# THE INHERITANCE OF COLOUR IN DIPLOID POTATOES

## II. A THREE-FACTOR LINKAGE GROUP

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(With One Text-figure)

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### 1. Introduction

In an earlier paper, we have described the types of anthocyanidins that occur in cultivated diploid tuberous *Solanums* ($2n=24$) and postulated two independent loci, *P* and *R*, concerned with their development (Dodds & Long, 1955). In this paper, three other loci, designated *B*, *I* and *F*, are considered. They are closely linked and concerned with the distribution of pigment on the body of the plant.

Loci *B* and *I*, although apparently physically distinct units, seem to function co-operatively in the determination of an effect. Their pseudo-allelic relationship was reported briefly in an earlier publication (Dodds, 1955).

### 2. The cultivated diploids

#### (*a*) *Species*

Cultivated diploid potatoes have an extensive range in the Andes from southern Peru to the north of Colombia, being grown chiefly between 8000 and 12,000 ft. Hawkes (1944) classifies them into nine species. They are all highly interfertile and, in those which have been examined cytologically, apart from occasional inversion bridges and univalents, the $F_1$ hybrids show a normal meiosis (Swaminathan & Howard, 1953). The species interbreed regularly, and no genetical evidence has yet been obtained in the experimental breeding which favours their delineation as species. For this reason they are treated as one pool of genetic variability in this account. The reference numbers, provenance and species of each of our parental clones are given in Table I. Most were collected as tubers in South America, and since then have been maintained by vegetative propagation.

#### (*b*) *Colours*

Colour throughout a diploid potato plant, that is, in tuber, sprout and flower, is controlled by two independent loci (at least), *P* and *R*. The former has a straightforward effect governing the presence and absence of an acylated glycoside of petunidin throughout the plant. It is epistatic to *R*. Two alleles of the *R* locus, namely, *R* and $R^{pw}$, have been identified and their effects throughout the plant are not the same. The former seems

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to control the occurrence of an acylated glycoside of pelargonidin in the tuber coupled with the presence of a glycoside of cyanidin in the flower, whereas with the latter in its homozygous phase, $R^{pw}R^{pw}$, these pigments do not occur. These plants are not without anthocyanin, however, for an acylated derivative of peonidin occurs in all cultivated diploids. Since all plants so far studied have this anthocyanin, no conclusions can be drawn about its inheritance. However, peonidin is suppressed in the flowers of genotypes homozygous for $R^{pw}$ (Table 2).

It is against this general background of pigmentation that the loci concerned with distribution produce their effects.

Table 1. *Parental clones of cultivated diploids*

| Reference | Species | Phenotype: Tuber | Phenotype: Sprout | Phenotype: Flower | Provenance |
|---|---|---|---|---|---|
| 827 | *S. Ascasabii* Hawkes | Blue purple | Blue | Blue | Ecuador |
| 836 | *S. Kesselbrenneri* Juz. & Buk. | White (very slight flush), red eyebrow | Red | Red | Ecuador |
| 979 | *S. Rybinii* Juz. & Buk. | Blue | Blue | Blue | Colombia |
| 1311 | *S. Rybinii* Juz. & Buk. | White | Red | Flecked | Colombia (via Bukasov Russia) |
| 1776 | *S. Cardenasii* Hawkes | White | Pink | White | Bolivia |
| 2171 | *S. Rybinii* Juz. & Buk. | White | Red | Flecked | Colombia |
| 2202 | *S. Rybinii* Juz. & Buk. | White (very slight flush) | Red | Red | Colombia |
| 2203 | *S. Rybinii* Juz. & Buk. | Patchy blue | Blue | Blue | Colombia |
| 2205 | *S. Rybinii* Juz. & Buk. | White | Red | Red | Colombia |
| 2207 | *S. Rybinii* Juz. & Buk. | Red | Red | Flecked | Colombia |
| 2211 | *S. Rybinii* Juz. & Buk. | Pink | Pink | White | Colombia |
| 2222 | *S. Rybinii* Juz. & Buk. | Weak spectacle,* pink | Pink | White | Colombia |
| 2358 | *S. Rybinii* Juz. & Buk. | Pink | Pink | White | Colombia |

* Self-coloured, but an area around each eye is unpigmented (Fig. 1).

Table 2. *The relation of genotype to colour and anthocyanidins*

| Genotype | Colours | Anthocyanidins |
|---|---|---|
| *P–R–I–* | Flower: blue<br>Tuber: blue | Petunidin, cyanidin, peonidin<br>Petunidin, peonidin |
| *P–R–ii* | Flower: blue<br>Tuber: white | Petunidin, cyanidin, peonidin<br>Nil |
| *ppR–I–* | Flower: red<br>Tuber: red | Cyanidin, peonidin<br>Pelargonidin, peonidin |
| *ppR–ii* | Flower: red<br>Tuber: white | Cyanidin, peonidin<br>Nil |
| $ppR^{pw}R^{pw}Ii$ | Flower: white<br>Tuber: pink | Nil<br>Peonidin |
| $ppR^{pw}R^{pw}ii$ | Flower: white<br>Tuber: white | Nil<br>Nil |

## 3. Plant characters

### (*a*) *Coloured and white tubers*

An independent locus *I* in its homozygous recessive phase *ii* is epistatic to the two colour loci and leads to the absence of pigment in the tuber, to which organ its main action is localized (Table 2, Fig. 1). Nevertheless, the haulms of plants homozygous *ii* are invariably lightly coloured with no more than speckled pigment on the internodes. This is in marked contrast to *P–R–I–* genotypes which are usually intensely self-coloured and deep blackish purple; *ppR–I–* genotypes are similarly self-coloured but the pigment is dark red.

A tuber variant designated 'spectacle' shows partial suppression of colour so that an area around each eye is unpigmented (Fig. 1). The inheritance of this character is not considered here.

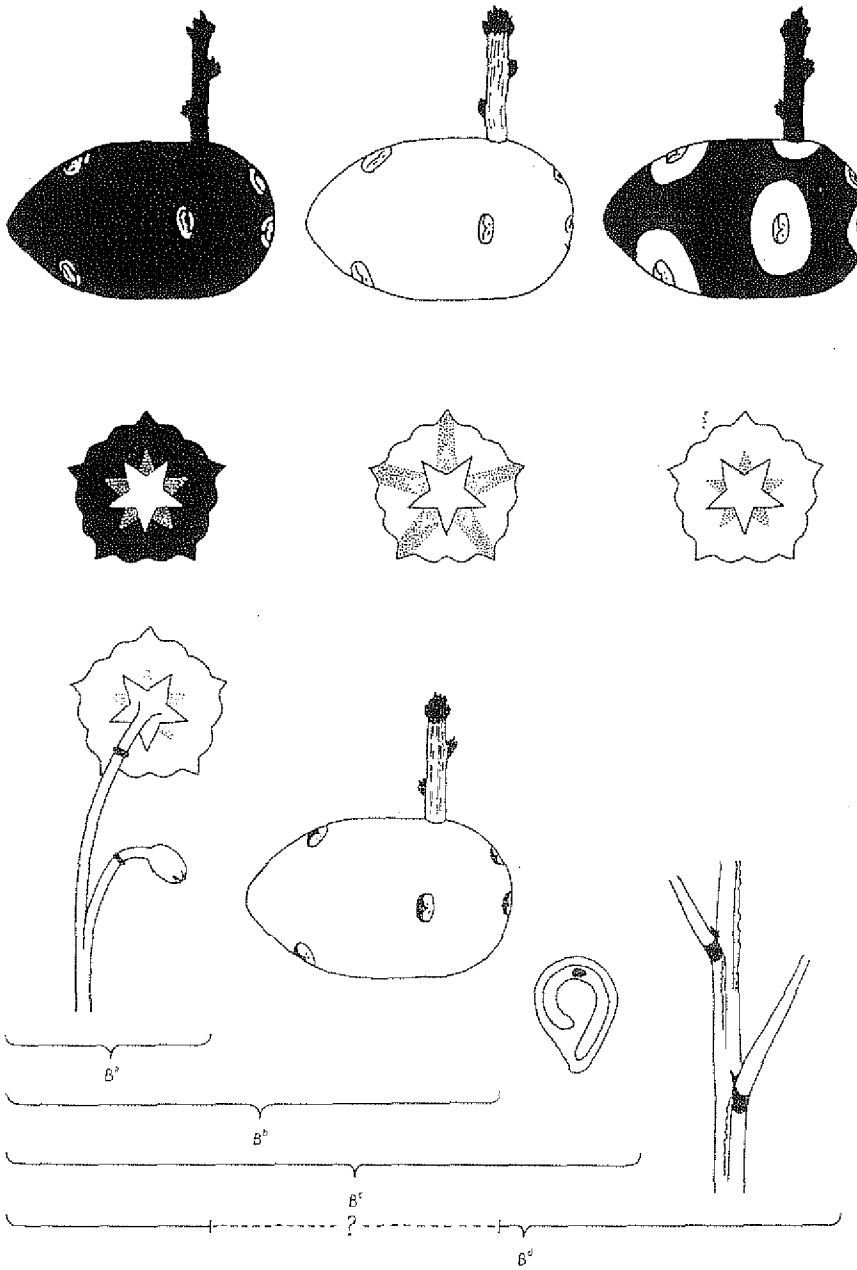


Fig. 1. Top row: tubers, self-coloured; white; 'spectacle'. Middle row: flowers, self-coloured; flecked; white. Bottom row: characters controlled by alleles at the *B*-locus.

*(b) Seed spot, nodal band, floral abscission layer and eyebrow on tuber*

Seeds may be marked with a single coloured spot owing to the presence of pigment on the cotyledonary node of the embryo. This spot is visible through the testa. A band of pigment may occur encircling the base of the petiole, and the floral abscission layer may be distinctly coloured with anthocyanin. In white tubers the eyebrow may be coloured or uncoloured, but the difference is masked in self-coloured tubers. These manifold expressions of the distribution of pigment occur in certain combinations and may be interpreted most satisfactorily by assuming a series of alleles, $B^d > B^c > B^b > B^a > b$, in order of dominance (Table 3, Fig. 1).

Table 3. *Phenotypic effects of alleles at locus B*

| | Pigment | | | |
|---|---|---|---|---|
| Allele | Floral abscission | Tuber eyebrow | Spot on embryo | Nodal band |
| $b$ | – | – | – | – |
| $B^a$ | + | – | – | – |
| $B^b$ | + | + | – | – |
| $B^c$ | + | + | + | – |
| $B^d$ | + | ? | + | + |

*(c) White and flecked flowers*

White flowers are completely without pigment in the corolla. They may occur on plants with either coloured or white tubers. With coloured tubers, in our present stocks, the tuber and haulm pigment is an acylated glycoside of peonidin. With white tubers this pigment is present only in the haulm. The two genotypes are $ppR^{pw}R^{pw}I\text{-}$ and $ppR^{pw}R^{pw}ii$, respectively (Table 2).

Flecked flowers are mainly white but show patches of colour on and around the vascular 'star' which is characteristic of the corolla (Fig. 1). In some genotypes the star may be completely coloured, whereas in others, only careful inspection reveals the presence of pigment. Flecked flowers may occur in association with all the genotypes given in Table 2, except with $ppR^{pw}R^{pw}$. This genotype produces only one anthocyanin and it is completely suppressed in the flower. Thus flecked blue and flecked red flowers may occur on plants with either self or white tubers.

## 4. The inheritance of white and flecked flowers

It will be seen from Table 4 that *white* by *white* gives white flowers, *flecked* by *flecked* gives flecked flowers but that *white* by *flecked* gives self-coloured flowers. This is not an uncommon situation in the genetics of floral pigments. Elsewhere (Dodds & Long, 1955), however, we have established the activity of major loci concerned throughout the potato plant with the production and suppression of a particular pigment. Clearly, then, if flowers and tubers owe their colour to the action of the same genetic loci, a plant with coloured tubers but white flowers has the inherent ability to produce pigment. Its absence in the flower must reflect localized inhibition or, conversely, its presence in the tuber shows localized development. We may suppose that in *white* and *flecked*, neither genotype lacks anything basic to the production of floral pigment—in one, localized inhibition is complete, and in the other only partial. Nevertheless, the factors controlling

inhibition are not allelic. It is necessary therefore to postulate an independent locus, say *F*, concerned in the flower with the contrast self-coloured versus flecked, homozygous recessives *ff* being flecked. Thus two alleles, *f* and $R^{pw}$, at independent loci have similar phenotypic effects on the same organ; effects which differ only in the degree of completeness.

Table 4. *The production of self red flowers in the cross* white × flecked

| | | | Flowers white | Progeny size |
|---|---|---|---|---|
| (1) White flower × White flower | | | | |
| C.P.C. 2358 white flower, pink tuber | × C.P.C. 2211 white flower, pink tuber | Tubers pink | 52 | 52 |
| C.P.C. 1776 white flower, white tuber | × C.P.C. 2211 white flower, pink tuber | Tubers pink | 41 | 41 |
| C.P.C. 2211 white flower, pink tuber | × C.P.C. 2222 white flower, pink tuber (spectacle) | Tubers pink {Self | 8 | |
| | | Tubers pink {Spectacle | 7 | 15 |
| (2) Flecked flower × Flecked flower | | | Flowers flecked | |
| C.P.C. 1311 flecked flower, white tuber | × C.P.C. 2207 flecked flower, red tuber | Tubers {Red | 7 | |
| | | Tubers {White | 8 | 15 |
| C.P.C. 2207 flecked flower, red tuber | × C.P.C. 1311 flecked flower, white tuber | Tubers {Red | 19 | |
| | | Tubers {White | 7 | 26 |
| (3) White flower × Flecked flower | | | Flowers red | |
| C.P.C. 1311 flecked flower, white tuber | × C.P.C. 2211 white flower, pink tuber | Tubers red | 83 | 83 |
| C.P.C. 2171 flecked flower, white tuber | × C.P.C. 2211 white flower, pink tuber | Tubers red | 20 | 20 |
| C.P.C. 2358 white flower, pink tuber | × C.P.C. 2171 flecked flower, white tuber | Tubers {Red | 39 | |
| | | Tubers {White | 29 | 68 |

## 5. Linkage between flower and tuber colour

During the course of this work it became obvious that the flecked flower and tuber colour loci, *F* and *I* respectively, are closely linked. Data which demonstrate this are shown in Table 5, in which the parental crosses show the origin of the $F_1$ hybrids that were used in the backcrosses to test linkage. For its estimation, blue and red tubers were classed together as self-coloured (Table 6). The two sets of data may be considered to be homogeneous ($\chi^2 = 2{\cdot}526$ for 1 D.F.) and the families agree in showing good single-factor segregations. A recombination percentage of $1{\cdot}68 \pm 0{\cdot}62$ was estimated from the combined backcross data by the method of maximum likelihood.

## 6. The series of alleles at locus *B*

*Alleles b and* $B^a$. In plants homozygous for the bottom recessive *b*, the seeds obtained by suitable crosses are without spots, the eyebrows of white tubers are uncoloured and there is no concentration of anthocyanin either on the nodes of the haulm or on the floral abscission layer.

$B^a$ produces a coloured floral abscission layer only and does so in the colour genotypes *P–R–* and *ppR–*. Its expressivity in genotypes $P\text{–}R^{pw}R^{pw}$ and $ppR^{pw}R^{pw}$ has not yet been determined.

These alleles were recognized in C.P.C. 979 which has blue tubers and flowers. Sometimes this clone selfs; its seeds are without spots. One such family of 103 plants gave 79 plants with self-coloured tubers (63 blue- and 16 red-coloured) and 24 plants with white tubers. The majority of the former seemed to have coloured floral abscission layers, but the condition of some was masked owing to the intense self-colouring of the haulms. All

Table 5. *Linkage between flecked flower and tuber colour (all clones of* Solanum Rybinii)

| Reference | Parental crosses | | | Self coloured tubers: Flowers: Self — Blue | Self coloured tubers: Flowers: Self — Red | Self coloured tubers: Flowers: Flecked — Blue | Self coloured tubers: Flowers: Flecked — Red | White tubers: Flowers: Self — Blue | White tubers: Flowers: Self — Red | White tubers: Flowers: Flecked — Blue | White tubers: Flowers: Flecked — Red | Progeny total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D. 51/114 | C.P.C. 979<br>blue flower, blue tuber | × | C.P.C. 1311<br>flecked flower, white tuber | 32 | 20 | — | — | 20 | 24 | — | — | 96 |
| D. 51/119 | C.P.C. 1311<br>flecked flower, white tuber | × | C.P.C. 2211<br>white flower, pink tuber | — | 83 | — | — | — | — | — | — | 83 |
| D. 51/121* | C.P.C. 2202<br>red flower, white tuber | × | C.P.C. 2203<br>blue flower, blue tuber | 15 | 20 | — | — | 8 | 20 | — | — | 63 |
| D. 51/123* | C.P.C. 2205<br>red flower, white tuber | × | C.P.C. 2203<br>blue flower, blue tuber | 30 | 14 | — | — | 11 | 10 | — | — | 65 |
| | Backcrosses | | | | | | | | | | | |
| | In coupling | | | | | | | | | | | |
| D. 53/41 | D. 51/114/16<br>*PpRR(FI)(fi)* | × | C.P.C. 1311<br>*ppRR(fi)(fi)* | 16 | 15 | — | — | — | — | 19 | 16 | 66 |
| D. 53/40 | D. 51/114/33<br>*ppRR(FI)(fi)* | × | C.P.C. 1311<br>*ppRR(fi)(fi)* | — | 20 | — | — | — | 1 | — | 20 | 41 |
| D. 53/42 | C.P.C. 1311<br>*ppRR(fi)(fi)* | × | D. 51/114/56<br>*PpRR(FI)(fi)* | 25 | 19 | 2 | 1 | — | — | 15 | 27 | 89 |
| D. 53/61 | D. 51/119/73<br>*ppRR$^{pw}$(FI)(fi)* | × | C.P.C. 1311<br>*ppRR(fi)(fi)* | — | 39 | — | — | — | — | — | 34 | 73 |
| D. 52/254 | D. 51/119/80<br>*ppRR$^{pw}$(FI)(fi)* | × | C.P.C. 1311<br>*ppRR(fi)(fi)* | — | 16 | — | 1 | — | 1 | — | 18 | 36 |
| | In repulsion | | | | | | | | | | | |
| D. 52/253 | C.P.C. 1311<br>*ppRR(fi)(fi)* | × | D. 51/121/21<br>*ppRR(fI)(Fi)* | — | 1 | — | 17 | — | 16 | — | — | 34 |
| D. 52/270 | D. 51/123/7<br>*ppRR(fI)(Fi)* | × | C.P.C. 1311<br>*ppRR(fi)(fi)* | — | — | — | 26 | — | 16 | — | — | 42 |
| D. 52/271 | D. 51/123/11<br>*PpRR(fI)(Fi)* | × | C.P.C. 1311<br>*ppRR(fi)(fi)* | — | — | 6 | 9 | 8 | 14 | — | — | 37 |

* Spectacle types in $F_1$ classed as self-coloured.

the plants with white tubers had uncoloured abscission layers. Neither nodal band nor coloured eyebrow appeared in the progeny.

In C.P.C. 979, linkage between loci *B* and *I* appears to be complete and in the coupling phase. The clone is $PpRR\,\frac{B^a\,I}{b\,i}$.

*Allele* $B^c$. This allele occurs in C.P.C. 827 and C.P.C. 836. It produces spots on seeds only in the presence of *P* and the spots are blue. It produces also pigmented eyebrows and coloured floral abscission layers and these effects are expressed in all three colour genotypes, namely, *P–R–*, *ppR–* and $ppR^{pw}R^{pw}$. The eyebrows of the tuber may be seen to be purple, red and pink, respectively.

Table 6. *Backcross data on the linkage of f and i*

| | Coupling phase | | Repulsion phase | |
|---|---|---|---|---|
| | *F* | *f* | *F* | *f* |
| *I* | 150 | 4 | 1 | 58 |
| *i* | 2 | 149 | 54 | 0 |

Both C.P.C. 979 $\left(PpRR\,\frac{B^a\,IF}{b\,iF}\right)$ and C.P.C. 827 $\left(PpRR\,\frac{B^c\,iF}{b\,If}\right)$ have blue tubers with blue sprouts and flowers. The cross between them demonstrates the presence of $B^c$, and shows close linkage between the two loci *B* and *I*. It does not allow an estimate of recombination.

With C.P.C. 979 as the female parent, 595 seeds were separable into 206 with blue spots and 389 without spots. In the reciprocal cross, 364 seeds gave 147 with blue spots and 217 without spots. Both these ratios are in close agreement with a 3 : 5 ratio ($\chi^2$ for pooled date = 0·20 for 1 D.F.; $P = 0{\cdot}6$). This is the expected ratio if both parents are heterozygous for *P*, but only one, namely C.P.C. 827, is heterozygous for an allele producing spots.

Fifty blue-spotted seeds and 50 seeds without spots were sown and the colours of the tubers and flowers of the plants they gave were recorded as follows:

| Seeds | Numbers: Sown | Numbers: Yielding | | Flowers: Blue | Flowers: Red | $\chi^2$ (to 1 : 1) | D.F. | *P* |
|---|---|---|---|---|---|---|---|---|
| Blue spot | 50 | 40 | Tubers Blue | 24 | 0 | 1·60 (to 6 : 3 : 1) | 1 | 0·20 |
| | | | Tubers White | 16 | 0 | | | |
| No spot | 50 | 43 | Tubers Blue | 28 | 0 | 0·64 | 2 | 0·7 |
| | | | Tubers Red | 0 | 12 | | | |
| | | | Tubers White | 0 | 3 | | | |

If the assumption is made that C.P.C. 827 is doubly heterozygous, with $B^c$ and *I* in the repulsion phase, then the observations agree with expectation. Purple-spotted seeds of the $F_1$ hybrid would be expected to give equal numbers of plants with either blue or white tubers but all with blue flowers. And seeds without spots would be expected to give plants with blue flowers and blue tubers, red flowers and red tubers and white tubers and red flowers in a 6 : 3 : 1 ratio.

All plants with white tubers, whether they came from spotted or non-spotted seeds, had coloured eyebrows. Apparently $B^c$ can produce a spot only in the presence of *P*, whereas the coloured eyebrow appears in both colour genotypes, *P–R–*$B^c$– and *ppR–*$B^c$–.

C.P.C. 836 $\left(ppRR^{pw}\ \frac{b\ if}{B^c\ iF}\right)$ has red flowers and white tubers with red eyebrows, a colour which shows the absence of *P*. Using C.P.C. 979 as a female parent, out of 343 seeds, 83 had blue spots and 260 were without spots. In the reciprocal, 11 out of 42 seeds were spotted. The combined segregations agree with a 1 : 3 ratio ($\chi^2 = 0{\cdot}70$ for 1 D.F.; $P = 0{\cdot}80$).

Once again, some of each of the two classes of seeds were sown separately and the colours of the flowers and tubers of the siblings were recorded:

| Seeds | Numbers: Sown | Numbers: Yielding | | Flowers: Blue | Flowers: Red | $\chi^2$ (to 1:1) | D.F. | *P* |
|---|---|---|---|---|---|---|---|---|
| Blue spot | 40 | 39 | Tubers: Blue | 23 | 0 | 1·26 (to 1:2:1:2) | 1 | 0·25 |
| | | | Tubers: White | 16 | 0 | | | |
| No spot | 80 | 77 | Tubers: Blue | 18 | 0 | 5·17 | 3 | 0·16 |
| | | | Tubers: Red | 0 | 19 | | | |
| | | | Tubers: White | 10 | 30 | | | |

All white tubers of plants raised from spotted seeds had blue eyebrows and the floral abscission layers were blue. Of the plants with white tubers raised from non-spotted seeds, the ten with blue flowers had no eyebrow colour and their floral abscission layers were not pigmented; of the 30 with red flowers, 17 had red eyebrows and coloured floral abscission layers and 13 had uncoloured eyebrows and non-pigmented floral abscission layers. In the non-spotted category, this is satisfactory agreement with an expected 1 : 1 : 1 ratio for these classes. The overall segregations agree with expectation on the assumptions that C.P.C. 836 is heterozygous at the *B* locus being $B^cb$ and a homozygous recessive *ii* at locus *I*. The two loci are completely linked.

*Allele* $B^d$. The source of this allele is C.P.C. 2211 $\left(ppR^{pw}R^{pw}\ \frac{B^d\ IF}{b\ IF}\right)$. This clone has pink tubers and white flowers. It is without a band of pigment at the base of the petiole, although it does carry this factor, the expression of which is absent in plants homozygous for $R^{pw}$. C.P.C. 2211 has a coloured floral abscission layer.

Using C.P.C. 979 as the female parent in a cross with C.P.C. 2211, 97 seeds gave 26 with purple spots, 21 with red spots and 50 without spots. In the reciprocal cross, the corresponding counts were 235, 181 and 411 seeds, respectively. Purple spots are easily seen with a hand lens, but red ones are distinguishable only with the help of a low-power binocular microscope, and even then some red-spotted seeds may be incorrectly sorted and scored as being without this character. Purple spots, red spots and no spots would be expected in a 1 : 1 : 2 ratio if it be assumed that C.P.C. 2211 is heterozygous for a dominant allele, $B^d$, which produces a blue spot only when *P* is present, that is, in genotypes *P*–*R*– and *P*–$R^{pw}R^{pw}$, a red one when *R* is present in the absence of *P*, genotype *ppR*–, and no spots in genotypes homozygous for $R^{pw}$ in the absence of *P*. As *P* and *R* control derivatives of petunidin and pelargonidin, respectively, this means that in appropriate crosses, spotted seeds may be of two kinds, one deep purple, the pigment being a derivative of petunidin (the presence of *P*) and the other red, the pigment being a derivative of pelargonidin (*R* in the absence of *P*). The data are homogeneous and the pooled totals give $\chi^2 = 7{\cdot}54$ for 2 D.F.; $P = 0{\cdot}02$ (Table 7). There is a slight deviation from the hypothetical ratio owing to an excess of purple-spotted seeds in each family. It is shown below, however, that red spots are probably underscored by about 6·6 %. With

this as a correction factor, $\chi^2 = 5{\cdot}85$ for 2 D.F.; $P = 0{\cdot}05$ and agreement with expectation is reasonable. Backcross progenies to C.P.C. 2211 were used to test expectations. Three out of four families were satisfactory when tested against a 6 : 3 : 7 ratio, and the fourth gave a reasonable fit when the segregation was adjusted by the correction factor. One family fitted neither of the two expected ratios, namely 6 : 3 : 7 and 2 : 1 : 5 (Table 7).

Table 7. *Segregation of seed spot in the cross D. 53/43 = C.P.C. 979 × C.P.C. 2211*

| | Spot | | | No. of families | Deviation | | | Heterogeneity | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Purple | Pink | None | | $\chi^2$ | D.F. | $P$ | $\chi^2$ | D.F. | $P$ |
| $F_1$ progeny D. 53/43 and reciprocal | 261 | 202 | 461 | 3 | (to 1:1:2) 7·54 | 2 | 0·26 | 0·70 | 4 | 0·95 |
| Backcrosses to C.P.C. 2211 | | | | | (to 6:3:7) | | | | | |
| D. 53/43/10 | 197 | 84 | 219 | 5 | 1·50 | 2 | 0·46 | 5·10 | 8 | 0·74 |
| D. 53/43/13 | 172 | 104 | 224 | 5 | 2·53 | 2 | 0·27 | 13·00 | 8 | 0·11 |
| D. 53/43/16* | 180 | 71 | 249 | 5 | 10·00 | 2 | 0·007 | 3·14 | 8 | 0·92 |
| D. 53/43/44 | 180 | 94 | 226 | 5 | 0·50 | 2 | 0·75 | 8·90 | 8 | 0·35 |
| D. 53/43/30* | 423 | 166 | 875 | 2 | (to 2:1:5) 12·20 | 2 | 0·0025 | 1·04 | 2 | 0·50 |

* Adjusted segregations.

As a check upon the supposition that the two kinds of spots entirely depend upon the status of the $P$ and $R$ loci, small backcross progenies of two $F_1$ siblings to C.P.C. 2211 were grown from seed which had been separated previously into the three classes blue spot, red spot and no spot.

The first of these progenies (D. 54/09) gave the following result:

| | Numbers | | Flowers | | | |
|---|---|---|---|---|---|---|
| Seeds | Sown | Yielding | Blue | Pale blue | Red | White |
| Blue spot | 19 | 15 | 7 | 8 | 0 | 0 |
| Red spot | 12 | 12 | 0 | 0 | 12 | 0 |
| No spot | 30 | 26 | 4 | 2 | 4 | 16 |

The segregation of blue- and red-spotted seeds to non-spotted seeds tested to a 6 : 3 : 7 gives $\chi^2 = 1{\cdot}09$, 2 D.F.; $P = 0{\cdot}58$. Blue-spotted seeds gave plants with blue and pale blue flowers, red-spotted seeds gave plants with red flowers; and seeds without spots gave plants with flowers of four colour classes—blue, pale blue, red and white. All tubers were self-coloured and coincided with expectations from the floral colour. Tubers of blue- and pale blue-flowered plants were visually the same and were blue. Red-flowered plants had red tubers and white-flowered plants had pink tubers. The results are those anticipated from a cross of the kind $PpRR^{pw}\frac{B^d\ I}{b\ \ i} \times ppR^{pw}R^{pw}\frac{B^d\ I}{b\ \ I}$, where the relations between genotypes and phenotypes are those given in Table 8.

The second progeny (D. 54/10) was as follows:

| | Numbers | | Flowers | | | |
|---|---|---|---|---|---|---|
| Seeds | Sown | Yielding | Blue | Pale blue | Red | White |
| Blue spot | 18 | 15 | 10 | 5 | 0 | 0 |
| Red spot | 7 | 7 | 0 | 0 | 7 | 0 |
| No spot | 35 | 27 | 3 | 9 | 7 | 8 |

The segregation for blue- and red-spotted seeds and non-spotted seeds tested to a 2 : 1 : 5 gives $\chi^2 = 0{\cdot}80$, 2 D.F.; $P = 0{\cdot}65$.

Presumably the cross is $PpRR^{pw}\frac{b\ IF}{b\ iF} \times PpR^{pw}R^{pw}\frac{B^d\ IF}{b\ IF}$, in which genotypes and their corresponding phenotypes are as given in Table 8.

Table 8. *Genotypes and corresponding phenotypes in D.54/09 and D.54/10*

| Genotype | Flower colour | Pigment: Spot on embryo | Pigment: Nodal band | Pigment: Floral abscission |
|---|---|---|---|---|
| $PpRR^{pw}B^d$– | Blue | + | + | + |
| $PpR^{pw}R^{pw}B^d$– | Pale blue | + | + | + |
| $ppRR^{pw}B^d$– | Red | + | + | + |
| $ppR^{pw}R^{pw}B^d$– | White | – | – | + |
| $PpRR^{pw}bb$ | Blue | – | – | – |
| $PpR^{pw}R^{pw}bb$ | Pale blue | – | – | – |
| $ppRR^{pw}bb$ | Red | – | – | – |
| $ppR^{pw}R^{pw}bb$ | White | – | – | – |

All plants raised from spotted seeds had bands of pigment at the bases of the petioles. The allele $B^d$ produces this effect only in the presence of $P$ or $R$ with the result that in plants coloured only by a derivative of peonidin, that is, homozygous $R^{pw}$ in the absence of $P$, this effect is not shown. But these plants have a coloured floral abscission layer.

The nodal band of pigment produced on the haulm by $B^d$ offers a ready means of distinguishing misclassifications in progenies from spots and non-spots. By this method, for example, four plants with nodal band and red flowers were transferred from the non-spotted class to the red-spot class in families D.54/09 and D.54/10; two from each total of 30 and 35 seeds. These corrections, together with others from unpublished data, have given an estimate of 6·6 % as a measure of the frequency with which red spots are missed in the initial separation of seed.

The allele $B^d$ seems also to control the pigmentation of the abscission layer of the flower and to be completely linked with allele $I$. The $F_1$ of the cross was not scored with regard to presence of coloured floral abscission layer. However, of the 16 white-flowered plants raised from seeds without spots in D.54/09, three had uncoloured abscission layers and, when tested to C.P.C.979 as a source of $P$, the seeds were without spots. The plants evidently were $\frac{b\ I}{b\ i}$. The remainder had coloured abscission layers and were either homo- or heterozygous for $B^d$ when tested similarly. This agrees with the expected 3 : 1 ratio of plants with and without $B^d$.

In D.54/10 there were eight white-flowered plants from non-spotted seeds. Four of these had coloured floral abscission layers and were either homo- or heterozygous for $B^d$ as judged by the occurrence of spotted seeds in suitable test crosses. The other four plants had uncoloured abscission layers, and no spotted seeds occurred in their progeny when suitably crossed with test plants carrying $P$ or $R$. They were evidently homozygous recessives $bb$. The expected segregation for $B^d$ was 1 : 1.

As yet we have not been able to determine whether a coloured eyebrow would develop in an otherwise unpigmented tuber in the presence of $B^d$. This is because $B^d$ and $I$ are completely linked in C.P.C.2211 so that all tubers from spotted seeds are invariably self-coloured and the effect of $B^d$ on eyebrow is masked.

### 7. Linkage between the three loci *B*, *I* and *F*

We have demonstrated separately linkage between loci *I* and *F* and between loci *I* and *B*. The close linkage of the three loci is further confirmed by a consideration of crosses in which joint segregation occurs. Suitable genotypes for giving a reliable estimate of its intensity are not yet available. C.P.C. 827 $\left(PpRR\,\frac{B^c\ iF}{b\ If}\right)$ is a triple heterozygote, whereas C.P.C. 836 $\left(ppRR^{pw}\,\frac{b\ \ if}{B^c\ iF}\right)$ is a double heterozygote, being homozygous *ii*. The cross offers a means of showing the close linkage between the three loci but is not informative for an estimation of recombination between them.

With C.P.C. 827 as the female parent, 174 seeds gave 69 spotted and 105 without spots. In the reciprocal, out of 50 seeds, 17 showed a blue spot and 33 were without it. The combined totals, 86 spotted : 143 non-spotted, are in close agreement with an expected 3 : 5 ratio ($\chi^2=0{\cdot}0002$ for 1 d.f.; $P=0{\cdot}99$).

As in the other crosses, seeds of both categories were sown and the plants scored for tuber and flower colour as follows:

| Seeds | Numbers: Sown | Numbers: Yielding | | Flowers: Blue | Flowers: Red | Flowers: Flecked | $\chi^2$ | d.f. | *P* |
|---|---|---|---|---|---|---|---|---|---|
| Blue spot | 38 | 37 | Tubers Blue | 12 | 0 | 0 | 0·013 (to 1:1:2) | 1 | 0·90 |
| | | | Tubers White | 25 | 0 | 0 | | | |
| No spot | 100 | 67 | Tubers Blue | 0 | 0 | 15 | 2·74 (to 1:1:1:2) | 3 | 0·44 |
| | | | Tubers Red | 0 | 15 | 8 | | | |
| | | | Tubers White | 0 | 29 | 0 | | | |

The results agree with those expected on the basis of the genotypes assigned to the parents. Flecked flowers only occurred in association with self-coloured tubers. All white tubers had coloured eyebrows.

*The cross* C.P.C. 827 $\left(PpRR\,\frac{B^c\ iF}{b\ If}\right)\times$ C.P.C. 1776 $\left(ppR^{pw}R^{pw}\,\frac{b\ if}{b\ if}\right)$

C.P.C. 1776 has white tubers with pink sprouts coloured by an acylated derivative of peonidin. The flowers are white.

Using C.P.C. 827 as the female parent, in 1951, 1953 and 1954 crosses between these two clones gave 55:291, 103:464 and 62:380 spotted and non-spotted seeds respectively; totals of 220 to 1135. Data for the reciprocal cross are only available for 1951 when 257 seeds gave 39 with spots and 257 without spots. The data are homogeneous (heterogeneity $\chi^2=3{\cdot}5$ for 3 d.f.; $P=0{\cdot}33$), but all the ratios deviate significantly from 1 : 3. It is not known why this occurs. Seeds were sown, 50 spotted and 50 non-spotted, and distribution of colour was recorded. Germination was poor and not all plants yielded tubers. The results were as follows:

| Seeds | Numbers: Sown | Numbers: Germinating | Numbers: Yielding | | Flowers: Blue | Flowers: Red | Flowers: Flecked | $\chi^2$ | d.f. | *P* |
|---|---|---|---|---|---|---|---|---|---|---|
| Blue spot | 50 | 26 | 19 | Tubers Blue | 0 | 0 | 1 | (to 1:1:1) | | |
| | | | | Tubers White | 18 | 0 | 0 | | | |
| No spot | 50 | 37 | 16 | Tubers Blue | 0 | 0 | 5 | 3·60 | 2 | 0·18 |
| | | | | Tubers Red | 0 | 0 | 2 | | | |
| | | | | Tubers White | 0 | 8 | 1 | | | |

These data agree with the assumptions that the three loci are closely linked and that parental genotypes are as given. All white tubers, whether from spotted or non-spotted seeds, had coloured eyebrows.

It will be seen that two apparent recombinants occur. One of these, $-(a)$, a plant with white tubers and red eyebrows and flecked flowers, raised from a non-spotted seed, must have resulted from a crossover between loci $I$ and $F$ which, as mentioned above, have $1{\cdot}68 \pm 0{\cdot}62\%$ recombination. That this individual is heterozygous $B^c b$ may be inferred from the presence of coloured eyebrows on the tuber.

The other recombinant, $-(b)$, is a plant with blue tubers and flecked flowers raised from a spotted seed. That it is indeed heterozygous $B^c b$ and not a misclassified non-spot is shown by its breeding behaviour in a backcross to C.P.C.1776. Expectation is a 1 : 3 ratio of spotted to non-spotted because the spotting effect of locus $B$ is expressed only in the presence of allele $P$ for which C.P.C.1776 is homozygous recessive. Out of 255 seeds, 60 had purple spots and 195 were without spots ($\chi^2$ to a 1 : 3 = 0·29 for 1 D.F.; $P = 0{\cdot}60$). The occurrence of this plant shows that the three loci are arranged in the chromosome in the sequence $B$–$I$–$F$.

A notable feature of recombinant $(b)$ is that pigment occurs on the bases of the petioles.

Thus it appears that when allele $B^c$ is brought into contiguity with dominant allele $I$, the characteristic banding effect of allele $B^d$ is developed (Table 1). Allele $B^d$ occurs in only one clone of our collection, namely, C.P.C.2211, of which the genotype is $ppR^{pw}R^{pw}\dfrac{B^d\ IF}{b\ \ IF}$. Here also the two dominant alleles are adjacent; and until they are separated by recombination it cannot be decided whether $B^d$ is merely the compound $B^cI$ or an allele in its own right.

## 8. The origin of allele $B^b$

Each $B$ allele with its manifold effects behaves as a unit in breeding and is usually stable. Nevertheless, we have observed one individual with attributes not present in its parents. This plant occurred in an $F_1$ progeny of the cross C.P.C.979 $\left(PpRR\dfrac{B^a\ IF}{b\ iF}\right) \times$ C.P.C.2205 $\left(ppRR\dfrac{B^a\ iF}{b\ iF}\right)$. The genotype of the male parent was established from a family of 45 siblings raised from seed from a selfed berry. All were white-tubered with uncoloured eyebrows, but plants with and without coloured abscission layer occurred.

The anomalous plant in the $F_1$ progeny had white tubers with blue eyebrows. The haulm was without pigment at the bases of the petioles, but the floral abscission layer was pigmented. This mutation has behaved as a stable unit and is distinguished, therefore, as allele $B^b$. The allele has not yet been encountered in the collection of clones.

## 9. Discussion

The manifold effects of locus $B$ on the distribution of pigment are not a simple illustration of pleiotropism if this term be restricted to examples where a single gene influences many apparently unrelated characters, thus indicating the possibility of multiple gene action (Tulpule, 1954). Eyebrow on tuber, spot on hypocotyl and band of pigment at the base of the petiole are at homologous locations on the plant body; they are related characters.

It seems reasonable to regard them as arising from the actions of a number of closely linked subunits. Each action is expressed at an homologous position on the plant body and jointly they reflect intragenic differentiation.

This genetic situation is similar in many respects to others concerned with distribution of anthocyanin. In rice, several distinct patterns involving the presence of colour in different organs occur constantly in varietal collections and breed true generally in crossing experiments. But aberrant types do occur occasionally in hybrid progenies. They are not satisfactorily explained as simple crossovers (Ramiah, 1945). Anthocyanin pigmentation in the Asiatic cottons is determined by an extensive series of multiple alleles with pleiotropic effect upon several organs of the plant. Anomalous segregates which rarely occur can only be adequately explained as having arisen by the recombination of parental attributes (Yu & Chang, 1948). Loci *A* and *B* in maize are known to be of a compound nature (Laughnan, 1949; Stadler, 1951). Thus it appears that in diverse families, the inheritance of the distribution of anthocyanin may be controlled by a compound gene.

The super-gene inferred here is usually stable, but the origin of allele $B^b$ in our experimental cultures demonstrates capacity for independent change in each component. One might suppose that complete inhibition of pigment in the tuber is achieved by two adjacent genic elements *te*. Acquisition of coloured eyebrow, that is, loss of suppressive element *e*, could then occur as a result of an unequal crossover similar to that suggested to account for mutants in $R^r/R^g$ progenies of maize (Stadler & Emmerling, 1954). However, as this rather implies that the dominant eyebrow effect is merely the loss of an element, a more attractive hypothesis from a formal genetical viewpoint is that true gene mutation is involved. Changes of individual components must have occurred during the evolutionary history of the super-gene and thus given rise to the series of alleles that can now be demonstrated in a collection of cultivated diploid potatoes.

Two levels of differentiation occur in the anthocyanin complex discussed here. At the first level are components of locus *B* which may be regarded as local modifications of a determinant. They are not as physically isolated from each other as are *B*, *I* and *F* which are on the second level. Crossovers between these three loci are sufficiently frequent to show that they are intergenic recombinations; that, in fact, the genetic novelty of the situation is that the three loci concerned with the distribution of pigment are close together on the same chromosome.

Sheppard (1953) points out that in a large proportion of animal species with extreme polymorphism, more of the genes responsible for the polymorphism are closely linked than would be expected by chance. It is suggested that this linkage has been evolved by the selective survival of translocations which move these genes onto the same chromosome. The chromosomal rearrangements have a survival advantage because of the selective value of their gene combinations. It is unlikely that any survival value would be gained in a domestic plant, propagated vegetatively, by an accumulation on one chromosome of genes controlling distribution of pigment. The more likely inference is that the loci are in their original position.

Lindegren & Lindegren (1952) suggest that the proximity of four genes controlling the fermentation of similar carbohydrates in *Saccharomyces* leads to the conclusion that they have a common origin and their number has been increased by unequal crossing-over and mutation of the 'extra' gene. As Laughnan (1952) observes, there is increasing evidence

for the widespread occurrence of closely linked genes with similar effects. Their proximity is not fortuitous but compatible with an origin through intrachromosomal duplication.

Apart from a bearing on mode of origin, it seems not unlikely that the closeness of loci *B*, *I* and *F* has functional value. This is implied by the observation that $B^c$ and *I* when adjacent give the banding effect of $B^d$. The example is similar to those interactions in *Drosophila* in which the heterozygotes represented as $\frac{+_1+_2}{m_1m_2}$ and $\frac{+_1m_2}{m_1+_2}$ (*m* designates the mutant form) are not equivalent in action (Lewis, 1948; Green & Green, 1949). The difference is understandable if a reaction sequence between adjacent elements is assumed. In like manner, $R^r$ in maize includes two components which, although structurally distinct, nevertheless are parts of a functional complex (Stadler, 1951; Stadler & Nuffer, 1953). Biochemical study of the anthocyanin pseudo-allelic series in *Gossypium* has suggested a close functional relationship in the action of adjacent loci (Stephens, 1948).

The closeness of these loci evidently facilitates intergenic co-operation either in reaction or timing sequences of the development of anthocyanins and the production of pattern effects.

## 10. Summary

1. Three loci, *B*, *I* and *F*, concerned with the distribution of anthocyanins in diploid cultivated potatoes, are identified. Alleles $B^d > B^c > B^b > B^a > b$, in order of dominance, are distinguished.

$B^d$ produces in the seed a band of pigment on the node of the hypocotyl of the embryo and, in the mature plant, a band of pigment at each node, a coloured floral abscission layer and a coloured eyebrow on the tuber.

With $B^c$, a band of pigment at each node of the mature plant is absent, but other effects are like those of $B^d$.

$B^b$ gives a coloured floral abscission layer and a coloured eyebrow on the tuber. With $B^a$ only the floral abscission layer is coloured.

Locus *I* in its homozygous recessive phase, *ii*, inhibits pigment in the tuber.

Locus *F* in its homozygous recessive phase, *ff*, gives flecked flowers.

2. The three loci are closely linked and probably in the order *B*–*I*–*F*.

3. $B^c$ and *I*, in coupling, give a positional effect so that the characteristic nodal bands of $B^d$ occur. Locus *B* appears to be a super-gene.

4. The suggestion is made that the proximity of these loci is original and functional. It may facilitate intergenic co-operation in the distribution of pigment.

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