Genetico-Physiological Studies on the Formation of Pigments in Several Organs of Japanese Morning Glories

(Preliminary Report)

By

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On the investigation of the relation between genetic factors and characters, the study to make clear whether the factor concerns to the formation of any substances or the factor gives rise to the chemical process of what kind is no less important than cytological studies at the foundamental study of genetic phenomena. The number of factors being responsible to exhibit characters can be determined at least by the genetic study of the character, but the physiological or chemical interpretation of these factors may be difficult to detect only by the genetic study without the chemical or physiological study.

From this stand point, the author undertook the genetico-physiological study on the formation of pigments in several organs of this plant. Indeed, the Japanese Morning Glory (*Pharbitis Nil*) having many white types and colour types may be better as the sample of studies of this category. Though this study is still proceeding, the author wants to report preliminarily so far as it has been obtained.

Four White Types

It has been already reported by TAKEZAKI,[1] IMAI[2] and MIYAZAWA[3] the fact that the colour in this flowers can be produced by two complementary factors. However, by the recent genetic study that contains researches of about eleven cross experiments carried out for some years, the author[4] obtained the result that three complementary factors are indispensable to produce pigments in corolla, and that the genetic formula of the following four white types are as follows.

1) Journal of the Japanese Breeders' Assoc. Vol. 1 1916 (Japanese).
2) Bot. Mag. Tokyo Vol. 35.1921 (Japanese).
3) Jap. Journ. Genetics. Vol. 3.1923 (Japanese).
4) Details of this experiment are to be maked public in the near future.

Table I

Type	Genetic formula	Distribution of pigments in organs			
		Corolla	Tube	Stem	Seed
I	C^a C r	white	colour	green	colour
II	C^a c R	white	light yellow	colour	colour
III	C^a c r	white	light yellow	green	colour
IV	c^a C R c^a C r c^a c r c^a c R	white	light yellow	green	white

These four white types are equally white in corolla, but they have the distinction at the pigmentation in several organs of those plants. When the pigmentation of several organs is compared with the genetic formula, it may be found that there is the correspondence between the factor and pigmentation, namely the factor **C** to tubes, the factor **R** to stems and the factor $\mathbf{C^a}$ to seeds.

The fact that most white flower turns to yellow in the vapour of ammonia, means the existence of flavone, according to WHELDALE[1] and SHIBATA.[2] Then the treatment with the ammonia vapour was worked out, giving the following results. Three whites out of four whites generally gave respectively the yellow colour though deep or light, but the remainder or the white with white seeds did not. These results are identical with the case of white flowers in *Antrrhinum* (WHELDALE),[3] *Phlox Drummondii* (WHELDALE)[3] and *Portulaca* (IKEÑO,[4] YASUI[5]). Details are shown in Table II.

Table II

Type	Genetic Formula	Colour given by ammonia vapour
I	C^a C r	+++
II	C^a c R	+
III	C^a c r	+
IV	c^a C R	−

1) The anthocyanin pigment of plants. Cambridge 1925.
Practical Plant biochemistry. Cambridge 1923.
2) Bot. Mag. Tokyo Vol. 29. 1915.
3) The anthocyanin pigment of plants. Cambridge 1925.
4) Journ. Coll. Agric. Tokyo Imp. Univ. Vol. VIII. 1921.
5) Bot. Mag. Tokyo Vol. 34. 1920.

Moreover, the alcohol extract of the corolla of four white flowers was reduced respectively by ClH and magnecium powders, and the colour given by this reduction was as follows.

Table III
Varietion of reduction colour

Type	colour * / formula	R-O	OR O	O	OY-O	Y-O	O-Y	YO-Y	O-YY	Y	YG-Y	no colour	experiment numbers
I	**C**^{a} **C r**	3	4	8	7	6	5	1					34
II	**C**^{a} **c R**					1	2	6	4	0	1		14
III	**C**^{a} **c r**						1	8	0	1			10
IV	**c**^{a} **C R**											6	6

* R....Red O....Orange Y....Yellow G....Green.

Considering the simple chemical experiment and the genetic experiment above mentioned, the following conclution may be draw, suggesting the essential fact. As the reduction colour is orange, the chromogenic substance of anthocyanin pigments in corolla and tube of the coloured flower and the I type white at least, may be one of colouring matters of the flavone group of which the side-benzene nucleus has OH or OCH_3 according to SHIBATA'S opinion.[1] And the flavone may be formed by the substances concerning respectively to the factor **C**, **C**^{a}, but the chemical nature of these substances is unknown at present. The another flavone differing from that of the I type white seems to be present in the II and III type white; while the IV type white lacks these fravones in corolla, stem and seed.

The flavone as the chromogenic substances of anthocyanin pigments was found as above mentioned in corolla or tube, but not in stem and seed by another qualitative reactions

Then, the chromogenic substances for the pigments of the stem and seed, may be those besides the flavone. By the chemical experiment undertaking to make clear this point, the substances of unknown chemical nature which SHIBATA[2] studied and considered as colourless anthocyanin (Pseudobase), was detected to exist in these organs. NAGAI[3] confirmed the fact that phlobaphenes and anthocyanins are derived from this substance on his chemical experiment. The phlo-

1) Journ. Biol. Chem. Vol. 28. 1916.
2) Bot. Mag. Tokyo. Vol. 31 1919 (Japanese).
3) Journ. Coll. Agric. Tokyo Imp. Univ. Vol. VIII. 1921.

baphene pigment with anthocyanins was detected to be present in stem of this plant. Therefore, the pigment of stems may be both phlobaphenes and anthocyanins. While, the phlobaphene alone was detected in seed.

The pigment in seed may be only the phlobaphene derived by the oxidation of same chromogenic substances with that of stem.

Then, the genetic factor concerning to the chromogenic substance and chromophelien which is indispensable to forming pigments in several organs, is tablated in Table IV.

Table IV

Factors \ Parts	Corolla	Tube	Stem	Seed
Chromogenic substances	C^a, C	C^a, C	C^a, X	C^a, X
Chromopheliens	R	R	R (O)	O
Chemical change	reduction	reduction	reduction oxidation	oxidation

The factor **R** and **R**^{t} for the chromophelien concerns to enzymes by which the chromogenic substance is reduced, and the factor **O.**, **O.** to enzymes to oxide the chromogenic substances to black or brown pigments.

The plant without the factor C^a has neither anthocyanins nor phlobaphenes in any organs, therefore, this factor may be presumed as the substance which plays the essential rôle for the production of these pigments. Here the genetic factor concerning to the substances of the unknown chemical nature was designated temporarily as **X**, but it is not certain whether **X** concerns to the same matter with C^a, or **X** is another substances having special relation to C^a.

By the above study, what the flavone in corolla may be one kind or probably two at least, was considered, but the observation to be shown in next paragraph may give the suggestion that there may be one more flavone.

Ground Colours and Yellow Flowers

The ground colour of corolla is divided into two kinds. However, in the normal coloured flower, the flower with white ground can not be distinguish seemingly from the flower with yellow ground. But, it is possible to distinguish the ground colour in such case that the formation of anthocyanin pigments is formed spotingly through the epidermal cell layer of corolla, resulting the so called spotted flower.

The recent genetic study worked out by the author gave the fact that two complementary factors S^1. S^2 may be required to produce the normal flower or unspotted flowers. In other words, the plant having the genetic formula $\mathbf{S}^1\ \mathbf{S}^2$ is the normal flower, but $\mathbf{S}^1\ \mathbf{s}^2$, $\mathbf{s}^1\ \mathbf{S}^2$ and $\mathbf{s}^1\ \mathbf{s}^2$ the spotted flower. Besides these factors, there may be a factor **S** which concerns to the distribution and area of anthocyanin spots. The function of the factor **R** seems to be governed by these factors $\mathbf{S}^1$, $\mathbf{S}^2$, **S**.

The yellow ground colour is developed by the coexistence of three factors **Y**, $\mathbf{C}^a$, **C** on the consideration of the genetic experiment. Then, the flower of the genetic formula $\mathbf{Y\ C^a\ C\ R\ s^1s^2s}$ gives a rarely-spotted flower having the appearance as yellow flowers because all these factors $\mathbf{S}^1$, $\mathbf{S}^2$, **S** are absent although the factor **R** exists.

On the chemical and microscopic study of such yellow flowers, the substance produced by the factor **Y**, $\mathbf{C}^a$, **C** may be considered to be another one of flavones which has more **OH** group than the flavone produced by the factor $\mathbf{C}^a$, **C**. The author did not treat the real cream or yellow flowers of this plant as those of Snapdragon, Porturaca and Sweet Pea, however, from the consideration of the genetic formula of yellow flowers in these plants which the genetic study worked out, the genetic formula of the yellow flower of this plant may be presumed to be perhapes $\mathbf{Y\ C^a\ C\ r}$.

Colour Series

As stated above, the colour of corolla owes to the anthocyanin pigment derived from flavones, and its colours are various in tint and shade. The colour series is possible to classify as indicated in Table V, based upon the genetic study of flower colours. Such classification of these colour series and genetical experiments of colour varieties have been already worked out by the author[1] some years ago.

Table V

	Genetic Formula	
Pure colour	$\mathbf{C^a\ C\ R\ K\ P\ B}$	Blue Group
	$\mathbf{C^a\ C\ R\ K\ P\ b}$ $\mathbf{C^a\ C\ R\ K\ p\ B}$	Purple Group
	$\mathbf{C^a\ C\ R\ K\ p\ b}$	Scarlet Group
Broken colour	$\mathbf{C^a\ C\ R\ k\ P\ B}$	Grayish Blue Group
	$\mathbf{C^a\ C\ R\ k\ P\ b}$	Grayish Purple Group
	$\mathbf{C^a\ C\ R\ k\ p\ B}$ $\mathbf{C^a\ C\ R\ k\ p\ b}$	Grayish Red Group

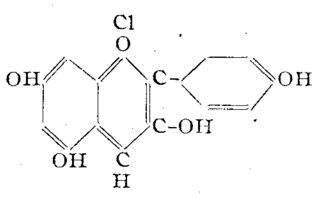
Broken colours are the shade of pure colours mixed with the gray colour. Basing upon author's recent studies,[2] complementary factors

1) Bot. Mag. Tokyo Vol. 37. 1923 (Japanese).
2) This report is to be maked public in the near future.

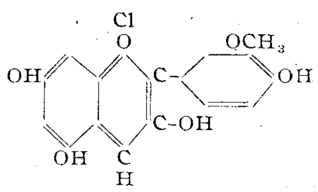
K_1 and K_2 consern to these shades, therefore, the development of pure colours requires the existence of two factors ; K_1, K_2.

The chemical interpretation of these genetic factors K_1, K_2, P, B is uncertain at present, and the further chemical study of anthocyanin pigments is required to solve this question.

Recently, KATAOKA[1) 2)] worked out the chemical study of anthocyanin in this flower, under the direction of Prof. SHIBATA. According to his study, the scarlet variety contains pelargonin, and the pigment of the purple variety is peonin; and moreover, the formula of pelargonidin chloride and peonidin chloride was respectively given as follows:—



Pelargonidin chloride



Peonidin chloride

Whether this flower contains or not the delphinin or delphinidin derivatives and other anthocyanins besides these pigments, is now being researching by him, but he told that delphinin is not probably present. The different colours of this flower, therefore, are due to the different anthocyanin as well as the blue or purple anthocyanin is cyanin, the pink is pelargonin, in cornflowers.

However we have the occurrence of different colours resulted by the same anthocyanin, i.e. in cornflowers, the dark purple-red variety contains neutral cyanin, and the blue the potassum salt of cyanin.

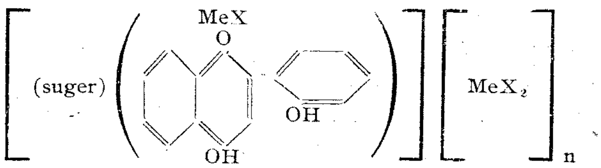
The colour variation in anthocyanin studied by Prof. K. SHIBATA, Y. SHIBATA and I. KASHIWAGI,[3)] giving the result which we believe to be more rational explanation than WILLSTÄTTER'S opinion.[4)] According to their opinion based upon experimental evidence, the production of flower colours is due to the fact that the metal organic or complex compounds of reduced flavonol glucoside, formulated generally here, exists in cell sap.

1) Pro. Imp. Academy Japan 1927. Vol. III.

2) The lecture read in the grand meeting of the scientific agricultural society at Tokyo. 1928.

3) Jour. Amer. Chem. Soc. Vol. 41. 1919.

4) Liebigs Ann: Chem. Leipzig. 1915 CCCCXIII.



Now, it may be possible to atempt the following assumtion on the studies so far described, of both genetical and chemical categories in this plant.

The pigment of flowers belonging to the scarlet group of which the genetic formula is $\mathbf{C^{a}CRKpb}$, may be pelargonin which is the state of the metalic complex compound with organic acids, and that of blue-purple flower in purple group having the genetic formula $\mathbf{C^{a}CRKPb}$, be peonin in the analogous state.

But the pigment of scarlet-purple flower in the purple group having the genetic formula $\mathbf{C^{a}CRKpB}$ may be, though unlikely, pelargonin which forms the complex compounds with the organic acid of the other metal, different from the metal concerned to the appearance of the scarlet flower, and supposed as Ca or Mg. The pigment of the blue flower in the blue group of which the genetic formula is $\mathbf{C^{a}CRKPB}$, may be peonin in the analogous state to pelargonin concerned to the appearance of blue-purple flowers. Then, if this assumption be true, it may be presumed that the factor **B** is probably related to the metal Ca or Mg.

The presence of delphinin or delphinidin derivatives is still uncertain as mentioned above, but if this is comfirmed to contain in this flower, the other explanation for genetic factors must be, of course as a mere assumption, taken as follows:—

The pigment of the scarlet of the genetic formula $\mathbf{C^{a}CRKpb}$ may be pelargonin, and that of the purple of $\mathbf{C^{a}CRKPb}$ may be peonin. And pigment of the blue of $\mathbf{C^{a}CRKPB}$ may be delphinin, or delphinidin derivatives, while that of the purple of $\mathbf{C^{a}CRKpB}$ is probably a mixture of the blue pigment and the scarlet pigment as well as the fact that was found in *Pelargonium* and *Dahlia*.

It is possible to say that $\mathbf{C^{a}}$ and **C** may be concerned to the chromogen, and **R** to the chromopheleins. But, it is uncertain whether **K** and **P**, and also **B** in the case of the latter assumtion, concern to the chromogen or to the chromophelein, though the suspicion is to the former.

Considering the evidence obtained by the qualitative reactions of four kinds of white flower, it may not be difficult to suppose the presence of two flavones at least in flowers of this plant, and that these flavones have **OH** or **COH_3** group in these side-benzene chains. In consequence, the supposition that these flavones may be kaempherol and isorhamnetin, corresponding to the anthocyanin, pelargonin and peonin, may be taken from WILLSTÄTTER'S opinion. Moreover, in case that delphinin is present, besides these flavones, myricetin may be added to them.

Thus, when these flavonols is isolated from this plant, as supposed above, correspondently to anthocyanins, a valuable contribution will be done on the chemistry and biochemistry of the anthocynin pigments.

Chlorophyll Inheritance

The consideration of genetic factors concerning to the production or modification of pigments in cell sap was studied as above mentioned, but in the next line, the author wants to do some genetic consideration with regards to the chlorophyll or plastid pigments.

Albino plants with white cotyledon seldom appear among normal individuals coloured to green or yellow. Of course, the chlorophyll of such individual is so abnormal that as soon as the food in the seed is exhausted, the individual perishes.

The yellow also is chlorophyll abnormality, but it grows up in safety. The albino seedling always lacks in chlorophyll, but not always in anthocyanins. The occurrence of such plant that has anthocyanins in its body but no chlorophylls, may suggest the physiological relation between the formation of chlorophylls and that of flavones.

En passant, both green and yellow leaves have colouring matters of flavone group.

Giving the conclution that the green behaves to the yellow as dominant, and the albino behaves to the normal as reccessive, the genetic researching of the leaf colour has been worked out respectively by TAKEZAKI,[1)] YASUI[2)] and IMAI,[3)] and the genetical and cytological study on the yellow and green has been attempted by Poof. FUJII.[4)]

These studies prove that plastid inheritance in this plant is Mendelian against *Mirabilis Pelargonium*, *Capsicum*.

Albino seedlings were found with the green and the yellow in F_2 and F_3 of some ones among many crossing between the green and the

1) Journ. of the Japanese Breeders' Assoc. Vol. 1 1916 (Japanese).
2) Bot. Mag. Tokyo Vol. 34, 1920 (Japanese).
3) Journ. Coll. Agric. Tokyo Imp. Univ. Vol. IX. 1927.
4) Bot. Mag. Tokyo Vol. 34. 1920 (Japanase).

yellow, and the segregation numbers was nearly in accordance with the 45:15:1 ratio of the green, the yellow and the albino. The observation of such segregation induced the following presumption. Three factors **G, G′, G″** may be important to explain this segregation, hence the genetic formula **GG′G″, GG′g″, Gg′G″** and **Gg′g″** concerns to green, **gG′G″, gG′g″** and **gg′G″** to yellow, **g′gg″** to albino.

Besides such genetical researches, the solubility of plastid pigments for the methyle alcohol was examined respectively in these leaves, as the chemical research.

These researches seem to give some suggestion on the relationship between these three factors for plastid pigments and four pigments in chloroplastid i.e. chlorophyll a, chlorophyll b, xanthophyll and carotin, but the further study must be required, because it is not enough to illuminate this relation. And also the study of the relation between these factors and the factor **C^a, C, R** demands the further research.

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(第 二 報)

早 田 文 藏

HAYATA, B. On the Systematic Importance of the Stelar System in the Filicales, II.

たいわんひめわらび (*Acrophorus stipellatus* (WALL. MOORE).

本屬ハ 1836 PRESL 氏ニヨリテ設立セラレタリシ以來ソノ分類學上ノ位置ニツキテハ議論紛々トシテ未ダ決定セラレザルモノナリ。PRESL 氏ハ之レヲ *Cystopteris* ニ連續セシメ、HOOKER 氏 (Sp. Fil. Vol. I. p. 157) ハ之レヲ *Davallia* 屬ト比較スベキモノトナシ (Syn. Fil. p. 92)、DIELS 氏 (p. 164) ハ矢張リ *Cystopteris* ニ最モ近キモノナリトセリ。