A CYTOGENETIC INVESTIGATION OF COLEUS SPP.

IN TRINIDAD, WITH PARTICULAR REFERENCE TO

COLOUR PATTERNS IN C. BLUMEI BENTH.

by

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Discussion: 12

Recommendations: 14

Illustrations: 17

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# 1. INTRODUCTION

The genus Coleus is native to the Old World, occurring naturally in Africa, Malaya, Australia and the Pacific islands. Its life form ranges from annual to perennial, and in habit from branching herbs to small shrubs, with a variety of growth patterns. As a result of the occurrence of colored foliage in various species, it has been distributed all over the world. This has occurred in gardens, where coleus can be commonly found under various shade, especially in bathrooms and other areas. The range of leaf color patterns occurring in the coleus is very wide. The intensity of pigments and their distribution considerably vary; leaf shape and texture also vary widely in Coleus blumei at least.

It was decided to investigate as many aspects of leaf coloration and pattern as possible while assessing the level of heterozygosity in the clones collected. Investigations of leaf morphology in Coleus blumei has not been possible as none of the abnormal types has yet flowered.

## 2. REVIEW OF LITERATURE

The first published work on Coleus leaf color patterns was that of Stout (1915). Stout worked on coleus variations and by selection of these variations he wished to establish new varieties. He also contended that in the case of Coleus, the products of asexual reproduction are
1. INTRODUCTION

The genus Coleus is native to the Old World, occurring naturally in Africa, Malaya, Australia and the Pacific islands. In life form it ranges from annual to perennial and in habit from branching herbs to small shrubs, the shrubby habit being attained by perennials after several years of growth.

As a result of the occurrence of coloured foliage, several members of the genus have found use as ornamental plants, and as such have been distributed all over the world. In places where climate and ecological conditions permit, garden escapes have become established in the wild. This has occurred in Trinidad, where two species of Coleus can be commonly found under cacao shade, namely C. blumei Benth. and C. atropurpureus Benth.

The range of leaf colour patterns occurring in the commonly grown varieties of Coleus is very wide. The intensity of pigments and distribution vary considerably, but variation is not confined to colour and pattern only; leaf shape and texture also vary widely in C. blumei at least.

It was decided to investigate as many aspects of leaf coloration and pattern as possible while assessing the level of heterozygosity in the clones collected. Investigation of leaf morphology in C. blumei has not been possible as none of the abnormal types has yet flowered.

2. REVIEW OF LITERATURE

The first published work on Coleus leaf colour patterns was that of Stout (1915). Stout worked on somatic variations and by selection of these variations he claimed to establish new varieties. He also contended that in the case of Coleus, the products of asexual reproduction may vary
Boye and Rife (1938) published data on leaf pattern genetics in two varieties or clones of Coleus. In one clone it was found that purple upper and lower epidermis segregated as a single dominant gene, the recessives being "patterned", that is having an upper leaf surface with a purple centre and green margin and an unpigmented lower epidermis. The purple gene was epistatic to another single gene controlling chlorophyll intensity, intense being dominant to dilute, use of high and low chlorophyll intensity or deficiency. In the second clone they found that green leaf colour was dominant to "pattern" and was controlled by a single dominant gene.

Rife (1940) showed that the leaf characters "lobing" and "crinkling" were due to single dominant genes, homozygous recessives being normal. Post (1939) published data on an unstable gene "ruffled" which behaved as a dominant, reversions to normal being frequent. In clone A the mosaic is greater at the base of the leaf and at the centre than at the apex or the margin; in clone J the mosaic is more marginal. Anthocyanin may be absent in some clones, for example P.

Fourteen clones have been collected all as cuttings. Clones A-F are ornamental stocks from San Antonio Nurseries, Santa Cruz, G, H and J are from a garden in St. Augustine, K, L, M, N and P are from wild populations growing under cacao. K is from River Estate, Diego Martin, L from the track to Mt. Tucuche, M, N and P are from Matura. Clones D and K agree with herbarium specimens of Coleus atropurpureus. Both have flowered freely but are more or less sterile. Clone D has not set any seeds. Clone K is evidently infertile although it bears some apparently
sound pollen; several selfed inflorescences have yielded only six seeds of which none have germinated. Of the remaining clones, identified in the herbarium as Coleus blumei, several have flowered and contributed data. The variations in leaf colour involve two components, the intensity and distribution of chlorophyll and the intensity and distribution of anthocyanin pigments when present. The nature of pigmentation in C. blumei and C. sansatropurpureus is fundamentally the same. Some Coleus clones have a uniform chlorophyll distribution in the leaf, in others, areas of high and low chlorophyll intensity or deficiency are distributed in the leaf. Areas of low chlorophyll intensity or deficiency are yellow in colour and may be distributed in either or both of two ways. The yellow area may be localised in the centre of the leaf around the midrib and the proximal portions of the lateral veins. The yellow area is usually widest at the base of the leaf and tapering towards the tip. Yellowness in some clones appears as a mosaic, which may be predominantly green or yellow. In clone H the mosaic is greener at the base of the leaf and at the centre than at the apex or the margin; in clone J the mosaic is mainly marginal. Anthocyanin may be absent in some clones, for example P, in others such as L almost the whole upper epidermis is pigmented, the only green areas occur at the tips of the crenations at the margin. Other clones such as F have a well defined and continuous green border which surrounds a solid central area of anthocyanin. In clones C and H anthocyanin is distributed in irregular blotches on both surfaces. A prominent feature of clones D, K and G is the occurrence of an anthocyanin reticulum on the upper surface arising from a central anthocyanin area and extending towards the margin.
The lower epidermis may or may not be pigmented. Pigmentation of the lower epidermis appears to be independent of that of the upper epidermis. Clones F and J have a uniformly pigmented lower epidermis while clones A, D, K, G and L show only a slight spotting near the veins. Only in clones C and H does pigmentation of lower and upper epidermis appear to be associated.

The genus is readily propagated by vegetative means. As soon as the growth of a clone permitted, lateral branches were removed and struck in ordinary potting soil in 6" pots in subdued light. The aim was to produce as many inflorescences as possible to allow for abundant selfing and hybridisation. Clones were found to vary in frequency of flowering and vegetative vigour. Clones A, C and H flowered freely and grew vigorously, F and J produced only a few inflorescences while B, G and E did not flower at all. Clones D and K have already been noted as free-flowering, the more recent acquisitions, L, M, N and P show promise of being free-flowering.

When an inflorescence appeared, it was bagged and allowed to self-pollinate in the first instance. The bags used were of cotton 5"-6" wide and 10"-12" long, they were closed with paper-clips round the base of the inflorescence. When flowering had finished, usually after three or four weeks had elapsed, the bag was removed and the seed allowed to ripen. Seed which had ripened before flowering had finished was removed in order to avoid loss by shedding. Ripe seed was germinated on moist filter paper in petri dishes. Seedlings were pricked out into seedling boxes approximately 18" x 12" at a density of 9 x 6 or 8 x 5; a box would contain 40-50 seedlings. These seedlings could be scored for segregating characters from six weeks after pricking out onwards, when the first two or three pairs of leaves have expanded. In some cases it was found necessary to keep
material up to 5 months in boxes but it has been found possible after 2 months to discard unwanted material.

It was found in the wet season to be more satisfactory to keep plants for selfing in the greenhouse to avoid wetting of the bags. High humidity seemed to have an adverse effect on seed setting. Further to this point it was found most satisfactory to keep flowering material in a dry part of the greenhouse.

In hybridisation the flowers were reduced to a convenient number i.e. six per whorl of the inflorescence, to facilitate emasculation and to ensure that no unemasculated flowers were overlooked. The emasculation had no adverse effect on seed setting. Seeds were allowed to ripen and were treated in the same way as selfed seed. The yield of hybrid seed obtained was much less than that of selfed seed on account of the reduction in flower number.

It was found that one could obtain four seeds per flower in relatively few cases, possibly a certain amount of shedding takes place on ripening, this does not account for all the reduction in the expected yield; however, as some seeds occurred singly in the calyx before maturity. It was found to be more satisfactory to germinate seed as soon as possible rather than store it for any length of time. Germination of fresh seed is usually very high 80-100% while older seed may fail to germinate or germinate very poorly, less than 10% in some cases.

Chromosome counts were from root-tip squashes made by the method of Tjio and Levan (1950). Excised tips were pre-treated for two hours in 8 hydroxy-quinoline, macerated and stained in N.HCl 1 part: 2% acetic orcein 9 parts, then mounted and squashed in a drop of the stain. Clear mitotic plates were generally obtained with contracted chromosomes.

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4. RESULTS

A. Cytological analysis

When the selfing programme was in progress, it was observed that in no circumstances could clone D be induced to set seed. Some cytological irregularity was suspected and the somatic chromosome complement was counted. The clone was found to have 2n = 72. Counts were then made of most of the remaining clones, all had 2n = 48. Furusato (1940, in Darlington and Janaki Ammal 1945) records Coleus blumei as having 2n = 24.

No study of meiosis has yet been undertaken in any clone. Preliminary work indicates that the material will prove difficult. Apart from the number and small size of the chromosomes, the buds at the meiotic stage are extremely small and yield few pollen mother cells. No active divisions were seen in the few buds examined.

B. Chromatographic analysis

It was observed that the colour of the anthocyanin areas varied between clones; some were tending to be purple, others to be red. Chromatograms showed that the differences in colour were apparent rather than real; this variation was not due to the anthocyanin itself, the same pigments being present in the same relative amounts, but rather to the underlying chlorophyll. The anthocyanin pigments were acylated cyanidin diglucoside and the unacylated pigment, which was present as a trace. In view of this result no attempt has been made to score such colour differences.

C. Genetic results in C. blumei

1. Parental types

Data are available from the following clones (see
Clone A is a "pattern" type having pigment on the upper leaf surface except at the margins, it has a narrow central pink area which lacks chlorophyll.

Clone C has small discrete angular blotches of pigment on both leaf surfaces. The ground colour is green with yellow central area.

Clone F is a "pattern" type but unlike A it has a uniformly pigmented lower epidermis. The ground colour is green with yellow veinal areas.

Clone H has large blotches tending at times to coalesce to an irregular pattern, blotching occurs also on the lower epidermis. The ground colour is green with a yellow mosaic mainly marginal and apical.

Clone J resembles a "pattern" type; the margin is green and broad with finger-like extensions of anthocyanin along the veins. The ground colour is a green-yellow mosaic. The lower epidermis is uniformly pigmented.

Clone L has a pigmented upper surface except for small green areas at tips of the crenations, pigment on the lower surface occurs as dots on or near the veins. The ground colour is green.

2. Inheritance of anthocyanin pigment
   a) No segregation for pattern in the clone A selfed progeny of 84 individuals.
   b) Clone C on selfing segregates 9:7 for pigment blotches versus no anthocyanin. (see plate 3).
Table 1: C selfed progeny

<table>
<thead>
<tr>
<th></th>
<th>Blotched</th>
<th>No pigment</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed</td>
<td>211</td>
<td>169</td>
<td>0.08</td>
<td>0.8-0.7</td>
</tr>
<tr>
<td>Expected (9:7)</td>
<td>213.75</td>
<td>166.25</td>
<td></td>
<td>0.53</td>
</tr>
</tbody>
</table>

Table 2: H selfed progeny

<table>
<thead>
<tr>
<th></th>
<th>Irregular pattern</th>
<th>Pattern</th>
<th>Blotched</th>
<th>No pigment</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed</td>
<td>57</td>
<td>25</td>
<td>25</td>
<td>7</td>
<td>1.95</td>
<td>0.7-0.5</td>
</tr>
<tr>
<td>Expected (9:3:3:1)</td>
<td>64.26</td>
<td>21.42</td>
<td>21.42</td>
<td>7.14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

c) Clone H on selfing segregates 9 irregular pattern: 3 regular pattern; 3 blotches (like clone C); 1 without anthocyanin. (The irregular pattern is broken by green angular blotches (see plate 2).

d) Clone F, on selfing a truncated inflorescence, produced nine progeny all of which showed pigmented lower epidermis and "pattern" anthocyanin. The marginal green area of the upper surface showed variation in size in different members of the progeny probably due to modifier action.

e) Clone J selfed yielded 40 progeny all of which were "patterns of varying type. Pigmented lower epidermis versus unpigmented approximates to a 3:1 ratio (see plate 4).
Table 3: J selfed progeny

<table>
<thead>
<tr>
<th>Observed</th>
<th>Lower epidermis pigmented</th>
<th>Lower epidermis non-pigmented</th>
<th>$X^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expected (3:1)</td>
<td>30</td>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

f) Clone L on selfing produced a small progeny of eight individuals, half of which reproduced the parental type exactly, the remainder had a reduced central anthocyanin area and a broader green margin.

The hybrid families raised are of rather limited usefulness due to the heterozygosity of the parent stocks. When it was realised that the parent stocks were heterozygous, hybridisation was discontinued. Small lateral inflorescences had been used for crosses and only small quantities of seed obtained.

a) The cross H x C yielded a progeny of 19 individuals as follows:

- 14 "blotched"; 3 "pattern"; 2 without anthocyanin.

b) The cross A x J produced 12 progeny, all "pattern".

Results for pigmented lower epidermis were as follows:

- 7 pigmented lower epidermis: 5 non-pigmented lower epidermis.

$X^2$ test of fit to a 1:1 backcross ratio would have been possible with a bigger progeny.
c) The cross J x C produced 11 progeny as follows: 
4 "pattern": 5 "reduced pattern": 1 "blotched": 1 pigmented on lower surface only.

C x L yielded a progeny of six as follows: 
4 "reduced pattern": 2 "broken pattern:"

Table 5: Yellow mosaic v. green, segregation

<table>
<thead>
<tr>
<th></th>
<th>Yellow centre</th>
<th>Green</th>
<th>$X^2$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Observed</td>
<td>77</td>
<td>7</td>
<td>0.63</td>
<td>ca 0.5</td>
</tr>
<tr>
<td>Expected</td>
<td>78.75</td>
<td>5.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C Observed</td>
<td>380</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Evidently there are at least two independent dominant genes controlling this character. Whether there are more or not can be determined by selfing F1s of crosses involving A and C.

In all the families the extent of the yellow central area showed marked and continuous variation from the outset, in different plants, indicating the action of a complex of modifier genes.

Superimposed on this, an environmental variation was shown, in the extent of yellow central area on different leaves of the same plant.
b) In the J progeny mosaic segregated on a 3:1 ratio. In H yellowness segregated on a 9:7 ratio, the yellows were of a range of types, the greens were uniform. (see plates 2 and 4).

Table 5: Yellow mosaic v. green, segregations

<table>
<thead>
<tr>
<th></th>
<th>Mosaic</th>
<th>Green</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>Observed</td>
<td>73</td>
<td>53</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Expected</td>
<td>71</td>
<td>55</td>
<td>0.7</td>
</tr>
<tr>
<td>J</td>
<td>Observed</td>
<td>27</td>
<td>13</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td>Expected</td>
<td>30</td>
<td>10</td>
<td>0.3</td>
</tr>
</tbody>
</table>

The yellow plants in the H progeny ranged from plants with only a few yellow spots to more or less albino plants, most of which died as seedlings. It was not possible to assign these to discrete sub-classes. They have been arbitrarily graded as follows:-


Once again a modifier complex is indicated.

The hybrid behaviour as regards yellowness was as follows:-

a) All nineteen H x C progeny showed "yellow centre"; this character in this cross is epistatic to mosaic variation.

b) The C x L progeny showed no yellow areas. This reverses the state of affairs in the H x C cross, since mosaic in H is dominant to green.
c) The J x C progeny gave the following results:

6 yellow mosaic: 3 yellow centre: 2 green.
No "yellow centre" epistasis is shown in this cross.

d) The A x J progeny gave the following results.

10 yellow centre: 2 green.

No mosaic was shown in this cross, epistasis can be inferred. On this basis the "yellow centre" offspring on selfing should segregate 12 yellow centre: 3 yellow mosaic: 1 green.

4. Linkage

No data suggestive of the operation of linkage were obtained from any progeny.

5. DISCUSSION

It the progenies obtained variation due to the action of major genes has been shown to be influenced by modifier complexes and also environmental factors.

1. Anthocyanin inheritance

At least four major genes controlling anthocyanin are apparent from the data obtained.

a) A single dominant gene Pe controls lower epidermis pigment.

b) "Pattern" is due to a single dominant gene Pp; blotch is due to a single dominant gene Pb or to two complementary dominants.
It is not possible to say whether the second gene found in clone C may not also be essential for the expression of "pattern", at the present moment. It may be that this mechanism parallels the complementary dominant interactions found in flower colour genetics, and first reported by Bateson, Saunders and Punnett (1905) in the sweet pea.

The action of modifier complexes on major genes affecting anthocyanin pigmentation, is to enlarge or reduce the pigmented area of the epidermis. From the fact that most of the H progeny had larger anthocyanin areas than the parent type, the indication appears to be that the break-up of a modifier complex may allow greater expression of a gene.

2. Inheritance of chlorophyll deficiencies

Two types of chlorophyll deficiency have been found. The first, "yellow centre" is due to at least two major independent dominants. Yellow mosaic is due to two complementary dominants.

Yellow centre in the H x C hybrids is epistatic to yellow mosaic, in the hybrid C x L green is dominant to yellow centre. These two hybrid populations alone show uniformity in any one character. There is not adequate data at hand to infer the relationship between yellow centre and yellow mosaic, whether in fact there are any genes common to both in the complement necessary for the expression of these two characters.

Modifiers act on genes producing chlorophyll deficiencies in a way similar to the anthocyanin modifiers. They affect the extent of the yellow central area in C, and the intensity of mosaic in H progenies. No previous work has been published on the action of modifier complexes.
The work on the major genes above has produced data comparable with those of Boye and Rife (1938), the material investigated has been of clones distinct from those of the latter.

3. Origin of cultivated forms of Coleus

Cytological evidence suggests that much cultivated material is tetraploid, probably allo-tetraploid (amphidiploid) derived from hybrids between species originally domesticated. It is not inconceivable that either C. blumei or C. atropurpureus may be autotetraploid. The evidence so far is that all the genetic data can be interpreted on a diploid basis; there is no indication of autotetraploid inheritance or of dosage effects. A final answer can only come from collection, comparison and possibly hybridisation of the original diploids together with meiosis studies of the material.

4. Data on leaf shape of clone R and pubescence characters of clone P should if possible be obtained.

5. An experimental-taxonomic study of the origin of garden material. Original Coleus blumei material and material from R. F. Pakenham should be considered.

6. RECOMMENDATIONS

In making recommendations for further work the following considerations must be borne in mind. The first is that the time from the parental flowering to progeny flowering is at least five months and usually rather more. The second is that certain strains vegetate more or less indefinitely while other clones are very free flowering. This may restrict or delay any programme. The third consideration, which is likely to weigh more heavily as time goes on, is that of space, it would not be feasible to raise more than two or three large progenies at any one time. Progenies may have to be kept for several months to ensure reliable scoring but after some experience of a particular
progeny it may be possible to discard material after two months.

1) It is recommended that a second selfed generation is obtained from the first, also that inter-sibling crosses are made to confirm the interpretations made, e.g. crossing as many of the non-pigmented progeny of clone C as possible to confirm the complementary dominant hypothesis advanced. Further generations will at the same time improve line purity.

2) Further F1's and F2's should be obtained to compare genes and interaction of genes from various clones. With increasing purity of line this should yield more useful results.

3) Search for new major genes should be made in unexplored material by first selfing and then proceeding by the methods outlined in 1) and 2).

4) Data on leaf shape of clone E and pubescence characters of clone P should if possible be obtained.

5) An experimental-taxonomic study of the origin of garden forms could be undertaken. Original Coleus blumei material and material of related species would be required. Hybridisation of these forms could be attempted and cytological examination undertaken at different stages.

7. SUMMARY

1. Coleus in Trinidad falls within the range of two species namely, Coleus blumei Benth and Coleus atropurpureus Benth.
2. The two clones available of *Coleus atropurpureus* are both more or less sterile and no genetical results have been obtained. One clone has $2n = 48$, the other $2n = 72$.

3. All *Coleus blumei* clones have $2n = 48$, in disagreement with a previously reported count of $2n = 24$.

4. All genes studied show diploid inheritance.

5. A minimum of four major genes for anthocyanin pigmentation in *C. blumei* are identified, and also four major genes for chlorophyll deficiencies. The expression of these genes is influenced by modifier complexes.

6. It is suggested that the *C. blumei* forms studied may be amphidiploids of hybrid origin.

ACKNOWLEDGEMENTS

The writer wishes to acknowledge gratefully the invaluable assistance received from Mr. K. Shepherd. The writer also wishes to acknowledge help received from the greenhouse staff.


Plate 1. Coleus blumei clones A-L
Plate 2: Clone H. segregations

Upper: green (1) versus yellow mosaic (2).

Lower: no anthocyanin (3) "blotched" (4)
"pattern" (5) "irregular pattern" (6).
Clone C segregations.

Upper: "blotched" (1) versus "non blotched" (2)

Lower: Range of yellow centre found in clone C progeny.

Plate 3: Clone C segregations.

Upper: (a) "blotched" (1) versus "non blotched" (2)

Lower: (b) Range of yellow centre found in clone C progeny.
Plate 4:

Upper: Clone A segregations. yellow centre (1) versus "green" (2).

Middle and Lower: Clone J segregations.
(a) pigmented lower epidermis (3) versus non pigmented (4).
(b) green (5) versus yellow (6).