INTERSPECIFIC AND INTERGENERIC HYBRIDS IN HERBAGE GRASSES

VIII. *LOLIUM LOLIACEUM*, *LOLIUM REMOTUM* AND *LOLIUM TEMULENTUM*, WITH REFERENCES TO '*LOLIUM CANADENSE*'

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Introduction

The three species, *Lolium loliaceum*, *L. remotum* and *L. temulentum*, have generally been recognized as being distinct. The differences between *L. loliaceum* and the other two are immediately evident, but those between *L. remotum* and *L. temulentum* are much less pronounced. Typical plants of *L. remotum* have short, weak and inconspicuous awns, but in the course of the present work a seed sample was received from Germany in which the inferior paleae were prominently and strongly awned.* As the plants grown from this seed were also conspicuously awned, but were otherwise typical *L. remotum*, this distinction between *L. remotum* and typical *L. temulentum*, which is strongly awned, breaks down.

In the course of the present work it has also been found that typical *L. temulentum* and its variety *arvense*, in which the awn is very weakly developed, are no more than types of the same species. They can be intercrossed to give fully fertile progeny.

Apart from the presence or absence of a strong awn the observable differences between *L. remotum* and *L. temulentum* are exceedingly difficult to describe except in relative terms. Although very frequently *L. remotum* plants are less tall than those of *L. temulentum* this is by no means always the case, so that tallness is quite an uncertain character. On the other hand, *L. remotum* is more slender than *L. temulentum* in all its parts, so that in particular the inflorescences are less bold and stiff. In *L. temulentum* the glumes are large, being both broad and long, usually extending beyond the spikelets, whereas in *L. remotum* the glumes and the spikelets which they subtend are much smaller, although the spikelets may consist of quite as many florets. These florets are, therefore, much smaller, and when the spikelet is mature it is more dense and less tightly pressed against the rachis than in *L. temulentum*.

On preliminary breeding evidence in conjunction with the comparison of the morphology of the two types, I was inclined to believe that they are 'merely genotypes of the same species, that have become separated by purely mechanical means through association with crops which are different in size of grain or seed', but further evidence showed that, at least in part, this suggestion could not be maintained (Jenkin, 1936), although it is still possible that the original divergence was one of size of seed and that the separation of the different sizes was due to association with different crops.

* I have met with no reference to this prominently awned *L. remotum* type in the literature.

A degree of affinity between *L. remotum* and *L. temulentum* and between these two (to a lesser extent) with *L. loliaceum* is also suggested by the somewhat parallel results obtained when each was intercrossed with the normally wind-pollinated *Lolium* species (Jenkin, 1954, *b*, *c*, *d*).

All three species are strict annuals and each normally sets seed from self-pollination. Pollen is produced and liberated very sparingly as compared with the wind-pollinated *Lolium* types, so that it might easily happen that an emasculated female unit would be inadequately pollinated. Moreover, the stigmas are generally very poorly exserted, but it may be recalled that when such emasculated units were artificially pollinated by dusting the spikelets (irrespective of whether stigmas were visible or not) satisfactory seed-setting resulted when the pollen supply was plentiful (Jenkin, 1935).

As a kind of appendix, some of the breeding reactions of a line of plants derived from seed described as '*L. canadense* Michx.' are discussed, although the exact taxonomic position of the plants involved is uncertain.

I. *LOLIUM LOLIACEUM* WITH *LOLIUM REMOTUM*

The same *L. loliaceum* plant was used as the pistillate parent in the only two crosses made between these two species. The two pollen parents were full sister plants of a standard *L. remotum* line.

The two female units consisted of a total of 369 florets, but it was eventually found that, presumably owing to faulty emasculation, six florets had set seed from self-pollination. As the pure *L. loliaceum* plants and the hybrids were easily distinguishable, this proved an advantage because a direct comparison could be made at any stage of plant development.

Omitting these six florets, average seed-setting for the two units was only 16 %. The dry seeds were described as 'probably normally developed'. The seeds were stored for about six months before they were incubated in the laboratory. All the seeds germinated in approximately normal time, but from the earliest stages of germination the six seedlings that were the result of self-pollination were different from the others in that the first leaf was perfectly normal and the seedlings developed quite normally and much more rapidly than the others.

The first leaf of the true hybrids was short and broader in relation to its length than in the non-hybrid seedlings. Progress was slow, and it was evident that the second and subsequent leaves found it difficult to emerge through the sheaths of the older leaves. When the shoots began to elongate, the leaves were seen to be curled and crinkled, and as the inflorescences emerged they became more or less badly deformed. Many of them failed to do more than show their tips, but an occasional inflorescence was able to emerge more or less normally. Even these were weak with few spikelets, but some spikelets were quite well developed. These more normal shoots did not closely agree with those of either parental species, being much shorter and harder than in *L. remotum*, yet much more slender in relation to their length than in *L. loliaceum*. At flowering time the spikelets were more reminiscent of *L. remotum* than of *L. loliaceum*.* Such abnormal development was not seen in extensive work with the parental species, yet all the hybrids without exception were greatly deformed although some produced subnormal inflorescences. Neither has this type of abnormal development been found in any *Lolium*

* For specimens of the hybrids, see sheets 481–488 of W.P.B.S. Herbarium, Exp. B143.

intraspecific crosses, the nearest, possibly, being the broad-eared variety *cristatum* of *L. perenne* which is sometimes met with in nature. Malformed segregates have also been obtained as segregates in inbreeding, but those usually form only a proportion of any particular family.

An exact parallel to the present case, however, is found in the hybrids of *L. loliaceum* with *L. temulentum* (§II below), so that it is evident that the abnormal development is somehow due to intercrossing *L. loliaceum* with the other two species.

Whether embryo development is perfectly normal up to the seed-resting stage is not known, but the abnormality is present at or very near the beginning of seed germination, and persists throughout the life of the plant except that, as if by accident, some inflorescences approaching normality may be produced. Even when the spikelets were large and well developed, the anthers were non-dehiscent.

Backcross

Some inflorescences were sufficiently near normal in development to allow an attempt to be made to use them as pistillate units in backcrosses.

One such unit consisting of 114 florets received the pollen of *L. loliaceum* and a single rather poor caryopsis was produced. This seed failed to germinate.

In the parallel backcross to *L. remotum* none of the 122 florets of the pistillate unit showed the slightest sign of stimulation.

As only a single test was made to either parental species, these results can only show that the hybrids are not highly fertile with the pollen of either parental species, but that the ovaries are not completely unresponsive to the application of *L. loliaceum* pollen.

II. *Lolium loliaceum* with *Lolium temulentum*

In three crosses with *L. loliaceum* as the pistillate parent, seed-setting was relatively very high at 79, 77 and 77 % respectively. The corresponding seed germination results were 95, 66 and 100 % respectively.

It had been observed that in the cross which only gave a germination of 66 % caryopsis development was much less regular than in another cross made in the same season, although the same *L. temulentum* plant had been used as the pollen parent in both crosses and the pistillate parents were also full sister plants. An adequate explanation of the cause of this difference between 66 and 100 % is not available.

The seeds of the odd cross had been grouped in the dry conditions into three classes, but such classification was necessarily arbitrary. In the best seeds (85 seeds out of 154) germination at 95 % was approximately the same as in the other two crosses. In the second class (47 seeds) it fell to 36 %, and in the third class seed (22 seeds) it fell to 18 %. Germinating capacity therefore did not exactly tally with the arbitrary classification.

The caryopses of the seeds that failed to germinate were described (at dissection) as 'less than normal length [for *L. loliaceum*], usually with good pulp development at the embryo end and sometimes a fair amount elsewhere, but some caryopses practically empty in the endosperm position'.

The condition of most of these caryopses suggested that they were dead before they were harvested, but why this should happen in one cross and not in the others cannot easily be explained. If one of the inflorescences forming the unit had died prematurely,

the caryopses would be expected to dry up in the condition they had reached rather than begin to disintegrate.

It seems possible that although the plants used as pistillate parents were full sisters in a line that was morphologically constant they still differed physiologically.

While this difference is certainly puzzling, the main fact is that both seed-setting and seed germination can be very high when *L. loliaceum* is pollinated by *L. temulentum*.

Only one cross in the opposite direction was attempted, and the female unit was a small one of only sixty-seven florets. This was perhaps an advantage because it is difficult to collect the pollen of *L. loliaceum* in quantities large enough to pollinate big units.

Caryopses developed in thirty-eight florets (57 %), so that seed-setting was again quite satisfactory. These caryopses were quite near normal for *L. temulentum* in thirty-four seeds; the others were a bit under-sized but still hard. This separation of the seed in the dry condition had no significance because two of the poorer seeds, and nineteen of those more fully developed, germinated. On dissection it was found that six other seeds had attempted to germinate, so that the total attempted germination was 71 %.

The condition of the contents varied in those seeds which had attempted to germinate and had then failed. In three of the six, endosperm was plentiful and well organized but was now showing early signs of degeneration. In the others there was little endosperm. Endosperm was also plentiful in some of the seeds that had made no attempt to germinate, but it was again showing signs of degeneration. In some others the endosperm contents were meagre and in an advanced state of disintegration.

The results were therefore appreciably different from those obtained in two of the reciprocal crosses, and also from the third of those crosses in the fact that in the present case the vigour of germination was more graded, and in some seeds that failed to germinate the endosperm was more perfectly developed and better preserved.

The Lolium loliaceum–Lolium temulentum F_1 *hybrids*

In those crosses in which germination was relatively low, some of the seedlings were initially weak with a short and slender first leaf, and there seems to be a suggestion that the endosperm present in some of the seeds was not readily available to the germinating embryo or else that the embryo was unable to make normal use of the endosperm available.

The seedlings generally were slow in becoming established, and further development was again slow. It was observed that the emergence of the third or fourth leaf was often not quite normal, the tip of the leaf being caught in the mouth of the sheath of the preceding leaf, causing a crinkling of the leaf blade. This became even more pronounced in subsequent leaves, and the inflorescences found great difficulty in emerging so that they were very badly deformed.* In fact, the development of these seedlings and the mature plants was as nearly as possible exactly similar to that of the *L. loliaceum–L. remotum* hybrids described in §I above. In the present case the species were intercrossed in both directions and no difference in plant development was found.

There is no doubt, therefore, that here *L. remotum* and *L. temulentum* have given parallel results, and it is reasonable to conclude that the abnormal development of the plants was due to the same cause. There was no real difficulty at fertilization, and many caryopses were apparently of fully normal development. Some deformity of the embryo

* See W.P.B.S. Herbarium, sheets 491–494, Exp. B143.

is suggested by the broad and short first leaf of the *L. loliaceum–L. remotum* hybrids, so that embryo development itself may not be perfectly normal. In any case the main difficulty in the way of the production of normal plants occurred during plant development and consisted fundamentally of the inability of the leaves and the inflorescences to progress within the sheaths of the older leaves and to emerge from them. This suggests that the sheaths were too narrow to allow the easy passage even of the young leaves, and that the difficulty was caused by a maladjustment of the plant parts, the effect being a purely mechanical one.

As such a condition has not otherwise been found in a wide experience of grass breeding (except possibly in relation to the emergence of the inflorescences in the *L. perenne* variety *cristatum*) it would seem that this maladjustment of plant parts is the direct result of the intercrossing of these particular species. In the individual species concerned no such difficulty exists, and the only possible conclusion therefore seems to be that the maladjustment of the plant parts is due to the manner in which the genetical factors carried by the species interact in the heterozygous or, perhaps, heterogeneous condition, a working balance not being created.

Backcross

Owing to the abortion of most of the inflorescences in the *L. loliaceum–L. temulentum* hybrids, little material suitable for backcrossing was available, but two backcrosses to *L. loliaceum* and one backcross to *L. temulentum* were attempted. The 315 florets pollinated by *L. loliaceum* yielded eleven caryopses (3·6 %). Seven of these germinated, but in three of the seedlings the first leaf was abnormally short and broad. One failed to become established on its own roots, but the others eventually became fairly strong. A severe greenfly attack, however, killed them before they could be studied.

The other four seedlings were initially stronger, but they also suffered severely from greenfly infestation. They showed none of the deformities of the F_1 hybrids, and at least superficially they were indistinguishable from *L. loliaceum*.

Only a small unit of seventy-two florets received *L. temulentum* pollen. Although it was judged that the unit received a fair pollination, the results were completely negative, so that evidently this hybrid was not highly fertile to *L. temulentum* pollen, but at the same time the results for the backcross to *L. loliaceum* show that the *L. loliaceum–L. temulentum* hybrids are not completely female-sterile.

III. *Lolium remotum* with *Lolium temulentum*

This cross was referred to on a previous occasion (Jenkin, 1936), but no details could then be given.

The incomplete results suggested that *L. remotum* and *L. temulentum* are merely genotypes of the same species, and that they had become separated in course of time through association with crop plants whose seeds differed in size. Before this suggestion was actually published, more complete results showed that this suggestion could not be maintained. On the one hand, the anthers of the F_1 hybrids were found to be non-dehiscent, while Mr (now Professor) P. T. Thomas found that meiosis proceeded quite normally up to the point of actual pollen-grain formation.

With *L. remotum* as the pistillate parent, two crosses were made. The 321 florets

yielded sixty-one seeds (19%). The caryopses in these seeds appeared to be quite normally developed and 95% of them germinated quite strongly.

Only a single cross was made in the opposite direction. The unit consisted of seventy-four florets which gave forty-three seeds (58%), of which thirty-nine (91%) again germinated strongly.

The F_1 hybrids

The seedlings from the reciprocal crosses did not differ in any respect, and development into mature plants was so normal that it was confidently anticipated that they would be fully fertile, but as already stated the expectation was not realized, the anthers failing to dehisce.

Seven units on five different plants were protected from external pollen for automatic self-pollination. The total number of florets involved would be about 4000, but none of the florets showed the slightest sign of fertilization.

Backcross

One backcross was made, the pollen parent used being *L. remotum*. The seventy-nine florets yielded thirty-four seeds (43%), and all except one germinated. The seedlings were quite normal, and they also developed into mature plants in a perfectly normal manner.

When full grown these backcross hybrids varied somewhat in type, and although no plants were weak they showed considerable variation in plant height (63–91 cm.). In general morphology, some of the plants would be classifiable as *L. remotum* but others showed *L. temulentum* influence in their broader leaves and stouter stems and inflorescences. As the differences between the parental species are mainly relative, it would obviously be very difficult to classify these plants with any degree of confidence so that the task was not attempted.

In one plant closely approaching *L. remotum* in type the anthers were observed to be more or less dehiscent. In several others grown in pots no dehiscent anthers were found. On two plants protected for automatic self-pollination units consisting of a total of 1082 florets yielded no seed. These two plants were therefore at least functionally self-sterile.

Mature inflorescences were harvested from a series of twenty-four plants grown in the open without protection from 'foreign' pollen. These plants were accessible to pollen from various *Lolium* types growing in the same general area.

The total number of florets examined was 7599, and the over-all average seed-setting was found to be 44·5%, with a range of 5·1–86·2%. The distribution within the range was, however, discontinuous with fifteen plants within the range 5·1–42·2% and nine plants within the range 64·4–86·2%. Actually, the concentrations were even more narrow than these figures suggest, because for fourteen plants the range was from 5·1 to 30·5% and for eight plants at the other end of the scale, 72·8–86·2%.

As far as could be judged all plants were about equally accessible to outside pollen, yet in two adjacent plants seed-setting was respectively 5·1 and 80·5%. This suggests that seed-setting depended on some condition or conditions other than accessibility to pollen from outside the family group.

Despite the fact that the only two plants tested were found to be functionally self-sterile these results obtained from open pollination suggest that in fact a proportion of these backcross plants were highly self-fertile.

When the results are examined in relation to the height of the plants it is found that in the main (but not without exception) the highest seed-setting occurred in plants of medium size. The actual distribution is shown below:

No. of plants	Average height (cm.)	Height range (cm.)	Average seed-setting (%)	Range of seed-setting (%)
8	67·3	63·0–71·2	29·9	11·8–80·5
6	74·1	72·1–76·1	77·6	64·4–86·2
10	80·1	77·6–91·1	31·0	5·1–77·9

The outstanding variants were plants whose respective heights were 69·2, 79·5 and 85·6 cm., and seed-setting again respectively 80·5, 77·9 and 77·5%. These are included in the averages shown above.

None of the plants was so weak that it would be expected that seed-setting would be adversely affected in plants of similar size of either of the parental species, but no direct comparison is available.

Five pure *L. remotum* plants grown in the same area ranged from 75·1 to 78·7 cm. in height, but they were not examined for seed-setting. With a greater number of plants it is probable that the height range would be wider, but it seems unlikely that the range would be as wide as in the backcross family. It may be no more than a coincidence that the heights of most of the high seed-setting backcross plants almost coincided with that of the *L. remotum* plants which, in fact, were of the same line as the *L. remotum* plants used in both the original cross and in the backcross.

From our present point of view, the important fact is that by backcrossing the *L. remotum–L. temulentum* hybrids to *L. remotum*, high seed-setting was restored in a proportion of the backcross hybrid derivatives. The conditions were such that the indications are that these plants were, in fact, highly self-fertile. This, in its turn, would suggest that failure of pollen-grain formation in the F_1 hybrids and in a proportion of the backcross hybrids is due to a gene complex having a dominant inhibiting effect. Such a complex might be created if the two parental species carried complementary factors, so that one may be represented by **AAbb** and the other by **aaBB**.

Discussion of §*III*

The further data now described show that *L. remotum* and *L. temulentum* do not behave in intercrossing as if they were only genotypes of one and the same species, but that a definite interbreeding barrier exists between them. There is no difficulty in the development of the F_1 hybrids such as was encountered when each was intercrossed with *L. loliaceum*, but the F_1 hybrids, as far as the data are able to show, are completely self-sterile and there is evidence that they are, in fact, pollen-sterile.

A single backcross to *L. remotum* showed that the hybrids were by no means highly female-sterile, and the backcross hybrids again developed quite normally. Although two backcross plants gave no seed from protected automatic pollination, in a series of plants grown in the open none showed complete sterility, and in a proportion of the plants seed-setting was so high as to suggest very strongly that they were in fact highly self-fertile. On the basis of seed-setting these plants could be separated into two rather well-defined groups such as might be expected if failure of pollen formation in the hybrids were due to the interaction of complementary dominant factors.

It is, therefore, possible that after all the primary divergence of the two types was

mainly one that gave a range of seed sizes. If then a mixture of such types became mixed with cultivated crops, and any serious attempt was made to clean the seed by screening those types in which the size of the seed coincided with that of any particular crop, the seed-size types would become mechanically separated. As the plants are normally self-fertilized it may have happened that even before this mechanical separation had taken place other mutations had intervened, or they may have occurred subsequent to such separation. If the original fertile type were represented by **aabb** a dominant mutation in either **a** or **b** might have no effect on pollen fertility, but if this occurred in both **a** and **b**, the new genotype would be pollen-sterile and therefore would not be perpetuated. Similarly, should the genotypes **AAbb** and **aaBB** intercross the F_1 plants would be pollen-sterile, but a proportion of the backcross plants would again be pollen-fertile. Unfortunately, in the present instance, the hybrids were not backcrossed to *L. temulentum*, so that it is not known whether such backcross hybrids would also show a similar segregation of fertility types.

In nature the first bar to interbreeding between *L. remotum* and *L. temulentum* is the fact that each is normally self-pollinated and self-fertilized. Even at flowering the stigmas are poorly exserted and little pollen is liberated outside the florets. It is probable therefore that even in a mixed population the chances of intercrossing would be small but not non-existent. Should hybrid seed be so produced they would germinate strongly and the hybrids would develop normally, but they would set seed only if pollinated by other plants. A proportion of the plants of the next generation would be self-fertile, but others would again depend upon pollen from outside for seed-setting. In this way it is conceivable that heterozygous plants could be perpetuated in a mixed population over an indefinite number of generations, particularly if heterozygosity were associated with some measure of heterosis.

The present results are indefinite on this point. The backcross hybrids actually graded in plant height with the highly fertile plants mainly but not exclusively in the medium height group, and those of lower seed-setting either of smaller or of greater stature.

It is not known to what extent (apart from botanical gardens) the two species occur in nature outside the crops with which they are usually associated, so that the future of the two species cannot clearly be foreseen. With the adoption of more effective seed-cleaning methods, it may be expected that both species will rather speedily be eradicated from such crops, and both *L. remotum* and *L. temulentum* may then become extinct unless they are intentionally preserved by botanists, because both species are regarded as undesirable weeds with *L. temulentum* even a dangerous one.

IV. '*Lolium canadense*' (Ba 2630)

The name '*L. canadense*' as here used carries no taxonomic implications. It is used only because the seed sample from which the plants now to be discussed were derived was described as '*L. canadense* Michx.'. The ultimate origin of that seed sample is unknown. When received at the Station, it was given the identification number Bs 1155.

This seed sample was used for purposes other than breeding, but a small lot of ten plants occurred in the gardens. They were very poorly grown and at first they were only casually observed. They gave the impression that they represented *L. temulentum*, but one plant was noticed to be 'awnless' while all the others were fully awned. On its 'awnless'

character this odd plant might be classifiable as either *L. remotum* or *L. temulentum* var. *arvense*, but otherwise it might again be either *L. remotum* with rather large seeds or *L. temulentum* with rather small seeds.

From this odd plant, seed that had been produced without protection from foreign pollen was harvested as 'Ba 2630'. It may probably be assumed that this seed was produced by self-pollination, although it is possible that it was not fully self-fertile, and in that case some or all of the seeds might be the result of cross-pollination.

Two plants raised from the Ba 2630 seed were used for breeding as also was one plant derived from one of these two plants by self-pollination. All three of these plants were 'awnless' and were otherwise substantially alike.

Apart from the two plants of Ba 2630 used for breeding, four others were grown to maturity, when two of them were found to be again 'awnless' while the other two were fully awned.*

In parallel work, it has been found that both in *L. remotum* and in *L. temulentum*, 'awnless' is dominant over 'fully awned', so that whether the plant from which seed Ba 2630 was harvested was self- or out-pollinated, it is evident that it was heterozygous for awnedness.

'Lolium canadense' *with* Lolium remotum

After pollination with '*L. canadense*' pollen a unit consisting of 163 *L. remotum* florets yielded thirty-four seeds (21 %). It was not observed that pollen was less abundant than in *L. remotum* or *L. temulentum* plants, nor that it was of poor quality, so that the '*L. canadense*' plant was presumably of normal pollen fertility. All the seeds germinated strongly and the seedlings developed quite normally to maturity.

In the reciprocal cross, with *L. remotum* as the pollen parent, seed-setting was appreciably higher at 49 %. Germination was also again high at 99 %.

All the F_1 plants were 'awnless' and approximately uniform in type, but as certain fertility results did not quite tally, the results for the reciprocal crosses are described separately.

Lolium remotum *as the pistillate parent.* Six F_1 plants were grown in pots and twenty-six in open ground. In all six plants grown in pots, the anthers were more or less dehiscent although apparently not fully so. In one of them the proportion of dehiscent anthers was estimated to be less than 25 %.

On each of these six pot plants, inflorescences were enclosed for automatic self-pollination. None of the six was completely self-sterile, but seed-setting was low in each case at 3·4, 5·3, 6·4, 13·8, 21·9 and 24·6 % respectively. It is possible that the highest result may represent full self-fertility.

From each of the twenty-six plants grown and allowed to set seed without protection in the open, mature inflorescences were harvested and carefully examined. In these seed-setting ranged from 19·2 to 87·9 %, with the relatively very high average of 62·5 %. Only in two plants of the twenty-six was seed-setting less than 42 % with some concentration of plants at the 40–50 % level and again at 65–75 %, with the suggestion of a third possible peak at 80–90 %, but the total number of plants involved was too low to allow more than a suggestion of the type of distribution.

Lolium remotum *as the pollen parent.* Six plants, taken at random in the seedling

* The 'awnless' type is represented by a progeny plant of Ba 2630 (5) on sheets 240 in the W.P.B.S. Herbarium, while the awned type is represented on sheets 239.

stage, were again grown in pots. From automatic self-pollination three of the six failed to set any seed, while in the remaining three seed-setting was very low at 1·6, 2·1 and 4·3% respectively. The average as well as the range was therefore considerably lower than in the pot plants of the reciprocal cross.

Sixty plants were also grown in the open in the same area as those of the other cross, and from each plant ripe inflorescences were harvested and examined. The total number of florets from the sixty plants was 17,364.

Average seed-setting was now very appreciably lower at 39% with the wider range of from 8·6 to 92·5%. The distribution of the plants was not continuous, definite concentrations being found at 10–35% and at 50–75%, with the suggestion of a third concentration at 80–95%. In form, therefore, the curve was not unlike that of the family from *L. remotum* as the pistillate parent, but the concentrations were at lower levels of seed-setting.

From the data available it is impossible to determine whether the differences between the families from the reciprocal crosses have any significance. A reconstruction of the lay-out on the ground from details available shows that the two lots were on adjoining ground and that adjacent plants might differ widely in seed-setting. A plant yielding 91·1% had as its nearest neighbours four plants yielding from 25·7 to 39·3%. The four nearest to another plant yielding 17·9% gave from 25·7 to 64·8%. It is, therefore, highly improbable that either the differences between the two lots or the variations from plant to plant within each was due to greater or lesser accessibility to outside pollen.

This suggests that while seed-setting was not necessarily in all cases the result of self-pollination, those plants in which a high proportion of the florets set seed did so mainly as the result of self-fertilization.

'Lolium canadense' *with* Lolium temulentum

Two emasculated units of *L. temulentum* received '*L. canadense*' pollen. The units were small (150 florets), and seed-setting was moderately good at 36 and 96% of the seeds germinated.

In two reciprocal crosses average seed-setting was somewhat higher at 50% and germination 99%. The differences recorded cannot be considered to have any significance.

The seedlings developed strongly and normally, and there was no observable difference of any kind between the plants according to the direction in which the crosses had been made.

Eight plants were given the opportunity to set seed from (automatic) self-pollination and the following seed-setting results were recorded:

ex *L. temulentum* ♀ × '*L. canadense*' ♂: 0·0, 3·5, 3·6 and 56·5% respectively;
ex '*L. canadense*' ♀ × *L. temulentum* ♂: 0·0, 13·3, 18·9 and 54·2% respectively.

The results are therefore roughly parallel, and except that two plants showed quite appreciably higher seed-setting the results do not differ greatly from those obtained in the '*L. canadense*' -*L. remotum* hybrids.

Seed setting in the '*L. canadense*'–*L. temulentum* hybrids under open-pollination conditions was not studied and therefore a comparison in this respect is not possible.

Discussion of §IV

As far as the available data are able to show, the odd *Lolium* line Ba 2630 ('*L. canadense*') gave very approximately similar results when intercrossed with either *L. remotum* or *L. temulentum*. As *L. remotum* and *L. temulentum* when intercrossed gave only self-sterile hybrids, it had been expected that when intercrossed with one of these two species, plants of line Ba 2630 would also give only self-sterile progeny. This expectation was not realized. Some self-sterile plants were produced in both crosses, but in both a majority of the progeny plants showed some degree of self-fertility and some may have been fully self-fertile. The present results therefore, equally with the examination of morphological characters, fail to place plants of line Ba 2630 definitely in either *L. remotum* or *L. temulentum*, although they have very close breeding affinities with both species.

The results for the cross with *L. remotum* are strongly reminiscent of those obtained when the *L. remotum–L. temulentum* hybrid was backcrossed to *L. remotum*, although the parallel is not complete, particularly in the distribution of the fertility grades as shown by open-pollination results. It has been found, however, that even in two crosses between line Ba 2630 and *L. remotum* such results did not quite agree.

In relation to *L. remotum* therefore the odd line seems to occupy a position approximately similar to a *L. remotum–L. temulentum* hybrid. A puzzling feature of the situation, however, is the fact that these '*L. canadense*' plants were sufficiently pollen-fertile to be used as male parents, while the *L. remotum–L. temulentum* hybrids were pollen-sterile. This suggests that the genetical explanation offered above to account for the failure of pollen formation in the *L. remotum–L. temulentum* hybrids is not adequate to cover the whole situation.

The present studies were not carried far enough to ascertain whether some of the backcross plants of low self-fertility could be effectively used as pollen parents, and it is therefore not known whether in fact these might be the exact parallel of the plants of the Ba 2630 line that were used. It seems possible that the full dominance of the sterility factors may be modified by other factors to allow a degree of pollen- (and self-) fertility in heterozygous plants. Such a suggestion seems to be supported by the fact that with both *L. remotum* and with *L. temulentum*, plants of the Ba 2630 line did give some completely self-sterile plants.

It is therefore possible that line Ba 2630 is ultimately derived from a cross between *L. remotum* and *L. temulentum*, but its impartiality towards one or the other with regard to breeding affinity is still puzzling, although it is true that the self-sterile *L. remotum–L. temulentum* hybrids could also be backcrossed to either of the two parental species.

The production of such a hybrid in nature would be expected to occur only very rarely, but it is conceivable that it could occur in a botanical garden where *L. remotum* and *L. temulentum* were grown side by side or possibly, by accident, even mixed. Under such conditions it has been shown that natural intercrossing can occur in other self-fertilized species such as wheat (Jenkin, 1925) and oats (Jones, 1933), and if in this particular case the F_1 hybrid was not completely self-sterile, similar hybrids could be perpetuated particularly again in a botanical garden.

This inevitably brings us back to a reconsideration of the independent specific status of *L. remotum* and *L. temulentum*. On all considerations, they have very close affinities, but their claim to independent specific rank is not eliminated by the present results,

because even though possibly some of their F_1 hybrids may be to some extent pollen-fertile, an interbreeding barrier does exist as proved by the fact that pollen-sterile plants are produced. If, however, as suggested, this is caused by relatively simple gene differences, the basis of specific distinction in this case would be paralleled by well-marked morphological features that are also produced by simple gene differences. It must be recognized, however, that an interbreeding barrier, however caused, effectively isolates the two types, and that henceforward further mutations will affect only the particular line in which it occurs. Parallel mutations may occur in the lines, but divergent mutations may also occur which further emphasize the specific differentiation of two such lines, and it would seem that in *L. remotum* and *L. temulentum* we are witnessing the separation of a prototype species into two independent species.

General discussion

The position of *L. remotum* and *L. temulentum* relative to one another has already been adequately discussed above, and the fact that they are closely related is strongly supported by the similarity of their breeding interactions with *L. loliaceum*. In particular, the hybrids entirely agreed in their abnormal development caused presumably by a maladjustment of the plant parts.

Little importance can be attached to differences in seed-setting because of the difficulty in obtaining an adequate supply of pollen from these normally self-pollinated plants.

In two of the crosses of *L. loliaceum* with *L. temulentum*, seed germination was relatively low, but in two other crosses of this type it was very high. It was also very high in both crosses of *L. loliaceum* with *L. remotum*, so that again *L. remotum* and *L. temulentum* run parallel. It is therefore evident that in so far as fertilization and caryopsis development is concerned there is a close affinity between *L. loliaceum* and both of the other species. On the other hand, in morphology and general growth it differs very widely from them, so that except largely for the long glumes and the self-pollinating habit it shows no close affinity to them. The relative (but not complete) failure of backcrosses may not be highly significant because of the deformity of the inflorescences, but the F_1 hybrids are not completely sterile to the pollen of *L. temulentum*.

In crosses with the wind-pollinated types also (Jenkin, 1954*b*, *c*, *d*) a parallelism between *L. loliaceum*, *L. remotum* and *L. temulentum* has been found, and particularly in the response of the maternal tissue of the ovaries to pollination by these other types.

With all three species, *L. rigidum*, whether used as the pistillate or as the pollen parent, gave a nominal seed-setting ranging from 42 to 91%, but none of the seeds germinated except two in the *L. loliaceum*–*L. rigidum* combination. As this amounted to less than 1% of the seeds it cannot be regarded as significant in relation to no germination in the other two combinations.

With *L. italicum*, the three self-pollinating annuals again showed a satisfactory nominal seed-setting irrespective of the direction of the cross, but with *L. italicum* as the pollen parent no seeds germinated. When these crosses were made in the opposite direction, however, germination ranged from 38% with *L. remotum*, 55% with *L. temulentum* to 93% with *L. loliaceum*, so that the response was of the same kind but not of the same order.

With *L. perenne* as a parent there was a further departure from the rule in that with

both *L. remotum* and *L. temulentum* as female parents, a seed germination of about 5% was obtained as compared with 26% with *L. loliaceum* as the pistillate parent. In the reciprocal crosses the results were essentially as with *L. italicum*, with germination still lowest with *L. remotum* and highest with *L. loliaceum*. With *L. perenne* therefore *L. loliaceum* stands clearly apart from *L. remotum* and *L. temulentum*, in that when used as the pistillate parent seed germination was substantially higher. *L. loliaceum* also gave particularly high germination when used as the pollen parent with both *L. italicum* and *L. perenne*.

With either *L. perenne* or *L. italicum* as the pollen parent there was no substantial difference in the response of *L. remotum* and *L. temulentum*, but in the reciprocal crosses seed germination was definitely lower with *L. remotum* as the pollen parent.

Thus, in spite of much that is parallel in the response of the three species to the wind-pollinated types, each shows a degree of individuality which is most definite in *L. loliaceum*. The breeding affinity this species has shown to *L. perenne* is remarkable because it was the only one which when used as the pistillate parent gave a reasonably good germination result. This is all the more striking because of the fact that when pollinated by *L. italicum*, *L. loliaceum* gave no germinable seed.

On all counts, therefore, *L. loliaceum* stands clearly distinct from both *L. remotum* and *L. temulentum*.

The distinction between the latter pair is far less definite, and it is possible that the difference in seed germination with both *L. italicum* and *L. perenne* is due to the individual plants used, but at least to an extent this argument could be applied to all cases of difference.

Although not directly relevant in the present connexion, the almost complete failure of *L. rigidum* types in crosses with each of the species now under consideration is quite remarkable because *L. rigidum*, in virtue of its strictly annual habit, might have been expected to have a closer affinity than *L. italicum* and *L. perenne* with the present group of species. Moreover, little more than a suggestion of incompatibility was found in the wind-pollinating *Lolium* species amongst themselves. This fact more clearly indicates a distinction between *L. rigidum* and both *L. italicum* and *L. perenne* than any of the results obtained by interbreeding these wind-pollinating types.

The high seed-setting and the high seed-germination results obtained when *L. loliaceum* was intercrossed with either *L. remotum* or *L. temulentum*, added to the common self-pollinating and strictly annual habits as well as to the morphology of the spikelet, undoubtedly combine to place it with these other two in a separate *Lolium* group, but its ready capacity to interbreed with *L. perenne* suggests that it is also closely allied to this species. Moreover, the stiff stems of the *L. perenne* type from Algiers suggests a transition. It is therefore conceivable that *L. loliaceum* now most nearly represents the primitive *Lolium* type from which all others are ultimately derived. This would not necessarily mean that the annual wind-pollinating types represent a reversion from primitive *L. perenne*, but that there were departures in several directions, including the loss of compatibility between *L. loliaceum* and the *L. rigidum* types.

BRIEF SUMMARY

In the present account of interspecific crosses in the herbage grasses attention is concentrated on the three annual species *Lolium loliaceum*, *L. remotum* and *L. temulentum*. The three have been found to be inter-fertile to the extent that the three possible hybrid types have been obtained as established plants.

L. loliaceum stands clearly apart from the other two species in some of its breeding reactions. With either *L. remotum* or *L. temulentum* it gave reasonably good seed-setting and high seed germination, but the seedlings developed very abnormally apparently because of lack of co-ordination between the different plant parts.

Apart from all other considerations, the fact that this was common to the hybrids with both *L. remotum* and *L. temulentum* brings these two species into a special class. In addition, these two species are easily intercrossed and the F_1 hybrids develop normally up to pollen formation. It is suggested that failure at pollen formation is caused by gene combinations. This possibility is not entirely removed by the results obtained by using for breeding an odd line ('*L. canadense*') which could not definitely be placed in either *L. remotum* or *L. temulentum*. A barrier to normal full fertility undoubtedly exists between the two types, but divergence has not yet proceeded very far.

The interaction of the three species with the normally wind-pollinated *Lolium* types is also reviewed.

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REFERENCES

JENKIN, T. J. (1925). Natural crossing in wheat. *Welsh J. Agric.* **1**, 104–10.

JENKIN, T. J. (1935). Interspecific and intergeneric hybrids in herbage grasses. II. *Lolium perenne* × *L. temulentum*. *J. Genet.* **31**, 379–411.

JENKIN, T. J. (1936). Natural Selection in relation to the grasses. In 'A discussion of the present state of the theory of Natural Selection'. *Proc. Roy. Soc.* B, **121**, 52–6.

JENKIN, T. J. (1954*b*). Interspecific and intergeneric hybrids in herbage grasses. V. *Lolium rigidum* sens. ampl. with other *Lolium* species. *J. Genet.* **52**, 252–81.

JENKIN, T. J. (1954*c*). Interspecific and intergeneric hybrids in herbage grasses. VI. *Lolium italicum* A.Br. intercrossed with other *Lolium* types. *J. Genet.* **52**, 282–99.

JENKIN, T. J. (1954*d*). Interspecific and intergeneric hybrids in herbage grasses. VII. *Lolium perenne* L. with other *Lolium* species. *J. Genet.* **52**, 300–17.

JONES, E. T. (1933). Natural crossing in oats. *Welsh J. Agric.* **9**, 115–32.