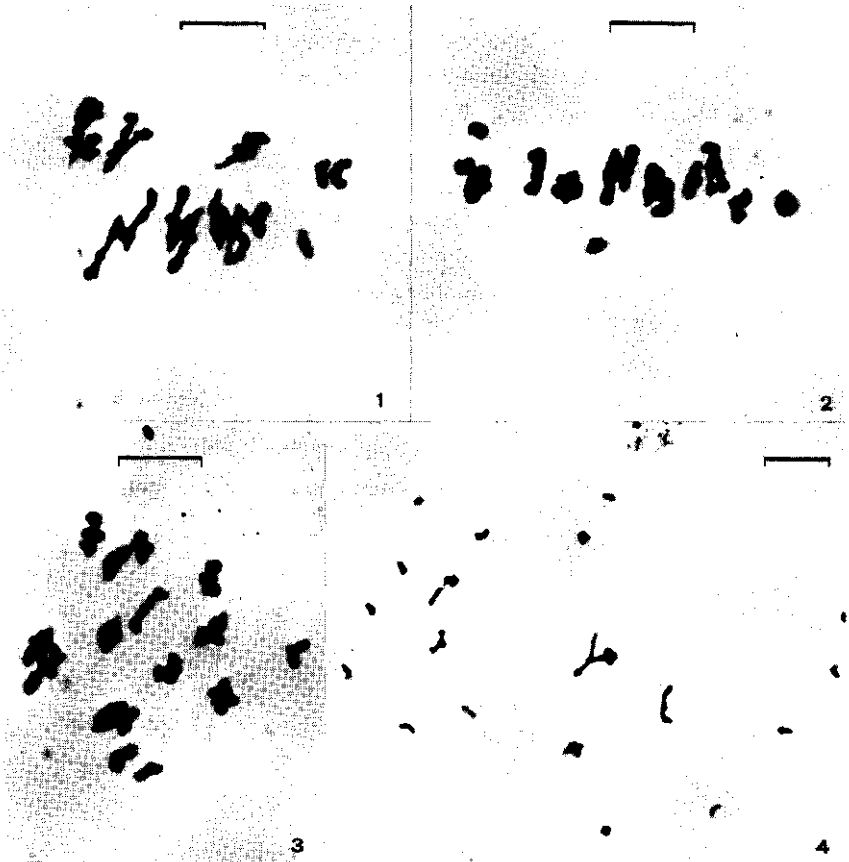
Fig. 4. Metaphase I of meiosis of the hybrids L.m. x F.a.: 1. Manawa x
Alpes with 6I, 4II, 2III and 2IV; 2. Manawa x Mayens with 4I,
10II and 1IV; 3. Lior x N.Z. with 2I and 13II; 4. Lior x
Alpes with 14I and 7II. The bars represent 10 µm

The first analysis of variance was set up to test the influence of the
parents on univalent formation in the hybrids. The analysis was carried
out with the average number of univalents per cross combination (the same
as presented in table 7 for the number of arms bound) and showed no si-
gnificant differences between the number of univalents of the combinations
with the different Festuca parents nor the three clones of Lior. This
means that no general influence of the genotypes of the different Festuca
varieties nor of the three genotypes of Lior on the number of univalents
in the hybrids could be detected.

The second analysis of variance was carried out to detect significant differences in numbers of univalents between the hybrids. Ten cells or more could be analysed in 36 hybrids. The analysis of these hybrids was carried out with individual cell data and showed that the differences between the hybrids were highly significant (1% level). The range in number of univalents was from 1.42 for a hybrid of Lior x Portugal to 8.35 for a hybrid of Lior x Mayens.

The third analysis of variance was carried out to see whether the hybrids within a cross combination showed significant differences. It involved six of the seven cross combinations with the same plants as used for the second analysis of variance. The number of hybrid plants within a cross combination varied between four and eight. Within all cross combinations highly significant (1% level) differences in numbers of univalents were found. Very large differences (high F values) were found in the cross combination Lior x Alta and Lior x Mayens. The range in average numbers of univalents in the hybrids of Lior x Alta was from 1.58 to 6.27. For the hybrids of Lior x Mayens this was 2.33 to 8.35. The high average number of univalents of Lior x Mayens (table 5) was mainly due to two hybrids with a very high number of univalents, 5.41 and 8.35 respectively.

The differences in numbers of univalents were also large between the hybrids of the cross combinations with the different clones of Lior, for instance two hybrids of Lior 3 x Alta had 2.21 and 7.88 univalents respectively and three hybrids of Lior 2 x Portugal had 1.25, 1.65 and 3.80 univalents per cell.

The within plant variation was rather high in some hybrids as indicated by the large standard deviation (s.d.) of the mean. For the hybrid of Lior x Alta with 7.88 univalents per cell the s.d. was 3.97 (in this hybrid the highest number of univalents, 18, was found). For another hybrid of Lior x Alta with 4.47 univalents the s.d. was 1.87. For two hybrids of Lior x Ludion with 4.60 and 1.75 univalents s.d. were found of 1.88 and 1.07 respectively. In almost all hybrids cells without univalents were found.

In these hybrids the three genomes of F.a. and the genome of L.m. are present in the haploid state. A very high number of univalents is thus expected because none of the chromosomes has its homologue. The number

of univalents found however is relatively low. The possible reason will
be discussed later. The large variation found in numbers of univalents
among the hybrids is probably due to genetic rather than environmental
factors, because analyses of ten hybrids over two years showed that for
only two hybrids the differences in number of univalents between the two
years were significant (at the 5% level).

### 3.3.2.2.2  Bi- and multivalents

The average number of bivalents of the hybrids presented in table 5 va-
ried between 9.98 and 10.90 with a maximum of 14 per cell. This maximum
was found in all cross combinations. Generally, more ring bivalents
(average 5.91) than rod bivalents (average 4.41) were found. The number
of trivalents varied between 0.41 and 0.52 with a maximum of four per
cell, and quadrivalents varied between 0.45 and 0.94, also with a maximum
of four per cell. Three cells of the hybrids of the cross Lior x Alta
showed a pentavalent. In two cases, hexavalents were found, one in a hy-
brid of Lior x Portugal and one in a hybrid of Lior x Ludion.

### 3.3.2.2.3  Number of arms bound

Because little variation was found between the cross combinations for the
number of bivalents, trivalents and quadrivalents, the variation of the
average number of arms bound was small (table 5). The same types of ana-
lysis of variance with the same hybrids as for the numbers of univalents
were carried out for the number of arms bound. The analysis to test the
influence of the parents will be discussed later (section 3.3.2.4, table
7).

The analysis to detect variation between the 36 hybrids was carried out
on the basis of individual cell data. This analysis showed that the dif-
ferences for the numbers of arms bound were highly significant (1% le-
vel). A range from 14.8 for a hybrid of Lior x Alta to 21.7 arms bound
for a hybrid of Lior x Alpes was observed.

The analysis of variance to detect differences between hybrids within
cross combinations showed highly significant differences for the number
of arms bound. For five of the six cross combinations analysed, the dif-
ferences were significant at the 1% level. Very large differences (high

F values) were found within the cross Lior x Alta and within Lior x
Mayens. The range of numbers of arms bound in the hybrids of Lior x Al-
ta was from 14.8 to 21.5. In the hybrids of Lior x Mayens, this was 15.2
to 21.1. The difference between the hybrids of Lior x Portugal was sig-
nificant at the 5% level.

### 3.3.2.2.3  Cells without univalents

Studying meiotic irregularities of the hybrids the percentage of cells
without univalents may also be important. These are not only cells with
14 bivalents but also cells with bi- and multivalents.

The percentage of cells without univalents varied from 4% for Lior x Ma-
nade to 18.5% for Lior x Mayens, both cross combinations with hybrids
with a high number of univalents. Although some hybrids of Lior x Mayens
had a high number of univalents, others, mainly of Lior clone 2 x Mayens,
had few univalents and a high percentage of cells without univalents.
The cells without univalents were of four types:  14 bivalents, 12 biva-
lents + 1 quadrivalent, 10 bivalents + 2 quadrivalents and some cells
with 8 bivalents + 3 quadrivalents. As the hybrids have 28 chromosomes,
a maximum of 14 bivalents can be expected and this was indeed found.
This means that pairing occurs between the chromosomes of the Lolium ge-
nome and a Festuca genome and between the chromosomes of the two other
Festuca genomes. Of the quadrivalents, 64% were open chains, the rest
were alternate rings. Quadrivalents can originate from translocation
heterozygosity, most probably between the Lolium genome and one of the
Festuca genomes, or can originate from homoeologous pairing between chro-
mosomes of the four genomes present in the hybrids. This will be dis-
cussed more in detail in section 3.3.9 and in chapter 4.

### 3.3.2.3  Hybrids of Manawa x Festuca

### 3.3.2.3.1  Univalents

The hybrids with Manawa as female parent (table 6, Fig. 4.1 and 4.2)
showed a range of average number of univalents from 1.80 for Manawa x
Alpes to 3.28 for Manawa x Manade. Here, the same analyses of variance
as for the hybrids with Lior as female parent were carried out. Neither
an influence of the genotypes of the Festuca varieties nor of the two

Table 6. Chromosomes associations of the hybrids Manawa x *F. arundinacea* (range in parentheses)

| | No. plants | No. cells | Uni-valents | Bivalents ring | Bivalents rod | Bivalents total | Tri-valents | Quadri-valents | No. arms bound | % cells without univalents |
|---|---|---|---|---|---|---|---|---|---|---|
| Manawa x Alpes | 5 | 71 | 1.80 (0-8) | 6.30 (2-11) | 4.94 (1-10) | 11.24 (5-14) | 0.34 (0-4) | 0.69 (0-2) | 20.63 | 40.0 |
| Manawa x Alta | 8 | 76 | 2.75 (0-12) | 7.09 (2-11) | 3.79 (1-8) | 10.88 (6-14) | 0.41 (0-3) | 0.57 (0-2) | 20.73 | 10.5 |
| Manawa x Ludion | 3 | 13 | 2.31 (0-6) | 6.92 (5-9) | 4.62 (3-8) | 11.54 (10-14) | 0.15 (0-1) | 0.54 (0-1) | 20.61 | 15.4 |
| Manawa x Manade | 3 | 65 | 3.28 (0-8) | 6.17 (1-11) | 4.34 (0-9) | 10.51 (6-14) | 0.42 (0-3) | 0.62 (0-3) | 19.46 | 10.8 |
| Manawa x Mayens | 9 | 75 | 2.72 (0-9) | 6.53 (2-12) | 4.17 (1-9) | 10.71 (6-14) | 0.55 (0-3) | 0.55 (0-3) | 20.17 | 25.3 |
| Manawa x N.Z. | 8 | 55 | 2.55 (0-6) | 5.75 (3-11) | 4.76 (0-9) | 10.51 (6-13) | 0.36 (0-2) | 0.84 (0-3) | 19.81 | 14.5 |
| Manawa x Portugal | 5 | 93 | 2.56 (0-11) | 5.75 (1-11) | 4.43 (0-12) | 10.18 (5-14) | 0.22 (0-2) | 1.11 (0-4) | 20.23 | 31.2 |
| Total and means | 41 | 448 | 2.59 | 6.29 | 4.39 | 10.68 | 0.37 | 0.73 | 20.22 | 21.2 |

clones of Manawa could be detected.  The second analysis to detect differences between hybrids was carried out with 17 hybrids with each ten cells or more analysed.  This analysis showed highly significant differences (1% level) between the hybrids.  The range in number of univalents was from 0.48 for a hybrid of Manawa x Alpes to 6.15 for a hybrid of Manawa x Portugal.

The analysis to test differences between hybrids within cross combinations was carried out for four of the seven cross combinations.  The number of hybrids per cross combination varied from three to four.  The differences in number of univalents within cross combination Manawa x Alpes and within Manawa x Portugal were highly significant (1% level).  The differences between the hybrids within the two other cross combinations were not significant.  Variation in number of univalents within a cross combination was sometimes very high, e.g. the three hybrids of Manawa x Portugal had 0.82, 1.29 and 6.15 univalents respectively.

### 3.3.2.3.2  Bi- and multivalents

The average number of bivalents was high and varied between 10.18 and 11.54 (table 6).  In almost all hybrids, some cells with 14 bivalents were found and more ring- than rod bivalents were found.  A t-test showed that the difference between the number of ring- and rod bivalents of the hybrids was highly significant (1% level).  The number of trivalents was low, although in some cells, up to four trivalents were found.  The hybrids of Manawa x Portugal showed the highest number of quadrivalents, average 1.11 per cell with a maximum of four.

### 3.3.2.3.3  Number of arms bound and cells without univalents

Again three analyses of variance were carried out with the number of arms bound.  The first analysis to detect the influence of the parents on the number of arms bound will be discussed later (section 3.3.2.4, table 7).  The second analysis to see whether differences occur between the hybrids  was carried out with the same 17 hybrids as the analysis for the number of univalents and showed highly significant differences (at the 1% level).  A variation in number of arms bound between 15.7 for a hybrid of Manawa x Portugal, and 22.8 for a hybrid of Manawa x Mayens was observed.

The third analysis showed highly significant differences (1% level) bet-
ween hybrids within a cross combination for three of the four analysed
cross combinations, Manawa x Alpes, Manawa x Mayens and Manawa x Portugal.
The highest variation within a cross combination was found for hybrids of
Manawa x Portugal with 15.7 to 22.0 arms bound. The differences between
the hybrids of Manawa x Alta were not significant.

The percentage of cells without univalents showed a large variation,
10.5% for the hybrids of Manawa x Alta to 40% for the hybrids of Manawa
x Alpes.

### 3.3.2.4  Comparison between the hybrids of Lior x Festuca and
####           Manawa x Festuca

A comparison between the hybrids with Lior as female parent and Manawa
as female parent showed on an average a higher number of univalents in
the hybrids with Lior but the difference was not significant. In both
cases, the hybrids with Alpes had the lowest number of univalents and
the hybrids with Manade the highest. The number of bivalents was almost
equal and the slightly higher number of bivalents in the hybrids with
Manawa was in favour of the number of ring bivalents. In the hybrids
with Manawa, less trivalents, more quadrivalents and no penta- and hexa-
valents were found in comparison with the hybrids with Lior as female
parent. In all hybrids cytological abnormalities were observed, such as
laggards at anaphase I and micronuclei in the tetrads (Fig. 5). In some
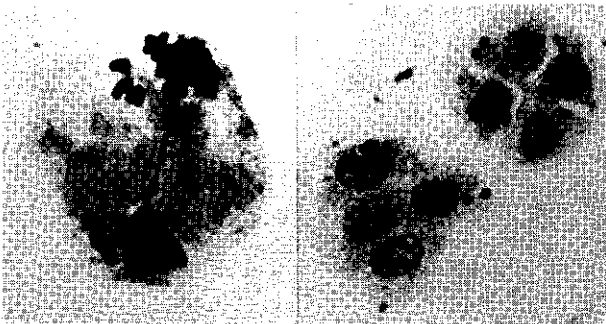


Fig. 5. Left: anaphase I with a bridge and a fragment of a hybrid of
        Lior x Ludion; right: tetrad with micronuclei of a hybrid of
        Manawa x N.Z.

hybrids, bridges with (Fig. 5) or without fragments were found. The ave-
rage number of arms bound and the average percentage of cells without uni-
valents was higher in the hybrids with Manawa. An analysis of variance
of the average number of arms bound for the hybrids of the cross combi-
nations of the three clones of Lior and the two clones of Manawa with the
seven Festuca parents (table 7) showed that the differences between the
Lolium parents were not quite significant ($P = 0.1$).

In the hybrids with Manawa as female parent, there is a tendency of less
variation in number of univalents and arms bound between the hybrids with-
in a cross combination than found between the hybrids within the cross
combinations with Lior. For the hybrids with Manawa, only four of the
seven cross combinations could be analysed and the number of hybrids ana-
lysed was smaller than that for Lior. This can be a reason for the
smaller variation found between the hybrids within the cross combinations
with Manawa as female parent.

There was no significant correlation between the number of arms bound and
the % of cells without univalents using data of hybrids of Lior and Mana-
wa ($r = 0.46$). The relationship between the parents and the hybrids will
be discussed in chapter 4.

The results of the meiotic analyses confirm in a general sense the find-
ings of several other investigations. CROWDER (1953a) found an average
of 2.52 univalents, 10.22 bivalents and the rest multivalents in his hy-
brids. He also found a maximum of 14 bivalents per cell. BUCKNER et al.
(1961) found a much lower number of univalents which varied between 0.66
and 2.44 per plant. These authors did not analyse the number of bivalents
and multivalents. LEWIS (1966), for the three plants he analysed, found
a range of univalents of 2.40 to 3.50. The number of bivalents varied
between 8.67 and 10.10. He also found a maximum of 14 bivalents per cell.
The number of tri- and quadrivalents were slightly higher than found in
this study.

Table 7. Average number of arms bound of hybrids of different cross combinations and analysis of variance

| Parents | Alpes | Alta | Ludion | Manade | Mayens | N.Z. | Portugal |
|---|---|---|---|---|---|---|---|
| Lior 1 | 20.31 | 16.33 | 18.53 | 21.08 | 16.27 | 19.42 | 19.60* |
| Lior 2 | 20.39 | 20.30 | 19.28 | 17.26 | 20.83 | 19.98 | 20.07 |
| Lior 3 | 20.36 | 16.19 | 20.82 | 17.04 | 21.20 | 17.97 | 21.35 |
| Manawa 1 | 19.52 | 20.76 | 20.00 | 18.60 | 19.64 | 19.59 | 19.83 |
| Manawa 2 | 22.24 | 20.66 | 21.00 | 21.05 | 22.00 | 20.06 | 20.91 |

* Calculated value

| Item | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Between Festucas | 6 | 12.99 | 2.16 | 1.03 | n.s. |
| Between Loliums | 4 | 21.35 | 5.33 | 2.55 | 0.1-0.05 |
| Error | 24 | 50.28 | 2.09 | | |
| Total | 34 | 84.63 | | | |

### 3.3.2.5 *Pollen stainability*

Pollen stainability was determined for most of the hybrids. The percentage of well stained pollen varied between 0 and 2%. In a number of pollen grains, some cytoplasma was present, but these were clearly different from the round, well stained, viable pollen grains. The percentage of this stained but abnormal pollen grains varied between 17.2% and 86.5%. The rest of the pollen grains were shrivelled and empty (range 13.5 to 89.2%). SULINOWSKY (1966) found a higher percentage of viable pollen, range 2.5 to 4.5%, in his hybrids.

### 3.3.3 The amphidiploids

### 3.3.3.1 *Introduction*

All amphidiploids examined had the expected chromosome number (56). The inflorescences were more of the festucoid type than Lolium like (Fig. 6). Doubled hybrids could be easely recognized because the anthers were dehiscent.

The results of the metaphase I analysis of different amphidiploids (the $C_0$ generation) are presented in table 8 for the cross with Lior as female parent, and in table 9 for those with Manawa as female parent. The results of all the plants analysed (generally two or three) of a cross combination are pooled. The number of cells analysed is low for the same reasons as for the hybrids: poor spreading and some stickiness of the chromosomes in most of the cells. Moreover, the high number of chromosomes, 56, complicates cytological analysis. The analyses were made over two years (1976 and 1977). During both years, the plants were grown under similar environmental conditions.
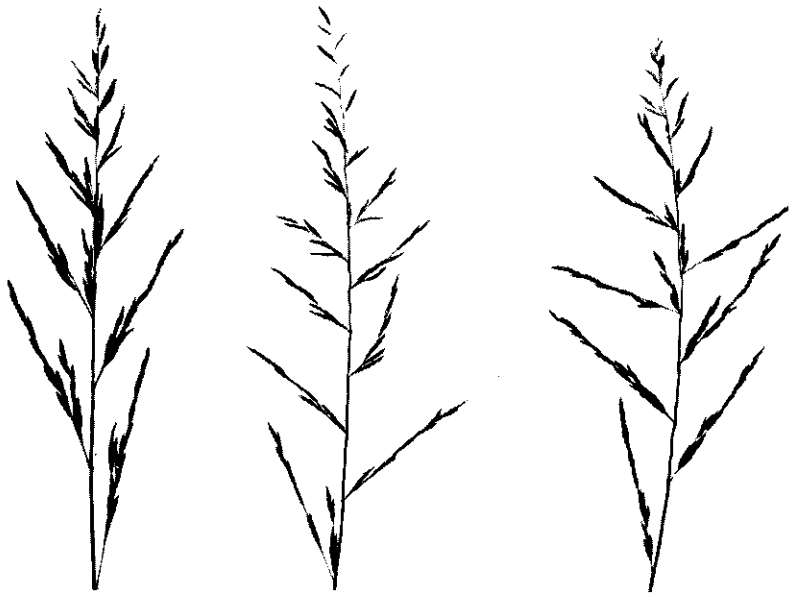
Fig. 6. Panicles of the amphidiploids L.m. x F.a. of Manawa x Ludion
(left) and Lior x Alpes (middle and right)

### 3.3.3.2 *Amphidiploids of Lior x Festuca*

#### 3.3.3.2.1 Univalents

The amphidiploids from the cross Lior x Alta and Lior x Mayens (table 8)
showed a high mean number of univalents per cell, 6,05 and 9,79 respect-
ively. In three cases, a low number of univalents was found, 2,20 for
the amphidiploids with Manade, 2.74 for Alpes and 3,00 for N.Z. The
number of univalents of the amphidiploids of Lior x Portugal was inter-
mediate, 3.92 per cell.

An analysis of variance using individual cell data of the amphidiploids
with ten cells or more analysed (nine of the 19 amphidiploids of table
8) showed highly significant differences (1% level) between the amphidi-
ploids for the number of univalents. Among these nine amphidiploids,

Table 8. Chromosome associations and number of arms bound per cell in the amphidiploids with Lior as female parent (range in parentheses)

| Cross | No. plants | No. cells | Uni-valents | Bivalents | | | Tri-valents | Quadri-valents | Penta-valents | Hexa-valents | No. arm bounds |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ring | rod | total | | | | | |
| Lior x Alpes | 4 | 19 | 2.74 (0-6) | 13.62 (7-22) | 9.53 (5-14) | 23.16 (18-28) | 1.11 (0-3) | 0.84 (0-3) | 0.05 (0-1) | | 42.00 |
| Lior x Alta | 4 | 22 | 6.05 (0-12) | 9.64 (6-15) | 10.59 (5-16) | 20.23 (16-25) | 1.91 (0-4) | 0.68 (0-2) | 0.14 (0-1) | | 36.27 |
| Lior x Manade | 2 | 15 | 2.20 (0-6) | 14.60 (10-19) | 9.06 (3-14) | 23.66 (17-28) | 0.86 (0-2) | 0.80 (0-3) | 0.13 (0-1) | | 43.63 |
| Lior x Mayens | 2 | 53 | 9.79 (2-18) | 8.96 (4-15) | 12.11 (7-17) | 21.08 (16-27) | 0.75 (0-3) | 0.43 (0-2) | | | 32.90 |
| Lior x N.Z. | 2 | 18 | 3.00 (0-8) | 14.17 (9-18) | 9.94 (0-13) | 24.11 (19-27) | 0.61 (0-2) | 0.56 (0-3) | 0.06 (0-1) | | 41.69 |
| Lior x Portugal | 5 | 129 | 3.92 (0-13) | 11.78 (5-21) | 10.81 (5-18) | 22.60 (13-28) | 0.79 (0-4) | 1.05 (0-5) | 0.02 (0-1) | 0.02 (0-1) | 39.62 |
| Total and means | 19 | 256 | 5.07 | 11.48 | 10.80 | 22.29 | 0.89 | 0.94 | 0.04 | 0.02 | 38.50 |

five were of the same cross combination, Lior x Portugal. A separate
analysis of variance with these five plants showed no significant differ-
ences in respect to number of univalents.

### 3.3.3.2.2  Bi- and multivalents

The number of bivalents varied from 20.23 for Lior x Alta to 24.11 for
Lior x N.Z. (table 8). Most of the bivalents were ring bivalents, ex-
cept for the amphidiploid of Lior x Alta and Lior x Mayens. These had
the highest number of univalents, the lowest number of bivalents, and
more rod than ring bivalents. For the number of univalents, ring- and
rod bivalents presented in table 8, the differences between the Festuca
parents were not significant (contingency chi-square = 2.89; P = 0.99).
Multivalents were present in all amphidiploids. Trivalents were very
frequent in Lior x Alta (1.91 per cell). Quadrivalents were most fre-
quent in Lior x Portugal (average 1.05 per cell) with one cell having
up to five quadrivalents (Fig. 7.3). Some pentavalents were observed
in almost all amphidiploids and a hexavalent in one.

### 3.3.3.2.3  Number of arms bound

Owing to the high number of univalents and the low number of bivalents,
the number of arms bound in the amphidiploids of Lior x Alta and Lior x
Mayens was low. An analysis of variance using individual cell data of
the nine amphidiploids with ten cells or more analysed showed highly
significant differences (1% level) for the number of arms bound. The
difference between the five amphidiploids of Lior x Portugal was signi-
ficant at the 5% level. There was a highly significant correlation bet-
ween the number of univalents and the number of arms bound (r = 0.94),
for the averages of the 19 plants of table 8.

Fig. 7.   Metaphase 1 of meiosis of the amphidiploids L.m. x F.a. of:
1.   Manawa x N.Z. with 3I, 23II, 1III and 1IV;   2.   Manawa x
Alta with 1I, 22II, 1III and 2IV;   3.   Lior x Portugal with
2I, 17II and 5IV;   4.   Manawa x N.Z. with 28II.   The bar re-
presents 10 μm

### 3.3.3.3 Amphidiploids of Manawa x Festuca

The highest number of univalents (5.79) in the amphidiploids involving
L.m. Manawa (table 9) was found for F.a. Ludion. Manawa x Alpes showed
a very low number of univalents (1.77). Analysis of variance with the
data of five amphidiploids with ten cells or more analysed, showed si-
gnificant differences between the amphidiploids (1% level) for the num-
ber of univalents.

The variation in number of bivalents was small (table 9). The majority
of bivalents were rings, with the highest number for Manawa x Alpes and
Manawa x N.Z. (16.46 and 14.79 respectively) (Fig. 7.1, 2 and 4). The
contingency chi-square for the Festuca parents in respect to the numbers
of univalents, ring- and rod bivalents was not significant (chi-square
= 4.57, probability between 0.8 and 0.9). Multivalents were present in
all amphidiploids. Manawa x Alpes had the highest number of quadriva-
lents (average 1.12 per cell). Here, one hexavalent was also found. In
an amphidiploid from Manawa x Alta, one cell was observed with two pen-
tavalents. Laggards at anaphase I and micronuclei in the tetrads were
frequently observed (Fig. 8).

The number of arms bound was generally high, except for Manawa x Ludion,
which had high numbers of univalents and rather low numbers of ring bi-
valents. An analysis of variance of five amphidiploids with ten cells
or more analysed showed highly significant differences (1% level) for
the number of arms bound. The correlation between the number of univa-
lents and the number of arms bound (r = 0.87; averages of the nine
plants of table 9) was highly significant.

### 3.3.3.4 Comparison between the amphidiploids of Lior x Festuca and Manawa x Festuca

On an average, the amphidiploids from the crosses with Manawa showed
better pairing (less univalents, more ring bivalents and a greater num-
ber of arms bound) than those from Lior but the differences on the ba-
sis of plant averages were not significant. Between the Festuca parents
the differences in pairing are sometimes large. Particularly, the
amphidiploids from the cross Lior x Mayens showed poor pairing compared

Table 9.  Chromosome associations and number of arms bound per cell in the amphidiploids with Manawa as female parent (range in parentheses)

| Cross | No. plants | No. cells | Uni-valents | Bivalents | | | Tri-valents | Quadri-valents | Penta-valents | Hexa-valents | No. arms bound |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ring | rod | total | | | | | |
| Manawa x Alpes | 3 | 26 | 1.77 (0-6) | 16.46 (10-22) | 7.46 (3-14) | 23.92 (21-28) | 0.46 (0-2) | 1.12 (0-3) | | 0.04 (0-1) | 45.31 |
| Manawa x Alta | 2 | 20 | 3.85 (0-10) | 13.85 (10-17) | 9.35 (5-14) | 23.20 (18-27) | 0.75 (0-2) | 0.70 (0-2) | 0.15 (0-2) | | 41.30 |
| Manawa x Ludion | 2 | 14 | 5.79 (1-14) | 12.00 (9-20) | 10.93 (6-14) | 22.93 (16-26) | 0.78 (0-2) | 0.57 (0-2) | | | 38.71 |
| Manawa x N.Z. | 2 | 56 | 3.02 (0-8) | 14.79 (9-25) | 10.45 (3-16) | 25.23 (15-28) | 0.27 (0-1) | 0.41 (0-2) | | | 42.03 |
| Manawa x Portugal | 1 | 3 | 2.67 (0-4) | 12.33 (10-17) | 11.67 (8-9) | 24.00 (18-28) | | 1.33 (0-4) | | | 40.00 |
| Total and means | 9 | 119 | 3.20 | 14.61 | 9.70 | 24.31 | 0.45 | 0.66 | 0.03 | 0.01 | 42.14 |
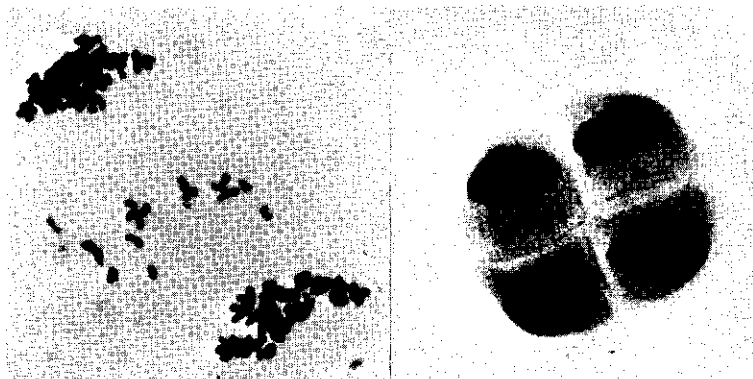
`Fig. 8.  Left:  anaphase I of an amphidiploid of Manawa x N.Z. with
         laggards;  right:  tetrad of an amphidiploid of Lior x Portu-
         gal with micronuclei

with the amphidiploids from Lior x Manade and Manawa x Alpes, which
showed a good pairing.  Based on the average data of cross combinations
(tables 8 and 9), no significant differences between the Festuca parents
could be detected but comparisons based on individual plant data showed
highly significant differences in numbers of univalents and numbers of
arms bound between the amphidiploids.  Differences within a cross combi-
nation were also found, but these were smaller than those between cross
combinations.  For instance, among the amphidiploids with ten cells or
more analysed, two plants of Lior x Mayens showed 33.70 and 32.18 arms
bound, two plants of Manawa x Alta 41.20 and 41.40 and two plants of Ma-
nawa x N.Z. 40.46 and 42.85 arms bound.  A t-test showed that these dif-
ferences were not significant, neither for the number of arms bound, nor
for the number of univalents.  The largest differences were found between
amphidiploids of Lior x Portugal, where five plants had 38.20, 38.70,
39.87, 40.42 and 42.38 arms bound respectively.  These differences were
significant at the 5% level.  The relationship between the hybrids and
their corresponding amphidiploids will be discussed in section 3.3.5 and
the relationship between the amphidiploids and their parents in chapter 4.

The analyses of pollen stainability in 1980 (table 10) showed large dif-
ferences between the amphidiploids of the different cross combinations.

Table 10.   Pollen stainability, seed fertility and seed germination of
the amphidiploids of the different cross combinations (1980)

| Cross | stained | Pollen, % partly stained | unstained | Grains/ spikelet | % Germination |
|-------|---------|--------------------------|-----------|------------------|---------------|
| Lior x Alpes | 23.7 | 27.4 | 48.8 | 0.04 | 58.8 |
| Lior x Alta | 12.7 | 27.4 | 59.9 | 0.07 | 44.2 |
| Lior x Ludion | 25.0 | 17.1 | 57.9 | 0.08 | 56.2 |
| Lior x Manade | 46.7 | 21.2 | 32.8 | 0.77 | 75.0 |
| Lior x N.Z. | 18.8 | 17.9 | 63.3 | 0.07 | 75.2 |
| Lior x Portugal | 41.3 | 34.3 | 24.4 | 0.21 | 70.7 |
| Means | 28.1 | 24.2 | 47.8 | 0.21 | 63.3 |
| Manawa x Alpes | 42.9 | 22.6 | 34.5 | 0.14 | 46.0 |
| Manawa x Ludion | 47.8 | 15.9 | 36.3 | 0.39 | 60.3 |
| Manawa x Mayens | 3.6 | 5.3 | 91.1 | 0.00 | |
| Manawa x N.Z. | 40.8 | 22.1 | 37.1 | 0.01 | |
| Manawa x Portugal | 23.6 | 31.3 | 45.1 | | |
| Means | 31.7 | 19.4 | 48.8 | 0.14 | 53.1 |

The percentage of well stained pollen varied from 3.6 for Manawa x Mayens
to 47.8 for Manawa x Ludion.  The percentage of unstained pollen was high
in all cases (variation from 32.8% for Lior x Manade to 91.1% for Manawa
x Mayens).  Pollen stainability of the low pairing amphidiploids of Lior
x Mayens had been studied two years earlier (1978).  The percentage of
stainable pollen was 12.7.

Seeds fertility, measured as the number of seeds per spikelet (table 10),
was low for all the amphidiploids flowering together in the greenhouse.
Only one plant (Lior x Manade) showed a number of seeds per spikelet
higher than 1 (1.13 seeds/spikelet).  No seeds were formed on the amphi-
diploids of Manawa x Mayens.  These had also the lowest percentage of
stainable pollen.  There was no significant correlation between pollen
stainability and seed fertility (r = 0.60), univalents and seed fertil-
ity (r = 0.10), arms bound and pollen stainability (r = 0.65) and arms
bound and seed fertility (r = 0.29).  These four correlations were based

on data of eight individual amphidiploids. The germination of the seeds
obtained was not high, maximum 75%. Germinability was low for Lior x Al-
ta and Manawa x Alpes, 44.2 and 46% respectively.

### 3.3.3.5 Results of other authors

Very few data have been reported in the literature on chromosome pairing
at metaphase I of meiosis of the amphidiploids. BUCKNER et al. (1961)
analysed only the number of univalents of one plant with 56 chromosomes
and found 2.16 univalents. LEWIS (1966) analysed three amphidiploids
and found a number of univalents varying between 1.73 and 5.46. In two
plants, he found more bivalents (25.13 to 25.35) than in this study. The
number of multivalents was similar. JAUHAR (1975b) analysed four other
amphidiploids. He found a range of univalents similar to those in this
study and a slightly higher number of bivalents (variation between 25.5
and 25.88). He found also multivalents but in lower frequencies than in
this study. ZWIERZYKOWSKY (1980) found a number of univalents varying
between 2.27 and 2.9. He did not find a relationship between the degree
of meiotic irregularity and the occurrence of viable pollen. BOWMAN and
THOMAS (1973) studied amphidiploids of L.p. and F.a. They found that in
the presence of B chromosomes of L.p. homoeologous pairing in the amphi-
diploids was suppressed and found in absence of B chromosomes 5.90 univa-
lents and 1.59 multivalents and in the presence of B chromosomes 2.39
univalents and 0.32 multivalents. However, due to their irregular trans-
mission the usefulness of B chromosomes in stabilizing amphidiploids is
very limited.

Pollen fertility was analysed by SULINOWSKY (1966) who found a range from
39.3 to 43.2% of stainable pollen for the four plants he analysed. The
56 chromosome plant studied by BUCKNER et al. (1961) had 25% stainable
pollen. BUCKNER et al. (1965) studied some 56 chromosome plants of the
$C_1$, $C_2$ and $C_3$ generations. Their variation in pollen stainability was
40.4 to 84.1%.

The seed fertility of the amphidiploids of the $C_0$ generation was studied
by WIT (1974) who found an average yield of 15.3 gram per plant and in $C_1$
14.0 g. BUCKNER et al. (1965) studied the $C_1$ and $C_3$ generations. They
measured fertility as seeds/plant or seeds/spike and found large differ-
ences between amphidiploids.

### 3.3.4  Influence of temperature on meiosis of amphidiploids

Because the analysis of meiosis was made on plants grown in the green-
house at a higher temperature (day temperature $20^{\circ}C$ and night tempera-
ture $15^{\circ}C$) than outside (average for Changins in May $13^{\circ}C$) and because
temperature may influence chromosome pairing and chiasma formation (for
an older review, see WILSON, 1959), it might be possible that some of
the meiotic irregularities are due to high temperature. For this reason
the influence of temperature on chromosome pairing was studied.

Three temperatures were chosen: $15^{\circ}C$, $20^{\circ}C$ and $25^{\circ}C$. A number of am-
phidiploids was cloned so that three plants per genotype were obtained.
The plants were vernalized outside and in the beginning of April 1978
the plants were placed in three different growth chambers at $15^{\circ}C$ during
the day and $10^{\circ}C$ at night. One week later, the temperatures were changed
so that the plants had constant temperatures of $15^{\circ}C$, $20^{\circ}C$ or $25^{\circ}C$ under
constant illumination.

The results of the meiotic analyses are presented in table 11. Although
on an average not many cells per plant could be analysed, a number of ob-
servations was made. For most plants, the chromosome associations at the
different temperatures were very similar. The greatest difference was
found for plant 20/34, where at $15^{\circ}C$ more univalents, fewer ring biva-
lents were observed, and fewer arms were bound than at the two other
temperatures. The differences in numbers of univalents and numbers of
arms bound at the three temperatures were not significant. The latter
was true also for the amphidiploids 20/28 and 20/107. Plant 20/103
showed a low number of univalents, average 1.70 for both temperatures.
There were differences between plants but these were not very large.
The overall results at the different temperatures were very similar,
e.g. the number of univalents at $15^{\circ}C$ was 2.42, at $20^{\circ}C$ 2.51 and at $25^{\circ}C$
2.68. The number of bivalents and the number of arms bound were also
very close to each other. More quadrivalents were found at $20^{\circ}C$, 0.75
versus 0.53 and 0.59. Some seeds were formed on the plants at the dif-
ferent temperatures. No detailed fertility studies were undertaken be-
cause most of the panicles were taken for meiotic analysis and not
enough were left for fertility studies. On an average, in all the
plants, a similar number of seeds was formed at the three different

Table 11. Chromosome associations of the amphidiploids cultivated at three different temperatures

| Cross | No. plant | Temp. °C | No. cells | Uni-valents | Bivalents ring | Bivalents rod | Bivalents total | Tri-valents | Quadri-valents | Penta-valents | No. arms bound | % cells with-out univalents |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lior x Alpes | 20/28 | 15 | 15 | 2.20 | 12.73 | 10.80 | 23.53 | 1.00 | 0.93 | | 41.03 | 7 |
| | | 20 | 17 | 2.94 | 13.18 | 10.06 | 23.24 | 1.06 | 0.88 | | 41.53 | 12 |
| | | 25 | 7 | 3.29 | 13.43 | 10.00 | 23.43 | 1.57 | 0.29 | | 40.85 | 0 |
| Lior x Alpes | 20/34 | 15 | 15 | 3.93 | 12.40 | 11.73 | 24.13 | 1.00 | 0.20 | | 39.20 | 13 |
| | | 20 | 7 | 1.71 | 13.86 | 11.14 | 25.00 | 0.57 | 0.71 | | 42.14 | 14 |
| | | 25 | 5 | 3.00 | 13.40 | 10.60 | 24.00 | 0.60 | 0.80 | | 41.00 | 40 |
| Manawa x N.Z. | 20/103 | 15 | 15 | 1.60 | 15.60 | 10.40 | 26.00 | 0.27 | 0.40 | | 43.40 | 27 |
| | | 20 | 12 | 1.83 | 14.58 | 10.75 | 25.33 | 0.50 | 0.58 | | 42.96 | 25 |
| Manawa x N.Z. | 20/107 | 15 | 6 | 1.17 | 15.50 | 9.83 | 25.33 | 0.50 | 0.67 | | 44.00 | 33 |
| | | 20 | 12 | 3.42 | 13.50 | 10.08 | 23.58 | 0.67 | 0.75 | 0.08 | 41.33 | 8 |
| | | 25 | 13 | 2.85 | 13.54 | 10.56 | 24.00 | 1.00 | 0.54 | | 41.38 | 15 |
| Manawa x Mayens | 20/135 | 15 | 2 | 2.50 | 13.00 | 12.00 | 25.00 | 0.50 | 0.50 | | 40.75 | 0 |
| | | 20 | 5 | 1.60 | 15.60 | 8.00 | 23.60 | 0.80 | 1.20 | | 44.80 | 20 |
| | | 25 | 9 | 1.78 | 15.67 | 9.56 | 25.22 | 0.22 | 0.78 | | 44.22 | 33 |
| Total | | 15 | 53 | 2.42 | 13.77 | 10.89 | 24.66 | 0.72 | 0.53 | | 41.51 | 19 |
| | | 20 | 53 | 2.51 | 13.89 | 10.17 | 24.06 | 0.75 | 0.75 | 0.02 | 42.20 | 15 |
| | | 25 | 34 | 2.68 | 14.06 | 10.15 | 24.21 | 0.85 | 0.59 | | 41.97 | 21 |

temperatures.  Generally, the number of seeds per plant was low.  For
this reason, the progeny data of different plants grown at the same tem-
perature were pooled.  The chromosome numbers of these progeny plants are
shown in table 12.  There was a large variation in chromosome number
among these plants which varied between 48 and 59 chromosomes for the
plants cultivated at $15^oC$, between 42 and 58 for the plants at $20^oC$, and
between 48 and 63 for the plants at $25^oC$.  At $15^oC$ and $20^oC$, the 56 chro-
mosome class was the most frequent.  The average chromosome number of
the progenies of the plants cultivated at the three different temperatu-
res was very similar, 54.57 for $15^oC$, 54.64 for $20^oC$, and 54.26 for $25^oC$.
Although the average number of chromosomes was similar for the three
temperatures, the percentage of euploids (plants with 56 chromosomes)
decreased with increasing temperature, and in the progenies of plants
cultivated at $15^oC$, twice as many euploids were found than in the pro-
genies of plants cultivated at $25^oC$.  The contingency chi-square, how-
ever, was only 3.66 (probability between 0.2 and 0.1).  Apparently, there
were no significant differences between the chromosome numbers of the $C_1$
plants at the different temperatures.

The influence of temperature was studied for *Tradescantia bracteata* and
*Uvularia perfoliata* by DOWRICK (1957).  He found that at higher tempera-
tures, the number and position of the chiasmata was affected.  Increase
of temperature resulted in an increase of the frequency of interstitial
chiasmata.  This increase was followed by a fall in both terminal and
interstitial chiasmata at higher temperatures (above $30^oC$ for *T. bractea-
ta*  and $20^oC$ for *U. perfoliata*).  High temperature in *Endymion non-
scriptus* led to reduced chiasma frequency, while decreasing temperatures
below $20^oC$ in *Hyachinthus orientalis* resulted in a progressive reduction
of chiasma frequency (ELLIOT, 1955).  Studies on the influence of tempe-
rature on meiosis of newly created amphidiploids have not been carried
out earlier.  The temperatures chosen in this study do not seem to in-
fluence chromosome pairing and chiasma formation in the unstable amphidi-
ploids of *L. multiflorum* x *F. arundinacea*.  This range of temperatures
was not excessive because only temperatures were chosen where eventually
seed production under natural conditions is possible.   It is pos-
sible that the temperatures tested were not extreme enough to influence
meiosis as found for *T. bracteata*, *U. perfoliata*, *E. non-scriptus*  and
*H. orientalis*.

Table 12. Chromosome numbers of progenies of $C_0$ plants cultivated at three different constant temperatures

| Temper-ature | No plants | 42 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 63 | Average | % euploids |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15°C | 51 | | 1 | 1 | | 3 | 5 | 6 | 5 | 6 | 17 | 4 | 2 | 1 | | 54.57 | 33.3 |
| 20°C | 45 | 1 | | | | | 5 | 7 | 6 | 7 | 10 | 5 | 4 | | | 54.64 | 22.2 |
| 25°C | 42 | | 1 | | | 5 | 2 | 9 | 8 | 3 | 7 | 2 | 3 | 1 | 1 | 54.36 | 16.7 |

## 3.3.5  Comparison between the hybrids and the amphidiploids

A comparison between the morphology of the hybrids and their correspond-
ing amphidiploids showed a great similarity in the general plant growth
(Fig. 9a and b).  The amphidiploids could be easily recognized because
their anthers at maturity were dehiscent in contrast to those of the hy-
brids (Fig. 10).



Fig. 9a.  Plants at flowering time of the hybrid (left) and correspond-
ing amphidiploid (right) of Lior x Alpes.

Fig. 9b.   Plants at flowering time of the hybrid (left) and correspond-
ing amphidiploid (right) of Lior x Manade

A general comparison of the number of arms bound per chromosome of the
hybrids (tables 5 and 6) with those of the amphidiploids (tables 8 and 9)
showed that the association per chromosome of the amphidiploids was si-
milar or only slightly better than that of the hybrids.  As these compa-
risons were made between averages of hybrids and amphidiploids which
were genotypically different, and as differences in numbers of arms bound
between the hybrids and between the different amphidiploids were found
(section 3.3.2 and 3.3.3), it is interesting to make a comparison between
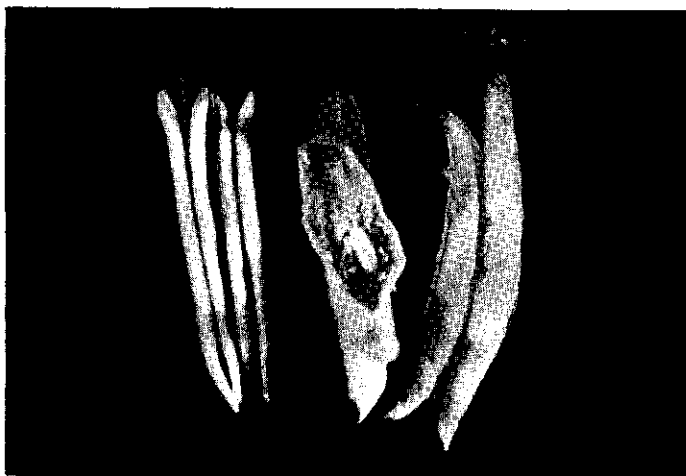hybrids and the amphidiploids derived by doubling a segment of the same

Fig. 10.  Anthers at maturity of Manawa x Alpes.  Left:  the hybrid
(non dehiscent);  right:  the amphidiploid (dehiscent)

plant.  Three hybrids and their corresponding amphidiploids were cloned
so that three or four plants per genotype were obtained.  Meiotic analy-
ses, number of micronuclei per tetrad (M/T), pollen stainability, germ-
ination of the pollen grains in vitro and seed fertility were studied on
these plants in 1981.  For six more hybrids and their amphidiploids,
chromosome association at metaphase I and the number of micronuclei per
tetrad (M/T) were analysed in 1981, in single, uncloned plants.

The results are presented in tables 13 and 14.  In table 13, the meiotic
analyses of six hybrids and amphidiploids and of two of the three (hybrid
21/26 with corresponding amphidiploid 20/28 and hybrid 21/100 with 20/103)
cloned genotypes are presented.  The number of univalents, bivalents and
arms bound was never significantly different between plants of the same
clone.  The results of the different plants of a clone were pooled.  An
analysis of variance with individual cell data showed significant differ-
ences (at the 1% level) for the number of univalents and the number of
arms bound among the hybrids and among the amphidiploids.  No significant
difference between the hybrids and their amphidiploids was found for the
number of univalents.  The differences between the hybrids and their am-
phidiploids for the number of arms bound per chromosome are shown in Fig.
11.  In most cases, the amphidiploid  showed a higher number of arms bound

Table 13. Chromosome associations per cell and number of micronuclei/tetrad (M/T) of hybrids and their corresponding amphidiploids

| Cross | No. plant | 2n | Number cells | Uni-valents | Bivalents ring | Bivalents rod | Bivalents total | Tri-valents | Quadri-valents | No. arms bound/chromosome | M/T |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Lior x Portugal | 21/8 | 28 | 14 | 3.21 | 5.71 | 5.43 | 11.14 | 0.36 | 0.36 | 0.67 | 3.41 |
| | 20/10 | 56 | 15 | 3.20 | 12.47 | 11.73 | 24.20 | 0.67 | 0.60 | 0.71 | 4.04 |
| Lior x Alpes | 21/26 | 28 | 37 | 3.19 | 5.70 | 4.94 | 10.64 | 0.59 | 0.40 | 0.68 | 4.65 |
| | 20/28 | 56 | 25 | 3.04 | 12.88 | 10.28 | 23.16 | 0.88 | 0.92 | 0.73 | 5.27 |
| | 21/27 | 28 | 20 | 5.20 | 5.50 | 3.70 | 9.20 | 0.80 | 0.50 | 0.63 | 1.87 |
| | 20/29 | 56 | 20 | 5.65 | 11.10 | 9.95 | 21.05 | 1.15 | 1.20 | 0.68 | 3.17 |
| | 21/32 | 28 | 20 | 2.20 | 6.30 | 5.25 | 11.55 | 0.40 | 0.35 1) | 0.71 | 2.02 |
| | 20/34 | 56 | 18 | 3.50 | 12.22 | 10.60 | 22.83 | 1.22 | 0.78 1) | 0.71 | 4.29 |
| Manawa x N.Z. | 21/100 | 28 | 32 | 1.47 | 5.85 | 6.27 | 12.13 | 0.09 | 0.50 1) | 0.71 | 2.27 |
| | 20/103 | 56 | 38 | 3.03 | 14.34 | 10.71 | 25.05 | 0.55 | 0.55 1) | 0.74 | 3.47 |
| | 21/104 | 28 | 20 | 2.80 | 5.65 | 5.25 | 10.90 | 0.40 | 0.55 2) | 0.69 | 3.09 |
| | 20/107 | 56 | 14 | 2.29 | 15.86 | 8.71 | 24.57 | 0.71 | 0.57 | 0.78 | 2.88 |
| | 21/101 | 28 | 20 | 3.10 | 4.60 | 6.20 | 10.80 | 0.50 | 0.45 | 0.64 | 3.98 |
| | 20/104 | 56 | 20 | 2.60 | 14.05 | 10.70 | 24.75 | 0.30 | 0.75 | 0.75 | 2.95 |
| Lior x Ludion | 21/152 | 28 | 20 | 4.80 | 5.00 | 5.60 | 10.60 | 0.20 | 0.35 | 0.61 | 3.66 |
| | 20/157 | 56 | 17 | 2.82 | 14.47 | 10.76 | 25.24 | 0.35 | 0.41 | 0.74 | 2.41 |

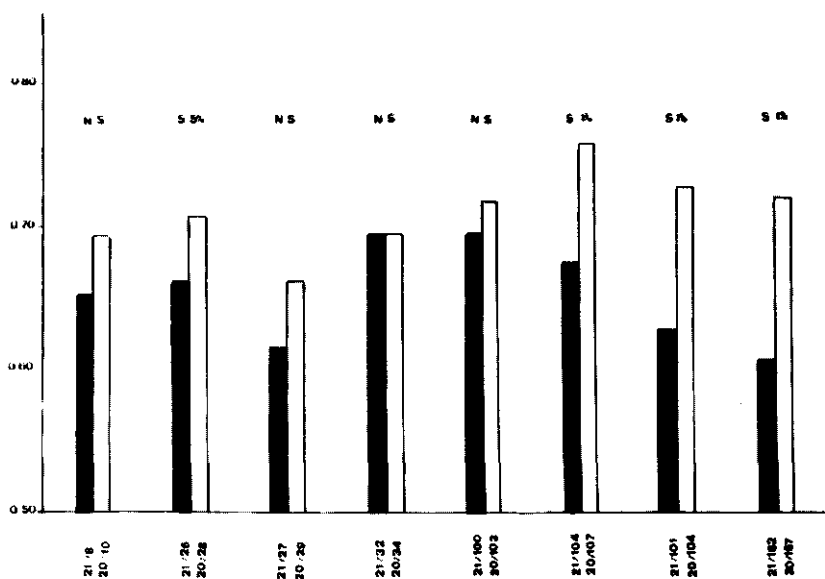1) one pentavalent

2) one hexavalent

Fig. 11.   Number of arms bound per chromosome of the hybrids ■ and their
corresponding amphidiploids □ , ns = difference not signifi-
cant, s 1%, 5% = difference significant at the 1% or 5% level

per chromosome than the hybrid.  For four comparisons, the difference
between the hybrid and the amphidiploid was not significant, for one the
difference was significant at the 5% level and in the other three cases,
the difference was significant at the 1% level.  A t-test with the aver-
age data of Fig. 11 showed significant (1% level) differences between
the hybrids and the amphidiploids.  This means that chromosome doubling
of the hybrids increases chromosome pairing and chiasma formation only
slightly in spite of the fact that each chromosome has a homologous
counterpart in the amphidiploid (see also section 3.3.9 and chapter 4).

The number of micronuclei per tetrad is also given in table 13 for the
different hybrids and amphidiploids (averages of 150 tetrads) and in
table 14 for plants of the different clones (averages of 100 tetrads).
The differences between plants were large and varied between 1.87 and
5.27 M/T (table 13).  An analysis of variance for the number of M/T in

Table 14.  Number of micronuclei per tetrad (M/T), average ($\bar{x}$) and stan-
          dard deviation (s);  % pollen stainability;  % germinated pol-
          len and seed fertility expressed as number of seeds/spikelet
          of plants of clones of different hybrids and their amphidiploids
          (21/26 with 20/28, 21/43 with 20/45 and 21/100 with 20/103)

| Cross | plant | 2n | M/T $\bar{x}$ | M/T s | Pollen stainabi- lity, % | Pollen germin- ation | Fertility seeds/ spikelet |
|-------|-------|----|------|------|------|------|------|
| Lior x Alpes | 20/28.1 | 56 | 5.50 | 2.69 | | 8.70 | 0.04 |
| | 20/28.2 | 56 | 4.18 | 3.63 | 52.47 | | 0.10 |
| | 20/28.3 | 56 | 6.65 | 3.19 | 42.04 | 3.67 | 0.10 |
| | 20/28.4 | 56 | 4.84 | 2.23 | 45.92 | | 0.01 |
| Manawa x Alpes | 20/45.1 | 56 | 1.72 | 2.00 | 80.63 | 47.42 | 0.19 |
| | 20/45.2 | 56 | 2.18 | 1.84 | | 45.24 | 0.16 |
| | 20/45.3 | 56 | 3.32 | 2.39 | 70.46 | 46.85 | 0.10 |
| | 20/45.4 | 56 | 4.84 | 3.59 | | 50.26 | 0.05 |
| Manawa x N.Z. | 20/103.1 | 56 | 3.13 | 3.02 | 66.66 | 37.02 | 0.08 |
| | 20/103.2 | 56 | | | 82.79 | 26.41 | 0.06 |
| | 20/103.3 | 56 | 3.82 | 4.21 | 79.36 | 29.13 | 0.05 |
| Lior x Alpes | 21/26.1 | 28 | 3.60 | 2.07 | 0.50 | | |
| | 21/26.2 | 28 | 5.71 | 2.86 | 0.80 | | |
| Manawa x Alpes | 21/43.1 | 28 | | | 3.33 | | |
| | 21/43.2 | 28 | 3.44 | 2.74 | 2.64 | | |
| | 21/43.3 | 28 | 3.50 | 2.42 | 0.00 | | |
| Manawa x N.Z. | 21/100.1 | 28 | | | 0.94 | | |
| | 21/100.2 | 28 | 2.47 | 2.26 | 0.31 | | |
| | 21/100.3 | 28 | 2.00 | 1.77 | 2.95 | | |
| | 21/100.4 | 28 | 2.28 | 2.56 | | | |

the hybrids showed highly significant differences (1% level).  The same
observation was made for the amphidiploids.  No significant differences
for the numbers of M/T were found between the hybrids and the amphidi-
ploids.  In the individual plants of the different clones (table 14), the

number of M/T varied between 1.72 and 6.65 M/T and the standard deviation
was high, indicating a high variability of this parameter. Significant
differences were found between plants of the same clone, except for the
hybrid Manawa x N.Z. (No. 21/100).

There was no significant correlation ($r = 0.08$) between the number of
univalents and the number of M/T using the data of table 13. CROWDER
(1953b) found a significant correlation at the 1% level ($r = 0.77$) bet-
ween univalents and M/T for F.a. The correlations calculated from the
data given by BUCKNER et al. (1961) and HILL and BUCKNER (1962) of hy-
brids between Lolium and F.a. and backcrosses were not significant: $r =
0.18$ and $r = 0.36$ respectively. This lack of correlation can be due to
the fact that univalents at metaphase I can behave in different ways.
At metaphase I - anaphase I, the two chromatids can already separate or
chromosome breakage can occur. The univalents may or may not be inclu-
ded in the newly formed nuclei of the dyad. When they are included in
the nucleus and the chromatids were already separated, there is a great
chance of chromosome breakage at metaphase II. The unequal size of the
micronuclei indicates that these events occur. All these events can be
the reason why the parameter M/T is very variable and why differences
between plants of the same clone were found and why there was no signi-
ficant correlation between the number of univalents and the number of
M/T.

Pollen stainability (table 14) corresponded with what was found earlier
(section 3.3.2.5 and 3.3.3.4). The average stainability in the hybrids
was 1.43% with a range of 0.00% to 3.33%, and the differences were not
significant. The amphidiploids showed good pollen stainability and the
percentages were higher than found earlier. The average of Lior x Alpes
(46.31%) was significantly lower (1% level) than the averages of the
other two cross combinations, 75.54% and 76.77% respectively.

Germination in vitro of pollen grains was studied only for the amphidi-
ploids. In table 14, the % of pollen grains with a normal pollen tube
is given. Two other types of pollen grains could be distinguished, one
type forming a globule at the pore and another with a bursting tube.
The differences between the three cross combinations for the percentage
of pollen with normal tubes (range 3.67% to 50.26%) were highly signi-
ficant.

Fertility of the amphidiploids measured as number of seeds per spikelet
was low (range 0.01 to 0.19) and the differences were not significant.

The number of M/T of the amphidiploids was significantly correlated with
pollen stainability ($r = -0.67$, 5% level) with the percentage of normally
germinating pollen ($r = -0.78$, 1% level) and with seed fertility ($r =
-0.67$, 5% level). The correlation between pollen stainability and pollen
germination ($r = 0.69$) was not significant. When the total percentage of
germinated pollen was considered, including pollen grains with a globule
and with burst tubes the correlation ($r = 0.85$) was significant at the 5%
level. Globule formation at the pore and bursting of the pollen tubes is
probably due to the nutrient medium and these pollen grains may be consi-
dered to be viable. JANSSEN and HERMSEN (1976) did not find a signifi-
cant correlation between coloration of the pollen grains with aceto-car-
mine or other stains and berry set or seed set in *Solanum*. Measuring
pollen stainability with aceto-carmine thus gives only an indication of
the pollen viability.

### 3.3.6 The $C_1$ generation

Seeds were collected on $C_0$ plants and chromosome counts of the $C_1$ plants
were made. The results of the chromosome counts of progenies of four $C_0$
plants with a high number of $C_1$ plants are presented in table 15. There
was a high frequency of aneuploids, some were hyperploid but most plants
were hypoploid. The range in chromosome number was somewhat smaller than
found in table 12 and averages were slightly lower. A contingency chi-
square test showed that the distributions of the different chromosomes
numbers in the progenies of the four amphidiploids were not significantly
different. The chi-square was 13.12 (P between 0.7 and 0.5).

$C_1$ plants with 56 chromosomes were selected and meiotic analyses carried
out on these plants in 1981. The results are presented in table 16. Ten
$C_1$ plants originating from open pollination of $C_0$ plants were analysed.
The plants designated 103/1, 103/4 and 103/5 originated from the same
mother plant, and 107/9 from another, but both were Manawa x N.Z. hybrids.
Similarly, plants 157/1, 2 and 9, and 163/1 and 4 were derived from two
mother plants of the cross Manawa x Ludion. One 56 chromosome plant
(157) was found after selfing of one of these amphidiploids, which was

Table 15. Chromosome numbers in progenies of four $C_0$ plants resulting from three different cross combinations

| Cross | N | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 63 | Average | % euploids |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Manawa x N.Z. | 42 | 1 | 4 | 4 | 9 | 8 | 6 | 10 | | | | | 53.80 | 23.8 |
| Lior x Alpes (1) | 32 | 1 | 2 | 5 | 5 | 9 | 7 | 3 | | | 1 | | 53.62 | 9.4 |
| Lior x Alpes (2) | 28 | 2 | 2 | 4 | 4 | 1 | 5 | 8 | | 1 | | | 53.61 | 28.6 |
| Lior x Portugal | 59 | 1 | 9 | 6 | 11 | 11 | 9 | 10 | | 1 | | 1 | 53.83 | 16.9 |

Table 16.  Chromosome associations per cell, number of micronuclei per tetrad (M/T) and seed fertility (number of seeds/spikelet) in plants of the $C_1$ generation, L = Lior and M = Manawa

| Cross | Plant | No. cells | Uni-valents | Bivalents | | | Tri-valents | Quadri-valents | No. arms bound | M/T | Seed fertility |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ring | rod | total | | | | | |
| L x Portugal | 103 | 5 | 3.20 | 13.20 | 10.60 | 23.80 | 1.20 | 0.40 | 40.80 | 4.45 | 0.74 |
| M x N.Z. | 103/1 | 6 | 5.16 | 14.33 | 9.33 | 23.66 | 0.50 | 0.50 | 40.58 | | |
| | 103/4 | 7 | 4.14 | 13.14 | 10.71 | 23.86 | 1.01 | 0.28[1] | 39.85 | 3.82 | |
| | 103/5 | 20 | 2.30 | 16.30 | 8.55 | 24.85 | 0.45 | 0.65[1] | 44.47 | 2.74 | 0.64 |
| M x N.Z. | 107/9 | 6 | 2.50 | 14.50 | 11.17 | 25.67 | 0.50 | 0.17 | 41.83 | 4.28 | 0.14 |
| M x Ludion | 157/1 | 14 | 2.64 | 15.78 | 8.50 | 24.28 | 1.21 | 0.28 | 43.53 | 2.92 | 0.54 |
| | 157/2 | 20 | 2.65 | 16.55 | 8.60 | 25.15 | 0.55 | 0.35 | 43.90 | 5.26 | 0.67 |
| | 157/9 | 16 | 3.75 | 15.37 | 8.62 | 24.00 | 0.75 | 0.50 | 42.59 | 4.85 | 0.12 |
| M x Ludion | 163/1 | 11 | 1.09 | 11.09 | 14.09 | 25.18 | 0.18 | 1.00 | 40.18 | 2.37 | 0.80 |
| | 163/4 | 20 | 1.85 | 11.85 | 13.85 | 25.70 | 0.25 | 0.50 | 39.45 | 2.00 | 0.31 |
| M x Ludion | 157 | 17 | 4.23 | 12.94 | 9.41 | 22.35 | 1.23 | 0.82[1] | 40.64 | 4.07 | 0.16 |
| x (L x Portugal) | 157x10 | 20 | 3.00 | 13.65 | 10.70 | 24.35 | 0.70 | 0.55 | 41.60 | 2.76 | 0.22 |
| x (M x N.Z.) | 157x103 | 20 | 2.40 | 15.65 | 9.15 | 24.80 | 0.60 | 0.55 | 43.42 | 4.03 | 0.39 |
| (L x Manade) x (M x Ludion) | 179x157 | 20 | 2.75 | 12.75 | 11.80 | 24.55 | 0.30 | 0.80[1] | 41.65 | 4.15 | 0.43 |
| (M x Alpes) x (M x Ludion) | 43x157 | 10 | 5.10 | 13.70 | 9.40 | 23.10 | 0.90 | 0.50 | 40.10 | 8.62 | 0.31 |
| Total | | 212 | 2.93 | 14.21 | 10.24 | 24.44 | 0.65 | 0.55 | 41.85 | 4.00 | 0.42 |

1) one pentavalent

also crossed with other amphidiploids. From these crosses two 56 chromo-
some plants (157x10 and 157x103) were obtained. Two more plants (179x157
and 43x157) from crosses between amphidiploids were analysed. The number
of univalents varied from 1.09 to 5.16. Significant differences (1% le-
vel) in number of univalents were found between the progenies of plant
103 (Manawa x N.Z.), and between the self progeny of plant 157 (Manawa x
Ludion) and the progenies of the two crosses 157x10 and 157x103. No si-
gnificant differences were found between the progenies obtained after
open pollination of plant 157 (plants 157/1, 2 and 9). The number of bi-
valents was high and varied between 22.35 and 25.70. Trivalents and qua-
drivalents were frequently present. In some plants, a rather large num-
ber of trivalents was found, up to 1.23 per cell. It might be possible
that some of the plants with a high trivalent frequency were not disomic,
but mono-trisomic, which also gives a chromosome number of 56. Most of
the trivalents were v-shaped, except in the self progeny of plant 157
(1.23 trivalents) where one third were 'frying-pan' trivalents, indicating
maximum chiasma formation in the trivalents. This may be explained by
the presence of three homologous chromosomes. Significant differences at
the 1% level in numbers of arms bound were found between progenies of
plant 103 and between the self progeny of plant 157, 157x10 and 157x103.
The differences between the progenies obtained after open pollination of
plant 157 (157/1, 2 and 9) were not significant. One plant was found
with different numbers of chromosomes in its cells, 53 and 54. This plant
was asynaptic, the average chromosome association of ten cells analysed
was 2.1 ring, 4.8 rod bivalents and 39.7 univalents.

Comparison of the average chromosome association in $C_1$ and $C_0$ plants
(tables 16, 8 and 9) showed a slightly lower number of univalents in the
$C_1$ plants than the average number found in the original amphidiploids
(table 9). The number of bivalents was similar to the average number of
bivalents of the amphidiploids in table 9. The average number of arms
bound (41.85) of the $C_1$ plants was higher than the average number of Lior
x Festuca (38.50) but lower than the average number of Manawa x Festuca
(42.14). However, $C_1$ plants originating from crosses with Lior partici-
pated only with 5 cells (plant 10/3) to the establishment of the average
number of arms bound (41.85).

The numbers of micronuclei per tetrad (M/T) in $C_1$ presented in table 16 are averages of 150 tetrads. Plant differences were large and varied from 2.00 to 8.62. The differences between plants were highly significant. There was a highly significant correlation ($r = 0.77$) between the number of univalents and M/T. This correlation was expected but was in contrast to the non significant correlation between univalents and M/T calculated from the data of table 13.

Seed fertility measured as number of seeds per spikelet is presented in the last column of table 16. It varied from 0.12 to 0.80. No significant correlation ($r = 0.18$) was found between the number of arms bound and seed fertility. The correlation ($r = 0.20$) between the number of M/T and fertility was also not significant. Compared to the fertility of the mother plants (averages in table 10), there was some increase of fertility of some of the $C_1$ plants. In 1976, for plant 157, 0.48 seeds/ spikelet were found. The progenies of this amphidiploid produced 0.16 seeds/spikelet in the plant obtained by selfing and 0.12, 0.22, 0.39, 0.54 and 0.67 seeds/spikelet in the other $C_1$ plants. Plant 163 had 0.06 seeds/spikelet in 1976. Two progenies of this plant had 0.31 and 0.80 respectively. Plant 103 had a fertility of 0.04 seeds/spikelet in 1976. In 1981, the fertility of the same plant was measured (table 14) and now 0.06 seeds/spikelet were found. A descendant of this plant had a ten times higher fertility.

On an average, the $C_1$ generation did not show an increase of chromosome association so homozygosity of the amphidiploids is not a cause of meiotic instability and irregularity. For inbred rye, *Secale cereale* L., (LAMM, 1936; REES, 1955 and SYBENGA, 1958) and inbred *L. perenne* (KARP and JONES, 1982) a reduction in number of chiasmata was found, Inducing heterozygosity had a positive influence on fertility in our amphidiploids: the plants of the $C_1$ generation were on an average more fertile than those of $C_0$, although the chiasma frequency was slightly reduced.

### 3.3.7  The hybrid and amphidiploid of the reciprocal cross

From the cross with the F.a. population Portugal as female and the L.m. variety Turilo as male parent, only one hybrid plant was obtained (table 2). The inflorescences of this hybrid were very similar to those of the hybrids L.m. x F.a. The anthers were not dehiscent.

Meiotic analyses of this hybrid were carried out in 1982. The following chromosome associations were found (averages of 20 cells): 5.20 I, 6.30 ring bivalents, 3.95 rod bivalents, 0.50 III and 0.20 IV. The average number of arms bound was 18.25. The percentage of well stained pollen was 0.78%.

The amphidiploid derived from a doubled segment of the hybrid plant had dehiscent anthers. Meiotic analyses carried out in 1982 showed 4.55 I, 13.65 ring bivalents, 10.2 rod bivalents, 0.65 III and 0.45 IV (averages of 20 cells). The number of arms bound was 40.25. The percentage of well stained pollen was 44.13%. Seed fertility could not be analysed yet.

The number of arms bound per chromosome was higher for the amphidiploid (0.72) than for the hybrid (0.65). A t-test showed that the difference was significant at the 1% level. Compared to the hybrids and amphidi- ploids with Lolium as female parent (tables 5, 6, 8 and 9), this hybrid and amphidiploid did not show a higher chromosome association and fell in the range found for the hybrids and amphidiploids of L.m. x F.a. So, obtaining of amphidiploids with cytoplasm of F.a., which is very diffi- cult (table 2), does not ensure a more regular meiosis than that of the amphidiploids with Lolium cytoplasm.

### 3.3.8  Hybrids and amphidiploids of tetraploid *L. multiflorum* x *F. arun- dinacea*

#### 3.3.8.1  *Introduction*

All hybrids examined (18 plants) had the expected 35 chromosomes. The inflorescences of these hybrids were of the panicle type but in compari- son to the hybrids between the diploid Lolium and Festuca, the influence of the extra Lolium genome on the morphology of the panicles could be distinguished by the fact that the panicles were more Lolium-like than those of the 28 chromosome hybrids. A large variation in panicle type was observed on the same plant (Fig. 12) where all intermediates between more or less branched panicles to the spike type of Lolium could be found. The anthers of these hybrids were dehiscent and 0 to 40 seeds per plant were formed, when the 18 plants flowered together. Chromosome counts of some of the progenies showed a range from 30 to 36 chromosomes with an average of 32.96 (table 17). The colchicine treatment was not very
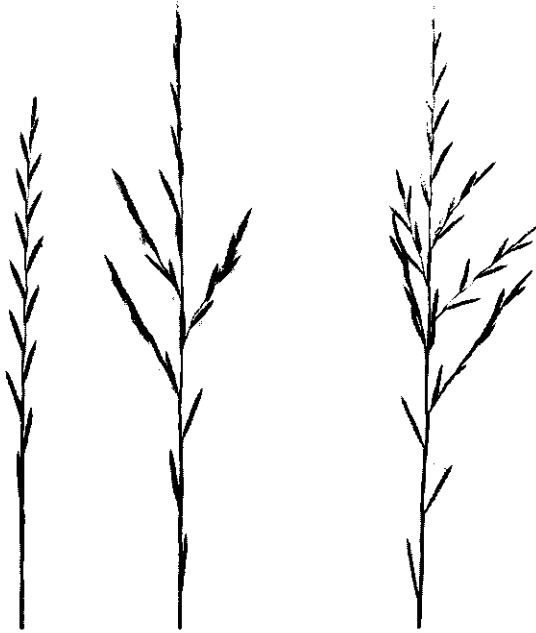
Fig. 12.  Panicles of an amphidiploid (2n = 70) of Amenda x Ludion.
The three panicles belong to the same plant

Table 17.  Chromosome numbers of the progenies of the hybrids (35 chromo-
somes) between the tetraploid Lolium and *F. arundinacea*

| Number of plants | 30 | 31 | 32 | 33 | 34 | 35 | 36 | Average |
|---|---|---|---|---|---|---|---|---|
| 27 | 2 | 3 | 7 | 4 | 6 | 3 | 2 | 32.96 |

successful, probably due to two reasons.  The first reason may be that
the doubled sectors could not be distinguished by the panicle character-
istics as both the anthers of the hybrids and those of the amphidiploids
were dehiscent.  The second reason may be that the phenomenon of 'loss'
of doubled sectors detected by chromosome counts was also observed in
these hybrids (cf. section 3.2).  Of the 18 hybrids, only seven plants
could be doubled in spite of repeated colchicine-treatment.  These dou-

bled plants will be designated as amphidiploids although they do not cor-
respond exactly to the definition of an amphidiploid, due to the presence
of four Lolium genomes originating from the tetraploid Lolium.

### 3.3.8.2 The hybrids

The results of the meiotic analyses of the hybrids are presented in table
18. As for the hybrids with the diploid Lolium x F.a., the level of
chromosome association is very high. Although the variation in the num-
ber of univalents is not excessive, an analysis of variance of the data
on the individual cells showed significant differences at the 1% level
between the hybrids. A t-test showed that for the number of univalents,
the differences within a cross combination were significant at the 5%
level. Little variation was found for the total number of bivalents
(range 12.15 for Amenda x Ludion to 13.90 for Amenda x New Zealand), but
rather large differences (significant at the 1% level) were found for the
number of ring bivalents (range from 4.65 for Amenda x Ludion to 8.75 for
Amenda x Alpes and Amenda x N.Z.). In all hybrids, multivalents were
found. Trivalents were more frequent (range 0.65 for Amenda x N.Z. to
1.40 for Amenda x Ludion) than quadrivalents (range 0.20 for Amenda x
N.Z. to 0.70 for Amenda x Ludion). Some pentavalents were also observed.
An analysis of variance of the number of arms bound (individual cells)
showed highly significant (at the 1% level) differences between the hy-
brids. The differences between the hybrids within a cross combination
for number of arms bound were also significant at the 1% level. Pollen
stainability was analysed for a number of hybrids but not in the same
year as the meiotic behaviour. The average percentage of stainable pol-
len of 14 analysed plants was 27.4% with a range from 1.25% for a plant
of Amenda x N.Z. to 54.2% for a hybrid between Amenda and Ludion. The
pollen grains were of irregular size.

### 3.3.8.3 The amphidiploids

The results of the meiotic analyses of the amphidiploids are presented
in table 19. Six of the seven doubled hybrids could be analysed. The
number of univalents is rather high and a large variation was found, from
3.29 (for Amenda x Ludion plant 35/35) to 7.08 (for Amenda x Alpes plant
35/20). An analysis of variance for the number of univalents (individual

Table 18. Chromosome associations per cell of the hybrids (35 chromosomes) of the tetraploid Lolium variety Amenda with different *F. arundinacea* varieties

| Cross | Plant | No. cells | Uni-valents | Bivalents | | | Tri-valents | Quadri-valents | Penta-valents | No. arms bound |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ring | rod | total | | | | |
| Amenda x Alpes | 35/1 | 14 | 4.28 | 7.28 | 5.64 | 12.93 | 1.00 | 0.28 | 0.14 | 23.68 |
| | 35/10 | 20 | 3.10 | 8.75 | 3.85 | 12.60 | 1.10 | 0.65 | 0.15 | 26.45 |
| Amenda x Ludion | 35/14 | 20 | 3.20 | 5.20 | 6.95 | 12.15 | 1.40 | 0.70 | 0.10 | 23.00 |
| | 35/16 | 20 | 4.15 | 4.65 | 7.80 | 12.45 | 0.95 | 0.65 | 0.10 | 21.47 |
| Amenda x N.Z. | 35/12 | 20 | 4.45 | 8.75 | 5.15 | 13.90 | 0.65 | 0.20 | | 24.75 |

Table 19.  Chromosome associations per cell of the amphidiploids (70 chromosomes) of the tetraploid
Lolium variety Amenda with different *F. arundinacea*

| Cross | Plant | No. cells | Uni- valents | Bivalents ring | rod | total | Tri- valents | Quadri- valents | Penta- valents | Hexa- valents | No. arms bound |
|-------|-------|-----------|--------------|----------------|-----|-------|--------------|-----------------|----------------|---------------|----------------|
| Amenda x Alpes | 35/20 | 13 | 7.08 | 10.92 | 14.85 | 25.77 | 1.13 | 1.46 | 0.23 | 0.08 | 45.23 |
|  | 35/29 | 32 | 4.34 | 12.00 | 15.81 | 27.81 | 1.50 | 1.06 | 0.16 | 0.03 | 42.33 |
| Amenda x Ludion | 35/33 | 11 | 6.27 | 8.36 | 17.18 | 25.55 | 1.64 | 1.55 | 0.09 | 0.18 | 43.59 |
|  | 35/35 | 7 | 3.29 | 8.43 | 19.71 | 28.14 | 1.71 | 1.14 | 0.14 |  | 44.28 |
|  | 35/38 | 11 | 6.09 | 10.54 | 18.27 | 28.73 | 1.00 | 0.73 |  | 0.09 | 44.36 |
| Amenda x N.Z. | 35/31 | 9 | 6.78 | 15.67 | 11.67 | 27.33 | 1.33 | 1.00 | 0.11 |  | 49.39 |

cells) of the amphidiploids showed significant differences at the 1%
level. Within the cross combinations Amenda x Alpes and Amenda x Ludion,
the differences between the amphidiploids were also significant (1% le-
vel). The majority of the chromosomes paired as bivalents. A t-test
showed that the differences within cross combinations, e.g. for Amenda x
Ludion 25.55 and 28.73 bivalents, were not significant. Most of the bi-
valents were rod bivalents, except for Amenda x N.Z. where more ring-
than rod bivalents were found. Only two of the 83 cells analysed had no
univalents. One of these cells had 35 bivalents, the other 33 bivalents
and one quadrivalent. Both cells were found in plant 35/29. Trivalents
and quadrivalents were found with a rather high frequency and a maximum
of four (Fig. 13) per cell was observed. In most amphidiploids, some
pentavalents (maximum two per cell) and hexavalents (maximum one per
cell) were found. Cytological aberrations such as laggards at anaphase
I (Fig. 13) and micronuclei in the tetrads were frequently observed.
The numbers of bound arms in the different amphidiploids were signifi-
cantly different (1% level), but not within the cross combinations Amen-
da x Alpes and Amenda x Ludion. Pollen stainability was difficult to
establish because most of the amphidiploids were still mixoploids so
that it was not certain whether the analysed pollen was from a doubled
sector. For three, not mixoploid, 70 chromosome plants analyses were
carried out. Pollen stainability varied from 25.6% (plant 35/20) to
58.7% (plant 35/29) and 56.2% (plant 35/33). These analyses were not
carried out in the same year as the meiotic analyses. The pollen grains
were of irregular size (Fig. 13).

Differences between the two clones of Amenda used for the crosses with
F.a. concerning meiotic behaviour could not be calculated because only
one hybrid (plant 35/12 of table 18) and its corresponding amphidiploid
(plant 35/31 of table 19) were available from the cross Amenda clone 2
x N.Z. The other hybrids and amphidiploids (tables 18 and 19) were from
the crosses between Amenda clone 1 with Alpes and Ludion.

### 3.3.8.4 Comparison between the hybrids and amphidiploids

A comparison was made between the number of arms bound per chromosome of
the hybrids and their corresponding amphidiploids. The results are pre-

Fig. 13.   1.   Metaphase I of an amphidiploid of Amenda x Ludion (2n = 70) with 5I, 22II, 4III, 1IV and 1V;   2.   Anaphase I of an amphidiploid of Amenda x Alpes (2n = 70) with laggards and bridges;   3.   Pollen at maturity of an amphidiploid of Amenda x Ludion.   The bars represent 10 μm

Table 20.   Comparison of the number of arms bound per chromosome in the
            hybrids (35 chromosomes) of tetraploid *L. multiflorum* x *F.
            arundinacea* and their corresponding amphidiploids (70 chromo-
            somes) using the data of tables 18 and 19

| Plant No. | Hybrids 35 chromosomes | Amphidiploids 70 chromosomes |
|-----------|------------------------|------------------------------|
| 1 35/1,35/20 | 0.67 | 0.64 |
| 2 35/10,35/29 | 0.75 | 0.60 |
| 3 35/14,35/33 | 0.65 | 0.62 |
| 4 35/16,35/35 | 0.61 | 0.63 |
| 5 25/12,35/31 | 0.70 | 0.70 |

sented in table 20.   All plants were grown together in the greenhouse in
1981.   This comparison showed that the number of arms bound per chromo-
some in the amphidiploids was not significantly different for plants No.
1, 3, 4 and 5, whereas  for plant No. 2, it was significantly lower (1%
level) than the number of arms bound per chromosome in the corresponding
hybrids.   This indicates that the chromosome doubling of the pentaploid
hybrids did not result in an improvement of chromosome pairing and chias-
ma formation in the decaploid products.

Although hybrids between tetraploid Lolium and F.a. have been produced
elsewhere (BADOUX, 1973;  GRÖBER *et al.*, 1974;  HERTZSCH, 1960), no meio-
tic analyses were made.   GRÖBER *et al.* (1976) analysed only pollen stain-
ability of their hybrids.   They found a higher percentage (72.6%) of pol-
len stainability than in this study.

### 3.3.9  Affinity between the genomes of Lolium and Festuca

Chromosome association in hybrids can be used as the basis for establish-
ing species relationships.   In a series of papers, Kimber and co-workers
of the University of Missouri (KIMBER *et al.*, 1981;  ALONSO and KIMBER,
1981;  KIMBER and ALONSO, 1981;  ESPINASSE and KIMBER, 1981) have de-
veloped a numerical method which allows the estimation of the relative
affinity (expressed as x) between genomes.   The term relative affinity
(ALONSO and KIMBER, 1981) was chosen to avoid confusion with homology or
homoeology of the genomes involved, for in some cases, it is a measure
of homology and in others of homoeology.

For tetraploid hybrids, such as those between L.m. and F.a., four types
of relative affinity can be taken into consideration (KIMBER and ALONSO,
1981). The first type was designated 2 : 2 with two genomes more closely
related to each other than they are to a similarly related second pair of
genomes (both relative affinity x). The second type of tetraploid hybrid
was designated 2 : 1 : 1. It is assumed to contain one pair of closely
related genomes (relative affinity x) and to more distant genomes to the
same degree related to each other and to the closely related pair (rela-
tive affinity y). The third type of tetraploid hybrids was designated
3 : 1 and contains three equally related genomes and a fourth more dis-
tantly related genome. In the fourth type, 4 : 0, all genomes have equal
affinity. The 4 : 0 hybrids can be of two types: autoploid when all ge-
nomes are (nearly) identical and 'alloploid' when all four genomes are
equally distant from each other and could be considered as the 1 : 1 : 1
: 1 type. The fourth type was considered as unrealistic for L.m. x F.a.
hybrids and was not taken into accound.

For the pentaploid hybrid from the tetraploid Lolium x F.a. cross, five
affinity types were taken into consideration (ESPINASSE and KIMBER, 1981),
type 2 : 2 : 1, type 3 : 2, type 2 : 1 : 1 : 1, type 3 : 1 : 1 and type
4 : 1. The relation between the genomes of these five types of hybrids
is similar to that explained for the tetraploid hybrids.

Equations were developed to calculate the expected relative frequencies
of meiotic configurations for the different types of affinities for te-
traploid and pentaploid hybrids. These equations were expressed in terms
of x, y and c. The variable x is the relative affinity of the two most
closely related genomes in the hybrids while y is the relative affinity
of all of the most distantly related genomes; c was defined by DRISCOLL
et al. (1979) as the probability of chiasma formation in a pair of chro-
mosome arms and redefined by ALONSO and KIMBER (1981) as the mean fre-
quency with which two related chromosome arms pair. The value of c is
calculated as the sum of the numbers of rod bivalents plus twice the
number of ring bivalents and v-shaped trivalents plus three times the
number of chain quadrivalents plus four times the number of ring quadri-
valents, the total divided by four times the basic chromosome number.
If the symbox Xt is used to indicate the number of pairs of arms connec-

ted by chiasmata than c = Xt/28 in a tetraploid hybrid with a basic chromosome number of seven. In a pentaploid hybrid c is also Xt/28.

The value of x, by definition ranges from 0.5 when all the chromosomes of a homoeologous group can pair with equal facility, to 1.0 when two or more genomes pair to the exclusion of the more distantly related genomes. The equations for calculating the expected meiotic configurations can be written in c or x as explained in detail by KIMBER and ALONSO (1981). Since y = 1-x and since c is obtained by observation, the value of x can be calculated. The affinity model that fits best has the smallest sum of squares of the differences between the calculated and observed meiotic figures.

On the basis of the data presented in tables 5 and 6 for the tetraploid hybrids and table 18 for the pentaploid hybrids the fit of the different models of affinity was calculated. The value of c, the relative affinity x and the sums of squares of the differences between observed and calculated values of the different models are given in table 21 for the tetraploid hybrids and in table 22 for the pentaploid hybrids. The c values of the hybrids with Lior (table 21) were lower than the number of arms bound per chromosome in table 5. This was because with the calculated meiotic figures of these hybrids all quadrivalents were considered to be chains; this was not entirely correct because ring quadrivalents occurred also. For the hybrids with Manawa, chain and ring quadrivalents were separated. Here, the c values were sometimes slightly lower than the number of arms bound per chromosome (table 6). This was due to some 'frying-pan' tri-and quadrivalents, which were considered as chains. From table 21, it could be concluded that the 3 : 1 model gave a very poor fit. The sums of squares were all in excess of 17. Except for the hybrids Lior x Manade and Manawa x Alta, the 2 : 2 model fits best. The values of x were not very high, which means that chromosome pairing is not exclusively within the two sets of genomes showing closest affinity but also between chromosomes of all four genomes. This, of course, agrees with the observation of tri- and quadrivalents in the hybrids.

GAUL (1958) developed a method which makes it possible to estimate the number of chromosomes (P) that in principle are able to pair on the basis of the relation between chiasma frequency and number of bivalents:

Table 21. The mean arm association frequency (c), relative affinity (x) and sums of squares (ss) between observed and calculated meiotic figures for three different genome models of the tetraploid hybrids between Lior (L) x Festuca and Manawa (M) x Festuca

| Cross | No. cells | c | 2 : 2 | | 2 : 1 : 1 | | 3 : 1 | |
|---|---|---|---|---|---|---|---|---|
| | | | x | ss | x | ss | x | ss |
| L x Alpes | 125 | 0.715 | 0.874 | 0.090 | 0.937 | 2.867 | 0.500 | 19.339 |
| L x Alta | 188 | 0.671 | 0.876 | 0.562 | 0.942 | 1.879 | 0.500 | 17.297 |
| L x Ludion | 169 | 0.675 | 0.910 | 0.030 | 0.937 | 5.521 | 0.500 | 20.540 |
| L x Manade | 75 | 0.682 | 0.879 | 2.578 | 0.959 | 0.573 | 0.826 | 21.475 |
| L x Mayens | 92 | 0.660 | 0.893 | 1.159 | 0.953 | 1.923 | 0.500 | 20.400 |
| L x N.Z. | 133 | 0.672 | 0.889 | 0.859 | 0.950 | 1.043 | 0.500 | 19.631 |
| L x Portugal | 116 | 0.715 | 0.878 | 0.192 | 0.945 | 2.138 | 0.500 | 20.747 |
| M x Alpes | 71 | 0.735 | 0.912 | 0.562 | 0.943 | 5.665 | 0.500 | 25.136 |
| M x Alta | 76 | 0.741 | 0.895 | 1.015 | 0.962 | 0.793 | 0.741 | 27.045 |
| M x Ludion | 13 | 0.736 | 0.927 | 0.137 | 0.960 | 3.444 | 0.500 | 28.995 |
| M x Manade | 65 | 0.695 | 0.892 | 0.532 | 0.951 | 1.956 | 0.500 | 21.376 |
| M x Mayens | 75 | 0.720 | 0.893 | 0.381 | 0.953 | 1.899 | 0.500 | 23.495 |
| M x N.Z. | 55 | 0.708 | 0.889 | 0.179 | 0.935 | 3.995 | 0.500 | 19.610 |
| M x Portugal | 93 | 0.723 | 0.864 | 0.376 | 0.932 | 2.627 | 0.500 | 17.691 |

$$P = \frac{X^2 + X - B}{(2X - B)C}$$

where X is the total number of chiasmata, B the total number of paired chromosomes and C the number of cells analysed. Applying this formula to the data of tables 5 and 6 showed that for the different cross combinations between 25.40 and 28.25 chromosomes were able to pair. This means that in the hybrids, all the 28 chromosomes in principle are capable of pairing. This calculation, however, does not take into consideration the possibility of the formation of chiasmata between more than two chromosomes in one meiotic configuration. The method developed by KIMBER and co-workers does this and allows the discrimination between different affinity models. The results of these analyses were not very surprizing. CROWDER (1953a) mentioned already on the basis of meiotic

analysis of his hybrids between L.m. or L.p. with F.a. that 'seven chro-
mosomes of F.a. are homologous enough to pair with the genome of Lolium
and the remaining 14 chromosomes of F.a. possessed enough homology, in
some instances, to pair among themselves'.  The calculation using the me-
thod of GAUL (1958) confirmed thus what was found in the meiosis of the
hybrids, that all the 28 chromosomes can pair.  This was found as 14 bi-
valents or as bi- and quadrivalents.  The calculations using the method
of KIMBER and co-workers showed that the conclusion of CROWDER (1953a)
was correct.  The 2 : 2 model which fits best indicates that the genome
of Lolium shows about the same affinity to one of the genomes of F.a. as
the other two genomes of F.a. show to each other.  The fact that F.a. is
still a good allopolyploid showing only bivalent pairing will be discuss-
ed in chapter 4.

Pairing was observed between 'homoeologous' chromosomes (affinity y) if
the chromosomes showing affinity (x) were considered as 'homologous'.
The frequency of this homoeologous pairing can be calculated (KIMBER and
ALONSO, 1981).  The actual frequencies of homologous (i) and homoeologous
(s) pairing events are conditioned both by the values of x and y.  Thus
the frequency of homologous pairing is ix and of homoeologous pairing sy.
Therefore, the proportion of homoeologous association is:

$$\frac{sy}{ix + sy}$$

The ratio of the number of ways that homologous and homoeologous pairing
can occur (i : s) can be derived by considering all possible arrangements
of pairing events between the chromosomes of a homoeologous group.  In
the 2 : 2 model i : s = 1 : 2.  The percentage of homoeologous associa-
tion calculated for the tetraploid hybrids of which the 2 : 2 model fits
best ranged from 16.24% for Lior x Alpes to 23.98% for Manawa x Portugal.
Many of the tri- and quadrivalents observed in the hybrids might be the
result of homoeologous chromosome association.

For Lior x Manade and Manawa x Alpes, the model 2 : 1 : 1 fits best.
The chromosome association results presented in tables 5 and 6 were ave-
rages of different hybrid plants.  After separating these hybrids into
three groups for Lior (the three different clones of Lior) and two groups
for Manawa (the two different clones), the analysis showed that for the

hybrids Lior 1 x Manade and Lior 3 x Manade the 2 : 1 : 1 model fits
best and for Lior 2 x Manade the 2 : 2 model. The hybrids of Manawa 1 x
Alta fit best the 2 : 1 : 1 model and Manawa 2 x Alta the 2 : 2 model.
The equations are similar to each other for the 2 : 1 : 1 and the 2 : 2
models at intermediate c values. Consequently, small changes in the rod
and ring bivalent ratio would cause better fit to one model or another.

From the five analysed pentaploid hybrids (table 22), three (two Amenda
x Alpes and one Amenda x N.Z.) fit best the 2 : 2 : 1 model and two (the
hybrids Amenda x Ludion) the 3 : 2 model. Discrimination between these
two models depends somewhat on the ratio of bivalents to multivalents
and a simple translocation or random variation in observation may account
for the differences (KIMBER, personal communication). Furthermore, the
number of analysed cells was rather small. The sums of squares for all
the cross combinations and all the models tested were rather high. The
difference between the sums of squares of the models 3 : 2 and 2 : 2 : 1
were not very large for the two hybrids of Amenda x Ludion. These re-
sults were not in contradiction with those of the tetraploid hybrids.
It seems that the two genomes of the tetraploid Lolium showed a good af-
finity in the pentaploid. The value of x was also slightly higher than
that found in the tetraploid hybrids. In both models, two genomes of
F.a. showed affinity. The third genome of Festuca, which showed affini-
ty with the Lolium genome in the tetraploid hybrids showed less affinity
in the pentaploid hybrids, probably due to the presence of two Lolium ge-
nomes.

Table 22. The mean arm association frequency (c), relative affinity (x) and the sum of squares (ss) between the observed and calculated meiotic figures for the 5 different genome affinity models of the penta- ploid hybrids between L.m. Amenda (A) and three Festuca varieties

| Cross | No. cells | Plant | c | 2 : 2 : 1 | | 3 : 2 | | 2 : 1 : 1 : 1 | | 3 : 1 : 1 | | 4 : 1 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | x | ss | x | ss | x | ss | x | ss | x | ss |
| A x Alpes | 14 | 35/1 | 0.843 | 0.935 | 8.583 | 1.000 | 14.151 | 0.982 | 12.836 | 0.958 | 39.897 | 0.880 | 41.640 |
| A x Alpes | 20 | 35/10 | 0.943 | 0.924 | 9.623 | 0.994 | 17.262 | 0.993 | 13.110 | 1.000 | 45.331 | 0.918 | 45.074 |
| A x Ludion | 20 | 35/14 | 0.818 | 0.888 | 18.533 | 0.979 | 16.367 | 0.939 | 22.761 | 0.691 | 34.166 | 0.500 | 34.326 |
| A x Ludion | 20 | 35/16 | 0.766 | 0.902 | 20.000 | 1.000 | 17.901 | 0.925 | 26.882 | 0.500 | 34.864 | 0.500 | 34.867 |
| A x N.Z. | 20 | 35/12 | 0.884 | 0.954 | 8.980 | 1.000 | 22.138 | 1.000 | 13.690 | 1.000 | 53.713 | 0.937 | 53.437 |

# 4. GENERAL DISCUSSION

Hybrids between L.m. and F.a. could be obtained rather easily. Some influence of the Festuca parent on the crossability was detected but the differences were rather small. The differences between the Lolium parents were not significant, but as only two varieties of diploid Lolium were used, no general conclusions about the influence of the Lolium parent on the crossability can be drawn. A very large difference in crossability between the reciprocal crosses was observed in this study and confirmed the findings of other authors (CROWDER, 1953a; HERTZSCH, 1960; LEWIS, 1966; GRÖBER et al., 1974). HERTZSCH (1960) and GRÖBER et al. (1974) found that, compared to diploid Lolium, tetraploid Lolium showed a lower crossability when used as pistillate parent, but a higher crossability than the diploid when used as pollen parent. None of these authors gave an explanation for these differences. These differences are probably, at least partially, due to abnormal endosperm development, or by an unbalance in the relation endosperm/embryo in some interspecific crosses. Both difficult crosses have 7x endosperm. The F.a. : L.m. ratio is 6 : 1 in the cross F.a. x L.m., 3 : 4 in the cross tetraploid Lolium x F.a., while in the cross L.m. x F.a. this ratio is 3 : 1. The fact that F.a. has already a genome very similar to the Lolium genome may be important too. It may be that in some crosses too much and in other crosses not enough F.a. genomes are present. An indication for poor endosperm development is that in both difficult crosses carried out in this study, F.a. x L.m. and tetraploid Lolium x F.a. most of the hybrid seeds were shrivelled and germination was poor. REUSCH (1959) explained the anomalous behaviour of endosperm in his crosses between L.p. and *F. pratensis* by an unfavourable nuclear-cytoplasmic relationship. GYMER and WHITTINGTON (1973), after testing several hypotheses, such as endosperm maternal tissue relationship, F.a. : L.m. genomic ratio in the endosperm, endosperm embryo relationship and genome : plasmon ratio in the endosperm found that the genome : plasmon ratio was the most acceptable hypothesis to explain their results of the crosses between L.p. and *F. pratensis*. The plasmon was not precisely defined. It may be partly determined by the nucleus. The differences in crossability presented here could not be explained by the nuclear-cytoplasmic relationship, nor by the genome : plasmon ratio.

The hybrids showed a surprisingly high level of chromosome association, taking into account that the homologous partner of each chromosome was lacking. A significant influence of the Festuca parents on chromosome pairing in the hybrids could not be detected in spite of large genotypic differences. The differences in numbers of arms bound between the hybrids with the Lolium varieties Lior and Manawa as female parent were not significant either. As only two diploid Lolium varieties were used in this study, no general conclusions can be drawn. The number of arms bound in the hybrids depends on the specific genotypic combination of the two parental plants rather than on the genotypes of the varieties used. An indication for this is the highly significant difference between the individual hybrids in number of univalents and number of arms bound.

In spite of rather good chromosome association and bivalent formation, the hybrids were completely male sterile. There did not seem to be a histological cause of this sterility (MOREL, personal communication) as is found in other male sterile plants (LASER and LERSTER, 1972). Only a few microspores complete first pollen mitosis. During meiosis RNA synthesis stops completely and starts again at the tetrad stage (MASCAREN-HAS, 1971; SAUTER, 1971) so that the genes controlling post-meiotic development and first pollen mitosis are probably active after meiosis. Genetically unbalanced microspores will stop their development at the uninucleate stage. If all the chromosomes of one of the parents must be present to assure normal development of the microspore, only the genome of Lolium (n = 7) can be completely present because with a segregation of approximately 14 chromosomes to one and 14 to the other pole the combination of the 21 chromosomes of F.a. in one gamete will never occur. If the chromosomes are distributed by chance over the gametes (14 chromosomes to one and 14 to the other gamete), 40,116,600 different gametes can possibly be formed. The whole Lolium genome will be present in 116,280 gametes which represent 0.29%. Owing to the presence of univalents in metaphase I, deficient gametes will be formed but the percentage of gametes in which the whole Lolium genome is present can be considered to be the same as for the gametes in which the 14 chromosomes are present. The percentage will be found when the chromosomes are distributed by chance over the gametes. When only bivalents are formed, the whole Lo-

lium genome will be present in $\frac{1}{2}^6 = 1.56\%$ of the gametes. As not only
bivalents are formed, the real percentage of gametes in which all the
Lolium chromosomes are present will be intermediate between 0.29% and
1.56%. The average number of viable pollen grains, colored with aceto-
carmine was 0.32% (average of 92 hybrids, 300 pollen grains analysed per
plant). Hybrids between the tetraploid Lolium and F.a. (2n = 35) which
have two complete genomes of Lolium, had an average number of viable pol-
len grains of 27.8%. This is close to the numbers found for the amphi-
diploids: 28.1% for Lior x Festuca and 31.7% for Manawa x Festuca. The
complete Lolium genome will also be present in 0.29% of the ovules.
SULINOWSKY (1966) backcrossed his $F_1$ hybrids with L.m. and with F.a. and
obtained 39 seeds from 7862 pollinated flowers (0.49%) and 34 seeds from
10731 pollinated flowers (0.32%) respectively. This suggests that the
sterility of the hybrids may be caused by the low percentage of gametes
which are well balanced for the genes controlling post-meiotic develop-
ment but the extent to which a full Lolium genome is required remains un-
certain.

Although most of the chromosomes of the amphidiploids paired as bivalents,
no amphidiploid with a significantly more stable and regular meiosis was
found. As among the hybrids also among the amphidiploids significant
differences were observed in numbers of univalents and of arms bound.
These differences between the amphidiploids originating from different
Festuca parents were only significant because of the deviating behaviour
of the two amphidiploids of Lior x Mayens, which showed a very high num-
ber of univalents and also a low number of arms bound. The amphidiploids
with Lior and those with Manawa as female parent were not significantly
different regarding number of univalents and arms bound. As observed in
the hybrids, chromosome pairing in the amphidiploids is dependent on the
specific genotypic combination of the parental species rather than on the
genotypes of the varieties used.

Although most tetraploid hybrids had a smaller number of arms bound per
chromosome than their corresponding amphidiploid, the difference was si-
gnificant. (t-test) only in four of the eight plants analysed (Fig. 11).
The reverse tendency was found when the pentaploid hybrids were compared
with their corresponding amphidiploids. Here the number of arms bound
per chromosome of the hybrids was generally higher (in one case signifi-

cantly as tested with a t-test) than that of their corresponding amphidi-
ploids (table 20). This implies that chromosome association increases
only slightly or not at all with chromosome doubling. This is surprising
because in the amphidiploids each chromosome now has a completely homolo-
gous partner.

The number of micronuclei per tetrad (M/T) was not correlated with the
number of univalents in hybrids and corresponding amphidiploids (table
13) but significantly so in $C_1$ plants (table 16). The high standard de-
viation illustrated its unstability. On the other hand, the number of
M/T can be established very reliably and is much less time-consuming than
analysis of metaphase I. In F.a. a good correlation between the frequen-
cy of univalents and M/T was found by CROWDER (1953b). The number of
univalents in F.a. was very low. It is possible that the correlation
between univalents and M/T is good with a low number of univalents. This
would mean that amphidiploids with a very low number of M/T should also
have a meiosis with a low number of univalents. A second feature of M/T
is its significant correlation with pollen stainability, pollen germin-
ation and seed fertility. Although a low number of M/T does not always
mean a low number of univalents, it is still a very useful measure of the
seed fertility of the amphidiploids.

A comparison of metaphase I chromosome association between the Festuca
parents, the hybrids and the amphidiploids is presented in table 23, in
which the data of tables 3, 8 and 9 are summarized. A t-test showed that
neither parents and hybrids nor parents and amphidiploids differed signi-
ficantly in number of arms bound per chromosome. The correlation coef-
ficients (table 23) showed that only between the Festuca parents and the
hybrids with Lior the correlation was significant but here a negative
correlation was found. The Lolium varieties showed a much higher number
of arms bound per chromosome ( in Lior and Manawa 1.85 and 1.86 respective-
ly) than the Festuca varieties. For each plant (parents, hybrids and
amphidiploids) anthers of several spikes were analysed so the average da-
ta were representative for the plant. Variation in bound arms and uni-
valents between varieties was not much greater than within varieties.
Meiosis in F.a. showed some lack of stability because in eight out of
ten analysed plants significant differences in number of arms bound
were found over two years and for some plants significant differences

Table 23. Average numbers of arms bound per chromosome of the 7 Festuca varieties, the hybrids and the amphidiploids of the crosses L.m. Lior x Festuca and L.m. Manawa x Festuca and correlation coefficients

| Festuca | Variety | Hybrids | | Amphidiploids | |
|---------|---------|---------|--------|------|--------|
|         |         | Lior    | Manawa | Lior | Manawa |
| Alpes    | 0.69 | 0.72 | 0.73 | 0.75 | 0.81 |
| Alta     | 0.79 | 0.67 | 0.74 | 0.65 | 0.74 |
| Ludion   | 0.70 | 0.68 | 0.73 | -    | 0.69 |
| Manade   | 0.75 | 0.69 | 0.69 | 0.78 | -    |
| Mayens   | 0.76 | 0.67 | 0.72 | 0.59 | -    |
| N.Z.     | 0.76 | 0.68 | 0.70 | 0.74 | 0.75 |
| Portugal | 0.68 | 0.73 | 0.74 | 0.71 | 0.71 |

Correlation coefficients:

Festuca - Hybrids Lior           $r = -0.82$ significant 1% level
Festuca - Hybrids Manawa       $r = -0.21$ not significant
Festuca - Amphidiploids Lior    $r = -0.39$ not significant
Festuca - Amphidiploids Manawa   $r = 0.02$ not significant

within clones could be detected. No significant correlation ($r = -0.28$) was found between the number of univalents and the number of arms bound in F.a. This can be due to a lack of variation in number of univalents within F.a. The number of univalents was very low. The correlation between univalents and arms bound was highly significant in the hybrids ($r = 0.86$) and the amphidiploids ($r = -0.94$ for the amphidiploids with Lior as female parent and $r = -0.87$ for those with Manawa as female parent). In the hybrids and amphidiploids, the univalents are due to a lack of chromosome pairing or chiasma formation. When comparing the Festuca parents with the amphidiploids, the same number of arms bound per chromosome was found although the Festuca parents have a stable and the amphidiploids an unstable meiosis. In the hexaploid F.a., two of the three genomes show affinity, but chromosome pairing is restricted to bivalent pairing, so that only pairing between homologous chromosomes may be assumed to occur. It is likely that this pairing between only homologous chromosomes is under genetic control. JAUHAR (1975a; b) proposed a rather simple model of genetic control of chromosome pairing. He stated that the gene or gene complex must be present at least in double dosage to be effective in suppressing homoeologous pair-

ing and therefore had no influence upon pairing in haploid complements
of the hybrids; it is hemizygous ineffective. He based his model on
meiotic analysis of one monosomic hybrid between two tall fescue varie-
ties and chromosome association in some aneuploid amphidiploids of L.m.
x F.a. These aneuploids showed more homoeologous pairing (more tri- and
quadrivalents) than other aneuploids or euploids. Compared to the aver-
age number of tri- and quadrivalents found in this study (tables 8, 9 and
13) with maxima of 1.91 trivalents and 1.12 quadrivalents the variation
found by Jauhar can be explained by the normal variation among amphidi-
ploids. It might be possible that the Lolium genome partially suppresses
the action of the pairing regulating genes in the amphidiploids because
the amount of homoeologous pairing in the amphidiploids indicates that
the genetically controlled diploidizing system does not function complete-
ly in the amphidiploids. This could be the origin of the meiotic insta-
bility of the amphidiploids. There did not seem to be a cytoplasmic ori-
gin for the meiotic instability although only one hybrid and one amphi-
diploid with F.a. cytoplasm could be analysed. Both showed a high number
of univalents and had about the same chromosome associations as found for
the hybrids and amphidiploids with L.m. cytoplasm.

The plants of the $C_1$ generation did not have more arms bound than the $C_0$
plants, but showed somewhat better seed fertility. $C_0$ plants originate
from chromosome doubling and thus are to a great extent homozygous. The
differences in seed fertility found between the $C_0$ plants were probably
due to genotypic differences of the hybrids from which the $C_0$ plants ori-
ginated. With increasing heterozygosity in $C_1$, a better seed fertility
might result. The fertility levels found in this study for the $C_0$ and $C_1$
plants are not high enough to assure normal seed production. WIT (1974)
found differences in fertility between $C_0$ and $C_1$ plants, with a slightly
higher fertility in $C_0$. WIT (1974) measured fertility as grams of seed
per plant which is less exact than number of seeds per spikelet. Differ-
ences in fertility in the $C_1$ generation and also in $C_1$-$C_3$ as found by
BUCKNER *et al.* (1965) may serve as a good selection criterion. However,
WEBSTER and BUCKNER (1971) after six generations of selection for fertil-
ity still obtained meiotically unstable plants. Therefore, it is very
difficult to overcome meiotic instability in this material. This is an
additional argument for the hypothesis that the Lolium genome partially

suppresses the action of the pairing regulating genes.  If this is real-
ly the case, it will be very difficult to obtain a meiotically stable
amphidiploid.

The high level of chromosome association in the hybrids and the fact that
in the amphidiploids chromosome association is only slightly better than
in the hybrids might be due to two reasons.  Firstly, there is a very
close affinity between the genomes of Lolium and F.a. and also between
the genomes of F.a. itself.  Secondly, the genetic control of pairing re-
gulation in F.a. is probably hemizygous ineffective (JAUHAR, 1975a, b)
such that the chromosomes of different genomes can pair easily.  There is
a better chromosome pairing in the hybrids than in the polyhaploid of
F.a.:  10.43 bivalents plus tri-and quadrivalents (tables 5 and 6) for
the hybrids with 28 chromosomes and 4.01 bivalents without multivalents
for the polyhaploid with 21 chromosomes (MALIK and TRIPATHI, 1971).  The
association in the polyhaploid is thus much lower than in the hybrids,
even when considering that in the polyhaploid only two of the three ge-
nomes can easily pair.  This clearly shows the influence of Lolium geno-
me on chromosome association in the hybrids.  Compared to the chromosome
pairing in hybrids between *Triticum aestivum* and *Secale cereale* much low-
er association was found in the latter hybrids.  In wheat, the genetic
regulation system of chromosome pairing depends on several genes located
on different chromosomes (RILEY, 1966) with a major dominant gene on
chromosome 5B.  In hemizygous condition, the pairing regulating system is
still active.  Polyhaploid wheat showed an average of 1.38 bivalents,
whereas with chromosome 5B absent 4.16 bivalents were found (RILEY and
CHAPMAN, 1958).  Hybrids between wheat and rye showed only 1.37 bivalents
per cell.  In the absence of chromosome 5B, this number increased to 3.18
bivalents and in addition some tri- and quadrivalents (RILEY *et al.*,
1959).  The same phenomenon was found by MELLO-SAMPAYO and CANAS (1973)
and ROOTHAAN and SYBENGA (1976).  Genomes of other species, e.g. *Aegilops
speltoides* and *Ae. mutica* can promote homoeologous pairing in wheat
(DOVER, 1973) as presumed for the Lolium genome in the present study.
There seems thus to be a difference between wheat and tall fescue in the
genetic control of chromosome pairing.  There are also similarities:  in
both cases, other species can influence the genetic control of chromoso-
me pairing.  The Lolium-like genome present in F.a. does not carry the

genes which modify the action of the pairing regulating gene(s) in F.a. This is comparable with the situation in wheat where diploid species with the B genome does not carry the pairing regulating gene on their chromosome 5B. The difficulties in obtaining stable amphidiploids between Lolium and F.a. are thus probably due to the close affinity between the Lolium and Festuca genomes and to the partial suppression of the gene(s) preventing homoeologous pairing in F.a.

The estimation of the number of chromosomes in the L.m. x F.a. hybrids which in principle are able to pair using the formula of GAUL (1958) is 28. The method developed by KIMBER and co-workers allows the estimation of relative affinities between genomes. For hybrids between L.m. and F.a., the model 2 : 2 fits best: two genomes are more closely related to each other than they are to a similarly related second pair of genomes. This means that the genome of Lolium has an affinity with one of the genomes of F.a. and that the two other genomes of F.a. have an affinity with each other. This conclusion is confirmed by the meiotic analysis of a polyhaploid of F.a. (MALIK and TRIPATHI, 1970), where an average number of bivalents of 4.01, with a maximum of 7 was found, indicating a close relationship between two of the three genomes of F.a. There are strong indications that the third genome of F.a. has originated from the diploid *F. pratensis* (MALIK and THOMAS, 1967; CHANDRASEKHARAN and THOMAS, 1971a, b). *F. pratensis*, *L. multiflorum* and *L. perenne* have a high degree of chromosome homology (PETO, 1933; LEWIS and JAUHAR, 1974). The genome in F.a. showing striking homoeology with L.m. may, therefore, well have been the *F. pratensis* genome. CHANDRASEKHARAN and THOMAS (1971a, b) suggested that *F. pratensis* and *F. arundinacea* var. *glaucescens* (2n=28) were the progenitors of the hexaploid F.a. (PPG1G1G2G2). This proposal agrees with the 2 : 2 model about the afinity between the genomes of the tetraploid hybrids. The hypothesis of JAUHAR (1975b) about the genomic structure of F.a. does not seem to be correct. He proposed AABBCC, with *F. pratensis* as the donor of the A genome, Lolium as the possible donor of the B genome and a yet unknown Festuca species as the donor of the C genome. If this genomic structure was correct, then in the hybrids with Lolium the 3 : 1 model should fit best. This was never the case. The hypothesis of CHANDRASEKHARAN and THOMAS (1971a, b) is subject to modification, because there are indica-

tions that the *F. pratensis* genome is related to one of the genomes of *F. arundinacea* var. *glaucescens* (MALIK and THOMAS, 1967; BOWMAN and THOMAS, 1976). The situation of the genomic relationships is still rather confusing, mostly because of affinity between most of the genomes of the diploid, tetraploid and hexaploid Festuca species.

Although no significant correlation could be found between meiotic regularity (expressed as number of univalents or number of arms bound) and fertility, a regular and stable meiosis is essential as a basis for starting selection. Selection for fertility is not an adequate means to obtain cytologically stable material. After several generations of selection for fertility, WEBSTER and BUCKNER (1971) still found a large variation in chromosome number (44 to 56). In some cases (SPECKMANN, personal communication), not one plant with 56 chromosomes was obtained. In this study, Festuca parents with significant differences in chromosome pairing were used, but no amphidiploids with a stable meiosis were found. The fact that significant differences between the amphidiploids were found in numbers of arms bound may indicate that certain genetic combinations may give a regular meiosis but the chance of finding such a genotype is very small.

Selection of amphidiploids from hybrids between *L. perenne* or L.m. and *F. pratensis* were more successful (see chapter 1. Introduction). This might be due to the lower chromosome number (2n = 28) of these amphidiploids. Meiotic analysis carried out by ESSAD (1956) on two plants showed some uni- and trivalents and a high frequency of quadrivalents. It is possible that in these amphidiploids, there is a strong selection against aneuploids which are less fertile than the euploids.

Most investigators used the amphidiploids of L.m. x F.a. in a backcross program. The variety Kenhy, released in the USA was bred by BUCKNER *et al.* (1977) and originated from a backcross of an amphidiploid with F.a. This variety has 42 chromosomes. Although with the backcross method a combination of all the good characters of both species will not be possible, introgression of some of the characters into one of the parental species has a great chance of success because homoeologous pairing occurs. This seems to be the most promising way of using the amphidiploids between *L. multiflorum* and *F. arundinacea*.

# 5. ABSTRACT

Plant breeders intercross *Lolium multiflorum* and *Festuca arundinacea* with the purpose of obtaining hybrids which combine agronomically inter-esting characters of the parent species. The end result can be an amphi-diploid, or the transfer of a limited number of genes from one species to the other. Especially in the first case, meiotic regularity often is a bottle neck. In the present study the influence of various Festuca and Lolium parents on chromosome pairing, further meiotic behaviour and fer-tility in the hybrids and amphidiploids was studied.

In *F. arundinacea* meiosis is somewhat irregular due to the presence of univalents and multivalents and lack of stability. For the same plant significant differences in number of chromosome. arms bound by chiasmata were found over two years, and a number of plants even showed signifi-cant differences within clones within years. Significant differences in meiotic chromosome association were found between the Festuca varie-ties. Between plants of the same variety, differences were also found, but were smaller than between varieties. Chromosome association seems to have little effect on pollen stainability and fertility.

Diploid Lolium had very regular meiosis and most of the chromosomes for-med ring-bivalents at first metaphase. Tetraploid Lolium showed some uni- and trivalents, and quadrivalents were found with very low frequen-cy (average 1.24 per cell), for an autotetraploid.

Hybrids were obtained easily between diploid *L. multiflorum* varieties Lior and Manawa (as female parent) and *F. arundinacea*. The percentage of seed set was high: 44% for the cross Lior x Festuca and 50% for Ma-nawa x Festuca. Some differences in seed set between the Festuca pa-rents were found with Ludion as the variety with the highest seed set. Diploid Lolium varieties did not influence seed set but influenced germ-ination. The crosses with the variety Manawa had a significantly higher percentage of germination (76%) than the crosses with Lior (50%). The reciprocal cross gave a very low percentage seed set (0.75%), and only one hybrid seed germinated. The seed set of the crosses with the te-traploid Lolium as female parent was much lower (19%) than that of the

diploid Lolium and the germination of the hybrid seeds was very low.

The hybrids (2n = 28) showed a high level of meiotic chromosome associa-
tion and averages of 10.31 bivalents for the hybrids Lior x Festuca and
10.68 bivalents for Manawa x Festuca were found. Univalents, tri- and
quadrivalents were observed frequently. The differences between the
hybrids in numbers of univalents and numbers of chromosome arms bound by
chiasmata were highly significant, but no specific influence of the Fes-
tuca nor the Lolium parent could be detected. Pollen stainability was
very low and the hybrids were completely male sterile. Pollen develop-
ment was almost normal until first pollen mitosis, after which the pol-
len grains degenerated. This might be due to a genetic unbalance and it
is possible that pollen grains completing first pollen mitosis had all
seven Lolium chromosomes present.

In the amphidiploids most of the chromosomes paired as bivalents (aver-
age 22.29 for the amphidiploids Lior x Festuca and 24.31 for Manawa x
Festuca). In comparison to the chromosome pairing in the hybrids a high-
er number of multivalents could be expected. This was not the case and
pairing was mostly limited to bivalents. All amphidiploids showed uni-
valents and multivalents. As for the hybrids, significant differences
in numbers of univalents and of arms bound were found between the amphi-
diploids. As observed for the hybrids, the chromosome pairing in the
amphidiploids depended on the combination of the parents rather than on
the specific genotype of the varieties used. Chromosome pairing in the
amphidiploids was not influenced by temperature in the range between 15
and $25^{\circ}C$. $C_1$ plants with 56 chromosomes did not show a more regular
meiosis than $C_0$ plants.

Comparison between the tetraploid and pentaploid (from tetraploid Lolium
x Festuca) hybrids with their corresponding amphidiploids showed that al-
though most amphidiploids had a higher number of arms bound per chromo-
some than their hybrid, only for four octoploid amphidiploids the differ-
ences were significant. Chromosome pairing thus increased only slightly
or not at all (decaploids) after chromosome doubling.

The number of micronuclei per tetrad (M/T) proved to be a highly variable
character and was not always correlated with the number of univalents.

However, this character can still be useful for selection. Plants with a very low number of M/T perhaps have a low number of univalents but most probably a relatively good pollen stainability, germination of the pollen grains and fertility.

Comparison between the Festuca parents, the hybrids and the amphidiploids in respect to the number of arms bound per chromosome (table 23) showed no significant differences between the parents and the hybrids and between the parents and the amphidiploids. For the same number of arms bound per chromosome, Festuca had a more regular meiosis with only homologous pairing and the amphidiploids an irregular meiosis with mainly homologous but probably also homoeologous pairing, or no pairing at all. It is likely that chromosome pairing in *F. arundinacea*, which is restricted to bivalents, is under genetic control. It seems that the Lolium genome partially suppresses the action of the pairing regulating genes of *F. arundinacea*. This could be the cause of the meiotic instability of the amphidiploids,

Calculation of the relative affinity showed that in the hybrids two genomes are more closely related to each other than they are to a similarly related second pair of genomes. The Lolium genome is closely related to the genome of *F. pratensis*, which probably is the donor of one of the genomes of *F. arundinacea*. The two other genomes of *F. arundinacea* are closely related so that in haploid state, their chromosomes can pair.

In this study, even with very distinct Festuca parents and significant differences in chromosome pairing, no amphidiploid with a stable meiosis was found. The fact that between the amphidiploids significant differences in numbers of arms bound were observed may indicate that certain genetic combinations give a regular meiosis but the chance of finding such a genotype is very small. If the amphidiploids cannot be used directly for breeding purposes, introgression of some characters from one into the other parental species seems to be the most promising way of using the amphidiploids between *L. multiflorum* and *F. arundinacea*. Since homoeologous pairing occurs, introgression is possible.

# RESUME

ÉTUDE CYTOGÉNÉTIQUE DE *Lolium multiflorum* LAM., *Festuca arundi-nacea* SCHREB., LEURS HYBRIDES ET AMPHIDIPLOIDES

Les sélectionneurs ont croisé *L. multiflorum* et *F. arundinacea* dans le but d'obtenir des hybrides combinant les caractères agronomiquement intéres-sants des espèces parentales. Le résultat final peut être un amphidiploï-de ou le transfert d'un nombre limité de gènes d'une espèce dans l'autre. Surtout dans le premier cas, la régularité méiotique est souvent difici-le à obtenir. Dans cette étude, l'influence de différents parents de Fes-tuca et Lolium sur l'appariement des chromosomes, le comportement méioti-que subséquent et la fertilité des hybrides et amphidiploïdes ont été étu-diés.

La méiose de *F. arundinacea* était un peu irrégulière en raison de la pré-sence d'univalents et de multivalents ainsi que du manque de stabilité. Pour une même plante, le nombre de bras liés des chromosomes variait si-gnificativement d'une année à l'autre. Un certain nombre de plantes ont même montré des différences significatives entre plantes d'un clone du-rant la même année. Des différences significatives dans l'association des chromosomes à la méiose ont été observées entre les variétés de *F. arundinacea*. Des différences ont également été trouvées entre plantes de la même variété, mais elles étaient moins importantes qu'entre variétés. L'association des chromosomes semblait avoir peu d'effet sur la colora-tion du pollen par le carmin acétique et la fertilité.

Le Lolium diploïde avait une méiose très régulière et la plupart des chro-mosomes formaient des bivalents en forme d'anneau en première métaphase. Le Lolium tétraploïde présentait quelques univalents et trivalents; des quadrivalents ont été trouvés à une fréquence assez basse (moyenne 1.24 par cellule) pour un autotétraploïde.

Des hybrides entre *L. multiflorum* diploïde (variétés Lior et Manawa com-me parent femelle) et *F. arundinacea* ont été obtenus facilement. Le pour-centage des semences formées était élevé: 44% pour le croisement Lior x Festuca et 50% pour Manawa x Festuca. Le nombre de graines formées va-riait selon les parents Festuca; Ludion était la variété qui a donné les

meilleurs résultats. Les variétés de Lolium diploïde n'influençaient pas la réussite du croisement, mais la germination des semences. Les graines issues des croisements avec la variété Manawa ont eu un pourcentage de germination significativement plus élevé (76%) que les graines issues des croisements avec Lior (50%). Le croisement réciproque avait un pourcentage de semences formées très bas (0.75%) et seulement une graine hybride a germé. Le pourcentage de semences obtenues à partir des croisements avec le Lolium tétraploïde a été beaucoup plus bas (19%) que celui du Lolium diploïde et la germination des graines hybrides était très faible.

Les hybrides (2n = 28) montraient à la méiose une association des chromosomes très élevée. Des moyennes de 10.31 bivalents pour les hybrides issus de Lior x Festuca et 10.68 bivalents pour Manawa x Festuca étaient trouvées. Des univalents, trivalents et quadrivalents étaient observés fréquemment. Les différences entre hybrides pour le nombre d'univalents et le nombre de bras liés par chiasmata étaient hautement significatives, mais une influence spécifique des parents Festuca ou Lolium n'a pas pu être démontrée. Le nombre de grains de pollen bien colorés était très bas et les hybrides complètement mâle stériles. Le développement des grains de pollen était pratiquement normal jusqu'à la première mitose pollinique; ensuite, les grains de pollen dégénéraient. Cela pourrait être dû à un déséquilibre génétique et il est possible que les grains de pollen qui achèvent la première mitose pollinique possèdent tous les chromosomes de Lolium.

La plupart des chromosomes dans les amphidiploïdes s'appariaient comme des bivalents (moyenne 22.29 pour les amphidiploïdes Lior x Festuca et 24.31 pour Manawa x Festuca). En comparaison avec l'appariement des chromosomes dans les hybrides, on pouvait s'attendre à un nombre plus élevé de multivalents dans les amphidiploïdes. Cela n'a pas été le cas et l'appariement s'est limité principalement à des bivalents. Tous les amphidiploïdes présentaient des uni- et multivalents. Comme pour les hybrides, des différences significatives entre les amphidiploïdes ont été observées pour le nombre d'univalents et bras liés. Comme remarqué dans les hybrides, l'appariement des chromosomes des amphidiploïdes dépendait plus de la combinaison des deux parents que du génotype spécifique des variétés utilisées. L'appariement des chromosomes dans les amphidiploïdes

n'était pas influencé par la température entre 15 et $25^{0}$C. Les plantes $C_1$ avec 56 chromosomes n'ont pas montré une méiose plus régulière que les plantes $C_0$.

La comparaison entre les hybrides tétraploïdes et pentaploïdes (issus du tétraploïde Lolium x Festuca) avec leurs amphidiploïdes correspondants a montré que la plupart des amphidiploïdes avaient un nombre de bras liés par chromosome plus élevé que leur hybride. Toutefois, la diffé-rence n'était significative que pour quatre amphidiploïdes octoploïdes. L'appariement des chromosomes n'a donc été amélioré que de très peu ou même pas du tout (décaploïdes) après doublement des chromosomes.

Le nombre de micronuclei par tétrade (M/T) s'est montré un caractère très variable et n'était pas toujours corrélé avec le nombre d'univa-lents. Cependant, ce caractère pourrait être utilisé pour la sélection. Des plantes avec un nombre de M/T très bas ont peut-être peu d'univa-lents et probablement un nombre de grains de pollen bien colorés, une ger-mination du pollen et une fertilité assez élevés.

La comparaison entre les parents Festuca, les hybrides et les amphidi-ploïdes en ce qui concerne le nombre de bras liés par chromosome (ta-bleau 23) a montré que les différences entre parents et hybrides ainsi qu'entre parents et amphidiploïdes n'étaient pas significatives. Pour le même nombre de bras liés par chromosome, *F. arundinacea* avait une méiose plus régulière avec seulement des appariements entre chromosomes homologues alors que les amphidiploïdes montraient une méiose irréguliè-re avec principalement des appariements entre homologues, mais proba-blement aussi entre chromosomes homoeologues, voire pas d'appariement du tout. Il est probable que l'appariement des chromosomes, limité à des bivalents chez *F. arundinacea* soit contrôlé génétiquement. Il sem-ble que le génome de Lolium supprime partiellement l'action des gènes régulateurs d'appariement de *F. arundunacea*. Ceci pourrait être la rai-son de l'instabilité méiotique des amphidiploïdes.

Les calculs d'affinité relative ont montré dans les hybrides que deux génomes étaient plus proches l'un de l'autre que ne l'était une deuxiè-me paire de génomes qui avait une relation similaire à la première paire. Le génome de Lolium est apparenté au génome de *F. pratensis* qui

est probablement le donateur d'un des génomes de *F. arundinacea*. Les deux autres génomes de *F. arundinacea* sont apparentés et dans l'état haploïde, leurs chromosomes peuvent s'apparier.

Dans cette étude, malgré des parents Festuca d'origines très différentes et en dépit de différences significatives dans l'appariement des chromosomes, aucun amphidiploïde n'a été trouvé avec une méiose stable. Le fait qu'entre amphidiploïdes, des différences significatives pour le nombre de bras liés aient été observées peut indiquer que certaines combinaisons génétiques donnent une méiose régulière, mais la chance de trouver une telle combinaison est très petite. Si les amphidiploïdes comme tels ne peuvent pas être utilisés pour la sélection, l'introgression de certains caractères d'une espèce à l'autre semble être la voie la plus prometteuse d'utilisation des amphidiploïdes entre *L. multiflorum* et *F. arundinacea*. Etant donné que des appariements entre chromosomes homoeologues ont lieu, l'introgression est possible.

# SAMENVATTING

## CYTOGENETISCHE ONDERZOEKINGEN AAN *Lolium multiflorum* LAM., *Festuca arundinacea* SCHREB., HUN HYBRIDEN EN AMPHIDIPLOÏDEN

Plantenveredelaars kruisen *L. multiflorum* met *F. arundinacea* met het doel hybriden te verkrijgen, die de gunstige landbouwkundige eigenschappen van de ouderplanten kombineren. Het eindresultaat kan een amphidiploïde zijn of de overdracht van een beperkt aantal genen van de ene soort naar de andere. Vooral in het eerste geval is de meiotische regelmatigheid vaak een knelpunt. In deze onderzoekingen is de invloed van verschillende Festuca en Lolium ouders op de chromosoomparing, het daarop volgend meiotisch gedrag en de fertiliteit bestudeerd.

De meiose van *F. arundinacea* is wat onregelmatig door de aanwezigheid van univalenten en multivalenten en gebrek aan stabiliteit. Significante verschillen in aantal chromosoomarmen, gebonden door chiasmata werden gevonden over twee jaar voor dezelfde planten en een aantal planten vertoonde zelfs significante verschillen binnen klonen, het-

zelfde jaar geanalyseerd. Tussen de Festuca rassen werden significante
verschillen in meiotische chromosoom associatie gevonden. Tussen planten
van hetzelfde ras zijn ook verschillen gevonden, deze waren echter klei-
ner dan tussen rassen. Chromosoom associatie schijnt weinig effekt te
hebben op de kleuring van de pollen korrels en de fertiliteit.

De diploïde Lolium had een zeer regelmatige meiose en de meeste chromo-
somen vormden ring bivalenten gedurende de eerste metafase. De tetra-
ploïde Lolium had enige uni- en trivalenten; quadrivalenten werden met
een erg lage frequentie (gemiddeld 1.24 per cel) voor een autotetraploï-
de gevonden.

Hybride planten van de diploïde *L. multiflorum* rassen Lior en Manawa
(als moederplant) en *F. arundinacea* werden gemakkelijk verkregen. Het
zaadzettings percentage was hoog: 44% voor de kruising Lior x Festuca en
50% voor Manawa x Festuca. Enige verschillen in zaadzetting werden ge-
vonden tussen de Festuca ouders. Ludion was het ras met de hoogste zaad-
zetting. De diploïde Lolium rassen beinvloedden de zaadzetting niet maar
wel de kieming. Manawa gaf een significant hoger percentage kieming
(76%) dan het zaad van de kruisingen met Lior (50%). De reciproke krui-
sing had een heel laag percentage zaadzetting (0.75%) en maar één hybri-
de zaad kiemde. De zaadzetting van de kruisingen met de tetraploïde
Lolium als moederplant was veel lager (19%), dan die van de diploïde Lo-
lium en de kieming van het hybride zaad was erg slecht.

De hybriden vertoonden een hoog niveau van meiotische chromosoom associa-
tie en gemiddelden van 10.31 bivalenten voor de hybriden Lior x Festuca
en 10.68 bivalenten voor Manawa x Festuca zijn gevonden. Univalenten,
tri- en quadrivalenten zijn veelvuldig waargenomen. De verschillen tus-
sen hybriden wat betreft aantallen univalenten en armen, gebonden door
chiasmata waren significant, maar geen specifieke invloed van de Festuca-,
noch van de Lolium ouder kon worden aangetoond. Het aantal goed gekleur-
de pollen korrels was erg laag en de hybriden waren volledig mannelijk
steriel. De ontwikkeling van het pollen was praktisch normaal tot aan de
eerste pollen mitose, daarna degenereerden de pollen korrels. Dit zou
kunnen komen door een genetische ongebalanceerdheid en het is mogelijk,
dat in de pollen korrels, die de eerste pollen mitose doorlopen, alle
zeven Lolium chromosomen aanwezig zijn.

De meeste chromosomen in de amphidiploïden paarden als bivalenten (ge-
middelden 22.29 voor de amphidiploïden Lior x Festuca en 24,31 voor Mana-
wa x Festuca). Vergeleken met de chromosoomparing in de hybriden kon
een hoog aantal multivalenten verwacht worden in de amphidiploïden. Dit
was niet het geval en de paring was hoofdzakelijk beperkt tot bivalen-
ten. Alle amphidiploïden hadden univalenten en multivalenten. Net als
voor de hybriden, zijn significante verschillen in aantallen univalenten
en gebonden armen gevonden tussen de amphidiploïden. Evenals waar-
genomen voor de hybriden hangt de chromosoomparing in de amphidiploïden
meer af van de specifieke kombinatie van de ouderplanten, dan van het
genotype van de gebruikte rassen. De chromosoomparing in de amphidiploï-
den werd niet beinvloed door de temperatuur (uitersten 15 - 25$^{O}$C). $C_1$
planten met 56 chromosomen hadden geen regelmatiger meiose dan de $C_o$
planten.

Een vergelijking van de tetraploïde en pentaploïde (van de tetraploïde
Lolium x Festuca) hybriden met hun korresponderende amphidiploïden toon-
de aan, dat, hoewel de meeste amphidiploïden een hoger aantal gebonden
armen per chromosoom hadden dan hun hybride, alleen maar voor vier octo-
ploïde amphidiploïden de verschillen significant waren. De chromosoom-
paring verbetert dus weinig of helemaal niet (dekaploïden) na verdubbe-
ling van het chromosoom aantal.

Het aantal micronuclei per tetrade bleek een erg variabele eigenschap te
zijn en was niet altijd gekorreleerd met het aantal univalenten,. Deze
eigenschap kan echter toch nuttig zijn voor selektie. Planten met een
erg laag aantal micronuclei per tetrade hebben mogelijk een laag aantal
univalenten, zeer waarschijnlijk een tamelijk goede pollen kleuring, kie-
ming van de pollen korrels en fertiliteit.

Een vergelijking tussen de Festuca ouders, de hybriden en de amphidi-
ploïden toonde aan, dat er geen significante verschillen waren in aan-
tal gebonden armen per chromosoom (tabel 23) tussen de ouders en de hy-
briden en tussen de ouders en de amphidiploïden. Voor hetzelfde aantal
gebonden armen per chromosoom, had Festuca een regelmatige meiose met
alleen maar paring tussen homologe chromosomen en de amphidiploïden had-
den een onregelmatige meiose met hoofdzakelijk homologe paring maar waar-
schijnlijk ook homeologe paring of helemaal geen paring. Waarschijnlijk

is de chromosoomparing in *F. arundinacea*, die beperkt is tot bivalenten, onder genetische kontrole. Vermoedelijk onderdrukt het Lolium genoom gedeeltelijk de werking van de genen die de paring in *F. arundinacea* regelen. Dit kan de oorzaak zijn van de meiotische instabiliteit van de amphidiploïden.

De berekening van de relative affiniteit toont aan, dat twee genomen in de hybriden meer verwant zijn aan elkaar dan ze zijn aan een soortgelijk verwant tweede paar genomen. Het Lolium genoom is nauw verwant aan het genoom van *F. pratensis*, dat vermoedelijk de donor is van één van de genomen van *F. arundinacea*. De twee andere genomen van *F. arundinacea* zijn nauw verwant, zodat in haploïde staat hun chromosomen kunnen paren.

In dit onderzoek, zelfs met erg verschillende Festuca ouders met significante verschillen in chromosoomparing, zijn geen amphidiploïden gevonden met een stabiele meiose. Het feit, dat tussen amphidiploïden significante verschillen in aantallen gebonden armen zijn waargenomen, kan aanduiden, dat bepaalde genetische kombinaties een regelmatige meiose geven, maar de kans om een dergelijk genotype te vinden is erg klein. Wanneer de amphidiploïde niet direkt gebruikt kan worden voor veredelingsdoeleinden, dan lijkt introgressie van enkele eigenschappen van één in de andere oudersoort de meest belovende weg om de amphidiploïden tussen *L. multiflorum* en *F. arundinacea* te gebruiken. Daar homeologe paring voorkomt, is introgressie mogelijk.

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# CURRICULUM VITAE

Geert Kleijer werd op 27 juni 1948 geboren te Ede. Hij bezocht het Christelijk Lyceum te Arnhem, alwaar hij in 1965 zijn einddiploma HBS-b behaalde. Aansluitend begon hij met de studie aan de landbouwhogeschool te Wageningen.

In juni 1972 behaalde hij het ingenieursdiploma met als
hoofdvak   : plantenveredeling
bijvakken : erfelijkheidsleer
            phytopathologie
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Vanaf december 1972 is hij verbonden als wetenschappelijk medewerker aan het Station Fédérale de Recherches Agronomiques de Changins, Nyon, Zwitserland, alwaar het gehele promotie onderzoek uitgevoerd is.