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SELF-INCOMPATIBILITY IN RYEGRASS VII. THE DETERMINATION OF INCOMPATIBILITY GENOTYPES IN AUTOTETRAPLOID FAMILIES OF *LOLIUM PERENNE* L.

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SUMMARY

The two locus gametophytic self-incompatibility system of the grasses entails a maximum of four different *S–Z* specificities in the diploid pollen of tetraploid individuals. It is not known for certain whether only one *S–Z* pair in such pollen needs to be matched by the same pair in the stigma for incompatibility to occur (hypothesis 1) or whether all such pairs have to be matched (hypothesis 2).

This paper, the first of three, concerns the results obtained from the application of the first stage of a three-stage procedure for the analysis of self-incompatibility in tetraploids. Pollen from diploids of known genotype is applied to the stigmas of related tetraploids in order to determine the incompatibility genotypes of the latter.

The results obtained from two families of tetraploid perennial ryegrass show that the 35 legitimate plants examined fell into 18 of the expected 81 different incompatibility classes, and that the gametic segregation ratios of the parents of these families were consistent with the assumption that the chromosomes segregate at random with respect to the incompatibility loci in tetraploid ryegrass. Furthermore, since it was possible to recognise plants of genotype $S_{3456}Z_{3456}$ and $S_{3356}Z_{3356}$, the alleles must act independently in the stigmas of tetraploids with no dosage effects.

1. INTRODUCTION

The effect of induced polyploidy in those dicotyledonous species of flowering plant which possess a one-locus, gametophytic system of self-incompatibility appears generally to cause a breakdown of the system. Thus an autotetraploid that has been produced from a self-incompatible diploid is usually at least partially self-fertile. This breakdown occurs because of competitive interaction between two different *S* alleles in a proportion of the diploid pollen of the tetraploid (Lewis 1943, 1947). Thus a tetraploid of genotype S_{1122} (*i.e.*, $S_1S_1S_2S_2$) is expected to produce two types of pollen, one of which is homogenic, S_{11} and S_{22}, and the other heterogenic, S_{12}. Because the *S* alleles act independently in the styles or stigmas of tetraploids, as well as in those of diploids, homogenic pollen of either genotype is incompatible on any style or stigma that contains the corresponding allele. The same is also true of heterogenic pollen, provided that the alleles act independently or the appropriate allele is completely dominant to the other. Where, however, the alleles of heterogenic pollen interact competitively, perhaps for a common substrate, neither is able to produce its characteristic

phenotype (Lewis, 1954). In these circumstances, a stigma carrying S_1 and S_2 apparently fails to recognise that the heterogenic pollen is carrying the same alleles; hence, such pollen is compatible.

Since most induced tetraploids of dicotyledonous species with one-locus gametophytic systems of self-incompatibility are at least partially self-fertile, competitive interaction between the alleles of heterogenic pollen appears to be the rule rather than the exception. It is presumably because of this that, in those genera which have a polyploid series, incompatibility is confined to species at the diploid level. This appears to be the case, for example, in the genus *Papaver* (Lawrence, Afzal and Kenrick, 1978).

In the monocotyledons, on the other hand, self-incompatibility appears to be quite immune to polyploidy. Thus autotetraploids of *Tradescantia paludosa* (Annerstedt and Lundqvist, 1967) and of *Ananas comosus* (Collins, 1961) are self-incompatible, though in both species self-incompatibility is determined by a single gene with gametophytic effect in the pollen (Annerstedt and Lundqvist, *loc. cit.*; Brewbaker and Gorrez, 1967). The same appears to be true of those monocotyledenous species in which control is exercised by a pair of multi-allelic loci; for example, autotetraploids of perennial ryegrass are as self-incompatible as their diploid progenitors (see, for example, Ahloowalia, 1977). Indeed, immunity to polyploidy appears to be a property of multilocus systems in general, for polyploids of the dicotyledonous species *Ranunculus acris* (Østerbye, 1975, 1977) and of *Beta vulgaris* (Larsen, 1977) are also self-incompatible. The disruptive effect of polyploidy on self-incompatibility appears, therefore, to be confined to dicotyledonous species with one-locus, gametophytic systems of incompatibility.

While these observations leave no doubt that the action of the incompatibility alleles remains unimpaired both in the stigma and pollen in polyploids of species with multi-locus systems, they tell us little about the circumstances in which a pollen grain is incompatible. Thus at the diploid level in a species with a two-locus system, a pollen grain carries one S and one Z allele and the stigma two alleles at each locus. Incompatibility occurs whenever a pollen grain alights on a stigma which carries the same S–Z pair of alleles as the pollen. A pollen grain has only one S–Z specificity, but in the case of a plant that is heterozygous at both of the incompatibility loci (*e.g.*, $S_{12}Z_{12}$), the stigma will form four S–Z specificities (S_1Z_1, S_1Z_2, S_2Z_1, S_2Z_2). The diploid pollen of the tetraploid, on the other hand, carries two S and two Z alleles and can, therefore, form as many as four S–Z specificities, as in the case with stigmas of diploids, whilst the stigmas of tetraploids can form as many as sixteen different S–Z specificities if the plant is tetra-allelic at both loci (*e.g.*, $S_{1234}Z_{1234}$).

It is reasonable to assume that the alleles at each locus act independently in the stigmas of tetraploids just as they do in diploids. Furthermore, since tetraploids are self-incompatible, it is also reasonable to assume, at the outset, that these alleles act independently in the pollen. The problem with tetraploids then reduces to the question of whether:

(a) only one S–Z pair in the pollen needs to be matched in the stigma (hypothesis 1); or
(b) all of the S–Z pairs must be matched in the stigma for incompatibility to occur (hypothesis 2).

The level of cross-compatibility among the members of a family of full sibs will be much lower on the first hypothesis than on the second. Indeed, such experimental evidence as is available from *Secale cereale* (Lundqvist, 1957) and *Festuca pratensis* (Lundqvist, 1962) favours the first rather than the second hypothesis, though this evidence is far from conclusive.

The solution of this problem is of considerable interest, both because of the light it would cast on the nature of the incompatibility mechanism and because it would allow us to investigate the dynamics of the polymorphism at the tetraploid level. Furthermore, since several tetraploid varieties of *Lolium perenne* and *L. multiflorum* and their hybrids have been introduced onto the temperate European agricultural market (Breese, 1983), both of these points have practical implications.

Now at the diploid level, it is possible to investigate the inheritance and expression of incompatibility by inter-crossing the members of one or more full-sib families. This method of analysis is not practicable with tetraploids, however, because a cross between two plants may be incompatible even though they are not of the same genotype. Thus with tetraploids, the simple analysis of the inter-relationships of the members of a full sibship is unlikely to meet with much success, particularly in those species with a two-locus system in which the expected number of genotypes in a family is large.

The solution to this methodological difficulty is to employ a crossing procedure in which pollen from diploids of known genotype is used on the stigmas of related tetraploids. In this way, the incompatibility genotypes of the tetraploids can be determined without any ambiguity caused by uncertainty about the mode of action of the *S* and *Z* alleles in their pollen. Once this has been done the procedure can be reversed by using the pollen from these tetraploids on the stigmas of their related diploids in order to ascertain whether only one (hypothesis 1) or all four (hypothesis 2) *S*–*Z* combinations have to be matched in the pollen by the *S*–*Z* combinations in the stigma for incompatibility to occur. Finally, pollinations may then be made between the tetraploids in order to check the classification of the two previous rounds of pollinations.

The present paper is concerned with the results obtained from the first stage of this procedure.

2. Materials and methods

Clonal replicates of a set of six pairs of plants from the *L. perenne* variety S23 were very kindly made available to us by Mr E. J. Lewis of the Welsh Plant Breeding Station, Aberystwyth. One member of each of these pairs was a diploid and the other an autotetraploid that had been produced from its partner by colchicine treatment of vegetative tillers. Three of these pairs were used in the present investigation, namely 1, 23 and 26. Two crosses were made between the diploids of these pairs, 26×23 and 23×1, and one between the tetraploids, 26×1 (fig. 1). To avoid ambiguity, the crosses giving rise to the two families of diploids will hereafter be referred to as $26^2 \times 23^2$ and $23^2 \times 1^2$ and that giving rise to the tetraploids, which was made in reciprocal, $26^4 \times 1^4$. Genotypes were assigned to the members of the diploid families in the summer of 1979 and to those of the tetraploid families in the spring of the following year by employing the same technical

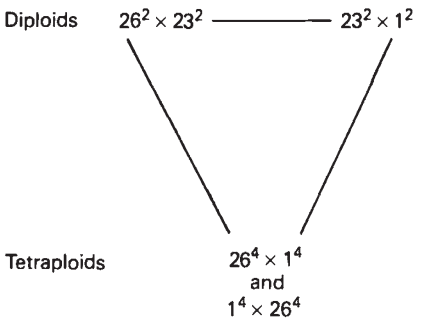

FIG. 1. Diagram showing the relationship between the pair of diploid families, on the one hand, and between these and the tetraploid families, on the other hand.

procedure as that used by Cornish, Hayward and Lawrence (1979). Further details of this work are given in Scarrott (1981).

3. RESULTS

(i) *The analysis of the diploid families*

A summary of the results obtained from the analysis of the pair of diploid families is shown in table 1. As in previous experiments (Cornish *et al.*, 1979; Fearon, Hayward and Lawrence, 1983), a proportion (22.3 per cent) of the partially compatible pollinations in each family were initially inconsistent with the overall classification of these plants and in one case,

TABLE 1

Summary of the results obtained from the two families of diploids. It should be noted that, while the assignation of plants to classes in the present experiment is consistent over families, there is no relationship between the labelling of the incompatibility alleles here and that of any previous experiment with ryegrass

Genotype *SSZZ*	Family $26^2 \times 23^2$	Genotype *SSZZ*	Family $23^2 \times 1^2$
1313	2	*1515*	4
1314	2	*1516*	0
1323	0	*1525*	3
1324	2	*1526*	2
1413	6	*1615*	5
1414	1	*1616*	1
1423	2	*1625*	2
1424	2	*1626*	1
2313	0	*2515*	3
2314	3	*2516*	0
2323	2	*2525*	1
2324	0	*2526*	2
2413	0	*2615*	2
2414	1	*2616*	0
2423	2	*2625*	2
2424	2	*2626*	1
Totals	27		29
No. of classes	12		13

a pollination that was fully compatible was initially classified as three-quarters compatible. Additional pollinations confirmed in each case that the overall classification was correct. Two plants in the family produced from the cross $23^2 \times 1^2$ when used as females turned out to be three-quarters compatible with each of the six testers used to classify the plants in this family, which suggests that they arose by self rather than cross-pollination. The phosphoglucoisomerase (*PGI*-2) phenotypes of these plants confirm this conclusion, for while those of the parents of this family were *aa* and *bc* for 23^2 and 1^2 respectively, those of the aberrant plants were identical to their female parent (23^2) and not *ab* or *ac* as expected. These plants were therefore excluded from further analysis. Once these minor difficulties had been overcome, the data from each family were entirely consistent with the hypothesis that incompatibility in ryegrass is determined by a pair of multi-allelic genes, *S* and *Z*, whose effect in the pollen is gametophytic (Cornish *et al.*, *loc. cit.*; Fearon *et al.*, *loc. cit.*).

No differences between reciprocal pollinations were observed in either of the families which indicates that their parents had no *S* or *Z* alleles in common. The results obtained from interfamily pollinations, following a procedure given by Fearon *et al.*, (*loc. cit.*), which exploits the fact that the members of these families have a half-sib relationship, enabled the progenies to be classified such that the genotype of their common parent, 23^2, is defined as $S_{12}Z_{12}$, and those of the other parents, 26^2 and 1^2, as $S_{34}Z_{34}$ and $S_{56}Z_{56}$ respectively.

There are three further points worth making about the results obtained from these families. First, only one of the eight single factor ratios in these families deviates significantly from the 1 : 1 expectation, namely $Z_5 : Z_6$ in the progeny of the cross $23^2 \times 1^2$ ($\chi^2_{(1)} = 7{\cdot}759$, $P = 0{\cdot}01 - 0{\cdot}001$). Second, there is no evidence in either family of linkage between *S* and *Z*. Third, there is, on the other hand, good evidence of linkage between *S* and *PGI*-2 in both families (see Cornish *et al.*, 1980). As was mentioned earlier, the phosphoglucoisomerase phenotypes of 23^2 and 1^2 were *aa* and *bc*, respectively; that of 26^2 was also *bc*. We can, therefore, test for linkage between *S* and *PGI*-2 in parents 26^2 and 1^2; both tests were highly significant ($\chi^2_{(1)} = 17{\cdot}640$, $P < 0{\cdot}001$ and $\chi^2_{(1)} = 12{\cdot}448$, $P < 0{\cdot}001$ respectively). The intensity of linkage was homogeneous over families and the joint estimate of linkage was $\hat{p} = 0{\cdot}1296 \pm 0{\cdot}0457$. Though this estimate is a little lower, it is nevertheless similar to the joint estimates obtained from other families of perennial ryegrass (Cornish *et al.*, *loc. cit*).

(ii) *The analysis of the tetraploid families*

Twenty-eight plants were used from the first ($26^4 \times 1^4$) and 21 from the second ($1^4 \times 26^4$) family giving a total of 49 in all. It is known that the chromosome number of synthetic autotetraploids of ryegrass is not completely stable (Evans and Davies, 1982). Examination of mitotic metaphases in root tips of tillers taken from each of the 49 plants revealed that in every case the chromosome number was the expected 28.

Since the genotypes of 26^2 and 1^2 are $S_{34}Z_{34}$ and $S_{56}Z_{56}$, those of their tetraploids, 26^4 and 1^4, must be $S_{3344}Z_{3344}$ and $S_{5566}Z_{5566}$ respectively. Each of these tetraploids is expected to produce nine distinguishable types of gamete (table 2), so that the expected number of different genotypes in the progeny of the cross between these tetraploids is $9 \times 9 = 81$.

TABLE 2

The gametic output of tetraploid parent 26^4 of genotype $S_{3344}Z_{3344}$ (left) and of tetraploid parent 1^4 of genotype $S_{5566}Z_{5566}$ (right). The column headed f shows the expected relative frequencies of the gametes on the assumption of random chromosome assortment at meiosis

Type	f	26^4 $S_{3344}Z_{3344}$ *SSZZ*	1^4 $S_{5566}Z_{5566}$ *SSZZ*
1	1	*3333*	*5555*
2	4	*3334*	*5556*
3	1	*3344*	*5566*
4	4	*3433*	*5655*
5	16	*3434*	*5656*
6	4	*3444*	*5666*
7	1	*4433*	*6655*
8	4	*4434*	*6656*
9	1	*4444*	*6666*

The procedure used to determine the incompatibility genotypes of the offspring of these tetraploids depends upon the fact that they have a half-sib relationship with the members of each of the diploid families, $26^2 \times 23^2$ and $23^2 \times 1^2$. Thus the allelic contribution of 26^4 to each of its offspring in the cross $26^4 \times 1^4$ can be ascertained if each is pollinated by a factorial set of four tester plants from the diploid family $26^2 \times 23^2$ which are known, from the analysis of the previous section, to carry S_3 and Z_3, S_3 and Z_4, S_4 and Z_3, and S_4 and Z_4 respectively, S_3, S_4, Z_3 and Z_4 being the alleles inherited from 26^2. Thus one quarter of the pollen from a diploid plant of genotype $S_{13}Z_{13}$, for example, is expected to carry the combination S_3Z_3 and hence to be incompatible on the stigmas of any tetraploid which also carries these alleles; that is, a three-quarters compatible reaction is expected in these circumstances. If, on the other hand, the tetraploid in question does not carry both of these alleles, the pollination will be fully compatible. It follows, therefore, that this factorial set of four testers can be used to separate the tetraploids of $26^4 \times 1^4$ and $1^4 \times 26^4$ into nine classes according to the alleles they received from their 26^4 parent (table 3). Similarly, an appropriate factorial set of four testers from the diploid family $23^2 \times 1^2$ can be used to determine the gametic contribution of 1^4 to each of the tetraploid offspring. Hence, between them these two sets of testers should separate each of the offspring into one or other of 81 zygotic classes, assuming, of course, that the *S* and *Z* alleles act independently in the stigmas of tetraploids. Wherever possible at least two plants were used of each tester genotype to pollinate the tetraploids. The use of pollen from diploids of known genotype on the stigmas of tetraploids in order to determine the genotype of the latter was first employed by Lewis (1943, 1947) with tetraploids of *Oenothera organensis.*

A summary of the results obtained with this procedure is shown in table 4. Eleven of the 28 plants analysed in the progeny of $26^4 \times 1^4$ turned out to contain no *S* or *Z* alleles from 1^4 and three of the 21 plants of the progeny of $1^4 \times 26^4$ contained no alleles from 26^4, giving a total of 14 aberrant plants in all. These plants almost certainly arose as a result of open-pollination

TABLE 3

The pattern of reactions expected when pollen from a factorial set of plants from the diploid family $26^2 \times 23^2$ is used on the stigmas of plants of the tetraploid family $26^4 \times 1^4$. Only the S and Z alleles derived from the common parent 26 are shown. + = fully compatible; T = three-quarters compatible. The entries in the column headed "Type" indicate the type of gamete (see table 2) from 26^4 that gave rise to each of the tetraploid zygotes

		Diploid ♂♂ $26^2 \times 23^2$			
	S	–	–	–	–
Tetraploid ♀♀	*S*	*3*	*3*	*4*	*4*
$26^4 \times 1^4$	*Z*	–	–	–	–
Type	*SSSSZZZZ*	*Z*	*3*	*4*	*3*	*4*
1	*33--33--*		T	+	+	+
2	*33--34--*		T	T	+	+
3	*33--44--*		+	T	+	+
4	*34--33--*		T	+	T	+
5	*34--34--*		T	T	T	T
6	*34--44--*		+	T	+	T
7	*44--33--*		+	+	T	+
8	*44--34--*		+	+	T	T
9	*44--44--*		+	+	+	T

of each tetraploid parent prior to them being bagged up, a conclusion which is confirmed by their enzyme phenotypes. Thus 25 of the 35 legitimate offspring and 12 of the 14 aberrant plants have been scored for their phosphoglucoisomerase (*PGI*-2) phenotype. The phenotype of both 1^4 and 26^4 was *bbcc*, so their offspring are expected to contain either *b* or *c* or both, as indeed was always the case. Eight of the 12 aberrant plants, however, contained a third allele, *d*, together with *b* and/or *c*, which for these plants, at least, puts the question of their origin beyond doubt.

The seventeen legitimate plants of the cross $26^4 \times 1^4$ fall into 10 incompatibility classes; the 18 of $1^4 \times 26^4$ into 12; pooling over reciprocals, the 35 plants which have been analysed fall into 18 different classes. If *S* and *Z* are considered independently, the outcome of tests of the agreement of the gametic segregation ratios with the 1:4:1 ratio expected on the hypothesis of random chromosome assortment are shown in table 5. The data from the aberrant plants were included in these tests whenever the gametic output of their female parent was under consideration. The observed segregation ratios of the *S* alleles in both parents and the *Z* alleles in 26^4 are in good agreement with the expected 1:4:1 ratio and the families are homogeneous in this respect. There is, however, a just significant overall departure from expectation for the segregation of the *Z* alleles of parent 1^4, due to there being an excess of Z_{56} and a deficit of Z_{66} gametes, the observed proportion of Z_{55} gametes being close to expectation.

Finally, though there is clearly not as much data as is desirable to investigate linkage between *S* and *Z* in the two tetraploid parents, estimates of the recombination frequency between these loci were obtained (see Fisher, 1947); the estimate of linkage for parent 26^4, based on 46 plants, was $\hat{p} = 0{\cdot}5 \pm 0{\cdot}0903$ and that for parent 1^4, based on 38 plants, $\hat{p} = 0{\cdot}5 \pm 0{\cdot}0993$, with the joint estimate of $\hat{p} = 0{\cdot}5 \pm 0{\cdot}0668$. In order to arrive at these estimates, it was necessary to assume that there were no cross-overs between

TABLE 4

Summary of the results obtained from the two families of tetraploids. The entries in the column headed "Type" indicate the genotype of the gametes that have given rise to each of the tetraploid genotypes, the number before the decimal point indicating the contribution of parent 26^4 and that after the point, the contribution of 1^4. The last six entries in the main part of the table show the partial genotypes of the L. multiflorum *contaminants*

Type	Genotype *SSSS*	*ZZZZ*	$26^4 \times 1^4$	$1^4 \times 26^4$	Totals
1·5	*3356*	*3356*	1	0	1
2·1	*3355*	*3455*	0	1	1
2·4	*3356*	*3455*	1	0	1
2·5	*3356*	*3456*	3	1	4
3·5	*3356*	*4456*	0	3	3
4·2	*3455*	*3356*	0	2	2
4·5	*3456*	*3356*	2	1	3
4·8	*3466*	*3356*	0	1	1
5·2	*3455*	*3456*	1	1	2
5·4	*3456*	*3455*	1	0	1
5·5	*3456*	*3456*	4	3	7
5·8	*3466*	*3456*	0	2	2
6·5	*3456*	*4456*	0	1	1
8·2	*4455*	*3456*	1	0	1
8·4	*4456*	*3455*	0	1	1
8·5	*4456*	*3456*	2	0	2
9·2	*4455*	*4456*	0	1	1
9·5	*4456*	*4456*	1	0	1
2·0	*33*--	*34*--	1	0	1
4·0	*34*--	*33*--	2	0	2
5·0	*34*--	*34*--	5	0	5
6·0	*34*--	*44*--	3	0	3
0·5	--*56*	--*56*	0	2	2
0·7	--*66*	--*55*	0	1	1
	Totals		17 (28)	18 (21)	35 (49)

these loci involving three chromosomes and that paired chromosomes undergoing crossing-over went to different poles. It follows from the second assumption that there can be no double reduction involving these loci.

Now, in theory, it is possible to distinguish between no linkage and very loose linkage ($p = 0{\cdot}5$) in a tetraploid (see de Winton and Haldane, 1931). Given the above assumptions, the relative frequencies of each of the nine distinguishable gametes may be derived in terms of the recombination frequency, p. Thus, the expected gametic frequencies when there is loose linkage may be found by substituting $p = 0{\cdot}5$ into this gametic series. The χ^2's testing for goodness of fit between the observed gametic numbers and those expected in these circumstances were $\chi^2_{(7)} = 11{\cdot}696$, $P = 0{\cdot}20 - 0{\cdot}10$ for parent 26^4 and $\chi^2_{(7)} = 10{\cdot}632$, $P = 0{\cdot}20 - 0{\cdot}10$ for parent 1^4. With no linkage, assuming random chromosome assortment, the χ^2's testing the goodness of fit of observed to expected data were $\chi^2_{(8)} = 7{\cdot}462$, $P = 0{\cdot}50 - 0{\cdot}30$ for parent 26^4 and $\chi^2_{(8)} = 10{\cdot}790$, $P = 0{\cdot}30 - 0{\cdot}20$ for parent 1^4. Hence, for both parents, the data fit the expectations assuming no linkage slightly better than those assuming very loose linkage.

TABLE 5

*Gametic segregation ratios, (S and Z considered separately), and tests of their agreement with the 1:4:1 ratio expected on the hypothesis of random chromosome assortment (*P = 0·02 − 0·01)*

Gamete	Family $26^4 \times 1^4$	Family $1^4 \times 26^4$	Pooled data		
S_{33}	6	5	11	Deviation	$\chi^2_{(2)} = 1{\cdot}902$
S_{34}	18	11	29	Heterogeneity	$\chi^2_{(2)} = 0{\cdot}348$
S_{44}	4	2	6		
Z_{33}	5	4	9	Deviation	$\chi^2_{(2)} = 0{\cdot}696$
Z_{34}	19	9	28	Heterogeneity	$\chi^2_{(2)} = 1{\cdot}846$
Z_{44}	4	5	9		
S_{55}	2	5	7	Deviation	$\chi^2_{(2)} = 1{\cdot}039$
S_{56}	15	12	27	Heterogeneity	$\chi^2_{(2)} = 4{\cdot}226$
S_{66}	0	4	4		
Z_{55}	2	3	5	Deviation	$\chi^2_{(2)} = 8{\cdot}934$*
Z_{56}	15	18	33	Heterogeneity	$\chi^2_{(2)} = 0{\cdot}045$
Z_{66}	0	0	0		

4. DISCUSSION

Three points emerge from the results obtained so far from this analysis of tetraploid perennial ryegrass. First, in discussing earlier in this paper the expression of incompatibility in tetraploids it was assumed that the alleles at each locus act independently in the stigma. The fact that we have been able to identify four alleles at each locus in plants of genotype S_{3456} Z_{3456} shows that this assumption is correct. Furthermore, the recognition of alleles in single dose when two copies of another allele are present, as in stigmas which are S_{3356}, indicates that there are no dosage effects either.

Second, when S and Z are considered separately, three of the four gametic segregation ratios are close to that expected on the hypothesis of random chromosome assortment and in the case of the fourth (Z_{55} : Z_{56} : Z_{66} in parent 1^4), the departure from expectation is only just significant. Moreover, because the marginally poor agreement in this fourth segregation ratio is due partly to an excess of Z_{56} and partly to a complete absence of Z_{66} gametes, this deviation cannot be ascribed to double reduction which is expected to increase the frequency of homoallelic gametes at the expense of heteroallelic ones. Although it is possible that the excess of Z_{56} gametes is due to preferential pairing between the chromosomes carrying Z_5 on the one hand, and between those carrying Z_6 on the other hand at meiosis in plant 1^4, it is more likely that this excess is due to chance and that the deficiency of Z_{66} gametes is genuine. This deficiency of Z_6 was also observed in the diploid family $23^2 \times 1^2$, which suggests that either this allele or that of another gene closely linked to the Z locus is at a selective disadvantage to Z_5 or, of course, to a gene linked to the latter. Furthermore, since the ratio of Z_{55} : Z_{56} : Z_{66} is homogeneous over the tetraploid families $26^4 \times 1^4$ and $1^4 \times 26^4$, it is clear that this disadvantage is transmitted both on the male and female side of the cross; the effect, therefore, is likely to be zygotic rather than gametic. The chief point that emerges from an analysis of these gametic segregation ratios, however, is that apart from the differential

viability associated with Z_6, the ratios are consistent with the hypothesis of random chromosome assortment.

The last point worth making about these results concerns the fourteen aberrant plants found in the tetraploid families which, it was argued, originated from open-pollination. Since each of these plants was known to be tetraploid from observation of the number of chromosomes in their root tips, it is clear that the stray pollen must have come from one or more unrelated tetraploids. The Welsh Plant Breeding Station records show that tetraploids of *L. multiflorum* were present in the glasshouse at the time that the tetraploid *L. perenne* cross was made. Furthermore, while the *d* allele of the *PGI*-2 gene, which was found in eight of the aberrant plants, is rare in populations of *L. perenne*, its frequency is higher in those of *L. multiflorum*. Lastly, though the extent of pollen contamination that this argument entails appears to be uncomfortably high, it is possible that the contaminants were unconsciously selected for analysis from an original pool of over one hundred plants, because of their vigour and early flowering, attributes which are, of course, particularly associated with *L. multiforum*. Thus though it is not possible to prove that the pollen in question came from tetraploids of *L. multiflorum*, the circumstantial evidence in favour of this supposition is nevertheless strong. While contamination on this scale is regrettable, it is perhaps worth pointing out that the method of analysis we have employed with these tetraploid families is sufficiently sensitive to have recognised these plants as contaminants; had this not been the case, it is likely that we should have been led to the false conclusion that incompatibility in tetraploids is less simple than it is.

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