THE CONTROL OF PLASTID INHERITANCE IN *PELARGONIUM*. III

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SUMMARY

Selfing two heterozygous green plants derived from crosses between the *Pelargonium* cultivars Dolly Varden and Flower of Spring produced solely green F_2 progeny in a ratio of 1 homozygote, Pr_1Pr_1 : 1 heterozygote, Pr_1Pr_2 instead of the expected mendelian 3 : 1 or 1 : 2 : 1 ratio. The fact that these F_2 are all green rules out the possibility that the ratio could be biased through loss of white or variegated embryos as was argued in previous experiments; the fact that the mean fertilisation and embryo survival is the same for families of homozygotes and heterozygotes contradicts zygotic selection; and the fact that embryo death is insufficient to swing the ratio from 3 : 1 to 1 : 1 all point to gametophytic selection against the Pr_2 allele on the female side. This implies that the conspicuous differences between the segregation patterns from heterozygous females after $G \times W$ plastid crosses occurs despite the fact that the female eggs are of uniform genotype Pr_1 . Hence the differences between the segregation patterns must be determined by the female parent before meiosis when still heterozygous and diploid.

1. INTRODUCTION

RECENT analysis of extranuclear plastid inheritance in *Pelargonium* has shown that two alternative patterns of segregation, following $G \times W$ plastid crosses, are under the control of the female nuclear genotype. The two segregation patterns have been called type I ($G > V > W$), in which most offspring are green, rather less variegated and a few are white, or occasionally the variegated are more frequent than green, and type II ($G > V < W$), in which green and white offspring are approximately equally frequent and variegated few (Tilney-Bassett, 1973). It is suggested that the two patterns are controlled by alleles of a nuclear gene, named Pr_1 and Pr_2 , with an effect on plastid replication at the time of fertilisation. Females whose offspring segregate as a type I pattern are homozygous Pr_1Pr_1 and females whose offspring segregate as a type II pattern are heterozygous Pr_1Pr_2 . Selfing the heterozygote cv. "Flower of Spring", and backcrossing it with the homozygote cv. "Dolly Varden", gives unexpected deviations from monohybrid and backcross ratios. There are two hypotheses to explain these observations:

(1) The deviations can be explained by assuming that the Pr_2 allele exhibits gametophytic lethality on the female side so that Pr_2 haploid cells after female meiosis do not usually develop into functional eggs that become fertilised. This idea comes from the observation of much greater deviations from the expected 1 : 1 ratios, owing to the deficit of type II plants, when the female is heterozygous in $Pr_1Pr_2 \times Pr_1Pr_1$ crosses than when the male is heterozygous (Tilney-Bassett, 1973). Further support is given by the

analysis of $G \times W$ and $W \times G$ crosses in D. Varden and F. of Spring, which showed that there is no difference in mean fertilisation, and no difference in survival during embryo development up to maturity and germination (Tilney-Bassett, 1970a); hence heterozygous embryos must survive just as well as homozygous ones. New data is presented in this paper for a large number of type I and type II segregations.

(2) An alternative interpretation is that the death after germination of all the white and some variegated seedlings is biasing the mendelian ratios which are based solely on scoring surviving variegated plants. Hence apparent gametophytic lethality of the Pr_2 allele on the female side is due in reality to selection against Pr_1Pr_2 heterozygous and, following $Pr_1Pr_2 \times Pr_1Pr_2$ crosses, Pr_2Pr_2 homozygous offspring. This is not difficult to imagine as about half the offspring from a heterozygous green female parent are variegated or white. It is only necessary to postulate a mechanism by which half the heterozygous and all the homozygous Pr_2Pr_2 offspring are closely associated with dying seedlings. My proposal for a mechanism to account for this association is to suggest that the Pr_1 allele favours replication of green plastids, and when all the eggs have this genotype a type I pattern results, whereas the Pr_2 allele favours the replication of white plastids, and when about half the eggs have the Pr_1 genotype and half the Pr_2 genotype a type II pattern results. In this manner most heterozygous offspring with the Pr_2 allele derived from a Pr_2 egg would be white as would homozygous Pr_2Pr_2 progeny, and these then die.

One way of testing, whether or not the Pr_2 allele is lethal on the female side, is to inbreed pure green heterozygotes and analyse the F_2 generation of pure green offspring so that there are no mutant white plastids to interact in any way whatsoever with the nuclear genotype. Moreover, in this way, an unbiased sample from the whole of the F_2 offspring is tested, instead of one biased by the problem of untested white embryos.

2. MATERIALS AND METHODS

The two parental cultivars used in these experiments were the green (G) and white-over-green (W) clones of Flower of Spring (FS) and Dolly Varden (DV) described in an earlier report, where the methods of pollination and of scoring embryos were also explained (Tilney-Bassett, 1970a). The crossing programme for the inbreds and hybrids from which fertility data are presented, and the methods of estimating the percentage green plastid contribution, were described in a more recent report (Tilney-Bassett, 1973); further fertility data are taken from crosses described in the present paper.

The crossing programme commenced with the choice of two F_1 hybrids derived from crosses between D. Varden and F. of Spring. These began as variegated seedlings but developed green shoots. The two plants, numbered DV $W \times G$ FS/28 and DV $W \times G$ FS/37, have been shown to produce a type II segregation in $G \times W$ crosses with white-over-green F. of Spring (Tilney-Bassett, 1973; table 5). On the basis of this segregation behaviour they were assumed to be heterozygotes; the present results confirmed this assumption. They were chosen from among many other possible F_1 hybrids because of their vigour and fertility. The two green F_1 plants were each selfed and a number of seeds were set, germinated and grown

up into green F_2 plants; as expected, there were no variegated or white F_2 seedlings. On flowering, about one year after germination, the green F_2 plants were used as female parents in $G \times W$ crosses with the white-over-green F. of Spring as the source of mutant plastids. The resulting embryos were scored, 3 weeks after pollination, as green, variegated or white in order to determine the nature of the segregation pattern produced from each green F_2 plant, and hence the genotype of the green F_2 plants. In the tables each separate F_2 plant is identified by one or two letters; the

TABLE 1

Comparison of the mean fertilisation of total and mean survival of mature embryos for families with type I or type II segregation derived from selfs or crosses within and between cultivars Dolly Varden and Flower of Spring

Selfs and crosses	Segregation type	Number of families	Number of flowers	Total embryos	Mean fertilisation	Mature embryos	Mean survival
<i>Inbreds:</i>							
(DV $W \times G$ DV) $G \times W$ DV	I	7	415	1385	3.337	1163	2.802
	II	None					
(DV $G \times W$ DV) $G \times W$ DV	I	10	717	2151	3.000	1932	2.695
	II	None					
(FS $W \times G$ FS) $G \times W$ FS	I	14	479	1351	2.820	1100	2.296
	II	11	309	884	2.861	723	2.340
<i>Hybrids:</i>							
(FS $G \times W$ DV) $G \times W$ FS	I	12	720	2869	3.985	2533	3.518
	II	1	31	116	3.742	111	3.581
(FS $W \times G$ DV) $G \times W$ DV	I	12	702	2383	3.395	2052	2.923
	II	None					
(DV $G \times W$ FS) $G \times W$ DV	I	9	680	2046	3.009	1915	2.816
	II	3	279	942	3.376	819	2.935
(DV $W \times G$ FS) $G \times W$ FS	I	20	1108	4152	3.747	3610	3.258
	II	15	626	2185	3.490	1888	3.016
<i>Selfed Hybrids:</i>							
(DV $W \times G$ FS/28) G self							
→ $G F_2 \times W$ FS	I	13	201	4616	3.065	559	2.781
	II	14	235	833	3.545	735	3.128
(DV $W \times G$ FS/37) G self							
→ $G F_2 \times W$ FS	I	9	154	471	3.058	411	2.669
	II	5	92	327	3.554	300	3.261
<i>Grand Totals:</i>							
	I	106	5176	17424	3.366	15275	2.951
	II	49	1572	5287	3.363	4576	2.911

absence of certain letters from the alphabet is caused by plants that failed to flower after 1 year, or produced too few flowers to obtain an adequate score.

3. FERTILITY OF HOMOZYGOUS AND HETEROZYGOUS PLANTS

In $G \times W$ plastid crosses the green clone of D. Varden produces a type I pattern, and that of F. of Spring a type II pattern, yet analysis of fertility data showed no difference between the two cultivars (Tilney-Bassett, 1970a, b). It is therefore interesting to compare the fertility of other plants producing a type I or type II pattern. Data from inbreds, hybrids and selfed hybrids are given in table 1 for total embryos fertilised, and for

surviving embryos when fully expanded 3 weeks after pollination. Individual families derived from the same cross are grouped together. In six cases it is possible to compare the fertilisation and survival means of type I and type II families derived from the same cross, in four of these the type II group shows the slightly higher fertilisation and in two the type I group. After adding the three crosses of the type I group, for which there are no type II families, means for the grand total of all type I and type II fertilities can be determined. As the table shows, these means are extremely similar and are clearly in agreement with the finding for F. of Spring and D. Varden. Hence there is no evidence of any differences in mean fertilisation or embryo survival of progeny derived from homozygous and heterozygous mothers. The 10 to 15 per cent of dead embryos include many green and variegated as well as white ones from heterozygous parents, while from homozygous parents they are mostly green in agreement with the majority of green offspring.

4. EMBRYO SEGREGATION

Solely green F_2 seedlings were obtained by selfing two heterozygous green plants derived from crosses between D. Varden and F. of Spring. The green F_2 plants were brought to flower and then crossed as females with pollen from the variegated clone of F. of Spring. The type I segregation for the progeny of some plants indicates that these F_2 parents are homozygous Pr_1Pr_1 , while the type II segregation for the progeny of other plants indicates that these F_2 parents are heterozygous Pr_1Pr_2 . The results are shown in tables 2 and 3. There are 12 type I and 13 type II F_2 plants derived from selfing DV W $\times$ G FS/28, and eight type I and five type II F_2 plants derived from selfing DV W $\times$ G FS/37; their combined segregation is 20 type I and 18 type II in close agreement with a 1 : 1 ratio. The type I segregations are remarkable for their very low frequencies of variegated and complete absences of white embryos. The type II segregations are more variable with quite large differences between the lowest and highest frequencies of white embryos, nevertheless these appear to be normally distributed and there is no segregation with a particularly high white phenotype, such as might be expected from a parent homozygous Pr_2Pr_2 .

Both F_1 plants were heterozygous for red flower colour and the F_2 s segregated into red and rose flowered plants. Both red type I and II, and rose type I and II F_2 plants were observed.

5. DISCUSSION

The fertility analysis shows that heterozygous plants are just as fertile as homozygous ones, and that embryos of segregating families are no less viable than embryos of true breeding families. It is therefore improbable that there is a strong selection against heterozygous plants between fertilisation and germination, or indeed during their growth to flowering. In other words, there is no reason to think that the ratio of homozygous to heterozygous seedlings grown up into green F_2 plants is any different from the ratio of homozygous to heterozygous zygotes immediately after fertilisation. Zygotic selection is therefore not the mechanism to account for the observed 1 homozygous : 1 heterozygous F_2 ratio, following the inbreeding of heterozygous plants, when a monohybrid 1 : 2 : 1 or 3 : 1 ratio is to be expected.

TABLE 2

Tests of green F₂ plants derived from selfing the green F₁ between white D. Varden and green F. of Spring (DV W × G FS/28)

(F₂ green plants female by white F. of Spring male)

Sibs arranged in order of percentage green plastid contribution		Percentage Embryos				Percentage green plastids	Segregation pattern type
		G	V	W	Total		
F ₂ : A	G × W FS	100.0	—	—	46	100.0	I
F ₂ : I	G × W FS	100.0	—	—	70	100.0	I
F ₂ : L	G × W FS	100.0	—	—	24	100.0	I
F ₂ : Q	G × W FS	100.0	—	—	39	100.0	I
F ₂ : Z	G × W FS	100.0	—	—	45	100.0	I
F ₂ : AB	G × W FS	100.0	—	—	91	100.0	I
F ₂ : AC	G × W FS	100.0	—	—	38	100.0	I
F ₂ : AE	G × W FS	100.0	—	—	31	100.0	I
F ₂ : AA	G × W FS	98.2	1.8	—	56	99.8	I
F ₂ : S	G × W FS	97.2	2.8	—	72	99.8	I
F ₂ : Y	G × W FS	96.4	3.6	—	28	99.7	I
F ₂ : K	G × W FS	96.8	3.2	—	63	99.1	I
F ₂ : R	G × W FS	71.4	—	28.6	35	71.4	II
F ₂ : C	G × W FS	69.6	—	30.4	23	69.6	II
F ₂ : T	G × W FS	58.9	9.6	31.5	73	65.9	II
F ₂ : U	G × W FS	60.4	1.0	38.6	96	61.1	II
F ₂ : X	G × W FS	57.6	3.0	39.4	66	60.2	II
F ₂ : P	G × W FS	55.3	2.7	42.0	112	56.9	II
F ₂ : G	G × W FS	53.8	2.1	44.1	93	55.7	II
F ₂ : V	G × W FS	54.8	2.4	42.8	42	55.2	II
F ₂ : J	G × W FS	53.5	1.7	44.8	58	54.3	II
F ₂ : M	G × W FS	51.3	2.6	46.1	76	53.2	II
F ₂ : B	G × W FS	52.0	—	48.0	25	52.0	II
F ₂ : O	G × W FS	51.9	—	48.1	81	51.9	II
F ₂ : AD	G × W FS	44.8	3.5	51.7	29	48.3	II

TABLE 3

Tests of green F₂ plants derived from selfing the green F₁ between white D. Varden and green F. of Spring (DV W × G FS/37)

(F₂ green plants female by white F. of Spring male)

Sibs arranged in order of percentage green plastid contribution		Percentage embryos				Percentage green plastids	Segregation pattern type
		G	V	W	Total		
F ₂ : A	G × W FS	100.0	—	—	38	100.0	I
F ₂ : H	G × W FS	100.0	—	—	84	100.0	I
F ₂ : I	G × W FS	100.0	—	—	34	100.0	I
F ₂ : J	G × W FS	100.0	—	—	55	100.0	I
F ₂ : L	G × W FS	100.0	—	—	95	100.0	I
F ₂ : E	G × W FS	98.3	1.7	—	58	99.9	I
F ₂ : C	G × W FS	96.5	3.5	—	58	99.6	I
F ₂ : N	G × W FS	96.7	3.3	—	30	99.2	I
F ₂ : D	G × W FS	62.5	13.6	23.9	88	70.1	II
F ₂ : G	G × W FS	68.6	—	31.4	35	68.6	II
F ₂ : O	G × W FS	65.6	—	34.4	32	65.6	II
F ₂ : F	G × W FS	58.5	4.3	37.2	94	61.0	II
F ₂ : M	G × W FS	54.8	2.7	42.5	73	56.3	II

Moreover, in order for zygotic elimination of all homozygous (Pr_2Pr_2) and half the heterozygous (Pr_1Pr_2) embryos to change the ratio from 3 : 1 to 1 : 1, there would have to be an elimination of 50 per cent of the embryos, whereas the observed death between fertilisation and germination in type II plants is usually not more than 30 per cent and often considerably less. The present ratio of 1 type I : 1 type II F_2 plants confirms that obtained by selfing F. of Spring (Tilney-Bassett, 1973) yet, on this occasion, the F_1 selfing programme has kept out all mutant plastids so that one cannot explain away the unexpected 1 : 1 ratio on the basis of the loss of heterozygous embryos through association with the death of white embryos. The weight of evidence therefore points overwhelmingly to gametophytic lethality of the Pr_2 allele on the female side, which would give a ratio of 1 Pr_1Pr_1 : 1 Pr_1Pr_2 corresponding to the observed segregation pattern. It is presumed that lethality is less important on the male side as found previously (see Introduction).

Agreement with the first hypothesis outlined in the introduction does not entirely rule out the second hypothesis as we still need to account for the differences between type I and type II patterns. The alternative patterns are now seen to be all the more remarkable because in a type I segregation all the eggs are identical Pr_1 because the parent is homozygous Pr_1Pr_1 , and in a type II segregation all the eggs are identical Pr_1 because, although the parent is heterozygous Pr_1Pr_2 , the Pr_2 gametophytes do not, or very rarely, produce any Pr_2 eggs. It has already been shown very clearly that the main control of the segregation pattern is dependent on the nuclear genotype of the female parent. I first suggested that this was because the female produced the eggs and from the heterozygous female half the eggs would be Pr_1 , and would encourage the replication of green plastids, and half would be Pr_2 and would encourage the replication of white plastids (Tilney-Bassett, 1973). But if, as now seems to be the case, the Pr_2 gametophytes seldom produce viable eggs, then the very conspicuous differences between a type I ($G > V > W$) and a type II ($G > V < W$) segregation pattern must occur despite the fact that the female eggs are of uniform genotype Pr_1 . This means, at least in the two families studied, that the differences between the Pr_1 eggs that decide whether it is the white or the green plastids that replicate in the zygote are pre-determined by the female before meiosis when still heterozygous and diploid. How this control operates is still unknown but comparison with another system is not irrelevant.

Sager and Ramanis (1973) have recently proposed a model for the control of maternal inheritance in *Chlamydomonas*. In their model, the female (mt +) parent contributes three essential components of the system: (1) a modification enzyme responsible for the observed density shift of female chloroplast DNA, (2) a regulator substance which activates the modification enzyme after zygote formation, and (3) a second regulator substance which activates a restriction enzyme produced in the male (mt -) parent. The restriction enzyme responsible for degrading male chloroplast DNA is postulated to be synthesised in the male gamete, but activated by a regulator from the female parent some time after mating. In the absence of any biochemical investigations, it is difficult to discuss a model for *Pelargonium* in such detail, but it is very doubtful if the two systems could be exactly the same. In *Chlamydomonas* the two isogametes fuse at fertilisation, and each contains a chloroplast, so that the zygote always begins with one chloroplast from

each parent, yet the male chloroplast DNA is normally inactivated with high efficiency (Sager, 1972). In *Pelargonium*, plastid transmission by the male is successful in $G \times W$ as well as $W \times G$ crosses so that there is probably no mechanism aimed at preventing male transmission. On the other hand, after most crosses with homozygous D. Varden, green plastids are more effectively transmitted than white, whichever way the cross is made (Tilney-Bassett, 1970b). Hence in *Pelargonium* we should probably be looking for a mechanism that seeks to recognise and remove abnormal plastids and plastid DNA irrespective of the parental source. Observation of a considerable number of type I segregating families derived from the crossing programme shows that the success with which mutant white plastids are removed varies from poor, with up to 40 per cent white plastid contribution, to highly efficient, with no white plastid contribution. A similar picture emerged from a study of 15 other green cultivars tested for their segregation pattern (Tilney-Bassett, 1974). This study also demonstrated the commonness of the homozygote and the rarity of the heterozygote, suggesting that the Pr_1 is the wild type and the semi-lethal Pr_2 the mutant allele.

The high proportion of white embryos in the offspring of heterozygous plants could be interpreted as a breakdown in the recognition system and is perhaps indicative of the inability of the Pr_2 allele to produce an essential component of this system. It is, however, to be expected that the heterozygote would produce the missing component from the Pr_1 allele, so how do the Pr_1 eggs come to differ? One possibility is that the informational molecules produced by the heterozygote to control plastid replication in the zygote are not produced in sufficient quantity from only one functional allele so that there is a segregation or dilution during the formation of the eggs. An alternative possibility would be for the heterozygote to behave as a functional mosaic so that some lineages become effectively Pr_1 in phenotype, and others effectively Pr_2 , and the Pr_1 eggs would receive the information dependent upon which allele was functional in the heterozygote prior to meiosis. The latter alternative more readily accounts for the remarkable switch that can allow the formation of either green or white embryos in approximately equal frequency with very few variegated. It might be tested by looking for significant heterogeneity of segregation within flowers, providing the switch in control occurred sufficiently early.

Another facet of the problem is the timing of the loss of the Pr_2 gametophytes. Hints as to what might be the mechanism for this loss can be gained from an examination of flower structure and fertilisation data. Examination of serial sections of whole ovaries 5 days after pollination revealed that on average more than half the ovules per flower were shrivelled. These were so distorted in shape, modified in cell structure and reduced in size that no embryo sac could be seen and I assumed they could not have been fertilised (Tilney-Bassett, 1965). After more detailed observations with cv. "Purple Heart", Tsai, Harney and Peterson (1973) showed that these ovules started with an apparently normal embryo sac at the time of pollination, which then rapidly collapsed through not being fertilised. Hence in Purple Heart, and perhaps F. of Spring and D. Varden, it is a failure of fertilisation that gives rise to the shrivelled ovules rather than failure to form a normal embryo sac. This being so, there are ten potentially functional ovules per flower of which only three to four are fertilised on average. This means that if all Pr_2 ovules are rejected there still remain

sufficient Pr_1 ovules that could be fertilised to maintain the observed mean fertilisation in type II families, providing there is enough flexibility in their position. In the cultivar "Paul Crampel", which behaves as a type I plant, only 3.5 per cent of the developing ovules were found in the lower position of the two superposed ovules per carpel, whereas in F. of Spring the value was 20.6 per cent (Tilney-Bassett, 1963). This suggests that there is a preference for upper ovules, but that this might be at least partly overcome by a preference for Pr_1 ovules instead of Pr_2 , indeed some observed frequencies of F. of Spring flowers with four or five developing embryos are much too high for them to be both all in the upper position and all Pr_1 ; either some of them must be Pr_2 or some must be developing in the lower position. Further analysis of the shifts in frequency from upper to lower positions after type I and type II segregations should reveal whether this is indeed the mechanism for sifting out the Pr_1 eggs from a mixture of Pr_1 and Pr_2 .

The present studies suggest that the Pr_2 allele has two major effects. The first appears to be initiated in the heterozygous female parent before meiosis, although the response is not observed until after fertilisation when the plastids replicate at the start of embryo development. The second effect appears to bring about the lethality of the female gametophyte sometime after meiosis and before fertilisation. Experiments to verify and pinpoint still more accurately the timing of these effects are now in progress.

6. REFERENCES

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