# THE INHERITANCE OF COLOUR IN DIPLOID POTATOES

## I. TYPES OF ANTHOCYANIDINS AND THEIR GENETIC LOCI

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(With One Text-figure)

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This investigation arose out of a general programme of inquiry into the genetic constitution of diploid ($2n = 24$) cultivated potatoes of South America.

The coloured plants of these diploids may be blue-purple or red, and there is a close correlation between the colours of the various organs of the individual plant; for example, a blue-purple tuber usually gives blue-purple sprouts, haulm and flowers, and a red tuber similarly gives red plant organs. No previous work has been done on the inheritance of these correlations in cultivated diploids, but several workers have studied them in domestic tetraploid potatoes (Swaminathan & Howard, 1953). Sirks (1929), Asseeva & Nikolaeva (1935) and Lunden (1937) agree to the extent of postulating two independent loci concerned with blue-purple and red-purple pigmentation, respectively, and Asseeva & Nikolaeva conclude that the colours only develop in tuber and flower in the presence of complementary factors specific to the two organs. Nevertheless, the situation is confused and it is evident that only combined genetical and biochemical investigations are likely to give a clearer understanding of the differences between colour classes.

In this paper, types of anthocyanidins in tuberous Solanums are identified. It is felt that an account is justified at this stage in order to place these on record and to enable particular facets of the problem of the inheritance of colour to be dealt with in future publications. Meanwhile breeding data, though not as extensive as could be desired, do clearly show that type of pigmentation in cultivated diploids is controlled by at least two independent loci. The activity of one locus is recognizable by the production of the same anthocyanidin in any organ of the plant, but the activity of the other locus leads to different anthocyanidin types in flower and tuber.

### The various colour classes

The Colombian diploid species *Solanum Rybinii* Juz. & Buk. ($2n = 24$) has been used mainly in this investigation, but two other diploid species, namely, *S. stenotomum* Juz. & Buk. and *S. Cardenasii* Hawkes have been used also in the crosses. These species are highly cross-fertile, and, indeed, no genetical evidence has yet been obtained in the experimental breeding which favours their delineation as species.

The phenotypes were as follows:

(1) Self-blue tubers, blue sprouts and blue flowers.

(2) Self-red tubers, red sprouts and red flowers.

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(3) White tubers, blue sprouts and blue flowers.
(4) White tubers, red sprouts and red flowers.
(5) Pink tubers, pink sprouts and white flowers.

No completely acyanic plants have been observed in cultivated diploids. The closest approach to a 'recessive white' is a plant with an unpigmented tuber and white flowers. This combination has been obtained as a genetic segregant, but invariably such an individual has coloured sprouts. The general impression is given that all individuals in the group are basically cyanic, but that acyanic organs may occur owing to the localized action of inhibitors for colour or, *mutatis mutandis*, the absence of localized developers for colour.

It was soon found that visual scoring for colour was unsatisfactory. Sometimes the difficulty was in deciding whether a flower should be classed as blue or red; at other times, the internal correlation seemed to be broken; for example, red flowers were scored on blue tubers. These problems were overcome by using chromatograms of the extracted pigments to supplement visual scoring.

Table 1. *Segregation for blue, red and white tubers in progenies from blue-tubered plants of* Solanum Rybinii

| | | | | White tubers | | | | |
|---|---|---|---|---|---|---|---|---|
| Ref. | Cross | Blue tubers sprouts flowers | Red tubers sprouts flowers | Blue sprouts flowers | Red sprouts flowers | $\chi^2$ (to 9 : 3 : 3 : 1) | D.F. | $P$ |
| D53/80 | C.P.C. 979 × C.P.C.2352 | 63 | 16 | 19 | 5 | 1·34 | 3 | 0·74 |
| D49/01 | C.P.C. 979 selfed | 54 | 24 | 14 | 4 | 7·26 | 3 | 0·03 |
| D53/148 | C.P.C. 979 selfed | 25 | 4 | 4 | 1 | — | — | — |
| D53/152 | C.P.C. 979 selfed | 33 | 8 | 1 | 1 | — | — | — |

## The genetic basis of the colour classes

Two clones of *S. Rybinii*, C.P.C. 979 and C.P.C. 2352, both with self-blue tubers, sprouts and flowers, were intercrossed and the progeny is recorded in Table 1. Despite the general occurrence of self-incompatibility in cultivated diploids, C.P.C. 979 not infrequently sets berries with viable seeds when artificially selfed. Three progenies from such seeds have been raised and their segregations for tuber, sprout and flower colour are given also in Table 1.

It will be seen that blue-, red- and white-tubered forms occur in the progenies, and that in the cross C.P.C. 979 × C.P.C. 2352 the proportions of the three colours are in agreement with expectations on the basis of a 9 : 3 : 4 ratio. The three selfed families diverge from this expectation largely owing to a deficiency of white-tubered phenotypes, but the irregularities are reasonably attributable to the effects of inbreeding on gametic survival and have no bearing on the genetical interpretation of the data. At this stage of the inquiry, the segregation for colour is explained satisfactorily by postulating one pair of alleles $P : p$ governing blue as opposed to red colour throughout the plant and an independent pair $I : i$ concerned with the contrast between self-coloured and white tubers. $I$ may be regarded as an allele which in the homozygous recessive phase $ii$ is epistatic to $P$ and leads to the absence of pigment in the tuber, to which organ its action is localized.

C.P.C. 2211 with pink tubers and sprouts and white flowers was crossed with C.P.C. 979, the self-blue form, and the segregations for flower and tuber colours observed in the progeny are given in Table 2. The $F_1$ gives a good approximation to a 1 : 1 ratio, there being 125 self-blues and 113 self-reds. Confining attention to flower colours, it will be seen that an $F_1$ blue backcrossed to C.P.C. 2211 segregates four classes of flowers, blue, pale blue, red and white, in equal numbers, whereas an $F_1$ red when backcrossed to C.P.C. 2211 segregates red and white flowers only, in good agreement with a 1 : 1 ratio. The former behaviour indicates a bifactorial system for flower colour, and the latter points to red and white flowers being allelomorphic characters within the system.

Table 2. *The cross blue flower and tuber* (C.P.C. 979) *by white flower, and pink tuber* (C.P.C. 2211)

Flower-score and tuber-score are recorded for each family.

| | | Blue* | Pale blue | Red | White | Pink† | $\chi^2$ (to 1 : 1) | D.F. | *P* |
|---|---|---|---|---|---|---|---|---|---|
| C.P.C. 979 × C.P.C. 2211 | Flowers | 125 | 0 | 113 | 0 | — | 0·60 | 1 | 0·44 |
| | Tubers | 125 | — | 113 | 0 | 0 | | | |
| $F_1$ blue × C.P.C. 2211 | Flowers | 29 | 23 | 22 | 31 | — | 2·24 (to 1 : 1 : 1 : 1) | 3 | 0·55 |
| | Tubers | 52 | — | 22 | 0 | 31 | (to 1 : 1) | | |
| $F_1$ red × C.P.C. 2211 | Flowers | 0 | 0 | 64 | 49 | — | 1·99 | 1 | 0·16 |
| | Tubers | 0 | — | 64 | 0 | 49 | | | |

* The tubers of plants in the blue and pale blue flower classes are indistinguishable visually.
† Pink and red tubers are not always visibly distinct; classification was done by chromatography.

Additional data on the segregation of the flower colours blue, pale blue, red and white, kindly supplied by Dr J. G. Hawkes, are given in Table 4.

If *P* and *R* are used to designate the two loci concerned with production of colour, red- and white-flowered plants are homozygous recessives at locus *P*, which controls the production of blue colour. There is no evidence of linkage between the two loci.

Table 3. *Backcross families showing the association of white flowers with pink tubers*

| Cross | Genotypes | Red flowers and tubers | White flowers and pink tubers | $\chi^2$ | D.F. | *P* |
|---|---|---|---|---|---|---|
| C.P.C. 2205 × C.P.C. 2211 | *ppRRii* × *ppR*$^{pw}$*R*$^{pw}$*II* | 110 | 0 | — | — | — |
| $F_1$ × C.P.C. 2211 | *ppRR*$^{pw}$*Ii* × *ppR*$^{pw}$*R*$^{pw}$*II* | 232 | 221 | to 1 : 1 (6 families) 2·69 | 6 | 0·85 |

The tubers of blue and pale blue flower types are the same colour and cannot be distinguished visually, and this is partially true of the tubers of the red- and white-flowered types. The latter qualification is added because although pink and red tubers may be distinguished visually when freshly harvested, the distinction tends to be lost with age as the pigments develop deeper tones. Nevertheless, pink tubers can always be segregated by means of chromatographic analysis, and when this is done, these tubers are found invariably to give plants with white flowers (Table 2). More extensive data concerning this phenotypic association are given in Table 3, in which the results obtained by crossing C.P.C. 2205 with C.P.C. 2211 are summarized. The former is a clone of *S. Rybinii* with white tubers, red sprouts and red flowers. Although usually self-sterile,

it has once set seed by selfing and a small family (forty-eight plants) was raised. All the individuals resembled the parent phenotypically, so that C.P.C. 2205 is evidently homozygous both for its colour alleles and the 'inhibitor' of tuber colour. In the $F_1$ with C.P.C. 2211, all the progeny had red tubers and red flowers. On backcrossing to C.P.C. 2211, a 1 : 1 ratio for red and white flowers was obtained, and all white-flowered plants had pink tubers. These data are consistent with the view that C.P.C. 2211 is homozygous at the $R$ locus for an allele $R^{pw}$ which gives pink tubers and sprouts and white flowers in the presence of $p$. At this juncture, it is clearly impossible to decide whether the $R^{pw}$ effect results from an allele in its own right or from the action of two closely linked but independent genes, one concerned with the development of a pink pigment throughout the plant body and the other with a suppressive action localized in the flower.

The concept of three independent loci $P$, $R$ and $I$, concerned with colour and its distribution throughout the plant, has been adopted, although an alternative interpretation might be to assume a series of multiple alleles at the $R$ locus. With red dominant to pink and the presence of pigment in the tuber dominant to its absence, the scheme might be as follows:

| Allele | Tuber | Sprout | Flower |
|---|---|---|---|
| $R_1$ | Red | Red | Red |
| $R_2$ | White | Red | Red |
| $R_3$ | Pink | Pink | White |
| $R_4$ | White | Pink | White |

| | *pp* | | | *P-* | | |
|---|---|---|---|---|---|---|
| Genotype | Tuber | Sprout | Flower | Tuber | Sprout | Flower |
| $R_1R_2$ | Red | Red | Red | Blue | Blue | Blue |
| $R_1R_3$ | Red | Red | Red | Blue | Blue | Blue |
| $R_1R_4$ | Red | Red | Red | Blue | Blue | Blue |
| $R_2R_3$ | Red | Red | Red | Blue | Blue | Blue |
| $R_2R_4$ | White | Red | Red | White | Blue | Blue |
| $R_3R_4$ | Pink | Pink | White | Blue | Blue | Pale blue |

Considering the segregation of tuber colour in Table 4, it will be seen that, although numbers are low, white- and self-coloured tubers occur in each of the four classes of flowers in approximately equal numbers. No genotypes within the limits of the scheme can be allocated to the parents to give these observed joint segregations of flower and tuber colours. Variations of the scheme have been tried, for example, a different dominance relation between alleles, but none fits the data as well as the hypothesis that $P$, $R$ and $I$ are independent loci. Certain other tests have been set up, but, until these have been completed, the view is held that production of colour throughout a diploid potato plant, that is, in tuber, sprout, haulm and flower, is controlled by two independent loci, designated $P$ and $R$. Furthermore, an epistatic allele in the homozygous recessive phase *ii* leads to the absence of pigment in the tuber, to which organ its action is localized. Genotypes that may be assigned to the main phenotypic classes are as follows:

| Tuber | Sprout | Flower | Genotype |
|---|---|---|---|
| Blue | Blue | Blue | *P-R-I-* |
| Red | Red | Red | *ppR-I-* |
| White | Blue | Blue | *P-R-ii* |
| White | Red | Red | *ppR-ii* |
| Pink | Pink | White | *pp*$R^{pw}R^{pw}$*I-* |
| Blue | Blue | Pale blue | *P-*$R^{pw}R^{pw}$*I-* |

Table 4. *Segregation of four colour classes in crosses of cultivated diploids. Discrepancies between flower-score and tuber-score arise where plants flowered but failed to form tubers*

| | | | | Blue* | Pale blue | Red | White | Pink | $\chi^2$ | D.F. | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | (to 3 : 3 : 1 : 1) | | |
| *S. stenotomum* (C.P.C. 322) × | *S. stenotomum* (C.P.C. 1878) | Flowers | | *29* | *31* | *5* | *13* | — | 3·50 | 3 | 0·32 |
| *PpRR*$^{pw}$*Ii* | *PpR*$^{pw}$*R*$^{pw}$*ii* | Tubers: | Self-coloured | 12 | 22 | 3 | — | 8 | | | |
| Blue flower, blue sprout, | Pale blue flower, blue | | White | 10 | 7 | 2 | — | 4 | | | |
| blue tuber | sprout, white tuber | | Total | *22* | *29* | *5* | — | *12* | | | |
| | | | | | | | | | (to 3 : 1 : 3 : 1) | | |
| *S. stenotomum* (C.P.C. 1558) × | *S. Rybinii* (C.P.C. 2212) | Flowers | | *28* | *10* | *26* | *11* | — | 0·48 | 3 | 0·92 |
| *PpRR*$^{pw}$*Ii* | *ppRR*$^{pw}$*ii* | Tubers: | Self-coloured | 11 | 4 | 8 | — | 3 | | | |
| Blue flower, blue sprout, | Red flower, red sprout, | | White | 10 | 5 | 11 | — | 7 | | | |
| blue tuber | white tuber | | Total | *21* | *9* | *19* | — | *10* | | | |
| | | | | | | | | | (to 1 : 1 : 1 : 1) | | |
| *S. Cardenasii* (C.P.C. 1776) × | *S. stenotomum* (C.P.C. 1558) | Flowers | | *19* | *18* | *17* | *17* | — | 1·63 | 3 | 0·64 |
| *ppR*$^{pw}$*R*$^{pw}$*ii* | *PpRR*$^{pw}$*Ii* | Tubers: | Self-coloured | 6 | 6 | 8 | — | 6 | | | |
| White flower, pink sprout, | Blue flower, blue sprout, | | White | 12 | 8 | 4 | — | 8 | | | |
| white tuber | blue tuber | Total | | *18* | *14* | *12* | — | *14* | | | |

* Self-coloured tubers from plants with either blue or pale blue flowers are indistinguishable visually. They are separated in the table to show that self-coloured and white tubers occur in about equal numbers within each of these two classes of flowers.

Table 5. *Comparison between* $R_F$ *values of anthocyanidins from diploid potatoes and authentic marker anthocyanidins*

| Component (Fig. 1) | $R_F$ values in hydrochloric acid-acetic acid-water (3 : 30 : 10) Aglycone | | Marker anthocyanidin |
|---|---|---|---|
| — | — | 0·30 | Delphinidin (via *Delphinium Ajacis*) |
| 0·09 | 0·43 | 0·42 | Petunidin (synthetic) |
| 0·41 | 0·45 | 0·46 | Petunidin (via dark blue *Petunia*) |
| 0·27 | 0·50 | 0·50 | Cyanidin (synthetic) |
| | | | Cyanidin (via Corn Poppy) |
| — | — | 0·56 | Malvidin (synthetic) |
| — | — | 0·60 | Malvidin (via *Lythrum*) |
| 0·15 | 0·62 | 0·69 | Peonidin (synthetic) |
| 0·53 | 0·65 | | |
| 0·16 | 0·70 | 0·72 | Pelargonidin (via scarlet *Pelargonium*) |
| 0·60 | 0·70 | 0·73 | Pelargonidin (via Corn Poppy) |

### Chromatography of the pigments

In the foregoing section a tentative genetic basis for pigmentation has been proposed; in what follows, an attempt is made to determine the nature of the chemical differences between the colour classes.

Crude extracts of the pigments were made in 1% ethanolic hydrochloric acid and paper chromatograms were run in butanol-2N-hydrochloric acid (1 : 1).

The extracts from blue tubers and sprouts of C.P.C. 979 (*PpRR*) gave identical runs so far as pigments were concerned. There were four coloured components on each chromatogram, two being purple in visible light ($R_F$ values 0·09 and 0·41)* and the other two pink in visible light ($R_F$ values 0·15 and 0·53; Fig. 1, column 1). The two higher valued components were suspected of being acylated derivatives of the lower valued ones. To test this supposition, crude extracts were run on Whatman no. 3 paper and the strips containing the components $R_F = 0{\cdot}41$ and 0·53 were eluted with 1% aqueous hydrochloric acid. The purified extracts were then given a mild alkaline hydrolysis (Beale, Price & Scott-Moncrieff, 1940), made just acid and run in butanol-2N-hydrochloric acid. This treatment successfully removed the acyl radicals and the bulk of the pigment now ran as the components $R_F = 0{\cdot}09$ and 0·15. In crude extracts, the non-acylated components are in very low concentration and, indeed, the possibility cannot be ignored that they arise during the processes of extraction and concentration of the extract for use.

Crude extracts of red tubers and sprouts (*ppRR*) similarly gave identical runs for pigments. The two pink components ($R_F = 0{\cdot}15$ and $R_F = 0{\cdot}53$) were present together with two components $R_F = 0{\cdot}16$ and $R_F = 0{\cdot}60$, yellow and yellow-orange in visible light, respectively (Fig. 1, column 3). On testing, the yellow-orange component appeared to be an acylated derivative of the component $R_F = 0{\cdot}16$ and, again, the latter was present only in low concentration in the crude extract.

The pink tubers and sprouts of C.P.C. 2211 ($ppR^{pw}R^{pw}$) contained only the pink components $R_F = 0{\cdot}15$ and $R_F = 0{\cdot}53$, with the former present in very low concentration (Fig. 1, column 5).

Like blue tubers, chromatograms of blue flowers showed the presence in low concentration of the purple and pink non-acylated components $R_F = 0{\cdot}09$ and $R_F = 0{\cdot}15$ and in higher concentration, their acylated derivatives $R_F = 0{\cdot}41$ and $R_F = 0{\cdot}53$. But a new component, magenta in visible light and peculiar to flowers, was present also with an $R_F$ value = 0·27 (Fig. 1, column 2).

The chromatograms of the red flowers lacked the yellow and yellow-orange components observed in the chromatograms of corresponding tubers and sprouts. They showed the pink components $R_F = 0{\cdot}15$ and $R_F = 0{\cdot}53$ and the magenta component $R_F = 0{\cdot}27$, and in this respect were similar to the blue flowers, but lacked, of course, the purple components of the latter (Fig. 1, column 4).

### Identification of the anthocyanidins

Crude extracts of the pigments were developed on Whatman no. 3 paper and the pigments were then eluted with 1% aqueous hydrochloric acid. The non-acylated components with $R_F$ values of 0·09, 0·15, 0·16 and 0·27 (Fig. 1) were purified in this way and then subjected

* The $R_F$ values given here were determined from papers run at 20±0·5° C. with equilibration for 24 hr. (Bate-Smith, 1951).

to the qualitative colour tests with saturated sodium carbonate solution, solid sodium acetate, dilute caustic soda and ferric chloride listed by Scott-Moncrieff (1936). They gave characteristic colour reactions compatible with the view that they are glycosides of petunidin, peonidin, pelargonidin and cyanidin, respectively. As mentioned earlier, the first three are present in the living plant mainly, if not completely, as acylated derivatives—the components with $R_F$ values 0·41, 0·53 and 0·60 (Fig. 1).

Each of the four purified components was hydrolysed and the distribution of its aglycone chloride between an aqueous phase and benzene was determined. The volumes of benzene required before the pigments passed into the aqueous layer were as follows: purple 4·6 vol., pink 8·0 vol., yellow 11·0 vol. and magenta (flower) 6·0 vol. These values are in accord with the tentative identifications given above as a result of the qualitative colour tests.

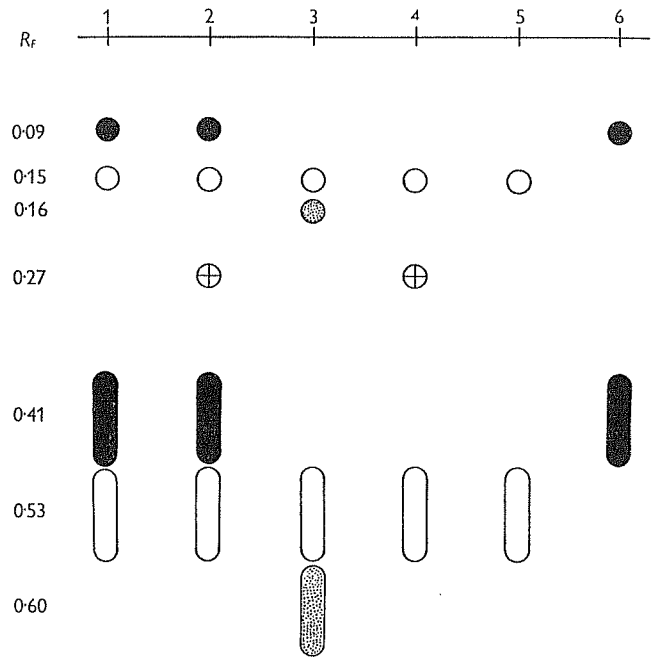



Fig. 1. $R_F$ values in butanol—2N-hydrochloric acid (1 : 1). (1) C.P.C. 979, *PpRR*: blue tubers and sprouts. (2) C.P.C. 979, *PpRR*: blue flowers. (3) D53/49/01 *ppRR*: red tubers and sprouts. (4) D53/49/01 *ppRR*: red flowers. (5) C.P.C. 2211, $ppR^{pw}R^{pw}$: pink tubers and sprouts. (6) Wild species, *P*-?-: blue tubers, sprouts and flowers; cultivated diploids with pale blue flowers, *P*- $R^{pw}$ $R^{pw}$.

| Component | Visible light | Ultra-violet light | Ultra-violet light and ammonia vapour |
|---|---|---|---|
| 0·09 | Purple | Purple | Dark blue |
| 0·15 | Pink | Pink | Reddens and fades |
| 0·16 | Yellow | Yellow | Scarlet |
| 0·27 | Magenta | Magenta | Blue purple |
| 0·41 | Purple | Purple | Dark blue |
| 0·53 | Pink | Pink | Reddens and fades |
| 0·60 | Yellow-orange | Orange | Scarlet |

Confirmation was obtained by running chromatograms of the aglycones in Forestal solvent, hydrochloric acid-acetic acid-water (3 : 30 : 10) and using authentic specimens of the anthocyanidins as markers. Close agreement between the $R_F$ values of natural and marker specimens was obtained in all cases except that of the aglycone believed to

be peonidin (Table 5). Its $R_F$ value (0·62–0·65) tends to be midway between those of malvidin (0·56–0·60) and peonidin (0·69). The $R_F$ values of malvidin and peonidin in this solvent are rather close but their colours help also to distinguish them; in amyl alcohol, malvidin is violet-purple whereas peonidin is crimson; and as spots on the chromatogram, the former is purple and the latter is pink. The aglycone was identical in colour with authentic peonidin in both solvents.

The nature of the anthocyanidins can be confirmed spectrographically since they all have well-defined peaks in the visible region (cf. Bate-Smith, 1954).* The spectra are the same whether measured in solution in ethanolic hydrochloric acid or when examined directly as a spot on a paper chromatogram by the method of Bradfield & Flood (1952), although in the latter case the values are accurate to no more than $\pm 2$ m$\mu$ (Table 6).

The free anthocyanidins obtained on hydrolysis of the extracts of the skins of pigmented tubers with aqueous hydrochloric acid were extracted from the digest with amyl alcohol and then either separated on paper chromatograms in the Forestal solvent and the spectra determined directly on the paper, or when in sufficient concentration, the amyl alcoholic extract diluted with ethanolic hydrochloric acid and the spectra of the solution measured in the normal manner. The results are given in Table 6. It can be seen that in all cases the

Table 6. *Absorption spectra of extracted anthocyanidins and reference compounds*

| | λ max (mμ) | |
|---|---|---|
| | Solution | Filter paper |
| Anthocyanidin from | | |
| Purple component $R_F$ 0·41* | 555 | 553 |
| Magenta component $R_F$ 0·27 | — | 543 |
| Orange component $R_F$ 0·60 | 530 | 528 |
| Pink component $R_F$ 0·53 | — | 545 |
| Reference compounds | | |
| Pelargonidin | 530 | 530 |
| Cyanidin | 545 | 544 |
| Peonidin | 545 | 545 |
| Delphinidin | 555 | 555 |
| Petunidin | 555 | 555 |
| Malvidin | 555 | 555 |

* See Fig. 1 and Table 5.

measurements agree with the identification obtained by chromatography and enable distinction to be made between peonidin and malvidin and petunidin and cyanidin which are less certain by the latter method alone.

The glycosidic components of the anthocyanins have not been identified. Robinson & Robinson (1932) state that the varieties of *S. tuberosum*, Mr Bresee and Cardinal, contain pelargonidin-3-pentose glycoside and 3:5-dimonoside, respectively. On chromatograms, however, extracts from tubers of these two varieties had identical runs in different solvents and contained the same derivatives of pelargonidin as those in diploid red tubers, namely, the components $R_F = 0{\cdot}16$ and 0·60 (Fig. 1, column 3). The $R_F$ values of the pink and yellow components (0·15 and 0·16, respectively) are lower in all solvents than the values for the diglycosides of peonidin and pelargonidin given by Bate-Smith

* Dr T. Swain of the Low Temperature Research Station, Cambridge, kindly carried out the spectrographic verification of the pigments.

(1949). In common with diglycosides and 3-biglycosides, they show low distribution between amyl alcohol and water.

Cyanidin-3-rhamno-glucoside has been identified in the magenta flowers of *Antirrhinum major* and *Papaver rhoeas* (Scott-Moncrieff, 1936). Extracts of this pigment from these two sources, after preparative purification on Whatman no. 3 paper as described above, were run in butanol-2N-hydrochloric acid (1 : 1), butanol-acetic acid-water (40 : 10 : 50) and *m*-cresol-acetic acid-water (50 : 2 : 48) (Bate-Smith, 1949), together with the glycoside of cyanidin peculiar to the flowers of diploid potatoes. $R_F$ values were similar, but these cannot be taken as proof of identity of chemical constitution without confirmation of the sugar residues by other means. Nevertheless, the comparison is suggestive.

## The distribution of anthocyanins

In addition to the cultivated diploids, the distribution of anthocyanin types in a wide range of tuberous wild species and cultivated polyploids has been determined. The results are given in Table 7, where the range is indicated without regard to the limitation of a pigment to a particular genotype of the species or organ of the genotype. It will be

Table 7. *The anthocyanins in tuberous Solanums*

| | Glycoside of cyanidin | Acylated glycoside of | | | |
|---|---|---|---|---|---|
| | | Pelargonidin | Peonidin | Petunidin | Malvidin |
| | | **Wild Species** | | | |
| Diploids ($2n=24$) | | | | | |
| *S. chacoense* | . | . | . | + | . |
| *S. verrucosum* | . | . | . | + | . |
| *S. infundibuliforme* | . | . | . | + | . |
| *S. macolae* | . | . | . | + | . |
| *S. maglia* | . | . | . | + | . |
| *S. vernei* | . | . | . | + | . |
| Triploid ($2n=36$) | | | | | |
| *S. vallis-mexicae* | . | . | . | + | . |
| Tetraploids ($2n=48$) | | | | | |
| *S. acaule* | . | . | . | + | . |
| *S. stoloniferum* | . | . | . | + | . |
| *S. sucrense* | . | . | . | + | . |
| Hexaploids ($2n=72$) | | | | | |
| *S. demissum* | . | . | . | + | . |
| Unidentified | . | . | . | + | . |
| | | **Cultivated Species** | | | |
| Diploids ($2n=24$) | | | | | |
| *S. Rybinii* | + | + | + | + | . |
| *S. stenotomum* | + | + | + | + | . |
| *S. Yabari** | + | + | + | . | . |
| *S. Ascasabii* | + | + | + | + | . |
| Triploids ($2n=36$)† | | | | | |
| *S. Juzepczukii* | . | . | + | + | . |
| *S. Chaucha* | . | . | + | + | . |
| *S. tenuifilamentum* | . | . | + | + | . |
| Tetraploids ($2n=48$)† | | | | | |
| *S. tuberosum* | . | + | + | + | + |
| *S. andigenum* | . | + | + | + | . |

* Only red phenotypes examined. † Tubers only.

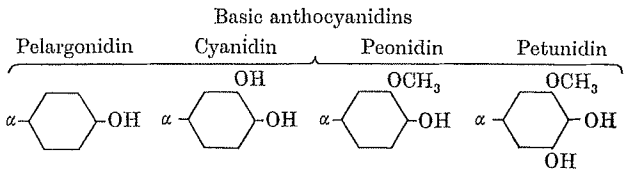
seen that an acylated glycoside of petunidin is the only pigment encountered in the tubers and flowers of wild species. Of interest is the extension of the range of methylated anthocyanin derivatives in potatoes by the confirmation of the presence of a glycoside of malvidin in *S. tuberosum*. This was observed to be a complex malvidin 3:5-dimonoside in the Congo variety by Robinson & Robinson (1932).

The distribution of the pigments within cultivated diploids according to genotype and plant organ is given in Table 8. It will be observed that the acylated glycoside of pelargonidin occurs only in tubers and similarly the glycoside of cyanidin is confined to flowers.

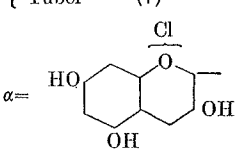
## Discussion

The observation that the same pigment may occur in all the organs of a particular genotype agrees with the indication given by genetical analysis that main loci are responsible for the production of certain pigments throughout the body of the plant. Locus *P* governs the presence or absence of an acylated glycoside of petunidin in cultivated diploids, and this same pigment occurs in the wild tuberous species of *Solanum*. No direct comparison of the main loci of wild and cultivated species has yet been made, but, from analogy with the genetics of wild and cultivated species of *Gossypium* (Silow, 1941), it seems highly probable that the main loci of the two groups will correspond.

Table 8. *Pigments and genotypes in cultivated diploids*

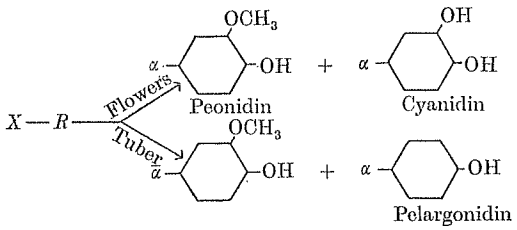


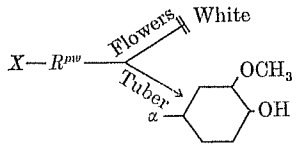

| | | | | | | |
|---|---|---|---|---|---|---|
| Wild type | *PP*?? | Flower | | | | + |
| | | Sprout | | | | + |
| | | Tuber | | | | + |
| White flower | *ppR*$^{pw}$*R*$^{pw}$ | Flower | | | | |
| | | Sprout | | | + | |
| Pink tuber | | Tuber | | | + | |
| Pale blue flower | *P*–*R*$^{pw}$*R*$^{pw}$ | Flower | | | | + |
| | | Sprout | | | + | + |
| Blue tuber | | Tuber | | | + | + |
| Red flower | *ppR*– | Flower | | + | + | |
| | | Sprout | + | | + | |
| Red tuber | | Tuber | + | | + | |
| Blue flower | *P*–*R*– | Flower | | + | + | + |
| | | Sprout | | | + | + |
| Blue tuber | | Tuber | (+) | | + | + |

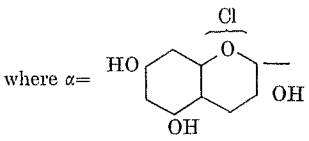



It has been seen that locus *P* has a straightforward effect governing the presence and absence of an acylated glycoside of petunidin (Table 8). The same clear-cut picture of

a main gene exerting its effects throughout the plant body is not obtained from a consideration of the *R* locus. The two alleles may be contrasted (with chromogens shown as chlorides) thus:



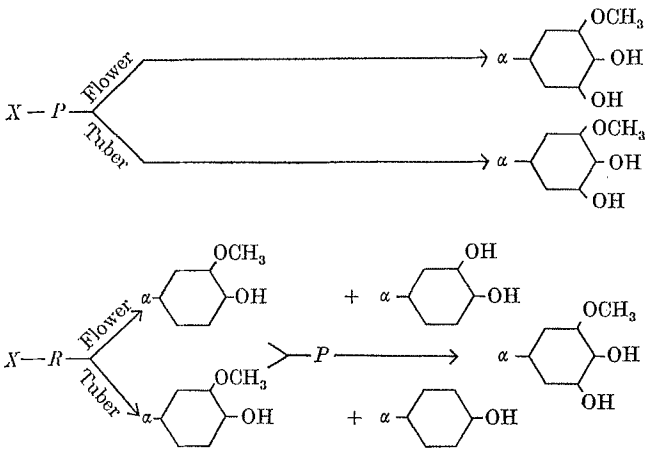




The effects of each of the two alleles *R* and $R^{pw}$ are not the same in tuber and flower. The former seems to control the occurrence of an acylated glycoside of pelargonidin in the tuber coupled with the presence of a glycoside of cyanidin in the flower, whereas the latter suppresses both these pigments at their respective sites and in addition the acylated glycoside of peonidin does not occur in the flower. Hence components which measure the activity of the *R* locus are: (1) the occurrence of a glycoside of cyanidin in the flower, (2) the presence of an acylated glycoside of pelargonidin in the tuber and (3) the suppression of an acylated glycoside of peonidin in the flower. Thus following the conventional outlook that every developmental step of an organism is controlled by genes, it becomes a matter for speculation whether this locus is in fact three closely linked genes or a compound structure of subunits not classifiable as genes within their own rights. The situation resembles that at the *R* locus of maize (Stadler, 1945). This apparent simplicity in heredity, however, does not reflect a corresponding simplicity in development. If the syndrome of products is the result of an integrated complex of reactions, it is clear that the branch concerned with floral pigment has a certain developmental independence. The glycoside of cyanidin occurs equally in flowers of genotypes *ppR-* and *P-R-*, but, in tubers, the acylated derivative of pelargonidin which occurs in the former genotype is usually completely replaced in the latter by the acylated derivative of petunidin. Thus the activity of the subunit of locus *R* concerned with floral pigment is not impaired by locus *P* nor is its product masked. Epistasy occurs only in the tuber.

The only modification observed in the universal occurrence of peonidin in cultivated diploids is its suppression in the white flowers of genotypes homozygous for $R^{pw}$– genotypes which have pink tubers and sprouts because peonidin is the only pigment that occurs in them. In effect, all families breed true for the presence of the acylated derivative of this anthocyanidin and no conclusions can be drawn about its inheritance.

Further search, however, might reveal the existence of a third allele $r$ which in the homozygous phase and in the absence of $P$ would be expected to suppress all pigments, at least in tubers, sprouts and flowers. The genotype $P$-$rr$ would be phenotypically 'wild-type' in contrast to $P$-$R^{pw}R^{pw}$ which has 'wild-type' flowers only, the sprouts and tubers containing the derivative of peonidin.

Locus $P$ may be incorporated within the scheme of pigmentation either as controlling an independent line of synthesis and competing at the outset with locus $R$ (*sensu lato*) for precursor $X$, or as governing simply the hydroxylation at position 5′ of the peonidin derivative to give a corresponding derivative of petunidin. The two alternatives can be represented as follows:




The first interpretation suggests that locus $P$ should be no less complex in structure than locus $R$, but as yet there is no evidence of this. Indeed, as mentioned above, the activity of locus $P$ seems to be clear-cut both in heredity and development. Evidence from other genera, however, suggests that the production of mixed pigments is simultaneous rather than successive (Scott-Moncrieff, 1936). Thus problems that await investigation are, first, to determine the counterpart of locus $R$ in wild species, and secondly, if loci $P$ and $R$ are of equal stature, to determine why allelic changes at the $R$ locus have taken precedence over direct changes at the $P$ locus as the main source of variability. Conversely, if locus $P$ is only controlling the hydroxylation of a derivative of peonidin, then a comparatively simple series of mutational events would lead to the exposure of the $R$ locus.

Evidence of competition between loci $P$ and $R$ has been obtained. In C.P.C. 979 ($PpRR$), $P$ is epistatic to $R$ and the tuber is self-blue; but in the $F_1$ of the cross C.P.C. 979 × C.P.C. 2211, epistasy is incomplete and in the majority of blue genotypes ($PpRR^{pw}$) the acylated glycoside of pelargonidin occurs in addition to the acylated glycosides of petunidin and peonidin normally present in blue tubers. Furthermore, inspection of chromatograms gives the impression on comparing petunidin and pelargonidin that as the concentration of one goes up, that of the other is reduced. This is the interaction that would be expected if $P$ and $R$ compete for a common precursor, but whether this competition occurs early or late in a synthetic chain is not obvious. It seems probable that the interaction occurs because of the substitution of an $R$ by an $R^{pw}$ allele and not as a result of lack of balance in the modifier background of the $F_1$; blue is epistatic to red in the

inbreds of C.P.C. 979. Here, then, is a suggestion that $P$, $R$ and $R^{pw}$ mutually compete for a common precursor.

As a mixture of pigments does not occur in wild species, the occurrence of one in cultivated species cannot be related to interspecific hybridity as, for example, in *Verbena* (Beale *et al.* 1940). The mixture of pigments is entirely the result of the establishment of the $R$ locus, and this must have occurred under human selection following domestication of a tuberous wild species. It is of interest to observe that the greatest variability of types of anthocyanins seems to occur in the tuber, the organ with a selection value to early agriculturalists. At the same time, flowers have changed from 'wild type', and further work may show whether or not the presence in them of a glycoside of cyanidin is an independent change or a correlated response to changes established in the tuber.

It has been observed that mixtures of pigments occur only in cultivated potatoes, and that the same types of anthocyanins are common to both diploids and tetraploids. An apparent difference is the presence of a derivative of malvidin in the Congo variety of *S. tuberosum*, but a more extensive survey may show this anthocyanin also to occur in some genotypes of cultivated diploids. As it seems highly unlikely that the same series of pigment mutations has occurred twice in the evolutionary history of domestic potatoes, it is a reasonable assumption that the $R$ alleles of domestic tetraploids are derived from the cultivated diploids; in other words, that tetraploidy followed domestication at the diploid level. Whether or not, however, the domestic tetraploids are autopolyploids of cultivated diploids or amphidiploids derived from crosses between cultivated diploids and wild diploid species must remain conjectural.

## Summary

1. Two independent loci are shown to be concerned with the development of certain anthocyanin pigments throughout the plant body of diploid cultivated potatoes. A third locus is epistatic to these in its homozygous recessive phase and suppresses the development of pigment in the tuber only.

2. One pigment locus, designated $P$, governs the occurrence of an acylated glycoside of petunidin in tuber, sprout and flower, and in this respect corresponds to the 'wild-type' locus of wild tuberous species in which the same derivative of petunidin occurs alone.

3. Two alleles of the other pigment locus, designated $R$, are identified and are hypostatic to $P$. The dominant, $R$, in the absence of $P$, has a dual effect: an acylated glycoside of pelargonidin occurs in the tuber and sprout only, and a glycoside of cyanidin in the flower only. Epistasy is incomplete in the flower where the glycoside of cyanidin occurs also in the presence of $P$. These multiple effects are inseparable in heredity. In individuals homozygous for the recessive $P^{pw}$, and in the absence of $P$, these pigments do not occur.

4. An acylated derivative of peonidin occurs in all cultivated diploids, and in consequence no conclusions can be drawn about its inheritance. This anthocyanin is suppressed in the flowers only of genotypes homozygous for $R^{pw}$. The suggestion is made that further search might reveal an allele $r$ which in the homozygous phase and in the absence of $P$ would be expected to suppress all pigments, at least in tubers and flowers.

5. Types of anthocyanidins other than petunidin occur only in cultivated potatoes but are similar in diploids and tetraploids. Presumably, this variability has been established by human selection at the diploid level and the evolution of tetraploidy was a later event in the genetic history of cultivated potatoes.

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