GENETICS AND PLASTID PHYSIOLOGY IN PELARGONIUM

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1. HISTORY OF BREEDING EXPERIMENTS

THE two important steps in the analysis of breeding variegated Pelargoniums were made by Baur and Imai. Baur found that on selfing, white-margined varieties give white offspring, green varieties give green offspring and reciprocal crosses between the two give various proportions of green, variegated and white offspring. The F_1 seedlings from reciprocal crosses are neither of one uniform type, nor of two or more types segregating in a Mendelian ratio; instead their proportions vary with the direction of the cross. Hence Baur (1909) concluded that the inheritance of the plastid character was not Mendelian and this prompted him to put forward (p. 349-350), as an alternative explanation, his theory of the sorting-out of plastids, as follows:

“ The fertilised egg, which originated through the union of a green with a white sex cell, contains two kinds of plastid, green and white. At cell division, by which the growing egg cell develops into the embryo, the plastids are distributed quite randomly into the daughter cells. Should a daughter cell have only white plastids, then this will have only white cell descendants, and a white area will develop; should a daughter cell contain only green plastids, then a constant green cell complex will be formed. Cells with both kinds of plastid will be able to segregate further, etc., indeed, I do not imply that segregation need occur at a single division. If segregation should have already occurred after one of the first divisions, such that the cells, which develop further into the cotyledons and apical growing-points, have only green plastids, then a pure green hybrid will develop; in the reverse case a pure white hybrid is developed, etc. It is now a simple matter, which I do not need to explain at length, to establish, that after a larger number of divisions the cells with both types of plastid must decrease in percentage in direct contrast to the cells with only one kind of plastid.”

Noack (1924, 1925), whose observations are useful, repudiated Baur's ideas on *a priori* grounds but Roth (1927) and Imai (1936) supported him. Their results confirmed those of Baur and at the same time revealed significant variations in the proportions of green, variegated and white seedlings from crosses between different varieties; three crosses by Imai gave only green seedlings whichever way the cross was made. I have included in table 1 only their reciprocal crosses and have grouped together varieties only when their results have been

similar. In addition, where the authors themselves have done so, I have separated their crosses according to whether the "white" parent is white (Ww), cream (Wc) or yellow (Wy) and the "green" parent green (G) or golden (g). In my own experiments I have not drawn this distinction between the "white" parents because I find such a classification to be misleading. In the absence of structural and biochemical analyses of the plastids and their pigments there is, at the moment, no evidence to correlate variations in the colour of

TABLE 1
Results of reciprocal crosses between "green" or "golden" and "white" pelargoniums

Author	White parent	Green or golden parent	Nos. F ₁ offspring							
			White female				White male			
			G	V	W	T	G	V	W	T
Baur 1909	3 vars. GWG	G	47	19	...	66	177	25	4	206
Noack 1924 1925	2 vars. GWwG	G	457	376	27	860	1238	15	1	1254
	" "	g	19	76	20	115	325	32	1	358
	1 var. GWyG	G	28	41	...	69	270	102	...	372
	" "	g	15	48	...	63	117	115	1	233
Roth 1927	1 var. GWG	G	45	19	3	67	148	...	5	153
	" "	g	19	14	2	35	47	...	...	47
Imai 1936	2 vars. GWwG	G	23	16	...	39	65	2	...	67
	3 vars. GWwG	G	75	...	...	75	193	...	...	193
	1 var. GWcG	G	15	18	1	34	262	72	...	334
	3 vars. GWyG	G	21	10	1	32	100	40	...	140

the leaf margins in *Pelargonium* with differences in the plastids. Similarly, there is no evidence that all the varieties of any one colour contain equivalent plastid types. At present, therefore, I think it sufficient to identify the plastids by their varietal names and to use the term white in a general sense.

The second important step was Imai's attempt (1936) to obtain fertility as well as breeding data, and to relate the two (table 2). He saw that the lethality of white embryos must be taken into account in explaining the proportions of offspring. In particular, on the assumption that fewer plastids came from the male than from the female, he concluded that this lethality must be more pronounced when the white parent was female rather than male; an explanation which admirably accounted for the lower fertility of this cross (W × G). The explanation of Baur's (1909) and Chittenden's (1926) results by Darlington (1949) is essentially the same.

The interest in these experiments has been maintained by Darlington who has pointed out that the predominance of the green plastids over the white is greater when they enter with the pollen; an observation which has led him to make the following suggestion (1958, p. 178):

“Thus the male germ cell makes a greater contribution of these cytoplasmic particles (plastids). And the female cell sometimes fails to contribute anything at all.”

Previously it has always been considered that in cases of biparental inheritance of plastids, but obviously to a less extent than with purely maternal inheritance, the egg cell predominated because it carried more plastids. But if, as Darlington has indicated, the female germ cell is not the predominant parent in some *Pelargonium* crosses, is this

TABLE 2

Aggregate percentages of offspring, and germination percentages, from selfing and reciprocal crosses of green and variegated pelargoniums (after Imai (1936))

Self or cross	Germination per cent.	Aggregate percentages of offspring		
		G	V	W
G self	51.5	100.0	0	0
{ G × W	49.3	84.1	15.9	0
{ W × G	21.3	71.0	27.5	1.5
W self	5.0?	0	0	100.0

because it really does carry fewer plastids than the male cell? Or is there a deeper, more obscure explanation connected, as already suggested by Imai and Darlington, with the lethality of white embryos? The published information is inadequate to answer these questions for the following reasons:

(1) The “green” parents in the crosses have usually been unnamed; presumably owing to the false assumption that they are of less importance than the “white” parents. It is therefore difficult to compare the results of different crosses, even when they are by the same author.

(2) The numbers of offspring from many crosses, especially those of Chittenden (1926), have been too small, and the many isolated, non-reciprocal crosses are of little value.

(3) Most workers have given no selfing and no fertility data and Imai, the exception, makes the mistake of giving only the aggregate fertility data even though the results of his crosses are heterogeneous.

(4) Imai’s fertility data do not separate bad seed from bad germination of good seed.

In order to overcome these difficulties I have begun my own series of experiments which I begin to describe in this paper.

2. MATERIALS AND METHODS

The horticultural varieties of *Pelargonium zonale*, which I have used in my experiments, are listed in table 3 and a selection of them illustrated in plate I. All were obtained from the collection in the Oxford Botanic Garden.

TABLE 3
List of pelargonium varieties used in breeding experiments

Description of varieties or bud variations	Abbreviation	Genetic combination* layer			
		L	I	II	III
<i>Pure green</i>					
Flower of Spring, green bud variation	FoS:G		G	G	G
Paul Crampel	PC		G	G	G
Mrs Pollock, green bud variation	MP:G		G	G	G
<i>Green-over-white chimeras</i>					
Freak of Nature, G-o-W bud variation	FN:R		G	G	W
J. C. Mapping, reversal bud variation	JCM:R		G	G	W
<i>Green-over-pale green chimera</i>					
A Happy Thought	HT		G	G	p
<i>Pure pale green</i>					
A Happy Thought, pale green bud variation	HT:p		p	p	p
<i>Golden-over-green chimeras</i>					
Crystal Palace Gem	CPG		g	g	G
Golden Harem	GH		g	g	G
<i>Pure golden</i>					
Golden Crampel	GC		g	g	g
Robert Fish	RF		g	g	g
<i>White-over-green chimeras</i>					
Lass o' Gowrie	LG		G	W	G
J. C. Mapping	JCM		G	W	G
Flower of Spring	FoS		G	W	G
Dolly Varden	DV		G	W	G
Lady Cullum	LC		G	W	G
Miss Burdett-Coutts	MBC		G	W	G
Foster's Seedling	FS		G	W	G
Mrs Pollock	MP		G	W	G
Kathleen Harrop	KH		G	W	G

* Pelargoniums have a 3-layered growing-point ; the classification is my own (Tilney-Bassett, 1963).

(i) Cultivation

Each plant has been grown in J.I. No. 1 Potting Compost in 5 inch pots, watered regularly, and kept in a greenhouse subject to day to day variations in light, temperature and humidity ; a minimum temperature of 14° C. has been maintained.

(ii) Flower structure and pollination control

Each flower has a single style with a 5-lobed stigma that unfolds, and becomes receptive, 2-3 days after the flower opens. At the base of the style, the ovary consists of 5 carpels each of which contains 2 superposed ovules to give a total of 10 ovules per flower ; occasionally there are only 4 carpels.

There are 6 stamens, 4 with long and 2 with short filaments. The anthers dehisce within a few hours of the flower opening, but the sticky pollen is not shed until visited by an insect pollinator.

The 2-3 day period between the maturation of the stamens and the carpels provides the opportunity for the pollen to be dispersed before the stigma is receptive; it thereby encourages cross-pollination. In a few varieties the stigma unfolds at the level of the anthers and is frequently self-pollinated by direct contact with sticky pollen remaining in the anthers. In most varieties the stigma unfolds above the anthers and natural self-pollination is less likely to occur. In either case, in order to achieve the highest seed set in fertility studies, it is necessary to pollinate the flowers by hand, and cross-pollinations are reliable only when the stamens have been removed before the stigma is receptive. I have therefore made all pollinations under controlled conditions.

(iii) Germination

Seeds were judged to be full if they were firm and the wall did not collapse when pressed gently between the fingers; all empty shrivelled seeds were discarded. A period of six weeks was allowed for the germination of the seeds which were sown, as soon as they were ripe, in J.I. Seed Compost, placed in a frame at 25° C. and kept well watered. After 2 weeks, when the first flush of germination was over, the non-germinated seeds were re-sown. I have found that this procedure, which was repeated after a further 2 weeks, greatly increases the seed germination. After 6 weeks further germination is negligible.

The total fertility is calculated from the product of the seed set and the germination percentage.

(iv) Pollen tube development

Flowers were removed 12 hours after pollination and boiled in 4 per cent. sodium sulphite solution for 10 minutes. The central strands were dissected out from the styles, stained with acid-fuchsin and light green, mounted on a slide under a cover slip and placed in an oven at 55° C. for 6 hours (Darlington and La Cour, 1963). The darkly stained pollen tubes were observed with the microscope. Pollen tubes were scored only if they had grown out of the stigma and were visible in the style.

(v) Sorting-out

All variegated seedlings that survived beyond the cotyledon stage were pricked out separately, grown in the heated greenhouse, and their development followed. Seedlings that died without producing any leaves were excluded from the sorting-out observations.

Each successive leaf from every individual seedling was recorded, by its appearance, as green, white, variegated or periclinal. The variegated leaves included all grades from predominantly green to predominantly white and also leaves with sectorial and mericlinal patterns. Leaves with periclinal chimera patterns were separated as white-over-green (W/G) or green-over-white (G/W). Scoring of an individual plant was stopped as soon as the first leaf had been produced, after which all later leaves were of the same type; *i.e.* when sorting out to a stable green, white or periclinal growing-point was complete. The leaves were numbered to include the cotyledons as the first two leaves.

(vi) Abbreviations for seedlings, leaves or germ layers

(i) General: nuclear or plastid type

G = Green

V = Variegated

W = White

GV = Green-variegated

gV = Golden-variegated

(ii) Nuclear types

p = Pale green

g = Golden

(iii) Periclinal chimeras

G/W = Green-over-white (G-o-W)

W/G = White-over-green (W-o-G)

3. BREEDING AND FERTILITY

(i) *Selfing*

Plant breeders have frequently and mistakenly ignored Mendel's example and used for crossing experiments, plants whose breeding and fertility behaviour on selfing they had not tested. I have therefore begun my experiments by selfing twenty varieties, or bud variations

TABLE 4
Results of selfing the pelargonium varieties listed in table 3

Vars. and L II	Total nos. and percentages						Total ferti- lity per cent.	Offspring		
	Infls.	Ovules Pollnd.	Seeds					G	p or g	W
			Full	per cent. full	Germd.	per cent. germd.				
<i>Green</i>										
FoS:G	7	850	203	23.9	172	84.7	20.2	172	...	...
PC	2	490	116	23.6	97	83.6	19.8	97	...	...
FN:R	6	580	107	18.5	85	79.4	14.7	85	...	...
HT	4	1100	158	14.4	131	82.9	12.0	92	39 p	...
JCM:R	3	140	12	8.6	4	33.3	2.9	4	...	...
MP:G	3	1020	9	0.9	3	33.3	0.3	3	...	...
<i>Golden</i>										
CPG	3	400	66	16.5	57	86.4	14.3	21	36 g	...
GC	5	840	85	10.2	74	87.1	8.9	28	46 g	...
GH	11	1180	157	13.3	73	46.5	6.2	19	49 g	5
RF	6	1000	2	0.2	0	...	...	...	...	...
<i>Pale green</i>										
HT:p	1	150	17	11.4	9	52.9	6.0	1	8 p	...
<i>White</i>										
LG	2	150	10	6.7	2	20.0	2.7	...	...	2
JCM	2	260	36	13.9	2	5.6	0.8	...	...	2
FoS	7	870	137	15.8	5	3.6	0.6	...	...	5
DV	4	1590	102	6.4	4	3.9	0.3	1	...	3
LC	4	1600	31	1.9	2	6.4	0.1	2	...	...
MBC	6	380	53	14.0	0	...	...	...	...	...
FS	5	450	11	2.5	0	...	...	...	...	...
MP	3	740	3	0.4	0	...	...	...	...	...
KH	*									

* KH is male sterile and only rarely produces good pollen.

from varieties, of *Pelargonium*. The results (table 4) show two important features as follows:

(1) Expected variations in the breeding behaviour of different varieties.

(2) Unexpected fertility losses of 80 per cent. or more for all varieties. I attribute these fertility losses to a number of different causes which I shall now discuss.

(a) *Male sterility*. I observed in 1962 pollen production by a few

flowers of K. Harrop from 11-13th July, by Mrs Pollock (MP and MP:G) from 6th July to 12th August, and by M. B.-Coutts from 8-24th August. For the remainder of the flowering season, from April till October, the three varieties were completely male sterile. In other varieties pollen production was good except at the beginning and end of the season.

(b) *Incompatibility*. The seed set in Mrs Pollock (MP and MP:G), R. Fish and L. Cullum is less than 2 per cent., and only from two clones have a few seeds germinated. The majority of flowers showed no sign of development, even though the pollen appeared to be good, which suggests that their ovules were not fertilised. Evidently these varieties are self-incompatible. It seems likely that in *P. zonale* self-fertility has arisen as the result of the breakdown of an incompatibility system by hybridisation, and by the unconscious selection for fertility in a cultivated plant.

(c) *Pollen tube failure*. In the green, diploid variety Meteor although many pollen grains germinated on the stigma, Philippi (1961) never observed more than seven growing down the style. My preliminary investigations with P. Crampel agree and have given an average of 4.25 pollen tubes from 28 flowers, with a maximum of eight pollen tubes in one style. Thus, on average, too few pollen tubes grow down the style to fertilise even half of the ten ovules available which therefore accounts for at least a 50 per cent. loss of fertility. Other varieties are probably similar to P. Crampel.

I have also dissected out under a binocular microscope, 4-5 days after pollination, the ovules from 63 flowers each of two green varieties. I found an average of 4.3 ovules per flower developing in P. Crampel and 4.2 ovules per flower in the green F. of Spring. The close agreement of these values, with those obtained by scoring the frequency of pollen tubes in the style, confirms my view that pollen failure is responsible for at least a 50 per cent. loss of fertility. This initial study has also revealed an interesting pattern of behaviour in ovule development.

(d) *Competition between ovules*. In most of the 126 flowers examined, I found only one of the two ovules in each carpel developing, while the other was shrivelled; the shrivelled ovule was most frequently the lower, and the developing ovule the upper of the two. The upper probably has a better chance of being fertilised than the lower because the pollen tube has a shorter distance to travel in order to reach it. Nevertheless, besides this positional preference, a genetic control is also suggested by a significant difference in the behaviour of the two varieties. Thus 3.5 per cent. of the developing ovules were found in the lower position in P. Crampel compared with 20.6 per cent. in the green of F. of Spring. Whenever I have found both ovules developing in the same carpel, the more rapid growth of one has severely restricted the growth of the other because the locule is large enough for only one ovule to develop to maturity.

At harvesting time, besides the good, I have always found many shrivelled seeds, which I believe may be equated with the dying off of numerous embryos observed by Philippi (1961). The death of these embryos, together with a small amount of germination failure, accounts for a further loss of 30 per cent. fertility. But in some varieties there are, in addition, other causes for fertility losses which we may now examine.

(e) *Plastid inviability, layer alterations.* The chimera F. of Spring normally has a white germ layer (FoS), but it can give bud variations that have a green germ layer (FoS:G); the nucleus remains the same in the two (plate I). When selfed, these two clones breed true, white or green. The fertility of the selfed white is, unexpectedly, much lower than for the selfed green plants. Owing to the constancy of the nucleus, the difference in fertility must therefore be caused by a defect of white plastids on one or more stages of development: pollen, eggs, endosperm, young or germinating embryo.

Seedlings, obtained by selfing 2 or 3-layered periclinal chimeras, normally correspond to the character of the second layer (L II). Thus the W-o-G mesochimeras in which L II is white usually give only white seedlings. But D. Varden has given one green in addition to three white, and L. Cullum has given two green and no white seedlings. Similar observations have been noticed in *Hydrangea* as well as other *Pelargonium* varieties (Tilney-Bassett, 1963). In these chimeras the green L I occasionally divides periclinally, as well as anticlinally, to give green flecks of mesophyll on the white leaf margins (plate I). Similar developmental variations can take place in the formation of the egg. Thus the placenta initials which are usually derived from L II are displaced as a result of periclinal division in L I. Consequently, if the placenta is to develop in its normal position, it must now develop from initial cells derived from L I and will, therefore, produce green seedlings corresponding to L I.

The formation of 2 unexpected, green seedlings, together with the complete absence of the expected white seedlings in L. Cullum, emphasises the contrast between the viability of green and the inviability of white plastids. At the same time it shows that the lethality of the white plastids is more pronounced in certain varieties than in others.

(f) *Segregation: gene inviability.* The golden genotype in *Pelargonium* was first analysed in the variety Verona by Baur (1907). When it was selfed, he observed segregation of the offspring in a ratio of 1 green: 2 golden, together with a few white seedlings. Baur concluded that the golden genotype was heterozygous; the Mendelian ratio was really 1:2:1 but the white seedlings were largely inviable. The results of Noack (1924), Roth (1927) and Imai (1936) obtained from selfing other golden varieties differed only in the varying proportions of white seedlings. In my experiments (table 4) a similar segregation is shown by the golden varieties C. P. Gem, G. Crampel and G. Harem in which the inviability of most of the potentially white seedlings causes a

corresponding fall in seed fertility. The high germination of C. P. Gem and G. Crampel demonstrates that the lethal genotype acts during seed development. But in G. Harem, in which the germination is much lower, and from which five white seedlings were obtained, the lethal genotype probably acts on germination, as Imai (1936) suggested for his varieties.

Segregation in a ratio of 3 green:1 pale green is shown by the chimera which had puzzled Chittenden (1926), the variety Happy Thought. Since the germ layer is evidently heterozygous, it is interesting to note that, when selfed, with the exception of one green seedling which could have arisen in many ways, the pale green core (HT:p) bred true (table 4). These results suggest to me that Happy Thought is a gene-differential chimera (Tilney-Bassett, 1963) with a heterozygous green skin covering a homozygous recessive pale green core.

(g) *Pollination period and flower density.* In G. Harem, green F. of Spring and the G-o-W bud variation from F. of Nature, I have compared the average number of germinating seeds produced by each flower of an inflorescence, with the number of its flowers, and with its pollination period. The wide variations in fertility, obtained for each inflorescence in a clone, appeared to have no relationship with the flower density. There was a slight suggestion that the fertility of G. Harem was significantly lower at the beginning of the flowering season in April; this was not the case with the other two clones.

What is certain, is that the fertility of different inflorescences from the same clone is liable to fluctuate considerably throughout the flowering season, probably owing to day to day environmental changes. A consistent average fertility is therefore likely to be obtained only after a high number of pollinations. Thus when comparing the results of selfing or crossing experiments we must be particularly cautious about attaching any significance to minor differences in their respective fertilities, especially if only a low number of pollinations has been made.

(ii) *Reciprocal crosses*

The effect on fertility of a mixture of green and white plastids, brought together by crossing, is more easily detected with a wide, than with a narrow difference between the self-fertilities of the parents. I have therefore chosen the one golden chimera and two pure green plants with the highest self-fertility and have crossed them with several white varieties having very low fertility (table 4). The results of these crosses are shown in table 5.

(a) *The isogenic reciprocal cross.* The unique, reciprocal cross between F. of Spring and its green bud variation (plate I) is extremely important because, owing to the constancy of the nucleus in all selfs and crosses, we may assume that differences in their progenies and fertilities are dependent upon differences in behaviour of the two types of plastid. The effects of the plastids on development can therefore be separated from the effects of the nucleus. We have already seen from selfing

TABLE 5
Results of selfing and reciprocal crosses between pelargonium varieties

A. CROSSES WITH GREEN FLOWER OF SPRING, FoS:G

Self or cross	Total nos. and percentages							Offspring		
	Infts.	Ovules pollnd.	Seeds				Total ferti- lity per cent.			
			Full	Per cent. full	Germd.	Per cent. germd.		G	V	W
G * self	7	850	203	23.9	172	84.7	20.2	172	...	
1. FoS:G × Flower of Spring : Isogenic cross										
{ G × W	5	800	149	18.7	121	81.2	15.2	60	11	50
{ W × G	5	660	117	17.7	114	97.4	17.3	88	24	2
W self	7	870	137	15.8	5	3.6	0.6	...	...	5
2. FoS:G × Mrs Pollock										
{ G × W	1	200	17	8.5	17	100.0	8.5	16	1	...
{ W × G	2	520	30	5.8	19	63.3	3.7	6	11	2
W self	3	740	3	0.4	0	...	...	...	...	...
B. CROSSES WITH PAUL CRAMPEL, PC										
G self	2	490	116	23.6	97	83.6	19.8	97	...	...
1. PC × Dolly Varden										
{ G × W	7	2370	446	18.8	330	74.0	14.0	108	139	83
{ W × G	5	1140	223	19.6	180	80.7	15.8	127	50	3
W self	4	1590	102	6.4	4	3.9	0.3	1	...	3
2. PC × Flower of Spring										
{ G × W	4	1570	432	27.5	380	88.0	24.2	276	76	28
{ W × G	7	1140	165	14.5	153	92.7	13.4	121	30	2
W self	7	870	137	15.8	5	3.6	0.6	...	...	5
3. PC × Miss Burdett-Coutts										
{ G × W	1	100	38	38.0	34	89.5	34.0	12	11	11
{ W × G	1	320	40	12.5	35	87.5	11.0	11	24	...
W self	6	380	53	14.0	0	...	...	...	...	...
4. PC × Kathleen Harrop										
{ G × W	†							3	...	...
{ W × G	5	520	70	13.5	63	90.0	12.1	63	...	...
W self	...	...	...	...	...	...	...	...	...	...

* The results for the three selfed "green" parents are given only once, at the head of each group of crosses.

† K. Harrop is male sterile; it has only once produced good pollen which was used in this cross.

TABLE 5 (*continued*)

Self or cross	Total nos. and percentages							Offspring				
	Infs.	Ovules pollnd.	Seeds				Total fertility per cent.					
			Full	Per cent. full	Germd.	Per cent. germd.						
C. CROSSES WITH CRYSTAL PALACE GEM, CPG												
g self . . .	3	400	66	16.5	57	86.4	14.3	21	36	...	...	...
1. CPG × Dolly Varden												
$\left\{ \begin{array}{l} g \times W \\ W \times g \end{array} \right.$ . .	3	810	94	11.6	71	75.5	8.8	31	36	4	...	...
W self . . .	4	1590	131	15.8	87	66.4	10.5	28	16	23	20	...
			102	6.4	4	3.9	0.3	1	...	...	...	3
2. CPG × J. C. Mapping												
$\left\{ \begin{array}{l} g \times W \\ W \times g \end{array} \right.$ . .	4	1730	239	13.9	213	89.1	12.3	104	104	...	5	...
W self . . .	5	1050	141	13.5	126	89.4	12.0	48	35	25	15	3
	2	260	36	13.9	2	5.6	0.8	...	...	...	...	2

experiments, that the white plastids cause a defect on one or more stages of development: pollen, eggs, endosperm, young or germinating embryo.

The reciprocal crosses $G \times W$ and $W \times G$ are similar in that they both give green, variegated and white offspring showing that plastids are contributed by both pollen and eggs. They differ from the expected behaviour in two ways:

(1) Instead of giving reciprocal proportions of green and white offspring, the majority of seedlings are green whichever way the cross is made.

(2) More white offspring are produced when the white plastids enter from the pollen ($G \times W$), than when they enter from the eggs ($W \times G$).

How can we interpret these anomalous results? The following possibilities should be considered:

(1) The proportions of offspring reflect the different plastid contributions from the two parents: I think this is unlikely for at least one of the crosses because it would mean that the number of plastids contributed is dependent not only upon whether they are transmitted by a male or female cell as expected, but also upon whether they are white or green, which I would not expect when the nucleus is the same in the two.

(2) The proportions of offspring do not reflect the different plastid contributions from the two parents owing to the green plastids multiplying more rapidly than the white: two observations, which I discuss later (section 5), suggest that the plastids do not behave in this way. I shall therefore assume that multiplication differences between green and white plastids do not occur.

(3) The proportions of offspring from the cross $W \times G$ do not reflect the parental plastid contributions owing to the inviability of numerous white embryos: how then can we explain the germination of numerous white seedlings from the cross $G \times W$, but only two white seedlings from the cross $W \times G$? I believe we must first consider what is happening to the endosperm as well as to the embryo.

TABLE 6

Relationship between observed and expected offspring and the fertility of the isogenic cross $W \times G$ (table 5) *

Isogenic crosses	Proportions of offspring								Changes in $W \times G$ cross	
	Observed				Expected				Extra white seedlings	New total fertility per cent.
	G	V	W	T	G	V	W	T		
$G \times W$ (control)	60	11	50	121	60	11	50	121	...	...
$W \times G$	88	24	2	114	88	24	105	217	103	32.9

* Based on the assumption that the proportions of offspring in the reciprocal cross $G \times W$ reflect their parental plastid contributions.

On the female side, it is quite likely that the number of plastids associated with the diploid endosperm nucleus is greater than the number associated with the haploid egg. On the male side, it is possible that more plastids go to the egg than to the endosperm nucleus. On either assumption, the endosperm will contain predominantly green plastids in the cross $G \times W$ and predominantly white plastids in the reciprocal cross. I suggest that white embryos can survive only if they have predominantly green endosperms to nourish them and this accounts for the low proportion of white seedlings in the cross $W \times G$, as well as from selfed white plants.

Assuming that the proportions of offspring from the cross $G \times W$ do reflect their parental plastid contributions, then the proportions from the cross $W \times G$ are expected to be their reciprocal. The results (table 5) show that this is not so owing, I believe, to the inviability of white embryos. In table 6 I have shown what would have been the proportions of offspring in the cross $W \times G$ had enough white embryos survived to give reciprocal results for the reciprocal cross. The table

also shows the rise in fertility up to 32.9 per cent. needed to account for the extra white seedlings. This value is well below the expected maximum limit of 42 per cent. of the ovules being fertilised, as found in the selfed green plants. The fertility data are therefore compatible with my plastid reaction hypothesis for the embryo-endosperm system.

About half of the embryos from the selfed green plants died during development, lowering the total fertility from 42 to 20.2 per cent. Presumably a similar number die in the reciprocal crosses. In the cross $G \times W$ if the proportions of offspring reflect the parental plastid contributions, the death of these embryos must be independent of whether they were potentially green, variegated or white seedlings. But in the cross $W \times G$ the white embryos have been almost entirely eliminated, while many of the green embryos that would otherwise have died, have evidently survived since the fertility is actually higher than for the reciprocal cross and only slightly lower than for selfed green (table 5). I suggest that this behaviour is understandable if the death of half of the developing embryos is because of competition between themselves. In the cross $W \times G$ there is the same competition as for selfed green or the reciprocal cross, but this time one of the contestants is the developing seed with the lethal combination of a white embryo and predominantly white endosperm. Hence the pressure of competition selects first against these weak, white embryos and thereby greatly reduces the competition amongst the remaining potentially green or variegated embryos.

(b) *Non-isogenic, intervarietal reciprocal crosses.* The present explanation of the results of the isogenic reciprocal crosses provides a working hypothesis which can be tested. The non-isogenic, intervarietal crosses (table 5) are more difficult to explain because the effect of the nucleus cannot be separated from the effect of the plastids. The results are extremely diverse, and often apparently contradictory. For example, in the cross P. Crampel $\times$ D. Varden, $G \times W$ gave 32.7 per cent. green, 42.1 per cent. variegated and 25.2 per cent. white offspring, yet when a golden parent is used, C. P. Gem $\times$ D. Varden, $G \times W$ gave 94.3 per cent. green and golden, 5.7 per cent. variegated and no white offspring. In the reciprocal cross the proportion of "green" offspring is raised to 70.6 per cent. with DV $\times$ PC but is lowered to 50.6 per cent. with DV $\times$ CPG. Thus as a result of changing the "green" parent the proportions of offspring have altered considerably; furthermore the effect of changing the direction of the cross has been to alter the proportion of "green" offspring in opposite directions. Similar striking differences have also occurred when the green parent is constant and the white parent is changed. These changes may be the result of a different hybrid nucleus or of the different plastid combination. Evidently many variable factors are to be considered and I think it unwise to attempt to draw conclusions from the breedings results alone.

The cross K. Harrop $\times$ P. Crampel may be quite different from the

other crosses since only green seedlings have been obtained. Similar results were obtained by Imai (table 1) for three of his W-o-G chimera crosses. It seems likely that these varieties are not plastid- but gene-differential chimeras, so that the crosses $G \times W$ or $W \times G$ produce purely green F_1 heterozygotes. I shall test this suggestion.

4. SORTING-OUT OF VARIEGATED SEEDLINGS

A detailed analysis of the sorting-out of variegated *Pelargonium* seedlings has not been made by earlier workers. My observations show that in every cross the variegated seedlings are not equal but form

TABLE 7

Analysis of the sorting-out, from mixed growing points, of pure green, pure white and chimerical leaves and shoots. Sorting-out is scored during the development of the primary stem of variegated seedlings derived from pelargonium crosses (table 5). It is classified, according to types of leaf formed, into three groups.

	Data for the three groups of sorting-out															
	G+V					G+V+W						V+W				
Sorting-out coefficient *	0.92					0.81						0.97				
Nos. of each type	G	V	W/G	G/W	T	G	V	W	W/G	G/W	T	W	V	W/G	G/W	T
Shoots . .	179	...	5	8	192	17	...	29	7	9	62	106	...	2	...	108
Leaves . .	686	1125	28	42	1881	243	826	248	50	67	1434	227	492	5	2	726

* Sorting-out coefficient = plants completely sorted-out/total no. of plants.

a continuous range from predominantly green to predominantly white. I have separated the plants along this range into three groups:

- (1) Seedlings which produce green and variegated leaves (G+V).
- (2) Seedlings which produce white and variegated leaves (W+V).
- (3) Seedlings which produce green, variegated and white leaves (G+V+W). Periclinal leaves occur in all groups.

(i) The three groups of sorting-out

My data for the sorting-out of variegated seedlings (plate II) showed no significant differences between each individual cross (table 5). I have therefore given their aggregate results (table 7 and fig. 1). The pattern of sorting-out in the G+V is paralleled by the W+V group. I have therefore summed their pure green and pure white shoots in the graph (fig. 1).

A comparison of the three groups (table 7) shows that most fall into the G+V group following the pattern of germinating seeds, in which the majority are green. The proportion of plants in the W+V group is higher than might be expected from the pattern of germinating

seeds. This is presumably because the white shoots can be observed before they die (plate II), whereas the white seedlings were frequently inviable. The $G+V+W$ group is the smallest because sorting-out in the direction of pure green or pure white is continuous throughout development, and in many crosses is approaching completion as early in development as at germination. The earliness of sorting-out

FIG. 1.—Relationship between the frequency of sorting-out of variegated seedlings to give pure green or white shoots or stable periclinal chimeras, their proportions of green : white tissue, and their stage in development.

- ——— Sorting-out of pure green, pure white and stable periclinal shoots from the $G+V$ and $W+V$ groups which have predominantly green or predominantly white tissue respectively (table 7).
 ○ ——— Sorting-out of pure green, pure white and stable periclinal shoots from the $G+V+W$ group which has roughly equal proportions of green and white tissue (table 7).

is further emphasised by the graph (fig. 1) which shows that most of the variegated seedlings of the $G+V$ and $W+V$ groups develop pure green or pure white shoots within the first few nodes. A particularly large number of pure shoots develop directly between the variegated cotyledons (classified as third leaf stage). Sorting-out from the $G+V+W$ group progresses gradually and steadily during development as also does the "tail" of the other two groups (fig. 1). How can the similarities of the $G+V$ and $W+V$ groups and the difference between them and the $G+V+W$ group be explained?

It is well known that sorting-out occurs in two phases:

- (1) Sorting-out of pure cells from mixed cells.
- (2) Sorting-out of pure shoots or stable periclinal chimeras from pure cells.

The first of these phases must be complete before the second. Since most seedlings of the G+V and W+V groups completely sort-out into pure shoots within the first few nodes, it is evident that the first phase of sorting-out has been completed during the development of the embryo, and only the second stage of sorting-out is apparent in the developing seedlings. The individuals of the "tail" take a long time to sort-out because the growing-points have often developed into a moderately stable sectorial chimera which, for example, has one sector pure green and the remainder a mixture of green and white cells (plate II).

It is possible that the first phase of sorting-out is still taking place in a few individuals of the G+V+W groups. But what is more important is that these plants must have approximately equal numbers of green and white cells since roughly equal numbers of green and white leaves are produced (table 7). It is easy to see that if the two types of cell are equally frequent in the growing-point it will be difficult for one type to displace the other and consequently to occupy the whole apex; hence sorting-out is completed later than in the other two groups.

Periclinal chimeras were formed intermittently from the ninth node onwards. Thus periclinal chimeras developed by the G+V and W+V groups occurred only among individuals of the "tail"; these plants are of course very similar to plants of the G+V+W group.

(ii) *Formation of periclinal chimeras*

The total results from all the crosses (table 7) show that shoots from the three sorting-out groups develop into periclinal chimeras (plate III) with the following frequencies:

(1) W+V group: out of 111 shoots, 108 have completed sorting-out of which two are W/G chimeras (approximately 2 per cent.).

(2) G+V group: out of 209 shoots, 192 have completed sorting-out of which five are W/G and eight G/W chimeras (approximately 7 per cent.).

(3) G+V+W group: out of 78 shoots, 62 have completed sorting-out of which seven are W/G and nine G/W chimeras (approximately 26 per cent.).

Possible periclinal chimeras involving the unseen epidermis have not been considered. In each group the proportion of chimeras may increase as there are some plants in which sorting-out is still incomplete. Such increases appear more likely to widen than to close the difference between the three groups.

Clearly the much higher proportion of periclinal chimeras in the G+V+W group is related to an approximately equal proportion of

green and white cells in the growing-points of these plants. Contrariwise, the low proportion of periclinal chimeras from the other two groups is evidently related to the unequal numbers of green and white cells in the growing-points of these plants.

Altogether there are 14 W/G and 17 G/W periclinal chimeras so it appears that the two types (plate III) are formed with about equal frequency. Furthermore, the two types of chimera appear to be evenly distributed between the reciprocal crosses as follows:

(1) $G \times W$: out of 210 shoots, 193 have completed sorting-out of which eight are W/G and ten G/W chimeras (9.3 per cent.).

(2) $W \times G$: out of 188 shoots, 169 have completed sorting-out of which six are W/G and seven G/W chimeras (7.7 per cent.).

The low numbers of chimeras produced make it impossible to say whether or not individual results agree with the combined results.

5. SELECTION: THE BASIS OF RECIPROCAL DIFFERENCES

It is difficult to say whether green and white plastids ever differ in their multiplication rate because any possible differences would be masked by the effect of inviable white embryos. Nevertheless certain observations suggest that multiplication differences probably do not occur:

(1) In whichever direction intervarietal crosses are made there are always more green than white seedlings (table 5). Yet when the number of green and white shoots, formed by the sorting-out of variegated seedlings, are scored, there is almost always a relative increase in the proportion of white (table 8). It seems most unlikely, however, that green plastids multiply more rapidly during embryo development and white plastids more rapidly after germination.

(2) Sorting-out of variegated seedlings to give stable periclinal chimeras indicates that pure green and pure white cells multiply at the same rate. Therefore any differences in the multiplication rate can exist only between plastids within the same cell.

A comparison between the proportion of green:white seedlings, obtained from reciprocal crosses, and the proportion of green:white shoots derived by the sorting-out of variegated seedlings is presented in table 8. In nearly every cross the change in the ratio of green:white from the seedlings to the shoots shows an increase in the proportion of white. In each pair of reciprocal crosses the increase in favour of white is small in the cross $G \times W$ but large in the reciprocal $W \times G$. On mathematical grounds, provided the plastids sort-out at random and multiply at the same rate, and provided all fertilised eggs survive, the ratio of green:white shoots will equal the ratio of green:white seedlings, which in turn will equal the ratio of green:white plastids introduced by the two sexes at fertilisation. On the present assumption that the green and white plastids multiply at the same rate and sort-out at random, the failure of many fertilised eggs to survive must therefore

be responsible for the observed ratios not being equal. This is perfectly plausible since we know that about half of the embryos die during development. On the basis of differential survival of the embryos, owing to the plastid reaction with the embryo-endosperm system, the direction of change in the ratios can now be explained. In the cross $G \times W$ there is either no, or only a slight, selection against white

TABLE 8

Comparison between the proportion of green : white seedlings, obtained from reciprocal crosses (table 5), and the proportion of green : white shoots derived by the sorting-out of variegated seedlings (table 7), obtained from the same crosses.

Reciprocal crosses		Seedlings from crosses			Shoots from variegated seedlings			
		Nos.		Ratios $G \div W$	Total no. seedlings	Nos. sorted-out*		Ratios $G \div W$
		G	W			G	W	
FoS:G $\times$ FoS Recip.	(G $\times$ W) (W $\times$ G)	60 88	50 2	1.2 44.0	11 20	9 12	2 7	4.5 1.7
PC $\times$ DV Recip.	(G $\times$ W) (W $\times$ G)	108 127	83 3	1.3 42.3	112 42	49 24.5	51 14.5	1.0 1.7
PC $\times$ FoS Recip.	(G $\times$ W) (W $\times$ G)	276 121	28 2	9.9 60.5	67 24	39 13.5	26 8.5	1.5 1.6
PC $\times$ MBC Recip.	(G $\times$ W) (W $\times$ G)	12 11	11 ...	1.1 ...	11 23	3 2	5 10	0.6 0.2
CPG $\times$ DV Recip.	(g $\times$ W) (W $\times$ g)	67 44	... ...	... ...	4 39	4 23.5	... 12.5	... 1.9
CPG $\times$ JCM Recip.	(g $\times$ W) (W $\times$ g)	208 83	... 3	... 27.1	5 40	5 22.5	... 12.5	... 2.0

* Each W/G and G/W periclinal chimera is scored as $\frac{1}{2}$ a white and $\frac{1}{2}$ a green shoot.

embryos; hence the small change in the ratios. In the reciprocal $W \times G$ crosses there is strong selection against white embryos and there may also be selection against variegated embryos; hence the large change in the ratios. The working hypothesis, based on the reaction of the white plastids with the embryo and endosperm, is thus equally applicable to the intervarietal crosses as for the isogenic cross. It is probable that selection against white embryos is greater in some crosses than in others, just as it was greater in some selfed varieties than in others.

The proportion of variegated seedlings in the $G \times W$ crosses, in which there is little or no selection against white embryos, ranges from 9.1 per cent. in the isogenic cross to 42.1 per cent. in the cross P. Crampel $\times$ D. Varden. Such significant differences are most

Plate I

Outside. $\times \frac{3}{4}$. A selection of periclinal chimeras.

1. Golden Harem, golden-over-green, ggG.
2. Crystal Palace Gem, golden-over-green, ggG.
3. Freak of Nature, G-o-W bud variation, GGW.
4. A Happy Thought, green-over-pale green, GGp.
5. Mrs Pollock, W-o-G mesochimera, GWG.
6. Miss Burdett-Coutts, W-o-G mesochimera, GWG.
7. Dolly Varden, W-o-G mesochimera, GWG. Notice the green flecks of mesophyll on the white marginal tissue near the petiole. They arise from division of the genetically green epidermis, L I...
8. Flower of Spring, W-o-G mesochimera, GWG.

Centre. $\times \frac{1}{8}$.

9. The origin of the isogenic clones with green and white germ layers. The W-o-G chimera Flower of Spring, GWG, has produced two bud variations, pure green, GGG, and pure white, WWW, in which the nucleus remains unchanged.

Plate II

A selection of variegated seedlings.

1. PC×DV. $\times 1\frac{1}{2}$. Three weeks. The two cotyledons and first foliage leaf (classified as 3rd leaf) are all variegated.
2. PC×DV. $\times 1\frac{1}{2}$. Eight weeks. Sorting-out of a mixed growing-point into pure green and pure white tissues has produced a sectorial chimera.
3. MBC×PC. $\times 1$. Five months. This seedling contains a roughly equal proportion of green and white cells. Hence sorting-out of a mixed growing-point into pure green and pure white tissues is still in progress.
4. FoS×PC. $\times 1$. Five months. This predominantly white seedling has sorted-out to give a pure white shoot.

Plate III

Reciprocal chimeras.

1. DV $\times$ PC. $\times \frac{1}{2}$. Six months. Sorting-out from a variegated seedling has produced a stable G-o-W periclinal chimera (G/W). The white core of the leaves is somewhat masked by the green skin.
2. FoS $\times$ FoS:G. $\times 1$. Six months. Sorting-out from a variegated seedling has produced a stable W-o-G periclinal chimera (W/G) similar to the female parent (plate I).

simply explained by the suggestion that the more rapid sorting-out of the isogenic cross is the result of the embryo having fewer plastids per cell. Differences in the number of plastids per cell are probably important in producing wide variation in the results of intervarietal crosses.

In the isogenic and two P. Crampel, $G \times W$ crosses (table 8) the ratio of green:white approaches 1:1 suggesting that almost as many plastids enter from the male as from the female parent. Variations in the proportions of plastids from the two sexes may be a further important factor in producing the large differences found between intervarietal crosses. But further experiments are needed before these important considerations can be discussed in more detail.

6. SUMMARY

1. By relating breeding and fertility results from selfs and crosses, I have begun to separate the viabilities of the pollen, eggs, endosperm, early and germinating embryos. By using an isogenic cross I have separated the influence of the plastids from that of the nucleus.

2. Twenty varieties were selfed (table 4). Nine factors were found to contribute to the breakdown of fertility, viz.:—male sterility; incompatibility; pollen tube, embryo and germination failure; competition between ovules; gene and plastid lethality and environmental conditions.

3. That white embryos are particularly lethal in $W \times G$ crosses but not in $G \times W$ was suggested by (i) the much greater lethality of white compared with green embryos, on selfing (table 4), (ii) the presence of numerous white seedlings from the $G \times W$ cross but their virtual absence from the $W \times G$ (table 5), (iii) the change, in favour of white, in the ratio of green:white between germination and the complete sorting-out of variegated seedlings was much greater for the $W \times G$ cross than for the reciprocal (table 8).

4. I conclude that there are more green offspring in the $G \times W$ cross because the female cells contributed more plastids than the male, but in the reciprocal $W \times G$ it is because white endosperms kill white embryos. Two factors account for this lethality. Firstly, reciprocal crosses are differential with respect to the proportions of white and green plastids in the embryo, and also their proportions in the embryo in relation to the endosperm. Evidently when the endosperm, with its double female contribution, has predominantly white plastids, it fails to nourish a white embryo. Secondly, half of all embryos die during development thus providing the opportunity for selection to operate against white embryos in competition with green or variegated.

5. Periclinal chimeras are most frequently formed from those variegated seedlings with roughly equal proportions of green and white cells (table 7). The two types W/G and G/W develop with equal frequency independently of the direction of the cross.

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