# オダマキのがく片におけるフラバノノールからアントシアニジンへの転換に関与する2遺伝子


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# Two Genes Controlling the Conversion from Flavanonol to Anthocyanidin in Sepals of Genus *Aquilegia*

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**Summary**

In order to make clear the conversion process from flavanonol to anthocyanidin, flavonoid components in sepals of *Aquilegia flabellata* with white flowers (Fw) and *A. hybrida* cv. McKana's Giant with creamy white flowers (Mw) were analysed. Isovitexin and populin were identified in both Fw and Mw while leucopelargonidin existed only in Mw. $F_1$ hybrids between Fw and Mw showed blue-violet sepals and $F_2$ progeny showed two-gene segregation of sepal coloration. Thus the conversion process is controlled by two genes. One recessive gene of Fw controls the reduction of flavanonol and the other recessive gene of Mw controls the dehydration of leucoanthocyanidin. Complementation of these two genes enables the synthesis of anthocyanidin in $F_1$ hybrids.

## Introduction

Genes controlling flower color expression and the enzymes related to flavonoid metabolism have been studied by many researchers and the pathway of anthocyanidin synthesis have been well explained by Grisebach(3) and Wong (11). However, the pathway from flavanonol to anthocyanidin is still obscure, although there have been a few reports on the conversion process(4, 6, 9, 10).

Recently, from feeding experiments of $^3$H-labelled leucopelargonidin to petals of two white mutant lines of *Matthiola incana*, Heller *et al.*(5) supposed that there were two steps in the conversion of flavanonol to anthocyanidin and the two steps should be controlled by two different genes, one controlling an enzyme which reduces flavanonol and the other controlling a dehydration of leucoanthocyanidin into anthocyanidin.

In order to make clear the conversion steps from flavanonol to anthocyanidin, the author analysed flavonoid components in sepals of a strain of *Aquilegia flabellata* Sieb. et Zucc. with white sepals and a strain of *A. hybrida* Hort. cv. McKana's Giant with creamy white sepals, because those strains produced $F_1$ hybrids having blue-violet colored sepals in which anthocyanidin was synthesized.

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## Materials and Methods

Two strains of *Aquilegia flabellata,* one of which had white flowers (Fw) and the other had blue-violet ordinal flowers (Fb), and a strain with creamy white flowers (Mw) of *A. hybrida* cv. McKana's Giant were used for the experiments. $F_1$ and $F_2$ progeny between Fw and Mw were also used for investigation of heredity of flavonoids. For the analysis of flavonoids, only sepals of the flowers were used.

The coloration of sepals was compared by a color difference meter (ND-101 DP, Nippon Denshoku Kohgyoh Co.). Absorption spectra of fresh sepals were measured by an integrating sphere attachment to Shimazu UV-240 apparatus.

The sepals of Fw, Mw and the $F_1$ hybrid, 40 g fresh weight each, were collected and extracted with ca. 400 ml of boiling methanol. The methanolic extract was evaporated at reduced pressure, the residue was extracted with hot water. The extract was shaken in a separating funnel with ether and subsequently with ethyl acetate. The ethyl acetate extract was evaporated to dryness, dissolved in a small volume of methanol and stored in a refrigerator at 4°C. The UV absorption spectrum in a part of the last methanolic solution was measured. The

remaining methanolic solution was examined by two-dimensional thin layer chromatography (Merck DC-Alufolien cellulose Art. 5552) using two kinds of solvents, n-butanol : acetic acid : water (6 : 1 : 2, v/v) and 15% acetic acid. The flavonoid compounds were separated by large scale cellulose thin layer chromatography (20×20 cm) using the above solvents. The method of Mabry *et al.*(8) was used for identification of flavonoids. Authentic samples were supplied by Dr. M. Hasegawa. The sample of leucopelargonidin used in this experiment was prepared by reduction of aromadendrin with sodium borohydride.

## Results and Discussion

The L. a. b. value of Mw was closer to y axis than that of Fw (Fig. 1). This means that the coloration of Mw is more yellowish than that of Fw. The $F_1$ hybrid and Fb were plotted in the fourth quadrant and showed blue-violet sepals.

The absorption spectra of fresh sepals of Fw, Mw and $F_1$ hybrids were compared in Fig. 2. Fw had a maximum absorption at 327 nm and Mw had a maximum at 336 nm. The spectrum of Mw showed a slight shoulder at 360–370 nm which was not observed in the Fw spectrum; this shoulder might be caused by the presence of flavonol. $F_1$ hybrids and Fb had three absorption maxima (533, 575 and 628 nm) in the visible region, which were caused by anthocyanins.

The absorption spectra of ethyl acetate soluble portions in hot methanol extracts are shown in Fig. 3. Fw had two absorption peaks at 293 and 328 nm which were similar to that of chlorogenic acid (296 and 327 nm), while Mw had similar absorption maxima to a group of flavones (270 and 333 nm).

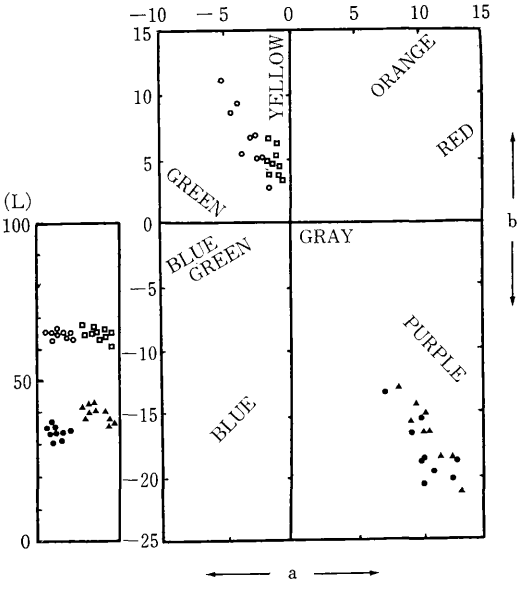


**Fig. 1.** L. a. b. values of sepals measured by color difference meter. ○ : Fw, □ : Mw, ● : Fb, ▲ : $F_1$ hybrids.

From the above results, it is clear that two kinds of white flowers of Fw and Mw differ each other in tint, and the soluble compounds in hot methanol extracts of Fw differ qualitatively and quantitatively from those of Mw. In the next experiment, ethyl acetate soluble

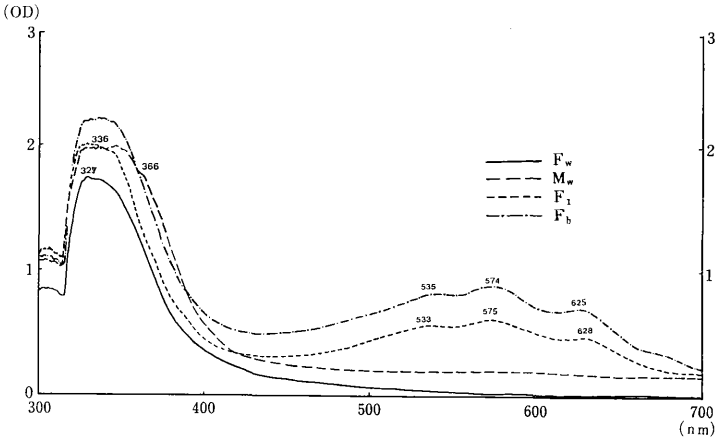


**Fig. 2.** Absorption spectra of sepals measured by integrating sphere.

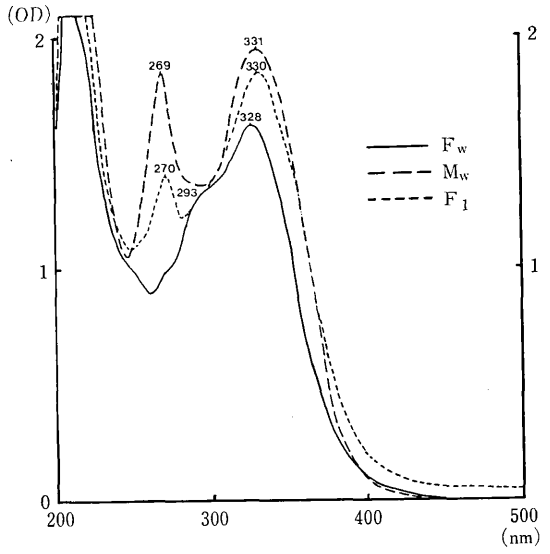


**Fig. 3.** Absorption spectra of ethyl acetate soluble portions.

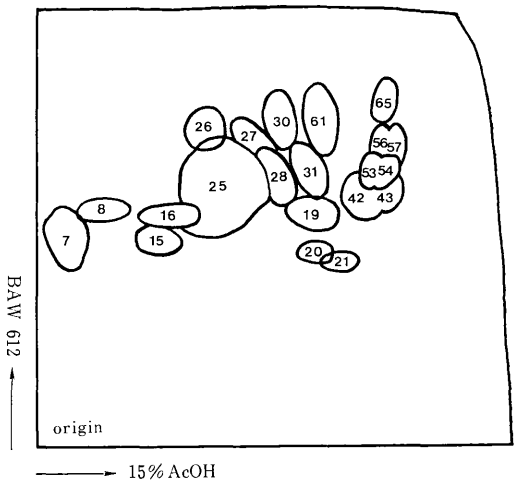


**Fig. 4.** Two dimensional chromatograms of ethyl acetate soluble portion in Mw. The spots were numbered for convenience.

compounds of Fw, Mw and $F_1$ hybrids were separated by two-dimensional chromatography (Fig. 4), and the main spots on the chromatograms were identified by comparison with sample substances (Table 1). Consequently, apigenin-6-C-glucoside (isovitexin) belonging to a group of flavones, and kaempferol-7-0-glucoside (populin) belonging to a group of flavonols, were identified as flavonoids in either Fw, Mw or $F_1$ hybrids. Caffeic acid, *p*-coumaric acid and ferulic acid were confirmed by thin layer chromatography after hydrolysis, and compounds of spot Nos. 35, 40, 42, 43, 53, 54, 56 and 57 were presumed to be respective derivatives of the above hydroxy-cinnamic acids. From the area of each spot using automatic area meter, Fw had a low content of flavonoids but a high content of organic acid esters. On the contrary, Mw had a high content of flavonoids but a low content of organic acid esters. It has been reported in garden snapdragon (*Antirrhinum majus*) that the albino flowers with semitransparent white petals did not have flavonoids, although the petals had accumulated a lot of esters of *p*-coumaric acid and caffeic acid(2). The white sepals of Fw had a low content of flavonoids and a high content of organic acid esters like the albino flowers of garden snapdragon. On the contrary,

**Table 1.** Characteristics of compounds isolated from sepals of Fw, Mw and $F_1$ hybrids.

| Compounds | Color | | | | Rf-values×100 in | | | | | Absorption maxima(nm) in MeOH | hydrolysis products |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | UV | +$NH_3$ | +$FeCl_3$ | +$NaBH_4$ +HCl | 612 | 15% | 22% | $H_2O$ | 5/3 | | |
| Spot No. 7 | Y | Y | Gr | | 45 | 12 | 6 | 1 | | 266, 321, 364 | kaempferol+glucose |
| (populin) | (Y) | (Y) | (Gr) | | (44) | ( 9) | ( 8) | ( 2) | | | |
| Spot No. 25 | Br+Pu | Br+Y | Br | | 58 | 40 | 41 | 21 | | 270, 300sh, 332 | isovitexin+vitexin |
| (isovitexin) | (Br+Pu) | (Br+Y) | (Br) | | (59) | (41) | (41) | (20) | | | |
| Spot No. 61 | t | p−Y | | R | 71 | 60 | | | 20 | 275, 283, 326sh | |
| (leucopelargonidin) | ( t ) | (p−Y) | | (R) | (71) | (61) | | | (22) | | |
| Organic acid esters | | | | | | | | | | | caffeic acid<br>*p*-coumaric acid<br>ferulic acid |

Y: yellow, Gr: green, Br: brown, Pu: purple, R: red, t: trace.
612: n-butanol/acetic acid/water (6:1:2) (v/v), 15%: 15% acetic acid, 22%: 22% isopropyl alcohol, $H_2O$: water, 5/3: nitrometane/methanol (5:3) (v/v) (used polyamide TLC).
Colors and Rf-values in parentheses indicate those of authentic samples.

creamy white sepals of Mw showed slight yellow coloration due to accumulation of flavone and flavonol.

As populin was produced, it is certain that two kinds of white flowers had the ability to synthesize flavanonol, an intermediate of anthocyanidin (Fig. 5). But, because of absence of anthocyanin, it is presumed that a genetic block exists in the process from flavanonol to anthocyanidin. Compounds in the ethyl acetate soluble portion were developed on cellulose TLC. The spots on this TLC were reduced by sodium borohydride and then the plate was exposed to HCl gas. Consequently, spot No. 61 (Fig. 4) which was specific only for extract of Mw changed color to red. The reaction suggests that spot No. 61 contains a kind of flavanonol or leucoanthocyanidin(1). After purification, the compound in spot No. 61 was determined as leucopelargonidin (Table 1). Thus Mw has the ability to reduce flavanonol to leucoanthocyanidin. Leucoanthocyanidin can be chemically changed to anthocyanidin by dehydration. In the feeding experiment to petals of *Matthiola incana*, $^3$H-labelled leucopelargonidin was converted to $^3$H-labelled pelargonidin(5). The $F_1$ hybrid of Mw with Fw produced anthocyanidin. Therefore, it appears that Fw is able to convert leucoanthocyanidin into anthocyanidin. The present results mean that the blocking parts of Fw and Mw are the process from flavanonol to leucoanthocyanidin and that from leucoanthocyanidin, respectively.

Sticklands and Harrison(10) in *Antirrhinum majus*, Kho and Bennink(6) and Kho *et al.* (7) in *Petunia hybrida* supposed that the conversion from flavanonol to anthocyanidin was controlled by one gene named *Pal* and *An*1, respectively. However, this process consisted of two steps, one being reduction and the other, dehydration. As mentioned earlier,

**Table 2.** Segregation of $F_2$ progenies in sepal color.

| $F_2$ strains from | colored | white | $\chi^2$-value (9 : 7) | P. |
|---|---|---|---|---|
| Mw×Fw | 30 | 21 | 0.137 | 0.71 |
| Fw×Mw | 15 | 18 | 1.561 | 0.21 |
| Total | 45 | 39 | 0.245 | 0.61 |

Heller *et al.* (5) suggested that the process from flavanonol to anthocyanidin consisted of two steps and was controlled by two genes.

The flower color segregation in $F_2$ progenies from Fw times Mw and the goodness of fit in $\chi^2$-test are shown in Table 2. The $F_2$ individuals were separated into two groups by their sepal colors, viz. colored and white, demonstrating a good fit to a ratio of two-gene segregation (9 : 7).

It can be concluded that two genes participate in two kind of blocks in Fw and Mw. One gene controlling the reduction of -CO- radical in flavanonol to leucoanthocyanidin exists in Mw, while the other gene controlling the dehydration of leucoanthocyanidin exists in Fw as shown in Fig. 5. The complement of these two genes prompts the synthesis of anthocyanidin in $F_1$ hybrids and then the sepals of $F_1$ hybrids express blue-violet color. Thus, the presumption of Heller *et al.* (5) was confirmed in the present experiments.

## Acknowledgement

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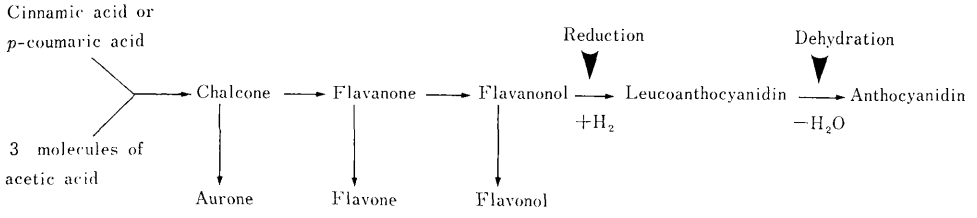


**Fig. 5.** The pathway of flavonoid biosynthesis.

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## オダマキのがく片におけるフラバノノールからアントシアニジンへの転換に関与する2遺伝子

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### 摘　要

フラバノノールからアントシアニジンへの転換過程を明らかにするために，紫青色花を呈する $F_1$ 雑種の両親である，ミヤマオダマキ(*Aquilegia flabellata*)白色花と，*A. hybrida* 品種マッカナジャイアント白色花のフラボノイド成分を分析比較した．2種類の白色花ともイソビテキシンとポプリンを持つが，マッカナジャイアント白色花には，ロイコペラルゴニジンが特別に存在した．$F_2$ の分離比よりこの転換過程は2遺伝子支配であった．したがって，ミヤマオダマキ白色花はフラバノノールからロイコアントシアニジンとの間で，マッカナジャイアント白色花は，ロイコアントシアニジンからアントシアニジンの間で，それぞれ遺伝的にブロックされていると考えられる．$F_1$ 雑種においては，この2遺伝子が補足し合い，アントシアニジンを生産し，紫青色花を呈したと考えられる．