

**HYBRIDS OF RYEGRASSES
AND MEADOW FESCUE AND THEIR
VALUE FOR GRASS BREEDING**

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HYBRIDS OF RYEGRASSES AND MEADOW FESCUE AND THEIR VALUE FOR GRASS BREEDING

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ABSTRACT

1. In attempts to intercross *Lolium perenne* and *L. multiflorum* with *Festuca pratensis* in a glasshouse, 13,284 emasculated flowers of diploid, triploid and tetraploid *Lolium* plants were provided with pollen of diploid and tetraploid *Festuca* plants. Only the combinations $2n \times 4n$ and $3n \times 4n$ produced hybrids, viz. on average 5.3 and 1.0 mature hybrid plants per 100 pollinated flowers. In crosses performed in winter these averages were 12.0 and 4.9 respectively.

2. Non-emasculated spikes of diploid, triploid and tetraploid plants of *L. perenne* and *L. multiflorum* in the field were enclosed in pergamine bags with panicles of diploid, triploid and tetraploid plants of *F. pratensis*. Apart from the above-mentioned combinations hybrids were also obtained of the crosses $2n \times 2n$ and $2n \times 3n$. The number of hybrids produced per inflorescence was on average nearly as high as in corresponding crosses in the glasshouse.

By bagging together spikes of self-incompatible diploid plants of *L. perenne* and *L. multiflorum* with panicles of tetraploid plants of *F. pratensis* in the field, large numbers of triploid hybrid seeds may easily be obtained. This opens the possibility of using seed treatment with colchicine for the production of hexaploids.

3. Triploid hybrids showed a better production in spring and a better aftermath growth in summer than either parent plants. Spontaneously obtained hybrids were more winterhardy than most winterhardy indigenous types of perennial ryegrass.

4. All artificial and most natural hybrids had no dehiscent anthers; the percentage of normal pollen varied in the artificial hybrids from 0 to 3.8 %, in the natural hybrids from 0 to 10 %. Several plants in both groups were slightly female fertile. Some plants with hybrid characters, collected from natural grassland, had dehiscent anthers and more than 75 % stainable pollen; their female fertility was fairly good.

5. Open pollinated seeds of triploid male sterile hybrids produced, apart from a majority of plants closely resembling the parent species, or the F_1 hybrids, also types with new combinations of characters. The male fertility was considerably higher than in the previous generation: several plants had dehiscent anthers. In spontaneous families of the male fertile hybrid forms greatly deviating types of *Lolium* occurred.

6. Some possibilities of the various cross combinations for the breeding of ryegrasses are discussed.

INTRODUCTION

Several species of the genus *Lolium* are important for grassland husbandry and for the production of fodder grasses. In permanent pastures and in leys of two years and longer perennial ryegrass (*Lolium perenne* L.) is the most valued grass species. For the establishment of one to two year leys Italian ryegrass (*L. multiflorum* LAM.) and its annual form Westerwolth's ryegrass are the most important grasses. All these ryegrass types excel in aggressiveness, high fodder value and great palatability. However, they exhibit some deficiencies which within the species are difficult to eliminate. In severe winters Italian ryegrass is often destroyed by frost; of perennial ryegrass even the indigenous types can be damaged to such an extent that a severely retarded growth in spring and a reduced contribution to the sward are the result. Other deficiencies of this species are a suppression of white clover in mixtures and a low production in mid-summer.

Meadow fescue (*Festuca pratensis* HUDS.) is less palatable and forms a more open sward as compared with perennial ryegrass, but it is more hardy in winter and more productive in summer. The species is very tolerant towards white clover and the less aggressive grasses such as timothy.

Plants with combinations of the favourable characters of ryegrasses ($2n = 14$) and meadow fescue ($2n = 14$) would offer new possibilities for grassland husbandry. In places where the two species occur side by side intermediate types are not rare. These types are considered as hybrids and are described under the names of *Festulolium* and *Festuca loliacea* CURT.

In several cases attempts to produce hybrids by means of artificial crossing have been successful. In broad outline these hybrids were similar to those found in nature. The most elaborate investigations in this field have been carried out by JENKIN (4, 10). The publication mentioned under 4 in the references gives a survey of the older literature.

LITERATURE

By reciprocal crossing of *L. perenne* and *F. pratensis*, JENKIN (10) obtained from approximately 8,000 emasculated flowers in total 12 mature F_1 hybrids. They were all diploid, male sterile and very weakly female fertile. Backcrossing to *L. perenne* yielded 19 F_2 plants. Most of them could not be distinguished from perennial ryegrass and were normally fertile. Some resembled this type in outward appearance but showed a reduced fertility. One plant had a clearly deviating appearance and was to a slight extent both male and female fertile; on cytological examination it was found to be a triploid. The two first-mentioned groups of backcross hybrids in further crosses behaved as normal plants of *L. perenne*. In some cases, however, there were strong indications that they carried some genes of *F. pratensis* and transferred these to their offspring. The triploid plant, after crossing with *L. perenne*, produced offspring with a wide range of variation in type.

CROWDER (2) crossed *L. perenne* and *L. multiflorum* with *F. pratensis*, excised the young embryos from the seeds and grew them on nutrient agar. In spite of his precautions only 0.3 % of these crosses resulted in hybrid plants. He obtained two hybrids which produced no offspring.

CARNAHAN and HILL (1) crossed diploid *L. perenne* with tetraploid *F. pratensis*. Some hundreds of emasculated flowers produced 12 triploid hybrids. These grew more vigorous and were leafier than the parent plants, but they appeared to be highly sterile. By treatment of rooted tillers with colchicine the authors obtained an autoallohexaploid from every triploid. It was expected that the hexaploids would possess a higher fertility than the triploids.

ESSAD (3), also by colchicine treatment of tillers, obtained two amphidiploids from two diploid hybrids. The percentage of stainable pollen was 1.3 in the triploid, nearly 40 in the amphidiploids. This would be an indication of the possibility to create fertile amphidiploids.

From the literature it appears to be possible to produce diploid and triploid hybrids and to create from these hybrids amphidiploids and autoallohexaploids by means of colchicine-treatment. Attempts to utilize these hybrids for practical breeding purposes may therefore be undertaken on a diploid, a tetraploid and a hexaploid level.

In all these cases it is desirable to combine a large number of genotypes of the parent species and to study the results of these combinations. This is only feasible when large numbers of hybrids can be obtained with relatively little work. The emasculation and pollination of thousands of florets and the production of amphidiploids and hexaploids by means of tiller-treatment are too laborious for practical application. A mass production of diploid and triploid hybrid seeds would enable us to apply seed treatment and to produce allopolyploids in an easier way and in larger numbers.

Crosses of the parent species on tetraploid level and crosses of tetraploids of one species with octoploids of the other might produce amphidiploids and hexaploids directly.

Finally a collection of hybrid types occurring in nature would constitute valuable supplementation of the experimental hybrids.

The above-mentioned possibilities are now studied more closely. The preliminary results of this investigation are given below.

THE PRODUCTION OF HYBRIDS

In the years 1955, 1956 and 1957 attempts were made to cross selected clones of *L. perenne* and *L. multiflorum* as female parents with clones of *F. pratensis* as male parents. For this purpose 20 diploids, 3 triploids and 33 tetraploids of *Lolium* were used; of *Festuca* these numbers were 8, 1 and 18 respectively.

The tetraploid clones were selected from C_1 and C_2 seedlings, obtained after colchicine treatment. The triploids were derived from C_0 plants by means of spontaneous pollination.

In the glasshouse 103 crosses were made by emasculating and pollinating 13,284 florets of 324 inflorescences. The seeds obtained were germinated on moist filter-paper and then planted in flats in the glasshouse. The resulting plants were planted in the field in the spring. The results of these glasshouse crosses are tabulated below.

TABLE 1. SOME RESULTS OF CROSSES BETWEEN RYEGRASSES AND MEADOW FESCUE IN THE GLASSHOUSE

Parent plants		Number of crosses	Number of spikes	Number of seeds	Number of plants		Number of successful crosses	Number of hybrids per successful cross	Number of hybrids per spike
<i>Lolium</i>	<i>Festuca</i>				<i>Lolium</i>	Hybrids			
2n	2n	18	58	6	4	—	—	—	—
2n	4n	21	64	188	5	138	10	13.8	4.1
3n	2n	2	6	—	—	—	—	—	—
3n	4n	9	26	19	1	11	3	3.7	1.0
4n	2n	17	52	3	2	—	—	—	—
4n	4n	36	118	5	3	—	—	—	—

From this table it is apparent that the crosses were successful only when diploid and triploid *Lolium* plants were pollinated with pollen of tetraploid meadow fescue. Of the first-mentioned combination on an average 5.3 mature hybrids were obtained per 100 florets. If the crosses which were not successful, for instance through incompatibility of the parental genotypes, are left out of consideration, 10% of the pollinated florets yielded hybrid plants. Part of these crosses were carried out in winter after flowering had been induced by long day treatment. Under these circumstances the combinations *Lolium* 2n × *Festuca* 4n produced on average 12.0 hybrids per 100 florets. The four most successful crosses produced 151 seeds (30.6%) on 492 florets; the seeds gave 116 mature plants, all of them hybrids. So in the most favourable cases 23.6 mature hybrid plants were obtained per 100 emasculated florets. The 15 *Lolium* seedlings are all derived from crosses made in summer. Presumably they resulted from spontaneous pollination by air-borne *Lolium* pollen, since the glasshouses could not be closed hermetically.

A number of crosses, partly made by means of the same parent plants, was tested in the field in summer by simply bagging together the non-emasculated inflorescences of both parent species. In the bags the inflorescences of the *Festuca* parents were always in positions above those of the *Lolium* parents, so that conditions were less favourable for the reciprocal crosses. As, moreover, sometimes inflorescences of meadow fescue had to be used which already showed a beginning of flowering, as a rule only the seeds produced by *Lolium* ears were sown. The results of the field crosses are presented in table 2.

TABLE 2. SOME RESULTS OF CROSSES OF RYEGRASSES AND MEADOW FESCUE IN THE FIELD

Parent plants		Number of crosses	Number of spikes	Number of seeds	Number of plants		Number of successful crosses	Number of hybrids per successful cross	Number of hybrids per spike
<i>Lolium</i>	<i>Festuca</i>				<i>Lolium</i>	Hybrids			
2n	2n	3	17	30	14	1	1	1.0	0.2
2n	3n	1	6	73	20	8	1	8.0	1.3
2n	4n	15	61	382	19	156	10	15.6	3.6
3n	3n	1	3	7	5	—	—	—	—
3n	4n	2	7	10	—	4	1	4.0	1.3
4n	2n	1	2	1	1	—	—	—	—
4n	4n	6	31	34	11	—	—	—	—

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From this table it is clear that the baggings $2n \times 4n$ and $3n \times 4n$ without emasculation produced about the same number of hybrids per spike as the crosses with emasculation and artificial pollination.

Although only a few combinations $2n \times 2n$ and $2n \times 3n$ were bagged, in both cases one cross was successful. The combinations $4n \times 4n$ and $4n \times 2n$ produced neither in the glasshouse nor in the field a single hybrid plant.

The number of hybrids from seeds of *Festuca* panicles will be no exact standard for the prospective success of the reciprocal crosses. However, the hybrids obtained in some cases show that certain cross combinations are possible. For instance four hybrids were obtained from 40 panicles of tetraploid meadow fescue which had been bagged together with ears of diploid *Lolium* plants. In the combination *Festuca* $3n \times$ *Lolium* $2n$ five panicles produced three hybrids, whereas the combination *Festuca* $2n \times$ *Lolium* $4n$ resulted in one hybrid from six panicles.

By treating tetraploid seeds with colchicine-solutions some octoploid plants were obtained in *L. perenne*, *L. multiflorum* and *F. pratensis*. So far no crosses have been made with these octoploids.

THE CHARACTERS OF THE F_1 -HYBRIDS

Morphological characteristics

When *L. perenne* was used as the female parent the hybrids could be easily distinguished from seedlings resulting from selfing by the "rolled" emergence of the youngest leaf, by the shorter ligule and by the broader leaf blade. This distinction was much more difficult when Italian ryegrass was used as the pistillate parent plant. However, it did not present any difficulties as soon as the plants started heading. Although the hybrids in a vegetative condition had a somewhat different appearance from meadow fescue, in many cases they could not be distinguished from the latter before the heading stage.

As a thorough investigation of our hybrids has not been carried out so far, only the most conspicuous characteristics will be mentioned here.

Descriptions of diploid F_1 -hybrids have been given by JENKIN (10), of triploid F_1 -hybrids by CARNAHAN and HILL (1). Judging from these descriptions and from our own observations, the influence of *Lolium* parent on mature plant characters is less pronounced in the triploid than in the diploid hybrids.

The inflorescence of the triploid hybrids is intermediate between the spike of *Lolium* and the panicle of *Festuca*. As a rule the lower nodes carry a side-rachis with 3 to 5 spikelets; towards the tip spikelets occur singly at the nodes. Only in weak plants and in weak tillers the inflorescences are sometimes unbranched. Also in these cases, however, they can easily be distinguished from pure *Lolium* spikes by their laxer structure and by the festucoid appearance of the spikelets. These are not compressed as in *Lolium*, but more cylindrical as in *Festuca*, elongated, rather lax and multiflowered. There are usually two glumes in the lateral spikelets, whereas there is only one in *Lolium*.

Vigour

The general impression is that the hybrids grow more vigorously than either parent.

In the first preliminary trials in which the former were compared with seedlings or clones of the parent plants, they distinguished themselves by more leaf production in the spring.

In table 3 the data of some of these trials have been summarized. The leaf production was estimated on 1 May 1958 according to a scale of 2 (very low) to 9 (very high). The figures for the aftermath growth in summer in rough outline exhibited the same trend.

TABLE 3. FREQUENCY DISTRIBUTION FOR SPRING PRODUCTION OF DIPLOID PLANTS OF PERENNIAL RYEGRASS, OF TETRAPLOID PLANTS OF MEADOW FESCUE AND OF THEIR TRIPLOID HYBRIDS

	Number of plants	Estimation of leaf production in spring							
		2	3	4	5	6	7	8	9
<i>Lolium</i> 2n	29	2	3	5	6	9	1	3	
<i>Festuca</i> 4n	14		2	6	4	2			
F ₁ Hybrid	85	1		2	3	12	25	28	14

So far no data are available of the winterhardiness of the artificial hybrids. Some spontaneous hybrids which were collected in 1955, in the winter of 1955/56 appeared to be equally winterhardy as indigenous plants of meadow fescue and to surpass clearly the best clones of perennial ryegrass in this respect. It was remarkable that a *Festulolium* hybrid which was obtained by spontaneous fertilization of a hybrid between perennial and Westerwolths ryegrass also showed a very high degree of winterhardiness.

Fertility

All artificial hybrids had non-dehiscent anthers; they were male sterile. The contents of pollen sacs of 17 plants were stained with aceto-carmin and observed through the microscope. In three hybrids of the cross *Lolium* 2n × *Festuca* 3n not a single normal-looking pollen grain was found. In 14 hybrids between diploid *Lolium* and tetraploid meadow fescue the percentage "good" pollen varied from 0 to 3.8.

In order to gain an impression of the female fertility, some spontaneously pollinated inflorescences of a number of hybrids were collected. In general seed setting appeared to be very poor, but of various hybrids between diploid *Lolium* and tetraploid *Festuca* a few dozens of seeds were obtained. They produced in total 31 mature plants.

HYBRID TYPES IN NATURAL GRASSLAND

As was already mentioned not seldom intermediate forms are found in places where *L. perenne* and *F. pratense* occur side by side. They seem to prefer the ditch-sides of meadows, but they are also found on other places in pastures and along way-sides. When in flower they can be easily distinguished from the pure species and largely resemble the artificial hybrids.

In total 93 of these intermediate types were collected from six habitats. On the basis of their outward appearance and their behaviour they could be grouped into two categories.

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The largest group comprised types as collected by JENKIN in Great Britain, variable in habit, in culm size, in degree of branching of the inflorescences and in leaf size. Plants from the same habitat were almost similar in their characters.

A small group of vigorous individuals derived from a meadow bordering the *Kaagse Plassen* (a lake near Warmond – province of South-Holland) differed from the first group in that the plants produced unbranched spikes even on the most vigorous tillers. They evidently deviated from *L. perenne* by their very lax inflorescences and by the cylindrical festucoid spikelets which were usually subtended by two glumes. On cytological examination they appeared to be triploid.

The pollen of 87 plants of the first group was examined; 75 plants produced less than 1 % normal pollen; in 12 plants this percentage varied from 1 to 10. In all these cases the anthers were non-dehiscent so that the plants were male sterile. However, the three plants derived from De Kaag had dehiscent anthers with 76–79 % normal pollen. Triploids of this type have not yet been found in nature, as far as the author is aware.

Seeds from open pollinated inflorescences were harvested from six of the first-mentioned male sterile hybrids; on cytological examination these plants were found to be triploids. The seeds produced 70 mature plants.

The three male fertile plants from de Kaag yielded a large number of seeds from which 650 plants were raised. Consequently plants of both groups can be used for the production of following generations. However, more investigations are needed to ascertain which plants are desirable as male parents.

PLANTS OF THE SECOND GENERATION

Only part of the spontaneously produced plants of the second generation has flowered so far; therefore only a few observations could be made and their results have only a preliminary value. The data on male fertility have been summarized in table 4.

TABLE 4. MALE FERTILITY OF NATURAL AND ARTIFICIAL HYBRIDS AND OF THEIR OPEN POLLINATED PROGENIES

Material	Number of plants examined	Percentage of stainable pollen			Anthers	
		Minimum	Maximum	Average	Non-dehiscent	Dehiscent
F ₁ <i>Lolium</i> 2n × <i>Festuca</i> 4n . .	14	0.0	3.8	1.3	14	0
F ₂ <i>Lolium</i> 2n × <i>Festuca</i> 4n . .	8	0.0	99.3	20.6	7	1
Natural hybrids Wageningen .	9	0.0	2.3	0.8	9	0
F ₁ Natural hybrids Wageningen	18	6.1	98.0	70.0	3	15
Natural hybrids de Kaag . .	3	75.7	78.5	77.0	0	3
F ₁ Natural hybrids de Kaag .	19	0.0	87.9	55.2	5	14

From this table it is clear that in the offspring of the artificial triploid hybrids the pollen fertility has greatly increased and in one case has become quite normal. In the offspring of the male sterile natural hybrids there was a far greater improvement of the pollen fertility; 15 plants had more than 46 % “good” pollen and fully dehiscent anthers. In the two plants with less than 40 % “good” pollen the anther-lobes

remained completely closed, while in one plant with 41 % "good" pollen they were partially dehiscent. This suggests that the anthers may be able to shed pollen when the percentage of normal pollen grains rises above a value of about 40.

The data from table 4 show that both the artificial and the natural triploid male sterile hybrids carry the potentiality of producing male-fertile types in their offspring. The value of such derivative types for practical breeding purposes will depend on the question whether they combine valuable genes from both parent species and whether they transfer such combinations to their progeny. In the present stage of the investigation this cannot be stated conclusively. However, a preliminary study of the material gives some indications in this direction.

In the offspring of the artificial hybrids seven individuals could not be distinguished morphologically from *Lolium* plants, two were similar to *Festuca pratensis* and four closely resembled the F_1 plants. In addition two *Festuca* plants occurred with awned glumes and two plants which showed inner glumes as a constant feature but were otherwise completely *Lolium* types.

Most derivatives of the male sterile natural hybrids were morphologically not clearly distinguishable from *L. perenne* or *F. pratensis*. A plant which had a very close resemblance to *Lolium* had the short ligule of meadow fescue.

Among the offspring of the plants derived from de Kaag only one individual was found with the characters of meadow fescue. The inflorescences of the other flowering plants all conformed well to the *Lolium perenne* type. However, there was far wider variation in plant type than that observed in earlier collections of thousands of plants sampled from various types of grasslands in the Netherlands. Apart from plants with very broad leaves there were other types with extremely narrow ones, resembling those from *Festuca rubra*. Such plants also occurred in some progenies from male-sterile spontaneous hybrids, though in smaller number. They corresponded in many respects to the description of JENKIN's plant 92-bE-2, obtained by crossing the triploid plant from his first backcross generation with *L. perenne* (JENKIN, 10).

DISCUSSION OF THE RESULTS

The data of tables 1 and 2 indicate that the breeding affinity of *L. perenne* and *L. multiflorum* with *Festuca pratensis* largely depends on the chromosome-numbers of the parent plants. For a closer examination of this question the results of the crosses have been summarized in table 5.

Although the various combinations are represented very disproportionately, the data of table 5 are a strong indication that by using *Lolium* plants as the female parents the crosses will be more successful as the number of *Festuca* chromosomes approaches more the double number of the *Lolium* chromosomes.

According to CROWDER (2) and JENKIN (10), after crossing diploid plants of *L. perenne* and *L. multiflorum* with *F. pratensis*, an embryo is often initiated. However, there is usually no further development and no germination of the seed, since no

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TABLE 5. RELATION BETWEEN THE CHROMOSOME-NUMBER OF THE PARENT PLANTS AND THE NUMBER OF HYBRIDS FOUND

Parent plants		Ratio of <i>Festuca</i> and <i>Lolium</i> chromosomes	Number of crosses		Number of hybrids	Number of hybrids	
<i>Festuca</i>	<i>Lolium</i>		Total	Successful		Per successful cross	Per spike
4n	2n	2.0 : 1	36	20	294	14.7	4.2
3n	2n	1.5 : 1	1	1	8	8.0	1.3
4n	3n	1.3 : 1	9	4	15	3.8	1.1
2n	2n	1.0 : 1	20	1	1	1.0	0.2
3n	3n	1.0 : 1	1	—	—	—	—
4n	4n	1.0 : 1	42	—	—	—	—
2n	4n	0.5 : 1	18	—	—	—	—

normal endosperm is produced. If the seeds germinate, the seedlings are very weak at first. Only in one case JENKIN obtained a hybrid which grew more vigorously from the beginning. It was produced by a well-developed seed which apparently had possessed normal endosperm and which had originated by pollination of meadow fescue with a mixture of ryegrass and meadow fescue pollen. JENKIN presumes that in this case male gametes of both species have entered the embryo sac. One of the *Lolium* gametes is supposed to have conjugated with the egg cell to lay the foundation of the hybrid embryo. The development of the endosperm would be due to a conjugation of the *F. pratensis* fusion nucleus with a gamete of meadow fescue. The fact that this particular cross did not produce meadow fescue seeds is an indication that the gametes of perennial ryegrass penetrated more rapidly than those of meadow fescue and thus rendered impossible a fertilization of the egg cells by the meadow fescue gametes.

By connecting the data of table 5 with the formation of endosperm they can be interpreted in the following way: A haploid *Festuca* gamete cannot conjugate with the diploid fusion nucleus of *Lolium*, or the triple nucleus is not capable of inducing a normal formation of endosperm. A diploid *Festuca* gamete combines fairly easily with the diploid fusion nucleus of *Lolium* and the nucleus produced – presumably tetraploid – effects a normal endosperm production. Triploid plants of meadow fescue may partly form superhaploid or subdiploid gametes which in this respect might be intermediate between haploid and diploid gametes.

Two sets of chromosomes of *F. pratensis* apparently have a similar – though weaker – effect than one set of *Lolium* chromosomes. The question now arises how the male gametes of the natural polyploids of the genus *Festuca* behave in crosses with *Lolium*. Table 6 presenting some results of the extensive breeding experiments of JENKIN (4, 5, 6, 7, 8, 9, 10) partly answers this question.

In these crosses also the number of viable seeds produced appears to be larger as the number of chromosomes of the father plant is increased. As under favourable conditions pollination of diploid *Lolium* by tetraploid *F. pratensis* plants produced on average 12.0 hybrids per 100 florets, it can be concluded that 14 chromosomes of *F. pratensis* are about as effective as 21 chromosomes of *F. arundinacea*, more effective

TABLE 6. RELATIONS BETWEEN THE NUMBER OF CHROMOSOMES OF THE FATHER PLANTS AND THE PERCENTAGE OF GERMINATED SEEDS AFTER CROSSING *Lolium perenne* WITH VARIOUS *Festuca* SPECIES

Male parent	Number of chromosomes	Germinated hybrid seeds per 100 florets
<i>Festuca capillata</i>	14	0
„ <i>pratensis</i>	14	0.2
„ <i>heterophylla</i>	28	1.6
„ <i>ovina</i>	28	1.2
„ <i>rubra</i>	42	8.3
„ <i>gigantea</i>	42	8.8
„ <i>arundinacea</i>	42	13.0

than 21 chromosomes of *F. rubra* and *F. gigantea*, and much more effective than 14 chromosomes of *F. ovina* and *F. heterophylla*. This gives the impression that the breeding affinities are less determined by the ratio between the number of chromosomes than by the ratio of homologous chromosomes, chromosome segments, or particular genes. Further speculations in this field, however, are beyond the scope of this paper.

The most important practical result of the breeding experiments is that hybrids of *Lolium* and *F. pratensis* can be easily produced on a large scale, by bagging together non-emasculated inflorescences of self-incompatible diploid *Lolium* plants and panicles of tetraploid *F. pratensis*. In selecting intercompatible parents, 5 to 10 hybrids per spike could be obtained. In as far as such hybrids were examined cytologically, they appeared to be triploids. Treatment of the germinated seeds with colchicine will then produce hexaploids. The laborious tiller treatment can be avoided and each duplication will result in a new hexaploid genotype.

The aim of producing large numbers of diploid hybrid seeds by the selection of intercompatible diploid parent types, and to create from these seeds amphidiploids by colchicine treatment, could not be realized so far. Nor was an attempt to produce amphidiploids by intercrossing tetraploid parent species successful.

However, this does not exclude the possibility to use hybrids for practical breeding purposes on a tetraploid level. Perhaps diploid hybrid seeds can be produced on a larger scale by pollinating, simultaneously or successively, diploid *Lolium* plants with haploid and diploid pollen of meadow fescue. A haploid gamete might conjugate with an egg cell and induce an embryo. A diploid gamete might conjugate with the fusion nucleus and thus initiate endosperm.

The hybrids of diploid *Lolium* and triploid meadow fescue offer other possibilities. Among them aneuploids may occur; colchicine treatment of hybrid seeds may create supertetraploids which after mutual crossing may produce pure amphidiploids.

The above-mentioned ways would lead to the creation of entirely new plant types with complete genomes of *Lolium* and *Festuca pratensis*. Such plants may demand a

treatment quite different from the diploid parent species. This will apply to a far lesser extent, if at all, when separate genes or gene complexes of *F. pratensis* can be combined with the genome of *Lolium*. The results of JENKINS' investigations strongly indicate that by backcrossing diploid hybrids to perennial ryegrass, genes of *F. pratensis* can be transferred to plants that are otherwise *L. perenne* and are interfertile with *L. perenne*. The same results may be obtained by backcrossing triploid and aneuploid hybrids, which can be produced on a larger scale, to diploid ryegrasses. JENKIN's triploid back-cross hybrid after further backcrossing to *L. perenne*, produced an offspring which showed a strong tendency towards a return to the diploid state and to normal fertility.

However fragmentary our own data may be, they show that from artificial and natural triploid hybrids new types can be developed which in addition to a majority of morphological characters of one species also possess some characters of the other species. Among them also highly male-fertile plants occur. It seems most probable, therefore, that fertile forms can be bred which combine some physiological characters of the two parent species. Further research must prove whether such forms can be developed in a stable condition and which line should be followed for that purpose.

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SAMENVATTING

Bastaarden van raaigrassen en beemdlangbloem en hun betekenis voor de grassenveredeling

1. In een kas werden 103 kruisingen beproefd tussen 56 planten van *Lolium perenne* en *Lolium multiflorum* als moeder en 27 planten van *Festuca pratensis* als vader. In totaal werden 13.284 gecastreerde bloempjes van diploide, triploide en tetraploide *Lolium*-planten bestoven met stuifmeel van diploide en tetraploide *Festuca*-planten. Alleen de combinaties $2n \times 4n$ en $3n \times 4n$ leverden hybriden op, nl. gemiddeld respectievelijk 5,3 en 1,0 volwassen bastaardplanten per 100 bestoven bloempjes. Bij de kruisingen, die in de winter werden uitgevoerd waren deze gemiddelden resp. 12,0 en 4,9.
2. Ongecastreerde aren van diploide, triploide en tetraploide planten van *L. perenne* en *L. multiflorum* werden op het veld in pergamine zakken ingehuld met pluimen van diploide, triploide en tetraploide planten van *F. pratensis*. Behalve van de bovengenoemde combinaties werden ook hybriden verkregen van de kruisingen $2n \times 2n$ en $3n \times 4n$. Het aantal per aar geproduceerde hybriden was gemiddeld ongeveer even hoog als bij overeenkomstige kruisingen in de kas.

Door aren van zelfincompatibele diploide planten van *L. perenne* en *L. multiflorum* op het veld samen in te hullen met pluimen van tetraploide planten van *F. pratensis* kunnen gemakkelijk grote aantallen triploide bastaardzaden worden verkregen. Dit maakt zaadbehandeling met colchicine, voor het verwekken van autoallohexaploiden, in de praktijk uitvoerbaar.

3. Triploïde hybriden vertoonden een grotere voorjaarsproductie en een betere na-groei in de zomer dan de ouderplanten. Spontaan verkregen natuurlijke bastaarden waren winterharder dan de meest winterharde inheemse typen van Engels raaigras.
4. Alle kunstmatige en de meeste natuurlijke hybriden hadden niet-openspringende meeldraden; het percentage normaal lijkend stuifmeel varieerde bij de eerste van 0 tot 3,8 %, bij de laatste van 0 tot 10 %. Verschillende planten van beide groepen waren zwak vrouwelijk fertiel. Enkele in de natuur gevonden planten met bastaardenkenmerken hadden openspringende meeldraden en meer dan 75 % „goed” stuifmeel; zij waren vrij goed vrouwelijk fertiel.
5. Spontane zaaïngen van triploïde manlijk steriele hybriden gaven naast een meerderheid van planten die sterk overeenkwam met de oudersoorten of met de F₁ generatie, ook typen met nieuwe combinaties van eigenschappen. Het percentage goed stuifmeel was aanmerkelijk hoger dan in de voorgaande generatie, verschillende planten hadden openspringende meeldraden. In spontane families van de manlijk fertiele bastaaardvormen kwamen sterk afwijkende vormen van *Lolium* voor.
6. Enkele mogelijkheden, die de verschillende kruisingscombinaties voor de veredeling van raaigrassen bieden, werden besproken.

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