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Complementary genes control biparental plastid inheritance in *Pelargonium*

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Summary. Zonal pelargoniums exhibit biparental plastid inheritance. After G × W plastid crosses the progeny are a mixture of green, variegated and white embryos corresponding to a maternal, biparental or paternal inheritance of plastids, respectively. There are two patterns of segregation: type-I females have families in which the majority of embryos are green, variegated are of intermediate frequency and white are the least frequent. Type-II females have families in which green and white embryos are present at about the same frequency and variegated are the least common. The results of many selfs and crosses made within and between 8 type-I and 8 type-II plants led us to conclude that the type of female was determined by its genotype with respect to a pair of complementary genes. Plants giving rise to the type-II pattern contained one or two copies of the dominant alleles of both genes, whereas in the absence of either one or both dominant alleles the plants were type I. The genes were called *Pr1/pr1* and *Pr2/pr2*, an adaptation of symbolism used previously. All 8 type IIs were double heterozygotes *Pr1pr1, Pr2pr2*, whereas we found 3 genotypes among the type Is, *Pr1Pr1, pr2pr2*; *pr1pr1, Pr2Pr2* and *pr1pr1, Pr2pr2*. In unrelated experiments we found type IIs of which some were again double heterozygotes and others single heterozygotes *Pr1pr1, Pr2Pr2* or *Pr1Pr1, Pr2pr2*. The model displaces an earlier model based on the proposed operation of a gametophytic lethal or incompatibility system.

Key words: *Pelargonium* – Plastid inheritance – Complementary genes

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Introduction

Evidence for the biparental inheritance of plastids has been found for many species (Kirk and Tilney-Bassett 1978; Hagemann 1979; Sears 1980; Corriveau and Coleman 1988; Smith 1988). In some experiments the results were derived from genetical studies, as in *Medicago sativa* (Smith et al. 1986), *Oenothera* species (Chiu et al. 1988) and *Petunia hybrida* (Cornu and Dulieu 1988); in other experiments the results of crosses were derived from the analysis of chloroplast DNA (cpDNA) by restriction fragment length patterns, as in *Daucus* species (Boblenz et al. 1990), *Medicago sativa* (Lee et al. 1986; Masoud et al. 1990; Schumann and Hancock 1989), *Petunia hybrida* (Derepas 1991) and the gymnosperm *Pinus monticola* (White 1990). In many other plants the possibility of biparental plastid inheritance has been revealed by the identification of plastids by electronmicroscopy and by the staining of plastid DNA within the generative and sperm cells of the pollen (Corriveau and Coleman 1988; Hagemann and Schröder 1989). Frequently such studies have revealed why in some species plastids are not transmitted by the paternal parent as in barley (Mogensen 1988) and wheat (Miyamura et al. 1987).

In *Petunia hybrida* the combination of genetic chloroplast DNA analysis has provided convincing evidence for the regular transmission of about 2% of the plastids from the male parent in the inbred line Tb1–3, whereas previously this plant had been seen as an example of purely maternal plastid inheritance (Cornu and Dulieu 1988). The character, called *Tp* (*Tp* symbol for plastid transfer through the pollen), appeared to be under nuclear control; however, it was expressed only if the male contribution was furnished by the Tb1–3 genotype (Dulieu et al. 1990). Analysis of one backcross generation supported the hypothesis that two major loci, *Tp1* and *Tp2*, deter-

mine the differences between Tb1–3 and the non-transmitter Skr4. Moreover, the testing of chimeral individuals as male parents suggested that genes favouring paternal transfer act at the male gametophytic level (Derepas and Dulieu 1992).

Variability with respect to the presence of plastid DNA in pollen was found in *Pisum sativum* (Corriveau et al. 1989). Alfalfa (*Medicago sativa*), which has a biparental inheritance pattern with a strong paternal predominance, also shows variations in plastid transmission behaviour that is influenced by both maternal and paternal genotypes with relatively insignificant environmental effects (Smith 1989). Smith's group compared three genotypes, one that is a strong transmitter of male plastids (genotype 301), one that is a weaker transmitter of male plastids (7W) and a third that is an even weaker transmitter (MS-5). Their results show that genotype MS-5 has significantly fewer plastids per generative cell than either of the other genotypes (Zhu et al. 1990) and significantly fewer plastid nucleoids per generative cell (Shi et al. 1991), which may account for it being a relatively poor transmitter of male plastids. However, number of plastids and plastid nucleoid frequency do not explain the known differences in male plastid transmission of the genotypes 301 and 7W (Zhu et al. 1991). In all of studies on *Oenothera*, in which many species have been crossed with one another, differences between the plastids themselves was a more important modifier of biparental plastid inheritance than differences in the nuclear genotypes (Chiu et al. 1988).

Pelargonium is another genus for which there is evidence of nuclear genes controlling the pattern of plastid inheritance, but in this case it is the maternal genotype that seems more variable. Crosses between cultivars in which the plastids carry either the normal wild-type allele – green phenotype (G) – or a mutant allele – white phenotype (W) – in their germ cells, produce progeny with a mixture of maternal zygotes (MZ), biparental zygotes (BPZ) and paternal zygotes (PZ) as defined by the presence or absence of green or white plastids in the young embryos into which the zygotes develop. The segregation of this mixture is scored after G × W crosses in which the maternal zygotes are green and the paternal zygotes white, or vice versa after W × G crosses, and the biparental zygotes are variegated. After G × W crosses there are two distinctive segregation patterns depending on the genotype of the female parent. The type-I female confers a segregation pattern among the progeny in which the occurrence of maternal zygotes are frequent; biparental, intermediate; and paternal zygotes, rare (MZ > BPZ > PZ). This contrasts strongly with the type-II female, which confers the segregation pattern of frequent maternal and paternal zygotes, with biparental zygotes being the least frequent class (MZ > BPZ < PZ). The type-I pattern was first recognized in the progeny of cv 'Dolly Varden', which bred true and was therefore assumed to be homozygous, and the type-II pattern was found in the progeny of cv 'Flower of Spring', which did not breed true and was therefore assumed to be heterozygous. The two patterns were considered to be under the control of a major nuclear gene, which carried the symbol *Pr*, with alternative alleles *Pr1* and *Pr2*, on the assumption that the gene controlled plastid segregation through an effect, direct or indirect, on plastid replication (Tilney-Bassett 1973). Further analysis of this behaviour may be separated into studies of the wide variability in gene expression (Tilney-Bassett 1976, 1984; Tilney-Bassett and Almouslem 1989; Tilney-Bassett and Birky 1981) and of attempts to understand the genetic behaviour of the gene itself (Kirk and Tilney-Bassett 1978; Tilney-Bassett 1974, 1975).

The initial limited study of the progeny of selfs and hybrids within and between the type-I and type-II cultivars produced rather unexpected results. When the heterozygotes were selfed, instead of segregating into type-II and type-I progeny in a Mendelian 3:1 ratio (assuming type II dominant to type I) they segregated in a 1:1 ratio. The alternative homozygote, *Pr2Pr2*, was not detected in the sample of progeny tested. Furthermore, when heterozygote and homozygote were crossed, the expected 1:1 ratio, when the *Pr2* allele was derived from the male, was observed, whereas there was often, but not always, a significant deviation from 1:1 when the *Pr2* allele was derived from the female. This led to the simple, but not wholly satisfactory, explanation (Tilney-Bassett and Abdel-Wahab 1982) that the *Pr2* allele was a gametophytic lethal on the female side. Alternatively, or additionally, it was suggested that an incompatibility mechanism was involved in which *Pr1* was a self-compatible allele, *Pr2* a self-incompatible allele and *Pr1-Pr2* cross-compatible alleles. Successful fertilization was then determined by sporophytic control on the male side and gametophytic control on the female side. Since that time new and additional crosses have led to the proposal of complementary gene model for biparental plastid inheritance (Tilney-Bassett et al. 1989).

In the new model we proposed that there are two independently assorting nuclear genes, each with a pair of alternative alleles. These are called *Pr1*/*pr1* and *Pr2*/*pr2*. We proposed further that the genes interact in a complementary manner; in other words the two recessives behave as if isoepistatic and are epistatic to the dominant genes: $(pr1 = pr2) > Pr2,Pr1$. Hence, a green maternal parent containing one or two copies of both dominant alleles produces the type-II plastid segregation pattern after being pollinated by a variegated male carrying a mutant white plastid in its germ line, whereas a maternal parent lacking the two dominant alleles of one or other or both genes produces the type-I plastid segregation pattern. Among type-II plants we ex-

pect four genotypes, *Pr1Pr1,Pr2Pr2*; *Pr1pr1,Pr2Pr2*; *Pr1Pr1,Pr2pr2*; *Pr1pr1,Pr2pr2*, and among type-I plants we expect five genotypes, *pr1pr1,Pr2Pr2*; *Pr1Pr1,pr2pr2*; *pr1pr1,Pr2pr2*; *Pr1pr1,pr2pr2*; *pr1pr1,pr2pr2*. After a 9×9 matrix of reciprocal crosses of type-II and type-I genotypes there are 81 combinations, but if we combine reciprocals the possibilities are reduced to 45, of which 9 are selfs and 36 crosses. Depending upon the precise genotype, selfs or crosses are expected to produce the following progenies.

Both parents type II:
progeny all type II, or
progeny segregate in a ratio of 9 type II: 7 type I, or
progeny segregate in a ratio of 3 type II: 1 type I.

Both parents type I:
progeny all type II, or
progeny all type I, or
progeny segregate in a ratio of 1 type II: 1 type I, or
progeny segregate in a ratio of 1 type II: 3 type I.

One parent type II and one parent type I:
progeny all type II, or
progeny segregate in a ratio of 3 type II: 1 type I, or
progeny segregate in a ratio of 1 type II: 1 type I, or
progeny segregate in a ratio of 3 type II: 5 type I, or
progeny segregate in a ratio of 1 type II: 3 type I.

Although we do not possess all nine genotypes, we shall show that our stocks include at least two type IIs and three type Is and that selfs within or crosses between them have given rise to enough of the expected segregation ratios to make a convincing case in support of the model.

Materials and methods

The parents used in this investigation included commercial cultivars and our own hybrids. Many of these have been described in previous publications or theses. Cultivars that exist as both green forms (G) and as white-over green chimeras (W) are 'Dolly Varden' (DV), 'Flower of Spring' (FS), 'Foster's Seedling' (FOS), 'Hills of Snow' (HS), 'Lass O'Gowrie' (LG), 'Miss Burdett-Coutts' (MBC) and 'Mrs J. C. Mappin' (JCM). The cv 'Verona' (VER) has golden leaves. Two cultivars, believed to be 'Alde' (DOR) and 'Fleurette' (DR), were originally unknown to us at the time and so named 'Darlington Orange Red' and 'Darlington Red' (Almouslem 1988) after Professor C.D. Darlington from whom they were a gift; we have subsequently retained the original abbreviations. The cv 'Eggshell' has the unusual feature of variegated petals – red flecks and sectors on a light salmon background; 'Pac Grosser Garten' (PGG) has rather dark green leaves, purple-red flowers, and is male sterile; 'Preston Park' has salmon flowers, and each leaf has a dark zone on the edge; 'Snowstorm' has pure white flowers and zoneless leaves. One of our hybrid parents MS1H (MBC:W × FS:G) has dark pink flowers and is male sterile, but the others are both male and female fertile. Two of these are CC2 (MBC: G × JCM:G) and CC4 (JCM:G × DV:W), both with white picotee flowers (a white flower with a coloured edge). Other white-flowered parents are H4, H5, H24 and H27, all (W2 × DR hybrids, or H6, a (DR × W2) hybrid, and W1 (JCM:G × JCM:G), W2 (JCM:G × FS:G) and W3 (JCM:G × LG:W). Finally, we used some plants which were the F_2 and F_3 generation of several crosses: (FS:W × MBC:G), (DV:W × FS:G), (DV:G × FS:W) and (LG:G × FS:W).

Each green parental cultivar or hybrid was classified as type I or type II by crossing it with the variegated form of 'Flower of Spring' as a source of mutant plastids (G × W) and then observing the progeny – usually a sample of embryos – to determine the frequencies of green, variegated and white embryos. The parent was then classified according to the pattern of embryos: Type I G > V > W, Type II G > V < W (see Introduction). In some type-I families there were green and variegated embryos but no white ones, and in others there were neither variegated nor white embryos. In type-II families, green and white embryos both occurred more frequently than variegated embryos. For over 95% of the families the classification worked well; for the remainder additional ways of separating type-I and type-II plants were required. Hence, when for a type-II classification the frequency of green embryos was very high, it was decided that at least 6% of the embryos should be variegated plus white and that there should be at least three more white than variegated embryos in order to qualify as type II; when variegated embryos were totally absent at least 6% of the embryos should be white. And, when the frequency of variegated embryos was high, there should be at least three more white embryos than variegated ones. At least 20 embryos were scored for each family (Tilney-Bassett and Almouslem 1989), and usually many more.

In order to determine whether the parents are homozygous or heterozygous type-I or type-II plants, and in order to find out the results of crosses between them, we germinated and grew to maturity a sample of their progeny and tested each of these progeny by crossing them as green females with variegated 'Flower of Spring' as the male parent. The segregation patterns of the embryos were scored just as the original parents were.

To determine whether all progeny are alike or whether segregation occurs does not require large numbers of progeny, but to distinguish with confidence between one segregation ratio and another often does (Mather 1951) and, regretably, our progeny sizes were usually too small. In order to overcome this problem we have put more emphasis on the interpretation of the patterns of results from selfs and from several crosses. By crossing one cultivar or hybrid with several others, we can usually recognize the genotypes involved by the patterns of segregation. According to the complementary gene model under test, the set of Mendelian ratios expected by crossing within type-I plants are not the same as by crossing within type-II plants, and yet another set of ratios is found after crosses between the two types (see Introduction). The occurrence of some patterns is so unique that it immediately defines the parental genotypes; in other cases the possible genotypes are reduced to alternatives, which further crosses may readily separate providing the right genotypes are present. As the range of genotypes was not known at the start, we were more likely to include several different and, therefore, several useful ones by including many cultivars in our programme. In making the chi-square tests of the goodness-of-fit between observed data and expected ratios, we have not tested progenies of less than ten individuals, and we have not applied the Yates correction to allow for the small size of many segregating families.

Results

Altogether, including data from a few publications (Tilney-Bassett 1973, 1974, 1988; Tilney-Bassett and Ab-

del-Wahab 1982), we have examined data from just over 150 selfs and crosses in which over 2700 plants were scored as type I or type II. A few isolated crosses in which the investigation of a parent was too limited to be of value were then excluded. Many of the remaining crosses were reciprocals or differed as to whether green or variegated forms of the parents were used. As there was no evidence of consistent significant differences, we were able to sum the data, reducing the total to 92 selfs and crosses. These were divided into two groups. The larger group of 2098 tested plants, which we shall describe first, is a matrix of 68 selfs and crosses, out of a theoretical maximum of 136, involving 16 parents. The smaller group of 58 tested plants consists of some informative data separate from the main matrix.

Of the 16 parents involved in the matrix of crosses 8 were type IIs and 8 type Is. Selfs and crosses were made within type IIs and type Is, and crosses were made between type IIs and type Is.

From among the 8 type-II parents there were none that bred true. All segregated after selfing, or after crosses between them, into a mixture of type-II and type-I progeny. According to the complementary gene model, the parents are expected to have the genotype *Pr1pr1,Pr2pr2* if double heterozygotes, and *Pr2pr1, Pr2Pr2* or *Pr1Pr1,Pr2pr2* if single heterozygotes. The progeny of double heterozygotes should segregate in the modified dihybrid ratio of 9 type II: 7 type I, and the progeny of single heterozygotes should segregate in the monohybrid ratio of 3 type II: 1 type I. Out of a possible 36 selfs and crosses within the matrix of 8 type-II parents, we tested 21. As we did not obtain much data from 'Hills of Snow' and as this cultivar appeared to be identical to 'Foster's Seedling', we added their data together under 'Foster's Seedling', so reducing the total to 18 tested (Table 1). Four of these have progeny sizes too small (<10) for the ratio to be worth testing, although the data were included in the total segregation. Of the remaining 14 progenies, the chi-square test of goodness-of-fit showed that 5 of these agreed with a 3:1 ratio and 8 agreed with a 9:7 ratio at the 5% probability level. This stayed at 5 agreeing with a 3:1 ratio at the 1% probability level, but improved to 12 agreeing with the 9:7 ratio. The progeny size was large enough (>67) to distinguish between the 3:1 ratio and the 9:7 ratio with 95% certainty in only 5 crosses (Mather 1951). In none of these 5 there was a fit with the 3:1 ratio even at the 0.1% probability level, whereas 1 cross fitted the 9:7 ratio at the 10% level, 2 at the 5% level, 1 at the 1% level and 1 at the 0.1% level (Table 1). Hence, although deviations from the expectations were sometimes rather high, the overall segregation pattern clearly fitted the 9:7 ratio better than the 3:1. Moreover, when all crosses were considered, the only cultivar that gave rise to a segregation in favour of 3:1 more often than 9:7 was 'Foster's Seedling'; however,

Table 1. Segregation into type-II and type-I progeny after selfing or intercrossing type IIs, all of which are interpreted as having the genotype *Pr1pr1, Pr2pr2*

Selfs and crosses	Type II	Type I	Total	χ^2	P
Pr1pr1, Pr2pr2 × *Pr1pr1, Pr2pr2*				(9:7)	
FOS × FOS	23	9	32	3.175	>0.05
FOS × FS	11	0	11	8.555*	>0.001
FOS × JCM	7	9	16	1.016	>0.30
FOS × MS1H	5	3	8		
FOS × PGG	25	8	33	5.103	>0.02
FOS × VER	35	47	82	6.133	>0.01
FS × FS	19	19	38	0.603	>0.30
FS × JCM	34	47	81	6.707*	>0.001
FS × MS1H	40	47	87	3.731	>0.05
FS × PGG	4	3	7		
FS × VER	34	39	73	2.776	>0.05
JCM × JCM	16	22	38	3.089	>0.05
JCM × MS1H	27	23	50	0.103	>0.70
JCM × PGG	3	5	8		
JCM × VER	52	52	104	1.651	>0.10
VER × VER	21	33	54	6.614	>0.01
VER × MS1H	34	12	46	5.832	>0.01
VER × PGG	6	2	8		
Total	396	380	776	8.589	>0.001
Total – * data	351	333	684	0.271	>0.50

overall the 'Foster's Seedling' data fitted the 9:7 ratio well ($\chi^2=0.294$) and the 3:1 ratio not at all ($\chi^2=27{,}260$). When the data from all crosses were pooled the overall fit with the 9:7 ratio showed an excess of type Is, but when the data from the crosses with the two highest deviations were removed the overall fit of the remaining 16 was excellent. We conclude that parents CC2, FOS, FS, HS, JCM, MS1H, PGG and VER are all type-II double heterozygotes *Pr1pr1,Pr2pr2*.

All 8 type-I parents bred true, but on further analysis proved not to be all alike. The key to their underlying differences came when the hybrids W1 and W2 were crossed with CC4, 'Fleurette' (DR) and 'Dolly Varden' (DV); all of the progeny turned out to be type II (Table 2). This was a clear indication of complementation. Each group of type-I plant was evidently homozygous for the dominant allele of one or other of the two complementary genes. When the two dominant genes were brought together they complemented each other and so gave rise to the type-II plants. This is predicted by the complementary gene model and, assuming the interpretation is correct, immediately defines one group of type-I plants, say W1 and W2, as having the genotype *Pr1Pr1,pr2pr2* and the other group, say CC4, DR and DV, as having the complementary genotype *pr1pr1,Pr2Pr2*. Further support for this interpretation occurred with the cross between W1 and W2 with 'Alde' (DOR), as the progeny segregated in good agreement with a 1:1 ratio (Table 2), thereby defining DOR as having the genotype

Table 2. Segregation into type-II and type-I progeny after selfing or intercrossing type Is. The results are classified in accordance with the interpretation that there are three different genotype *Pr1Pr1, pr2pr2* or *pr1pr1, Pr2Pr2* or *pr1pr1, Pr2pr2*

Selfs and crosses	Type II	Type I	Total	χ^2	*P*
Pr1Pr1, pr2pr2 × *Pr1Pr1, pr2pr2*				(All I)	
W1 × W1	0	17	17		
W2 × W2	0	35	35		
W1 × W2	0	49	49		
Total	0	101	101		
pr1pr1, Pr2Pr2 × *pr1pr1, Pr2Pr2*				(All I)	
DR × DR	0	18	18		
DV × DV	0	17	17		
DR × DV	0	8	8	–	
DV × LG	0	9	9	–	
Total	0	52	52		
pr1pr1, Pr2pr2 × *pr1pr1, Pr2pr2*				(All I)	
DOR × DOR	0	20	20		
Pr1Pr1, pr2pr2 × *pr1pr1, Pr2Pr2*				(All II)	
W1 × CC4	60	0	60		
W1 × DV	47	(1)	48		
W2 × DR	52	0	52		
W2 × DV	29	0	29		
Total	188	(1)	189		
Pr1Pr1, pr2pr2 × *pr1pr1, Pr2pr2*				(1:1)	
W1 × DOR	29	28	57	0.017	>0.80
W2 × DOR	7	9	16	0.250	>0.50
Total	36	37	73	0.014	>0.90
pr1pr1, Pr2Pr2 × *pr1pr1, Pr2pr2*				(All I)	
DR × DOR	0	23	23		
DR × MBC	0	1	1		
DV × DOR	0	11	11		
DV × MBC	0	1	1		
LG × MBC	0	9	9		
Total	0	45	45		

Table 3. Segregation into type-II and type-I progeny after crosses between type Is ant type IIs. The results are classified in accordance with the interpretation that there is one type-II genotype *Pr1pr1, Pr2pr2* and that there are three different type I genotypes *Pr1Pr1, pr2pr2*; *pr1pr1, Pr2Pr2* or *pr1pr1, Pr2pr2*

Selfs and crosses	Type II	Type I	Total	χ^2	*P*
Pr1Pr1, pr2pr2 × *Pr1pr1, Pr2pr2*				(1:1)	
W1 × JCM	6	0	6		
W1 × PGG	15	10	25	1.000	>0.30
W2 × CC2	17	13	30	0.533	>0.30
W2 × PGG	18	12	30	1.200	>0.20
Total	56	35	91	4.846	>0.02
pr1pr1, Pr2Pr2 × *Pr1pr1, Pr2pr2*				(1:1)	
DR × FOS	2	8	10	3.600	>0.05
DR × FS	12	15	27	0.333	>0.50
DR × JCM	31	35	66	0.242	>0.50
DR × PGG	6	7	13	0.077	>0.70
DR × VER	1	1	2		
DV × FOS	12	13	25	0.040	>0.80
DV × FS	20	56	76	17.043*	<0.001
DV × JCM	21	28	49	1.000	>0.30
DV × PGG	13	15	28	0.143	>0.70
DV × VER	40	46	86	0.419	>0.50
pr1pr1, Pr2Pr2 × *Pr1pr1, Pr2pr2*				(1:1)	
LG × FOS	2	3	5		
LG × FS	50	38	88	1.636	>0.20
LG × JCM	0	1	1		
LG × PGG	4	10	14	2.571	>0.10
LG × VER	5	5	10	0.000	1.00
Total	219	281	500	7.688	>0.001
Total – * data	199	225	424	1.594	>0.20
pr1pr1, Pr2pr2 × *Pr1pr1, Pr2pr2*				(3:5)	
DOR × FOS	5	6	11	0.297	>0.50
DOR × FS	5	8	13	0.005	>0.90
DOR × PGG	2	10	12	2.222	>0.10
DOR × VER	5	10	15	0.111	>0.20
MBC × FOS	4	7	11	0.533	>0.30
MBC × FS	22	44	66	0.489	>0.30
MBC × JCM	37	31	68	8.298*	>0.001
MBC × VER	23	32	55	0.438	>0.50
Total	103	148	251	1.339	>0.20
Total – * data	66	117	183	0.161	>0.50

pr1pr1,Pr2pr2. Finally, crosses between type Is with the genotypes *pr1pr1,Pr2Pr2* and *pr1pr1,Pr2pr2* gave wholly type I progeny, as expected.

Crosses between representative type IIs of genotype *Pr1pr1,Pr2pr2* and the type Is W1 and W2 of genotype *Pr1Pr1,pr2pr2* segregated in a 1:1 ratio (Table 3). Similarly, crosses between representative type IIs and type Is 'Fleurette' (DR) and 'Dolly Varden (DV) of genotype *pr1pr1,Pr2Pr2* segregated in a 1:1 ratio. At this stage the behaviour of 'Lass O'Gowrie' in crosses suggested that it also had the genotype *pr1pr1,Pr2Pr2*. Out of 15 crosses 3 had progeny sizes too small to test their segregation ratios. Of the 12 remaining, only 1 did not fit the 1:1 segregation ratio tested. This exceptional cross was the sum of two reciprocal crosses. When 'Flower of Spring' was the female parent we obtained 1 type-II and 27 type-I progeny, yet when 'Dolly Varden' was the female parent we obtained 19 type-II and 29 type-I progeny (Tilney-Bassett 1973; Tilney-Bassett and Abdel-Wahab 1982; Almouslem 1988). The expected ratio was 1:1 in both cases. We cannot account for the difference, but do note that 'Flower of Spring' did not behave in such an exceptional

Table 4. Segregation into type-II and type-I progeny after: (a) Selfing dihybrid type IIs; (b) crossing the dihybrid type IIs with type I *pr1pr1, Pr2Pr2*; (c) crossing the dihybrid type IIs with type I *Pr1Pr1, pr2pr2*; (d) selfing dihybrid type IIs of F_2 and F_3 origin; (e) selfing monohybrid type IIs

Selfs and crosses	Type II	Type I	Total	χ^2	*P*
(a) Type IIs *Pr1pr1, Pr2pr2* selfed				(9:7)	
H4 self	14	12	26	0.061	>0.70
H5 self	15	12	27	0.005	>0.90
H6 self	4	3	7	0.002	>0.90
H24 self	11	12	23	0.663	>0.30
H27 self	6	14	20	5.600	>0.01
Total	50	53	103	2.486	>0.10
(b) Type II *Pr1pr1, Pr2pr2* × Type I *pr1pr1, Pr2Pr2*			(1:1)		
H4 × DR	12	11	23	0.043	>0.80
H5 × DR	12	11	23	0.043	>0.80
H6 × DR	5	0	5	5.000	>0.02
H24 × DR	8	12	20	0.800	>0.30
H26 × DR	9	10	19	0.053	>0.80
Total	46	44	90	0.044	>0.80
(c) Type II *Pr1pr1, Pr2pr2* × Type I *Pr1Pr1, pr2pr2*			(1:1)		
H4 × W2	12	10	22	0.182	>0.50
H5 × W2	28	9	37	9.757*	>0.001
H6 × W2	18	6	24	6.000	>0.01
H24 × W2	5	7	12	0.333	>0.50
H27 × W2	13	12	25	0.040	>0.80
Total	76	44	120	8.533	>0.001
Total – * data	48	35	83	2.036	>0.10
(d) Type IIs *Pr1pr1, Pr2pr2* selfed				(9:7)	
(DVW × GFS/28):F_2	13	12	25	0.183	>0.50
(DVW × GFS/37):F_2	5	8	13	1.672	>0.10
(DVW × GFS):F_2	20	22	42	1.271	>0.20
(DVW × GFS):F_3	29	25	54	0.142	>0.70
(DVG × WFS):F_2	1	0	1		
(FSW × GMBC):F_2	20	17	37	0.072	>0.70
(LGG × WFS):F_2	18	14	32	0.000	1.00
Total	106	98	204	1.525	>0.20
(e) Type II *Pr1Pr1, Pr2pr2* or *Pr1pr1, Pr2Pr2* selfed (3:1)					
Eggshell self	11	3	14	0.095	>0.30
(SS × PP/7,8)	50	17	67	0.005	>0.90
Total	61	20	81	0.004	>0.90

manner in other crosses. When these exceptional data were excluded, the pooled data had a good fit with the 1:1 ratio. These results fit the predictions of the model. Finally, crosses between representative type IIs and the third type I 'Alde' (DOR) of genotype *pr1pr1,Pr2pr2* segregated in a ratio of 3 type II:5 type I, again as expected. At this stage the behaviour of 'Miss Burdette-Coutts' in crosses suggested that it also had the genotype *pr1pr1,Pr2pr2*. Out of 8 crosses 7 segregated with a good fit to the ratio of 3 type II:5 type I. When the data from the 8th and poorly fitting cross was removed the good fit of the overall data was improved (Table 3). Hence, these crosses between the two types confirmed the genotypes deduced from selfs and crosses within each type.

The smaller group of plants analysed outside of the matrix of selfs and crosses were all type-II plants for which, in the light of the complementary gene model, some further analysis is possible.

Upon selfing, the hybrids H4, H5, H6, H24 and H27 segregated in overall agreement with the ratio of 9 type II:7 type I, and upon crossing to the complementary type Is, DR and W2, these hybrids segregated in reasonable agreement with the ratio of 1 type II:1 type I (Table 4)., Out of the 15 selfs and crosses 11 had a good fit with the ratios tested at the 5% probability level and 14 at the 1% probability level. So all 5 hybrids would appear to be double heterozygotes with the genotype *Pr1pr1,Pr2pr2*. A second group of plants consisted of the 7 hybrids DV:W × FS:G (F_2 and F_3), DV:W × FS:G/28 and 37 (F_2), DV:G × FS:W (F_2), FS:W × MBC:G (F_2) and LG:G × FS:W (F_2). All segregated in a ratio of 9 type II:7 type I with not a poor fit at the 5% probability level and a good fit overall (Table 4). Hence, these too appear to be double heterozygotes, *Pr1pr1,Pr2pr2*. Finally, type II 'Eggshell' and the 2 hybrids between type IIs 'Preston Park' and 'Snowstorm' (SS × PP/7 and 8) segregated upon selfing into good fits with the monohybrid ratio of 3 type II:1 type I (Table 4), suggesting that their genotypes were either *Pr1Pr1,Pr2pr2* or *Pr1pr1,Pr2Pr2*. Further tests to separate these alternatives are desirable.

Discussion

The overall pattern of our results is consistent with the expectations of the complementary gene model. Although all of the type-II plants used within the matrix of crosses appeared to be identical double heterozygotes, we were more fortunate in finding 3 type Is. So we had four genotypes, enabling us to make ten tests – four selfs and six crosses. For every test the overall results were consistent with the model. In the light of our current findings, the results which led to our former model of a gametophytic lethal or incompatibility system (Tilney-Bassett and Abdel-Wahab 1982) seem increasingly anomalous. We had five results, out of 47 segregating families with 10 or more progeny, in which the segregation frequencies did not fit the expected ratios at the 0.1% level of significance. In only one case, in a small family of 11 progeny (Table 1), were all the progeny of the same kind – type II. Similarly, out of 12 true-breeding families with 10 or more progeny, we had only one case of a single anoma-

lous type I amongst 47 type II (Table 2). Hence, even when the data had a poor fit with the expected ratios, the expectation of segregation, or of no segregation, was met.

A single anomalous plant among otherwise identical progeny is most likely a contaminant – an accidental self. The rarity of this event indicates that our emasculation 1 or 2 days before cross pollination was normally very effective. An occasional mutation of type II to type I is also conceiveable. The quite large deviations from expectation are more difficult to explain. No doubt we owe some deviations to chance. In other cases, deviations may partly arise from misclassification in which one type is too frequently grouped with the other. An important reason why this might happen is because we are using the same criteria for distinguishing type IIs and Is throughout. This method is logical and practical, but it ignores the underlying differences in the background genotypes of the maternal parents. Tilney-Bassett and Almouslem (1989) regressed the percentage maternal plastid transmission of variable numbers of type-II offspring on nine selfed cultivars and found a significant heritable component. At one side of their distribution type-II embryo ratios sometimes overlap type Is, and this tendency is stronger in some genetic backgrounds than others. If in some crosses the real border between type IIs and Is is shifted away from the border used in our classification, then some misclassification is to be expected. Another possible cause of large deviations from the expected is selection. We have already referred to the cross between 'Dolly Varden' and 'Flower of Spring' in which there was a great deficit of type IIs with 'Flower of Spring' as the female parent but not with 'Dolly Varden'. Although the expected outcome of this pair of reciprocal crosses is the same, the crosses are not identical. When 'Flower of Spring' (*Pr1pr1,Pr2pr2*) is the female parent the haploid eggs may be any of four genotypes, *Pr1,Pr2*; *Pr1,pr2*; *pr1,Pr2*; *pr1,pr2*; whereas when 'Dolly Varden' is the female all eggs are of the same genotype, *pr1,Pr2*, with respect to the two independently assorting genes. The difference in the outcome of the reciprocal crosses suggests that there could be selection against eggs of genotype *Pr1,Pr2*; *Pr1,pr2* in competition with eggs of genotype *pr1,Pr2*; *pr1,pr2* when 'Flower of Spring' is the female parent, but there can be no comparable selection when 'Dolly Varden' is the female because now the eggs are all of the same kind. As many eggs are not fertilised, or do not develop, resulting in an overall fertility of about 25% (Kubba and Tilney-Bassett 1981 a, b), there is certainly an opportunity for selection. There remains, however, the puzzle that this has not happened in every cross in which 'Flower of Spring' is the female parent.

According to the complementary gene model, we should be able to identify any type-II genotype by the segregation pattern of the progeny after crossing with the two type Is of genotype *Pr1Pr1,pr2pr2* and *pr1pr1,Pr2Pr2*. Similarly, we should be able to identify any type-I genotype by the segregation pattern of the progeny after crossing with two type IIs of genotype *Pr1Pr1,Pr2pr2* and *Pr1pr1,Pr2Pr2*. Hence, we should be able to find more of the nine genotypes in the future. We also hope to be able to submit a report on the results of a thorough examination of a further set of crosses designed to provide an additional test of the model (Amoatey 1991). When we have isolated additional genotypes, we should be able to determine whether or not the differences between them within each type affect the outcome of crosses. If they do this might indicate a gene dosage effect, if not the observed effects of differences between cultivars would be indicative of polygenic effects, as previously suggested (Tilney-Bassett and Almouslem 1989).

The demonstration of complementary gene action, which is a common phenomenon in genetical studies, emphasises that both the fate of the plastids within the zygote and early embryo development are under cellular control; it is not just due to chance. It tells us little, however, about what happens to the plastids, so at this time we have nothing to add to our previous discussions (Tilney-Bassett and Almouslem 1989; Tilney-Bassett 1991). Nevertheless, the interesting results obtained with other plants encourage us to believe that our understanding of the behaviour of plastids in inheritance is going to continue to improve.

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