Biochemical and Genetical Investigations of Flower Color in Swiss Giant Pansy, *Viola × Wittrockiana* Gams. III. Dominance Relations in F_1 Hybrids, with Special Reference to Flower Color and Anthocyanin Pigment Constituents.*

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In the previous papers of this series (Endo 1954, 1959), a perliminary determination was reported of aglycones and glycoside types of six anthocyanins present in cyanic flowers of Swiss Giant Pansy. The present paper deals with dominance relations among the genes responsible for the flower coloration and the production of the anthocyanin pigments. The analysis of the genes concerned is in progress.

The chromosome number of cultivated pansies is still an interesting problem. According to a historical survey of Wittrock in 1897 (quoted by Clausen 1926 and Crane 1951), cultivated pansies arose by crossing between two wild pansies, *Viola tricolor* L. ($n=13$) and *V. lutea* Hudson ($n=24$) some time between 1830 and 1840. Clausen (1927, 1931) reported that cultivated pansies had chromosome numbers very close to those of the parent with the larger number, that is, $n=24$, but the numbers varied among the different individuals tested. Recently, Horn (1956), however, found that forty-four varieties of cultivated pansy collected in Europe have a constant number ($n=24$), though some irregularities were still observed in meiosis. He concluded from his cytological and genetical experiments that an auto-allo-octoploid genome constitution, AAAA BBBB, should be assumed for this plants.

Materials and Methods

Materials: Pansies are originally allogamous. Offsprings after three generations of selfing have shown distinctly a decrease of vigor and seed-fertility. On the contrary, inter-varietal hybrids have shown heterosis in vigor.

During the present work, since 1954 till 1958, more than 7,000 plants from selfing, sib-mating and crossing have been examined. Small numbers of variants in color tone were segregated in the progenies of all varieties, for instance, bluish white flowers

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in Lake of Thun (purplish blue) and reddish white in Raspberry Rose (purplish red).

The characters of the materials used are listed in Table 1. All except variety

Table 1. Flower colors and pigment constituents of pansy varieties.

Variety name	Flower color	Main characteristics of pigment constituents
Mont Blanc	White	Two kinds of quercetin-glycoside present, of which one is rutin and the other probably quercetin-triglycoside.
Rhinegold	Yellow	Three kinds of xanthophylls or their esters responsible for the flower coloration.
Raspberry Rose	Reddish purple	The major anthocyanin: keracyanin.
Fire Beacon	Yellowish red	Comparatively small amount of anthocyanins and xanthophylls present.
Alpenglow	Deep red	The flower color is due to the blending effect of xanthophylls and a comparatively large amount of keracyanin.
Lake of Thun	Purplish blue	The flower color is due to the blending effect of an anthocyanin, aD_2, and a large amount of quercetin-glycosides.
Berna	Deep purple	Large amount of aD_2 is present.

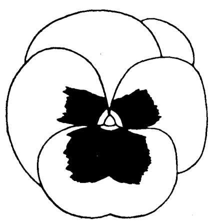

Fig. 1. Typical flower pattern of Swiss Giant Pansy. Black areas represent the blotched parts. ×ca. 0.5

Berna show almost the same pattern in the blotched parts of the lateral and anterior petals, as shown in Fig. 1.

Cytological method: The chromosomes of all varieties were observed in root tips which were treated with 0.002 M aqueous solution of 8-hydroxyquinoline for about 3 hours, placed in a mixture of 45% acetic acid and 90% ethanol (1 : 3, $^{V}/_{V}$) for about 10 minutes, stained with Feulgen and squashed in 45% acetic acid.

Paper-chromatographic analysis: For the analysis of the anthocyanin constituents, about 2g. of fresh posterior petals (not blotched) were immersed in 1% methanolic hydrochloric acid for two hours and filtered. The filtrate was concentrated under reduced pressure, and immediately spotted or streaked of filter paper, and chromatographed with a solvent, *n*-butanol/conc. hydrochloric acid/water, (5 : 1 : 2 or 5 : 1 : 4, $^{V}/_{V}$). One-way and circular paper-chromatography (Fig. 2) were used. In these chromatograms, a rough quantitative analysis of all anthocyanin constituents was made visible.

Results

Cytological observation: All varieties have the same chromosome number, $2n=48$, so far as examined (Fig. 3). Two chromosome pairs have somewhat globular satellites, which are not always clearly seen because of their small size.

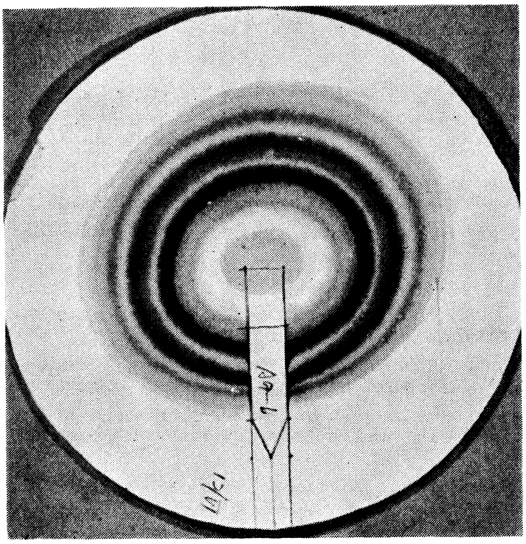

Fig. 3. Photograph of circular paper-chromatogram of extract from flower petals (a mixture of anterior and posterior petals) of Alpenglow. Solvent: *n*-butanol/conc. hydrochloric acid/water, 5 : 1 : 2. ×ca. 0.4

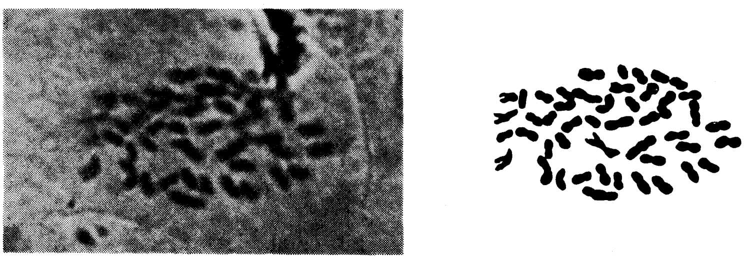

Fig. 2. Photograph of chromosomes of Swiss Giant Pansy (Alpenglow). ×ca. 2400

Description of flower color in F_1 hybrids: Thirty eight cross combinations in 1955 and thirty five in 1957 were made amon gseven varieties which have been selfted at least for two generations. About 1,300 F_1 plants were observed. In both years, almost the same results were obtained with regard to flower color. The following description chiefly deals with the results obtained in 1957.

1) *Acyanic versus Acyanic*:

Mont Blanc × Rhinegold 13 }
Reciprocal 6 } All yellowish white or of intermediate color. It was paper-chromatographically established that the pigment system of the hybrids is the same as that of Rhinegold which consists of three kinds of xanthophylls.

2) *Acyanic versus Cyanic*:

Mont Blanc × Raspberry Rose 17 }
Reciprocal 37 } 38 reddish purple and 16 pale reddish purple.

Mont Blanc × Fire Beacon 15 10 pale reddish purple with yellowish parts in the posterior petals and 5 pale yellow with reddish areas around the blotched parts and at the margin of the petals.

Reciprocal 12 5 reddish purple and 7 pale reddish purple with yellowish parts.

Mont Blanc × Alpenglow	31	25 deep red or purplish red to yellowish red purple and 6 pale yellow with reddish areas around the blotched parts and at the margin of the petals.
Reciprocal	41	28 yellowish red purple and 13 pale yellow with reddish parts.
Mont Blanc × Lake of Thun	29	23 bluish purple and 6 bluish white.
Reciprocal	0	
Mont Blanc × Berna	5	All deep purple similar to Berna.
Reciprocal	7	
Rhinegold × Raspberry Rose	10	9 pale yellow with reddish parts and 2 reddish purple with yellowish parts. One deep red similar to Alpenglow.
Reciprocal	2	
Rhinegold × Fire Beacon	20	14 yellow, but somewhat paler than Rhinegold and 6 yellow with reddish margin.
Reciprocal	17	16 yellow and one yellow with reddish margin.
Rhinegold × Alpenglow	16	7 yellow, but reddish on the lower surface of the petals and 8 yellow with reddish parts. One deep red.
Reciprocal	28	9 yellow and 19 brownish to deep red.
Rhinegold × Lake of Thun	11	All pale yellow turning to yellowish blue-purple or grayish purple.
Reciprocal	2	
Rhinegold × Berna	17	All deep purple, but different from Berna in having distinguishable blotched parts; 8 among them reddish around the blotched parts.
Reciprocal	28	All deep purple and 11 among them have distinguishable blotched parts.

3) *Cyanic versus Cyanic*:

Raspberry Rose × Fire Beacon	10	7 purplish red with yellowish parts and 3 reddish yellow similar to Fire Beacon.
Reciprocal	33	24 purplish red with yellowish parts and 9 reddish yellow.
Raspberry Rose × Alpenglow	2	All deep reddish purple similar to Alpenglow.
Reciprocal	5	
Raspberry Rose × Lake of Thun	14	All bluish purple and 6 among them purplish blue.
Reciprocal	32	
Raspberry Rose × Berna	7	All deep purple and 5 among them have distinguishable blotched parts.
Reciprocal	42	
Fire Beacon × Alpenglow	4	All deep red, but somewhat lighter than Alpenglow.
Reciprocal	2	
Fire Beacon × Lake of Thun	18	8 bluish purple, 7 purplish blue and 3 pale yellow.
Reciprocal	16	10 bluish purple and 6 yellow with bluish purple margin.
Fire Beacon × Berna	11	51 deep purple and 2 purple.
Reciprocal	42	
Alpenglow × Lake of Thun	9	7 purple, very similar to Berna in color tone and 2 grayish brown.
Reciprocal	0	
Alpenglow × Berna	2	All deep purple to purplish black.
Reciprocal	4	
Lake of Thun × Berna	4	All bulish purple with distinguishable blotched parts.
Reciprocal	38	

The F_1 hybrids are roughly classified into four groups, (1) those which have a flower coloring similar to one of the parents, (2) those which have an intermediate color between the parental flower colors, (3) those showing a continous variation and (4) those which segregate deviating colors or color patterns.

The first group is represented by crosses between deep purple (Berna) and all other flower colors, probably due to complete dominance of the genes responsible for the pigmentation of the deep purple variety.

Among the second group, a truly intermediate type was obtained from a cross between acyanic flowers, white and yellow.

To the third group belong the crosses between acyanic and cyanic varieties. They showed many types of variation in size and pattern of reddish parts on yellow ground or yellowish on red ground.

In the fourth group, the deviating segregants are due to a residual heterozygosity of one or both parents and the tetrasomic constitution of the plants (Horn 1956). However, it is noteworthy that the differences in color tone are mainly caused by quantitative difference of the pgiment constituents.

The dominance relations in F_1 hybrids, with reference to the flower coloration, may be represented in the diagram of Fig. 4.

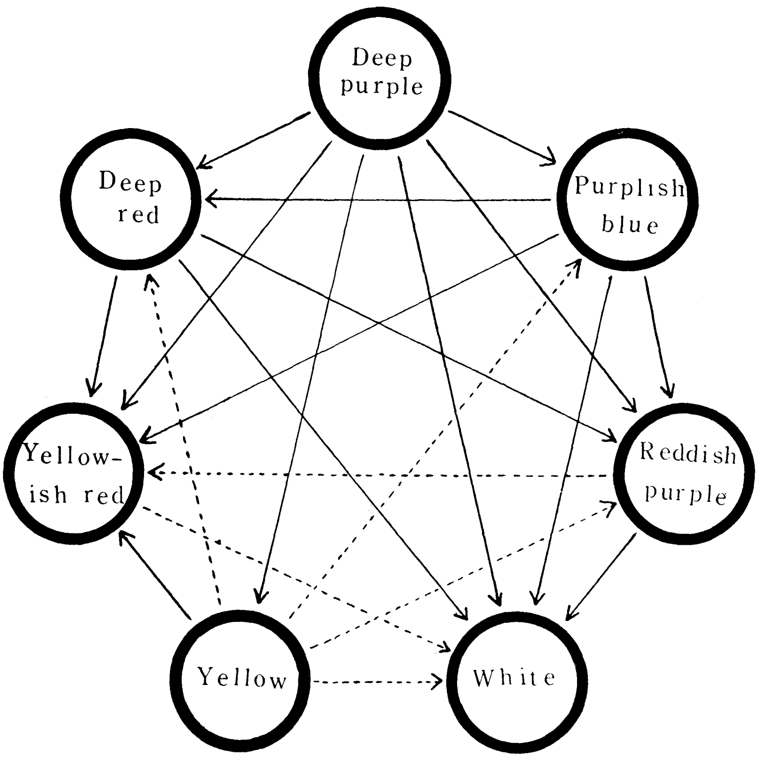

Fig. 4. Approximate dominance relations of flower colors in F_1 hybrids. Solid lines : dominance (epistasis) relation ; broken lines : partial dominance.

Pigment analysis: In a preceding paper (Endo 1959), six anthocyanins present in pansy flowers were arbitrarily designated as aC_1, aD_2, C_3, D_4, C_5 and D_6 and were preliminarily determined as follows:

aC_1: cyanidin-*p*-coumarylglycoside

aD_2 : delphinidin-3 : 5-*p*-coumarylglucorhamnoside
C_3 : cyanidin-3-glucorhamnoside (keracyanin)
D_4 : delphinidin-3-glucorhamnoside (tulipanin)
C_5 : cyanidin-3 : 5-glucoglucorhamnoside
D_6 : delphinidin-glucorhamnoside

Most of them are represented on a chromatogram, as shown in Fig 5.

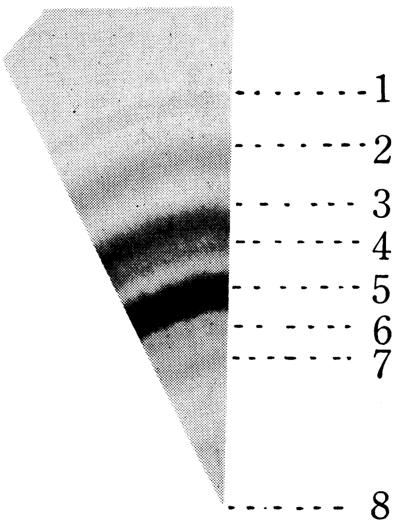

Fig. 5. Designation of anthocyanins on the chromatogram. Material and method are the same as those represented in Fig. 4. 1=solvent front, 2 = flavonol (yellowish brown), 3=aC_1 (purpliah red), 4 =aD_2 (reddish purple), 5=C_3 (red), 6=D_4 (reddish purple), 7= C_5 (red), 8 = center of circular filter paper.

In the present experiment, the anthocyanin constituents of the posterior petals of F_1 hybrids were examined paper-chromatographically and were compared with those of the parental varieties. The identification on the chromatograms of every one of the anthocyanins of F_1 hybrids with one of the anthocyanins of the parents was carried out. The analysed results are summarized in Table 2.

1) *Acyanic versus Cyanic* :

It has been so far recognized that these anthocyanins form three groups : aC_1, C_3, D_4 and C_5 (for reddish flower color), aC_1 and aD_2 (for purplish blue) and aC_1, aD_2, C_5 and D_6 (for deep purple). Among them, aD_2 and C_3 are the major constituents in bluish and reddish flowers, respectively, and the other anthocyanins are the minor ones.

All three anthocyanin groups appeared in all F_1 hybrids between cyanic and acyanic varieties (except Fire Beacon × Rhinegold), even though the amounts of the pigments were decreased. From these results, it is concluded that the genes controlling the production of the pigment groups of the cyanic flowers are in many cases dominant over those of the acyanic flowers. Epistasis could be considered in some cases.

2) *Cyanic versus Cyanic* :

The anthocyanin constituents of F_1 hybrids from the present cross combinations are fairly complex, especially when the flowers contained different anthocyanin groups.

Table 2. Anthocyanin constituents of parental varieties and F_1 hybrids.

Parental variety	Flower color	aC_1	aD_2	C_3	D_4	C_5	D_6
Mont Blanc	White	(+)*	(+++)*	·	·	·	·
Rhinegold	Yellow	(+)*	(+++)*	·	·	?	·
Raspberry Rose	Reddish purple	+	·	+++	+	+	·
Fire Beacon	Yellowish red	+	·	++	+	+	·
Alpenglow	Deep red	+	·	+++	+	+	·
Lake of Thun	Purplish red	+	++	·	·	·	·
Berna	Deep purple	+	+++	·	·	+	+
F_1 of acyanic × cyanic							
Mont Blanc × Raspberry Rose	Reddish purple	+	·	+++	+	+	·
Mont Blanc × Fire Beacon	Reddish purple	+	·	++	+	+	·
Mont Blanc × Alpenglow	Yellowish red purple	+	·	+++	+	+	·
Mont Blanc × Lake of Thun	Bluish purple	+	++	·	·	·	?
Mont Blanc × Berna	Deep purple	+	+++	·	+	·	+
Rhinegold × Raspberry Rose	Reddish yellow	+	·	++	+	+	·
Rhinegold × Lake of Thun	Yellowish blue	+	+	·	·	·	·
Rhinegold × Berna	Deep purple	+	+++	·	+	·	+
F_1 of cyanic × cyanic							
Raspberry Rose × Fire Beacon	Purplish red	+	·	+++	+	+	·
Raspberry Rose × Alpenglow	Deep reddish purple	+	·	+++	+	+	·
Raspberry Rose × Lake of Thun	Bluish purple	++	++	·	·	+	?
Reciprocal	Bluish purple	++	++	·	·	+	?
Raspberry Rose × Berna	Deep purple	+	+++	·	·	+	+
Reciprocal	Deep purple	+	+++	·	·	+	+
Fire Beacon × Alpenglow	Deep red	+	·	+++	+	+	·
Fire Beacon × Lake of Thun	Bluish purple	++	++	·	·	+	+
Reciprocal	Bluish purple	++	++	·	·	+	?
Fire Beacon × Berna	Deep purple	+	+++	·	·	+	+
Alpenglow × Lake of Thun	Purple	++	++	+	·	+	+
Alpenglow × Berna	Deep purple	+	+++	+	+	+	+
Lake of Thun × Berna	Deep bluish purple	+	+++	·	·	+	+
Alpenglow × Lake of Thun	Purple	++	++	+	·	+	+
	Purple	++	++	+	+	+	+
	Grayish brown	++	+	++	+	+	+

Notes; + = trace, ++ = minor, +++ = major constituent, ? = irregular appearance.
* anthocyanin present in the blotched parts.

Crossing among reddish varieties (Raspberry Rose, Fire Beacon and Alpenglow) having the same anthocyanin constituents gave the same results as the parental varieties. On the other hand, crossing of purplish (Lake of Thun) with the reddish varieties gave a much larger amount of acylated anthocyanin, aC_1, than any one of the parental varieties. The flowers of these hybrids contained the same amount of aC_1 as that of aD_2. Furthermore C_3 was not found in F_1 hybrids of Lake of Thun × Fire Beacon or Raspberry Rose, but a trace of C_3 was found together with a larger amount of aC_1 and aD_2 in F_1 hybrids of Lake of Thun × Alpenglow, as shown in Fig. 6.

One of the other minor constituents D_4, is always present together with C_3 in the parental varieties, but it was not necessarily found together with C_3 in the hybrids between bluish and reddish varieties. The appearance of D_6 was somewhat irregular, while C_5 was found in all F_1 hybrids.

It was expected that most of the flower color variations within a family of F_1 hybrids resulted from variations of their anthocyanin constituents. This proved to be true in the hybrids of Alpenglow × Lake of Thun, as shown at the bottom of Table 2. It is evident that this variation is chiefly caused by different amounts of the major constituents, aD_2 and C_3.

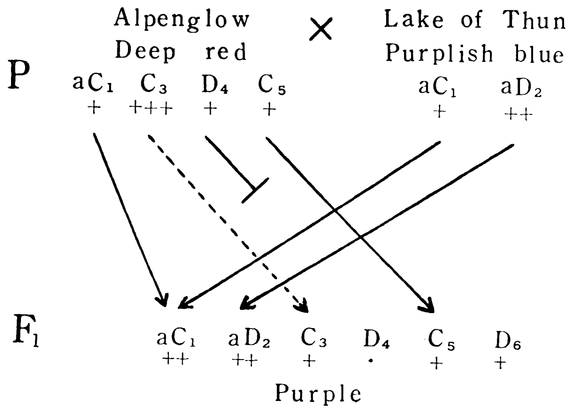

Fig. 6. Anthocyanin constituents in F_1 hybrid between Alpenglow and Lake of Thun.

Discussion

It is well known from many experiments that some of the crosses between plants with different flower colors give other kinds of color than those of the parental flowers, due to interaction of genes responsible for flower coloration. For example, in *Pharbitis* (Hagiwara 1931) crossing of purple genotype *pr Mg*) with magenta (*Pr mg*) produces blue flower color (*Pr Mg*). In *Dianthus* (Mehlquist & Geissman 1947) crossing between lavender (*s R m*) and deep pink (*S r M*) give magenta-purple (*S R M*).

In the present materials, however, the flower color of all F_1 hybrids is more or less similar to that of one of the parents or intermediate between the parental flower colors, even though there some segregants or continuous variations are found. A seeming exception was found in the F_1 hybrid from a cross of deep red (Alpenglow) with a purplish blue variety (Lake of Thun). The flower color of this hybrid was more similar to Berna than to the parental varieties, but its pigment constitution was considerably different from that of Berna.

Moreover, every one of the anthocyanin constituents found in the F_1 hybrids corresponds to one present in the parental varieties. Therefore, interaction of the genes is not so strong as to produce flower colors different from those of the parents.

Nevertheless the results obtained here resemble in general many findings reported by other workers. From our present knowledge concerning the dominance relations of genes responsible for flower coloration to which Scott-Moncrieff (1936), Beale (1941) and some authors have contributed, it is highly probable that the following general features may be drawn :

1) Cyanic flower colors are generally dominant to acyanic, due to dominance of the basic gene for the production of anthocyanins which, in most cases, cooperates with one or more additional genes.

2) In modifications of anthocyanidin molecule such as hydroxylation, methylation, glycosidation and acylation, higher degree of the process is usually dominant over the lower. Thus, bluish flower colors behave, in many plants, as dominant over reddish

flower colors, since the hydroxylation and glycosidation of anthocyanidin tend to produce a blue tone.

The present experimental results regarding two major anthocyanins, aD_2 and C_3, are in fair agreement with the above-mentioned features. On the other hand, some noteworthy results have been reached in regard to the minor anthocyanins. All anthocyanins present in cyanic flowers reappear as a group in the flowers of F_1 hybrids between cyanic and acyanic varieties. Some anthocyanins are increased or decreased in quantity or are inhibited completely in F_1 hybrids between reddish and purplish varieties. Thus, it is concluded that there are various types of interaction of genes responsible for the production of all anthocyanin constituents.

Summary

1. Chromosome numbers of Swiss Giant Pansy, *Viola* × *Wittrockiana* Gams were counted to be $2n=24$ in the somatic tissues of seven varieties.

2. The seven varieties with different flower colors were crossed and approximate dominance relations were observed.

3. Anthocyanins present in flowers of parental varieities and their F_1 hybrids were paper-chromatographically analysed. Every one of anthocyanin constituents found in the F_1 hybrids corresponds on the chromatograms to one present in the parental varieties.

4. The genetic background responsible for the production of a major anthocyanin, aD_2, is dominant over that of another major anthocyanin, C_3.

5. Anthocyanins present in cyanic flowers reappear as a group in F_1 hybrids between cyanic and acyanic varieties.

6. Some anthocyanins are quantitatively increased, decreased or inhibited in F_1 hybrids among the cyanic varieties.

Literature

Beale, G.H. 1941. Gene relations and synthetic processes. J. Genetics **24**: 197-214.

Clausen, J. 1926. Genetical and cytological investigations on *Viola tricolor* L. and *V. arvensis* Murr. Hereditas **8**: 1-156.

——— 1927. Chromosome number and the relationship of species in the genus *Viola*. Ann. Bot. **41**: 677-714.

——— 1931. Cytogenetic and taxonomic investigations in Melanium violets. Hereditas **15**: 219-308.

Crane, H.H. 1951. Pansies and violas. 101 pp Collingridge, London.

Endo, T. 1954. Biochemical and genetical investigations of flower color in Swiss Giant Pansy, *Viola* × *Wittrockiana* Gams. I. Inter-relationships of pigment constituents occurring in ten varieties. Jap. J. Bot. **14**: 187-193.

——— 1959. *Ditto* II. Chromatographic studies of anthocyanin components. Bot. Mag. Tokyo **72**: 10-19.

Hagiwara, T. 1931. The genetics of flower colors in *Pharbitis Nil*. J. Coll. Agric. Tokyo Univ. **11**: 241-262.

Horn, W. 1956. Untersuchungen über die cytologischen und genetischen Verhältnisse beim Gartenstiefmütterchen *Viola tricolor maxima* hort. (=*V. wittrockiana* Gams), einer polyploiden Bastardart. Züchter **26**: 193-207.

Mehlquist, G. A. L. and Geissman, T.A. 1947. Inheritance in the carnation *Dianthus caryophyllus*. III. Inheritance of flower color. Ann. Missouri Bot. Gard. **34**: 39-74.

Scott-Moncrieff, R. 1936. A biochemical survey of some Mendelian factors for flower color. J. Genetics **32**: 117-170.