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The control of plastid inheritance in *Pelargonium*

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SUMMARY

Twenty-four reciprocal crosses between two green and six chimera cultivars, containing respectively normal green and mutant white plastids in their germ layers, were analysed for their segregation patterns of green, variegated and white embryos, and for their fertility data.

Analysis of variance of fertility data showed that W × G crosses were generally less fertile than the reciprocal G × W crosses, but this was not correlated with selection against any particular class of embryo.

Analysis of segregation patterns showed that they were essentially the same with a constant female and varying male parent, but change the female and the pattern could be radically altered. The six different mutant females with both constant green males could be arranged in a sequence in which white plastids were increasingly successful, and white and variegated embryos increasingly frequent. A similar sequence was demonstrated with three green females and two constant white males. It was concluded that the major control of plastid inheritance was determined by the female nuclear and plastid genotypes, with the male having only a minor, modifying influence even when male plastids were more successfully transmitted than female ones.

The very low frequencies of variegated embryos in some crosses led to a rejection of the classical hypothesis of sorting-out from mixed cells, and its replacement by the hypothesis that pure green or pure white embryos arise by the replication of only one plastid at a time, whereas variegated embryos arise by replication of both plastids.

1. INTRODUCTION

The classical experiment of Baur (1909) on reciprocal crosses between *Pelargonium* cultivars containing normal and mutant plastids in their germ layers, led him to put forward his hypothesis on the genetic continuity of plastids, with a non-mendelian, biparental pattern of inheritance, and sorting-out from mixed cells during embryo development. This was a notable achievement and laid a solid foundation for subsequent plastid transmission studies in higher plants. Later workers confirmed his results (see Tilney-Bassett, 1963; Hagemann, 1964) and, at the same time, revealed a surprising variability and discrepancy between observed and anticipated results. Reciprocal crosses between cultivars containing normal green and mutant white plastids in their germ cells gave rise to a mixture of green, variegated and white seedlings. Reciprocal crosses did not, however, give rise to reciprocal results, instead green seedlings tended to predominate just as much

when the male donated the green plastids as when these were donated by the female parent. White seedlings were usually absent or rare, but just occasionally became quite frequent. Variegated seedlings were sometimes more frequent, sometimes less frequent than green seedlings and sometimes, like the white, were absent altogether. All these varying patterns of segregation have been confirmed by my own results (1963, 1964, 1965) without, so far, revealing the underlying causes.

Initially progress was greatly handicapped by the labour of producing a useful number of seedlings from a plant that normally produces only about two seedlings from one pollination. The method of scoring developing embryos (Kirk & Tilney-Bassett, 1967) has reduced this problem, and has the added advantage, over scoring germinated seedlings that one can examine dead or dying as well as living embryos. Use of this new method of scoring has enabled me to test two hypotheses that might account for the variability between crosses.

The selection hypothesis (1970*a*) assumed that white embryos and to a less extent variegated embryos were strongly selected against during development so that few of these reached maturity, and germination was mostly of green ones. The combination, in all G × W and W × G crosses within and between the cultivars Flower of Spring and Dolly Varden, of no difference in mean fertilization, no difference in embryo survival between fertilization and 3 weeks after, and no change in the proportion of green, variegated and white embryos demonstrated conclusively that there was no selection against white embryos in the earliest days of development. The selection hypothesis must therefore be rejected when analysing the results of young embryos; in two crosses there was selection against white embryos between maturation and germination, but as the white embryos formed the smallest class from the outset the effect of this late selection on their segregation ratios was fairly mild.

The second hypothesis (1970*b*) assumed that comparisons between crosses were unreliable because the segregation patterns were extremly susceptible to changes in the environment. Again the hypothesis has proved to have no foundation. For a number of crosses it has not been possible to demonstrate clear statistically significant differences in the variations of their segregation patterns either between years or between the environmental treatments tested.

Until these two hypotheses could be disproved they have acted as barriers to the now very real suggestion that significant differences between crosses are due to their underlying genetic make-up. The results can no longer be explained away, instead we must look for a wholly genetic mechanism to account for the variations obtained. It is the purpose of this paper to begin to investigate how such a control mechanism might operate.

2. MATERIALS AND METHODS

As source of mutant white plastids (W), the chimera cultivars used in the breeding experiments are cl. 'Dolly Varden' (DV), cl. 'Flower of Spring' (FS), cl. 'Foster's Seedling' (FoS), cl. 'Mrs J. C. Mappin' (JCM), cl. 'Miss Burdett-Coutts'

(MBC), and cl. 'Lass o' Gowrie' (LG), all supplied by Telston Nurseries, Kent. As source of green plastids (G), I have isolated two green clones derived from the above cultivars Flower of Spring and Dolly Varden. The green clones are isogenic with these two cultivars, differing only in the plastids of their germ layers. Their description is given in an earlier report (1963), except that the abbreviations for Flower of Spring and Foster's Seedling have since been reversed.

In order to convert varying numbers of embryos into effective plastid contributions from the two parents, it is necessary to take account of the fact that variegated embryos contain plastids from both parents usually in disproportionate amounts. This is done by classifying variegated embryos into ten percentage groups according to their relative proportions of green and white tissues. Then, assuming that wholly green or wholly white embryos effectively received only one kind of plastid, an average of the relative numbers of green and white plastids received by the offspring can be determined. Some white embryos (W^g) actually have green tips, or at least a few cells containing green chloroplasts, at the suspensor end of the embryo; these are scored as white along with the pure white (W^w) because white is how they would appear had they been scored at germination. Moreover, the reverse condition, a green embryo with an apparently white tip, is extremely rare.

For crosses within and between the cultivars F. of Spring and D. Varden some results have already been published (1970*a*, *b*); in the present paper only the more recent results are shown in which the variegated embryos were visually classified into the ten percentage groups.

3. EMBRYO SEGREGATION

The six cultivars containing mutant plastids in their germ layers were crossed reciprocally with the two green clones from F. of Spring and D. Varden. The segregation frequencies for good and bad embryos are shown in Table 1. The results show that the proportion of bad embryos is less amongst the variegated than for either green or white. This is undoubtedly indicative of a failure to score the dead or dying embryos as accurately as the larger healthy ones. Amongst bad embryos there is no mistaking a green for a white, but one can mistake a 5% variegated for a green, or a 95% variegated for a white. This interpretation is supported by the observation that a disproportionately few bad variegated embryos were scored at the extremes of the variegation range compared with the centre, even though the total frequency of all variegated embryos is disproportionately high at the extremes.

The difficulty of scoring variegateds makes any analysis less certain, nevertheless it is clear the overall failure of white embryos is more severe than failure of green. Is this significant? A closer look at the crosses shows that in six, selection is actually against green, and in eight, against white by no greater amount; in six crosses one class, green or white, represents less than 4% of all embryos and with such small numbers apparent strong selection against one class may be simply due to chance,

and certainly has little effect on the segregation pattern; finally in six crosses the few bad embryos of all classes form less than 5 % of the good embryos so that again apparent stronger selection against one class involves very few embryos and is of little significance. On the basis of these arguments a convincing selection against white embryos is shown by only four crosses, two are G × W and two W × G. The lack of any apparent relationship between them makes it seem doubtful if even here there is any real genetic basis for the selection against white. An alternative possibility is that they may have been subjected to a sudden rise in temperature up to 40 °C or above for a short period, an occasional greenhouse hazard to which white embryos seem more susceptible than green embryos.

Table 1. *The segregation frequencies of good and bad embryos for twelve G × W crosses and their corresponding reciprocal W × G crosses*

Crosses (♀ × ♂)	Good embryos				Bad embryos			
	G	V	W	Total	G	V	W	Total
	(*a*) Green female × white male crosses							
FS × FoS	136	5	107	248	20	0	24	44
FS × FS	156	12	136	304	21	1	34	56
FS × JCM	211	9	174	394	13	1	33	47
FS × DV	100	47	83	230	4	0	1	5
FS × LG	139	37	136	312	4	0	5	9
FS × MBC	157	60	131	348	6	0	5	11
DV × FoS	193	45	16	254	17	0	0	17
DV × FS	195	57	23	275	12	2	6	20
DV × JCM	333	60	4	397	31	2	4	37
DV × DV	148	85	24	257	27	3	4	34
DV × LG	149	78	13	240	11	1	2	14
DV × MBC	206	70	15	291	20	2	2	24
	(*b*) White female × green male crosses							
FoS × FS	104	38	3	145	66	3	6	75
FS × FS	180	71	10	261	26	3	5	34
JCM × FS	128	74	23	225	22	3	5	30
DV × FS	104	63	18	185	47	0	5	52
LG × FS	74	187	58	319	1	1	3	5
MBC × FS	12	180	52	244	1	2	19	22
FoS × DV	113	15	0	128	43	3	0	46
FS × DV	203	48	3	254	14	1	7	22
JCM × DV	150	66	5	221	9	0	2	11
DV × DV	85	71	8	164	39	8	3	50
LG × DV	68	180	20	268	2	1	3	6
MBC × DV	1	251	44	296	0	5	18	23

The good embryo segregation data is expressed in Table 2; firstly as percentages of green, variegated and white embryos, and secondly as the average effective plastid contribution. Table 2(*a*) shows the result of crossing six white cultivars as male parent with a constant green female parent, on the left F. of Spring, on the right D. Varden. The crosses with F. of Spring show a remarkable consistency with respect both to the ratio of green to white embryos and the ratio of green to white plastids; approximately 1:1. As male parent, the mutant types can scarcely be

distinguished from each other with the constant green female parent, except that between some cultivars there are differences in the proportion of variegated embryos. When, instead of F. of Spring, D. Varden is used as the constant green female parent, we note the same similarity of the segregation pattern with all six mutant males, although the pattern itself is quite different. The balance of green to white embryos and plastids is now tilted very much in favour of green for all six of the crosses, and the proportion of variegated embryos, which was always the

Table 2. *Percentages of good embryos from the distributions in Table 1, and the corresponding estimate for the average percentage contribution of green plastids*

Source of white plastids	Green plastids: Flower of Spring				Green plastids: Dolly Varden			
	Embryos (%)			Green plastids (%)	Embryos (%)			Green plastids (%)
	G	V	W		G	V	W	
	(*a*) Green female × white male crosses							
FoS	54·8	2·0	43·2	56·3	76·0	17·7	6·3	89·1
FS	51·3	4·0	44·7	54·6	70·9	20·7	8·4	83·8
JCM	53·5	2·3	44·2	55·0	83·9	15·1	1·0	93·6
DV	43·5	20·4	36·1	54·5	57·6	33·1	9·3	77·2
LG	44·5	11·9	43·6	50·1	62·1	32·5	5·4	80·3
MBC	45·1	17·3	37·6	55·0	70·8	24·1	5·1	87·6
	(*b*) White female × green male crosses							
FoS	71·7	26·2	2·1	89·1	88·3	11·7	—	98·3
FS	69·0	27·2	3·8	85·2	79·9	18·9	1·2	95·5
JCM	56·9	32·9	10·2	77·3	67·9	29·9	2·2	88·1
DV	56·2	34·1	9·7	71·6	51·8	43·3	4·9	78·3
LG	23·2	58·6	18·2	51·3	25·4	67·2	7·4	62·6
MBC	4·9	73·8	21·3	34·6	0·3	84·8	14·9	42·0

least frequent class with F. of Spring, is always intermediate in frequency with D. Varden as the green female parent. These results show us that with a constant green female parent a change in the white male modifies the segregation pattern only slightly or not at all, but change the constant female and the whole pattern of segregation can be completely altered. Evidently, in these crosses, the green females exert a far greater control over the pattern of segregation than the white males. But is it being green, or being female that matters? We may begin to answer this question by studying the reciprocal crosses.

In Table 2 (*b*) we seet hat when F. of Spring is the constant green male andwe have six different white females, the results are not all alike, instead we are able to arrange the crosses in a sequence showing a steadily decreasing percentage of green embryos coupled with a corresponding increase in variegated and a slightly less well marked increase in white embryos. Consistent with this order is a decrease in the effective contribution of green plastids and an increase in white. When D. Varden is the constant green male parent, the six white females give results that allow them to be placed in exactly the same order as with F. of Spring, with the corresponding decrease in green, increase in variegated and increase in the percentage white

embryos. Evidently the relationship of the white females to each other is not upset by changing the green male parent, for the same white female gives a similar segregation pattern with the two green males, but change the white female and the segregation pattern can be altered drastically.

In answer to the above question, it is clear from the data of Table 2 (*a*) and (*b*) that changing the female parent, whether green or white, can radically alter the segregation pattern, whereas changing the male parent, whether green or white, has far less effect. This generalization must, however, be treated with caution because the white males were only tested against two green females, and conversely only two green males were tested against the white females. Other cultivars may yet be found in which the male influence is much greater than in the present series of crosses. That a green male can at least have some influence is clear from a comparison of the effect of F. of Spring and D. Varden on the six white females. For each of the six pairs of crosses compared, the effective contribution of green plastids is always higher for D. Varden than for F. of Spring, and of course white plastids correspondingly lower. The range of percentage variegation covered by D. Varden is greater than for F. of Spring. Thus at one extreme, Foster's Seedling, gives fewer variegated with D. Varden than with F. of Spring, but at the other end of the sequence Miss Burdett-Coutts gives more variegated with D. Varden than with F. of Spring. In other words, throughout the sequence of the six white females the increments in variegation frequency are in the same order, whether the male is F. of Spring or D. Varden, but they are larger with D. Varden. Hence, although the difference between D. Varden and F. of Spring as green males is not great it is consistent; they do not alter the basic pattern determined by the female, but they clearly modify it.

The major control exerted by the female on the segregation pattern compared with that of the male parent does not mean that the plastid itself has no effect. It may mean that all green plastids behave alike, and even that all white plastids behave alike, but not that green and white plastids behave alike. I have already shown (1970*a*) that in the two cultivars D. Varden and F. of Spring, in which we have both green and chimera clones of the same genotype, the green plastids are always more successful than the white irrespective of the direction of the isogenic cross. The cultivar Miss Burdett-Coutts is the first example, in my experience, in which the white plastids are clearly more successful than the green, but even this success is only partial since white plastids only gain the upper hand when they are donated by the female. Alsc the white plastids from Miss Burdett-Coutts have not been tested against its own green plastids with an isogenic background, but against the green plastids of D. Varden and F. of Spring.

4. FERTILIZATION AND EMBRYO SURVIVAL

The number of visible embryos plus the occasional empty seed were counted for each flower of the twenty-four crosses; empty seeds outwardly appear of normal size but on dissection no embryo is found within. Each embryo scored was classi-

fied as good, if growing healthily, or bad, if dead or dying. From these values, the mean total fertilization and the mean frequency of good, surviving embryos per flower and their standard errors are given in Table 3. Since all means are based on a maximum of ten ovules per flower they may also be expressed as percentages of total ovules simply by moving the decimal place one digit to the right. The means from Table 3 are converted to angles and the transformed data analysed as a 6×4 factorial (Table 4). Normality is assumed as each mean is the average of a large number of observations. There is no correlation between the means and their standard deviation.

Table 3. *Mean total fertilization, and mean embryo survival at approximately 3 weeks post fertilization, for the twenty-four crosses*

Crosses (♀ × ♂)	Nos. flowers pollinated	Mean fertilization	S.E.	Mean survival	S.E.
		(*a*) Green female × white male crosses			
FS × FoS	93	3·301	0·102	2·667	0·102
FS × FS	100	3·720	0·112	3·040	0·136
FS × JCM	122	3·730	0·103	3·230	0·090
FS × DV	73	3·247	0·147	3·151	0·146
FS × LG	87	3·747	0·105	3·586	0·108
FS × MBC	103	3·631	0·113	3·379	0·120
DV × FoS	98	2·776	0·136	2·592	0·137
DV × FS	80	3·762	0·143	3·437	0·140
DV × JCM	101	4·327	0·068	3·931	0·081
DV × DV	96	3·094	0·132	2·677	0·129
DV × LG	65	3·969	0·157	3·692	0·162
DV × MBC	85	3·800	0·149	3·424	0·137
		(*b*) White female × green male crosses			
FoS × FS	98	2·337	0·097	1·480	0·103
FS × FS	91	3·352	0·130	2·868	0·139
JCM × FS	108	2·444	0·100	2·083	0·090
DV × FS	89	2·921	0·112	2·079	0·137
LG × FS	94	3·468	0·095	3·394	0·105
MBC × FS	90	3·000	0·115	2·711	0·110
FoS × DV	66	2·742	0·145	1·939	0·164
FS × DV	93	3·022	0·143	2·731	0·136
JCM × DV	97	2·412	0·114	2·278	0·113
DV × DV	66	3·318	0·150	2·485	0·191
LG × DV	86	3·244	0·120	3·116	0·126
MBC × DV	102	3·147	0·105	2·902	0·108

As the twenty-four crosses have not been replicated it is not possible to determine an error sum of squares in the usual way from the variance between replicates. Instead the theoretical variance associated with the angular transformation analysis is used. This is determined by the formula (Fisher & Yates, 1963)

$$\frac{820{\cdot}7}{n},$$

where for unequal samples, n is the harmonic mean of the sample size. The theoretical error has an infinite number of degrees of freedom. Each sum of squares

to be tested is divided by the theoretical error to give a χ^2 value based on the number of degrees of freedom associated with the sum of squares in the numerator. The resulting χ^2 are referred to the statistical table for judging significance.

The analysis of variance for the mean fertilization of total embryos is summarized in Table 4. The analysis shows that the W × G crosses are significantly less fertile than the reciprocal G × W crosses. There is no significant difference in fertility when F. of Spring is the source of green plastids as compared with D. Varden, nor is there any significant interaction.

Table 4. *The analysis of variance for the angular transformed data of the mean fertilization for total embryos*

	Analysis			
Source of variance	s.s.	d.f.	χ^2	P
Green female × white female	61·937	1	6·70	0·01–0·001
Green F. of Spring × Green D. Varden	0·499	1	0·05	0·9–0·8
(FS♀ + DV♂) × (DV♀ + FS♂)	0·009	1	0·001	0·98–0·95
Residual interaction	167·567	20	18·13	0·7–0·5
Total	230·012	23	—	—
Theoretical error	9·242	—	—	—

Table 5. *Comparison between transformed means for total fertilization of green × white and white × green crosses by Scheffé's method*

	Group means			
Cultivar chimera	High (G × W)	Low (W × G)	Difference	Significance (P*)
FoS	66·87	60·48	6·39	0·05–0·01
FS	74·68	67·51	7·17	0·05–0·01
JCM	78·77	59·04	19·73	< 0·001
DV	68·53	67·89	0·64	Not
LG	76·79	70·80	5·99	0·05–0·01
MBC	75·12	67·33	7·79	0·01–0·001

* Values $\sqrt{(FS^2)}$ at five probability levels: 20%, 3·893; 10%, 5·004; 5%, 5·957; 1%, 7·834; 0·1%, 10·004.

The difference between G × W and W × G crosses was not previously observed in the analysis of crosses within and between F. of Spring and D. Varden (Tilney-Bassett, 1970*a*). I have therefore analysed the contrasts between means in more detail (Table 5) by the method of Scheffé (1959). The analysis shows that the difference in total fertilization between G × W and W × G crosses is significant or highly significant for five of the cultivars but not for D. Varden. I had previously argued (1970*a*) that since there was no significant difference in total fertilization between the G × W and W × G crosses, one could not attribute the observed differences in frequency of white embryos to their loss during the earliest stages of embryo development. At first sight, the present findings might seem to weaken this conclusion, in fact they support it. The six chimera cultivars can be arranged

in sequence with a steadily increasing contribution of white plastids (Table 2). If this order was dependent upon the degree to which white embryos survived at the earliest stages of embryo development, then there should be a corresponding steady increase in the fertility values obtained (Table 3). As there is no such correlation the observed fertility differences cannot be correlated with selection against white embryos at a stage when they are too small to be visible in the stereomicroscope. Perhaps the lower total fertilization of W × G crosses is related to the chimera structure of the maternal parents in these crosses, and hence to a slightly lower nutritional fitness compared with pure green plants. The better fertility of D. Varden may be due to the narrow white margins of its leaves compared with the much wider white margins of the other five cultivars.

When the frequencies of good and bad embryos for the twenty-four crosses were similarly analysed no significant differences were found either between the G × W and W × G crosses, or between the crosses with D. Varden and F. of Spring as the green plastid parent. Instead most of the variance resulted from interactions, reflecting wide differences both within and between the varying hybrid crosses. Again there is no general correlation between high frequencies of bad embryos and the loss of large numbers of white embryos. Thus analysis of the fertility data does not provide any reason for wishing to alter the observed constitution or sequence of the segregation patterns.

5. DISCUSSION

The analysis of the present fertility data for the twenty-four crosses tested confirms my earlier conclusion (1970*a*) that differences in the proportions of green, variegated and white embryos are not the result of strong selection against any one class of embryo during development. Although not specifically tested, there was no reason to suspect that factors of the external environment had any more significant influence in the present crosses than previously (1970*b*). It is therefore clear that any explanation of the crossing results cannot be based on selection or environmental factors.

Any interpretation of the varying proportions of green, variegated and white embryos with different crosses must consider not only possible control by the nuclear genotype—female, male and hybrid, and by the plastid genotype—normal and mutant, but must also relate the behaviour of the plastids with the sequence of cell division during embryogenesis. After fertilization, the probable course of cell division in *Pelargonium* is that the zygote divides into a two-celled embryo consisting of a basal and terminal cell; the basal cell develops into the suspensor. The next three divisions of the terminal cell are one transverse and two vertical, to give an octet of eight cells. The basal quadrant nearest the suspensor eventually develops into the root and hypocotyl and the upper quadrant into the cotyledons and shoot. Evidently the green and white plastids of a mixed celled zygote must sort-out at the very first division to produce a terminal cell with only one type of plastid, green or white. Sorting-out from a mixed terminal cell by a vertical division would give rise to a sectorial chimera splitting the embryo longitudinally, and these

are sometimes seen, whereas sorting-out by a transverse division would, for example, split the embryo into a white cotyledonary half and a mixed celled radicle half; one sometimes sees embryos with green or white cotyledons and a variegated, striped radicle or, if sorting-out has followed another cell lineage, the radicle may also be white with only the tip green. Since pure green or white embryos must apparently arise by sorting-out of one type of plastid from a mixed celled zygote at the first zygotic division, how is it possible for there to be such a high proportion of non-variegated embryos following so many crosses? And also, how is it possible for the same mutant plastid to be so successful in one cross and so unsuccessful in another? Let us consider some models.

(i) Upon fertilization, male and female plastids mix and then sort-out from one another during embryogenesis. Pure embryos result from rapid sorting-out, while variegated embryos are the residual in which sorting-out has not been so quick. This is essentially the classical explanation first put forward by Baur. But if, as I have argued above, pure embryos arise by sorting-out at the first zygotic division, one would not expect over 95 % of the embryos in some crosses to be pure green or white even if the zygote consisted of only one green and only one white plastid, and it would indeed be surprising if there was no more than one plastid from each parent.

(ii) Varying segregation patterns are dependent upon different numbers of plastids contributed by each female and each male parent. This would only be workable for crosses in which a high proportion of embryos are variegated since, according to the theoretical frequency distributions (Michaelis, 1955*a*, *b*), immediate random segregation of plastids to give pure embryos is not to be expected at a high frequency whether the ratio of green:white plastids is 1:1, 8:2 or 18:2. Moreover, there seems no logical reason why different green varieties should each have their own specific number of plastids within an egg; it is difficult to see how such a situation could arise and of what benefit it could be.

(iii) The position of the male and female plastids in the zygote in relation to the plane of cell division determines the segregation patterns. This model is also unpalatable. When green and white embryos are equally frequent, are we to assume that the plastids are consistently alternating in their positions? Or when the green plastids are strongly favoured in both reciprocal crosses, is it likely that they manage to occupy the same advantageous position when they are donated by the male as when they are donated by the female, as would have to be assumed in the isogenic D. Varden crosses?

(iv) Of the two types of plastid only one is replicated, except in a proportion of zygotes which give rise to the variegated embryos. This new hypothesis is free of the objections of the first three models. It is also conceptually the simplest of the models, and is based on the assumption that within a given zygote each kind of plastid has a specific probability of replication first, and the first to replicate, if allowed to go to completion, effectively inhibits the propagation of the other, thus giving rise to a pure-celled embryo. Opposing this tendency is the particular probability of the replication of both plastids overlapping or coinciding, in which

case variegated embryos arise. In other words, the varying patterns of segregation could be determined simply by the readiness of the two kinds of plastid in the zygote to divide synchronously or not. The control of this synchrony would presumably operate through the varying interactions between different nuclei and different plastids in which, as we have already concluded from the crossing results, the female nucleus and the colour of the plastids play particularly important roles.

Of course this new model is by no means proven, but it has the great advantage over the classical model that it is no longer necessary to assume extremely rapid rates of sorting-out to account for low frequencies of variegated offspring. Nor is it necessary to have to attempt to explain these rapid rates in terms of low plastid numbers, non-random sorting-out or differences in the multiplication rates of the two types of plastid, hardly likely in isogenic crosses. When we regard the sequence of six chimera cultivars with a steadily increasing proportion of variegated offspring, this can now be visualized as a gradation in the extent to which synchronous plastid division occurs in the zygote, whereas on the classical model, we should have to assume a gradation in the rapidity of sorting-out along with all the other supporting assumptions which do not seem to stand close examination. Again on the classical model, we might expect the peak frequency among variegated embryos to be about 50 % variegated in those crosses with approximately the same proportions of green and white plastids. Instead, in the crosses with the green female F. of Spring, the peaks are towards predominantly green or predominantly white. On the new model this is to be expected. Most embryos are pure white or pure green, hence the variegated embryo represents the exceptional condition when one or two green plastids manage to get into the terminal cell along with many white or vice versa. It is fully to be expected that mixing of plastids, which normally does not take place at all, should be very incomplete when it does occur. It also follows that any analysis of sorting-out during the development of variegated embryos is complicated by the likelihood that the terminal cells will vary in the starting ratios of green : white plastids that they contain. Those with predominantly green plastids will give largely green, variegated embryos and those with predominantly white plastids in the starting ratios will give rise to largely white, variegated embryos. Finally, the few embryos with approximately equal numbers of green and white plastids will account for the occasional variegated seedlings in which sorting-out may still not be complete in the growing point many months after germination.

The six pairs of W × G crosses (Table 2*b*), with two constant green male parents, could be arranged into a sequence with respect to the patterns of green, variegated and white embryos. Evidently the interaction between the nucleus and the plastids must differ for each cross. Similarly, with the same white male, the cross with a green female F. of Spring gives quite a different result to the cross with a green female D. Varden (Table 2*a*). These results can also be compared with seedling data for two sets of crosses using the nuclear-differential chimera cl. 'Crystal Palace Gem' (CPG) (Kirk & Tilney-Bassett, 1967).

FS G × W DV: 43·5 % G, 20·4 % V, 36·1 % W embryos and 54·5 % G:45·5 % W plastids
DV G × W DV: 57·6 % G, 33·1 % V, 9·3 % W embryos and 77·2 % G:22·8 % W plastids
CPG G × W DV: 94·3 % G, 5·7 % V, — seedlings and 98·6 % G: 1·4 % W plastids

and

FS G × W JCM: 53·5 % G, 2·3 % V, 44·2 % W embryos and 55·0 % G:45·0 % W plastids
DV G × W JCM: 83·9 % G, 15·1 % V, 1·0 % W embryos and 93·6 % G: 6·4 % W plastids
CPG G × W JCM: 97·7 % G, 2·3 % V, — seedlings and 99·4 % G: 0·6 % W plastids

In both sets of crosses we are able to arrange the three green varieties in the same order of an increasing contribution of green plastids or decreasing contribution of white plastids. The differences between the results are highly significant, and there can be no doubt that the three green females are each behaving quite differently from one another with a constant white male. Does this mean that there are perhaps as many as three different mutant plastids, or might it be one mutant plastid with three different nuclei, or a combination of different plastids and nuclei? And we may ask the same questions of the W × G crosses. It would be no surprise if the mutant plastids were not all alike, but if the green plastids are all different, then the cultivated zonal Pelargoniums must be polymorphic for their chloroplasts. This idea cannot be dismissed outright because it is well known that the modern garden cultivars have arisen by hybridization between two or more wild species, and this could be the source of entry of genetically different plastids. Provisional analysis of backcrosses between F_1 hybrids and their parents suggests, however, that it is the nucleus rather than the plastids that differ. We have therefore to recognize the paradoxical situation in which the male plastids can be the more successful, and give rise to a predominantly paternal inheritance, even though it is the female parent that allows this to happen. Even before fertilization has taken place the plastid inheritance is predetermined to be largely paternal, or maternal, or about equally dependent upon the nuclear and plastid genotypes of the female.

REFERENCES

BAUR, E. (1909). Das Wesen und die Erblichkeitsverhältnisse der 'Varietates albomarginatae hort' von *Pelargonium zonale*. *Zeitschrift für Vererbungslehre* **1**, 330–351.

FISHER, R. A. & YATES, F. (1963). *Statistical Tables for Biological, Agricultural and Medical Research*, 6th. ed. Edinburgh and London: Oliver and Boyd.

HAGEMANN, R. (1964). *Plasmatische Vererbung. Genetik Gründlagen, Ergebnisse und Probleme in Einzeldarstellungen*. Jena: Veb Gustav Fischer Verlag.

KIRK, J. T. O. & TILNEY-BASSETT, R. A. E. (1967). *The Plastids: Their Chemistry, Structure, Growth and Inheritance*. London and San Francisco: W. H. Freeman and Company.

MICHAELIS, P. (1955*a*). Modellversuche zur Plastiden- und Plasmavererbung. *Züchter* **25**, 209–221.

MICHAELIS, P. (1955*b*). Über Gesetzmässigkeiten der Plasmon-Umkombination und über eine Methode zur Trennung einer Plastiden-, Chrondriosomen-, resp. Sphaerosomen-, (Mikrosomen)- und einer Zytoplasmavererbung. *Cytologia* **20**, 315–338.

SCHEFFÉ, H. (1959). *The Analysis of Variance*. New York and London: John Wiley and Sons Inc.

TILNEY-BASSETT, R. A. E. (1963). Genetics and plastid physiology in *Pelargonium*. *Heredity* **18**, 485–504.

TILNEY-BASSETT, R. A. E. (1964). Failure to transmit mutant plastids in a *Pelargonium* cross. *Heredity* **19**, 516–518.

TILNEY-BASSETT, R. A. E. (1965). Genetics and plastid physiology in *Pelargonium*: II. *Heredity* **20**, 451–466.

TILNEY-BASSETT, R. A. E. (1970*a*). Genetics and plastid physiology in *Pelargonium*. III. Effect of cultivar and plastids on fertilisation and embryo survival. *Heredity* **25**, 89–103.

TILNEY-BASSETT, R. A. E. (1970*b*). Effect of environment on plastid segregation in young embryos of *Pelargonium* × *Hortorum* Bailey. *Annals of Botany* **34**, in Press.