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Studies on the Flower Colours in the *Camellia*

II. On the Anthocyanin Constitution in the Cultivars of *C. japonica*, *C. japonica* subsp. *rusticana*, *C. sasanqua*, *C. hiemalis*, *C. vernalis* and *C. wabisuke*

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Introduction

Concerning the constitution of anthocyanin pigments in the genus *Camellia* with purplish red to creamy pink petals, there were few experimental evidences available until recently. In 1974, using synthetic hybrids and back-crosses involving *C. japonica* and *C. saluenensis*, Parks *et al.*[6] showed the presence of two anthocyanins which were characteristic of *C. japonica* or of *C. saluenensis*, respectively.

In 1975, Yokoi[10] demonstrated that almost all the cultivars of *C. japonica* examined contained only one anthocyanin, cyanidin 3-monoglucoside, as a main pigment, and the other species and hybrids, such as *C. sasanqua, C. hiemalis, C. reticulata, C. saluenensis* × *C. reticulata* and *C. saluenensis* × *C. japonica,* had a more complex constitution than that of *C. japonica*. However, the number of anthocyanins he could detect were only some 6.

In the previous work[7] on the constitution of anthocyanins in *C. japonica, C. sasanqua* and *C. vernalis* surrounding Hirado Island, situated in the north-western part of Kyushu, the authors observed two major anthocyanins and seven minor ones, and disclosed a difference between the distribution patterns of anthocyanins in *C. japonica* and *C. sasanqua*. Furthermore, it was ascertained that the constitution of anthocyanins in *C. vernalis* originated in Hirado were intermediate between *C. japonica* and *C. sasanqua,* and differed with the difference in ploidy, from triploid to pentaploid, which was interpreted to substantiate, together with other morphological characteristics, its hybrid origin between these two species.

The present investigation was conducted to obtain more detailed and comprehensive figures concerning the constitution of anthocyanins in genus *Camellia*. This paper deals mainly with the results confined to the species of Japanese origin, and the results concerned with other species and interspecific hybrids will be presented elsewhere in a forthcoming paper.

Materials and Methods

1. Plant material

The places where the experimental materials were collected were as follows: Faculty of

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Agriculture, Kyushu University (Fukuoka); Faculty of Agriculture, Niigata University (Niigata); Faculty of Agriculture, Tokyo University of Agriculture and Technology (Tokyo); the Botanic Gardens in Kodomo-no-Kuni (Yokohama); Hirado Island; Kurume Camellia Lovers Association (Kurume) and Nagashima Tropical Botanic Gardens (Kagoshima), respectively.

One hundred and one cultivars of *C. japonica*, 21 cultivars of *C. japonica* subsp. *rusticana*, 42 cultivars of *C. sasanqua*, 5 cultivars of *C. hiemalis*, 22 cultivars of *C. vernalis* and 8 cultivars of *C. wabisuke* were used for this experiment. In addition to these, *C. reticulata* cv. 'Captain Rawes' and the wild form of *C. saluenensis* were examined.

The fresh petals collected during the period from late autumn to the following spring were quickly immersed in boiling water for approximately 15 to 20 seconds, in order to destroy the enzyme activity involved in the oxidation of the pigments. Then, they were air-dried at room temperature and stored in a desiccator.

2. Extraction and chromatography of anthocyanins

Each dried and powdered sample was allowed to stand overnight in cold 1% methanolic hydrochloric acid at room temperature and was filtered. After evaporation of the methanol within the solvent mixture, the crude extract was purified by repeated washings with petroleum ether and ethyl acetate.

The concentrated residual extract was applied to a corner of a 20×20 cm glass plate coated with micro crystalline cellulose powder (Art 2330, Merck), and was developed two dimensionally, using the following solvent systems, n-butanol-acetic acid-water 1: 2: 7 (miscible) for first dimension, and n-butanol-acetic acid-water 4: 1: 5 (upper layer) for the second.

The relative concentration of the constituent anthocyanins on the chromatograms were directly measured by a dual-wavelength TLC scanner (Shimadzu CS-900) at a wavelength of 530 nm, and the percentage of the respective anthocyanins were calculated.

Furthermore, the optical density of the extracts at a wavelength of λ_{max}, 535 nm, was measured by using a double-beam spectrophotometer (Shimadzu UV-200). Then, the total amount of anthocyanins was determined by using pure cyanidin 3-monoglucoside as a standard substance for the calculation. This amount was expressed in mg of cyanidin 3-monoglucoside per 100 mg of dry petal weight.

Results and Discussion

Fig. 1 is a stylized drawing of the chromatographic distribution of the anthocyanins found in this analysis of petal extracts. The genus *Camellia* examined was revealed to contain 14 kinds of anthocyanins, spot 1 to spot 14.

The details of the distribution of these anthocyanins in the respective cultivars are presented in the appendix. Table 1 shows summarized data for the respective species prepared by dividing the sum total of the percentages of the respective pigments by the total number of cultivars examined in each case.

As seen in the distribution pattern of pigments shown in the appendix and Table 1, the genus seemed to be divided into two different groups, one with pigments of lower Rf values, spot 1 (cyanidin 3-monoglucoside) to spot 9, and the other with those of higher Rf values, spot 10 to spot 14. On the basis of their chromatographic behaviours, the former pigments are chemically simpler than the latter ones, probably due to the difference of sugar residues attached.

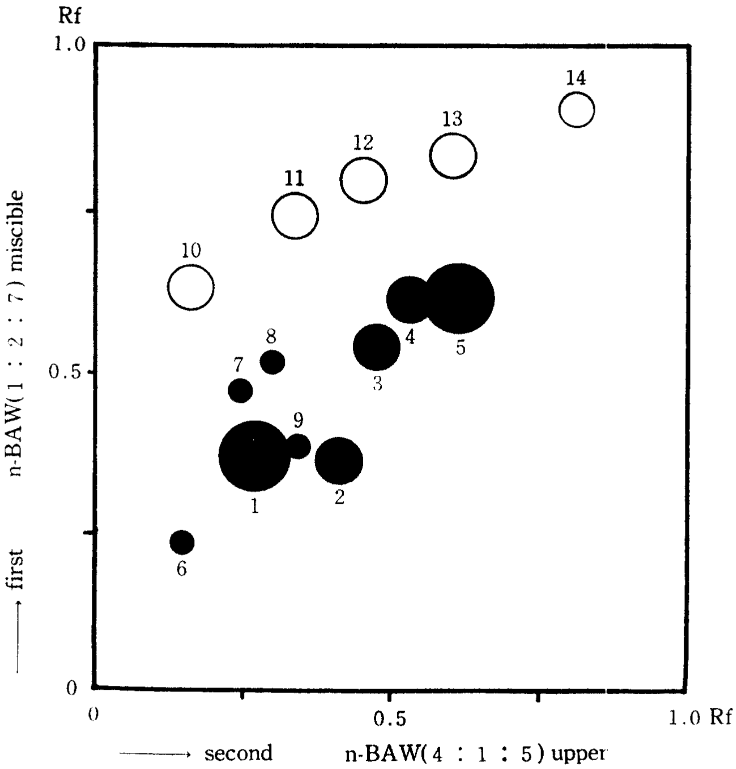

Fig. 1. Schematic representation of pigment-spots appearing on chromatograms in the anthocyanins of genus *Camellia*. Spot 1 is identical with cyanidin 3-monoglucoside.

Table 1. Summarized constitution of anthocyanins in genus *Camellia*

Species	Number of cultivars examined	Percentages of constituent anthocyanins														Spot 1: Spot 5 ratio
		1*	2	3	4	5	6	7	8	9	10	11	12	13	14	
C. japonica	101	65		4	7	24		+	+	+						2.7
C. japonica subsp. *rusticana*	21	88		2	1	5				2		2				17.6
C. sasanqua	42	15	1	14	3	62	5									0.24
C. hiemalis	5	17	2	9	3	67	2									0.25
C. vernalis	22	39		4	7	50										0.78
C. wabisuke	8	27		2	3	18					14	5	29	2		1.5
C. saluenensis	1										35		65			—
C. reticulata	1	1		2		2					6	37	7	45		—

* Numbers used are corresponding to the spot numbers represented in Fig. 1.

1. *C. japonica*

As seen in the appendix, almost all the *C. japonica* cultivars contained exclusively the anthocyanins of lower Rf values, among which the commonly occurring ones were spots 1, 3, 4 and 5. The only exceptions to this rule, were 'Daikagura' and 'Mura-Musume'. In spite of their being found in quite low concentrations, they contained one or two pigments of higher Rf values, that is, spot 14 in 'Daikagura' and spots 11 and 13 in 'Mura-Musume'.

Of the 10 kinds of anthocyanins appearing in this species, both spots 1 and 5 were major components. As shown in the appendix and Fig. 2, however, a considerable, intraspecific variation was evident, that is, from the cultivars exclusively confined to spot 1, such as 'Nanban-Kō' (100%) and 'Christmas Beauty' (100%), to those predominated by spot 5, such as 'Sheboshushi' (62%) and 'Tiffany' (59%).

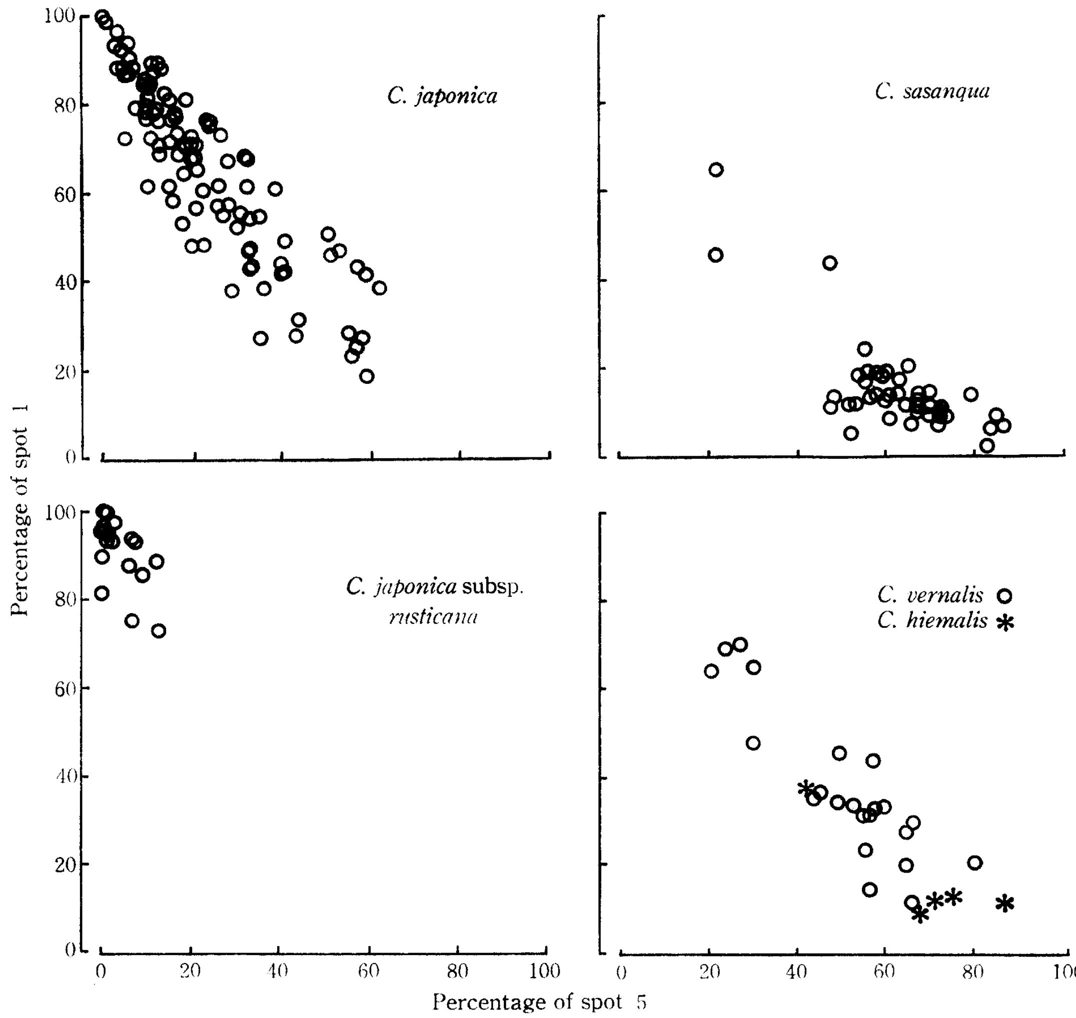

Fig. 2. Interrelationship between the relative concentration of spot 1 and that of spot 5 in genus *Camellia*. One dot represents one cultivar examined.

As seen in Table 1, the ratio of spot 1 to spot 5 in the pigment constitution was 2.7. Thus, *C. japonica* might be considered to be in a group essentially having spot 1 as a main pigment.

The reason why *C. japonica* showed a wide range of variation in pigment constitution is not clear. The authors, however, suppose that it might be due to the wide geographic distribution and to the fact that this species has been cultivated for a long time in Japan. Hence, the cultivar of *C. japonica* itself is likely to have developed these variable characteristics in the gene level.

2. *C. japonica* subsp. *rusticana*

As apparent from the appendix, this subspecies was also a group with pigments of lower Rf values. However, the constitution of the anthocyanins were fairly different from those in *C. japonica*. That is to say, in addition to major spots 1, 3 and 5, the cultivars contained spot 9 and spot

11, the latter of which was one of the pigments of the higher Rf values. The frequency of occurrence of these two anthocyanins was 48% in the former and 14% in the latter, as compared with the figures 3% and 1% in *C. japonica.*

Furthermore, spot 1 was found in quite high concentrations, and the variation in pigment constitution was not as wide as that in true *C. japonica,* as seen in the appendix and Fig. 2. Moreover, the ratio of spot 1 to spot 5 in the pigment constitution was 17.6 (Table 1). Thus, this subspecies might be considered to be in a group substantially confined to spot 1.

As Tsuyama[8)] described, the habitat of *C. japonica* subsp. *rusticana* is restricted within the snowy mountain areas in the Hokuriku district. This much narrower distribution, as compared with true *C. japonica,* might have resulted phylogenetically in the relatively narrow range of variation in pigment constitution.

3. *C. sasanqua*

C. sasanqua which comes under the section *Paracamellia,* is different from *C. japonica* and *C. japonica* subsp. *rusticana* both in its morphological characteristics and in the number of chromosomes. As presented in the appendix, the constitution of the anthocyanins was also different from that in the above mentioned species and subspecies. In addition to the main spots 1, 3, 4 and 5, this species contained spot 2 and spot 6, which never occurred neither in *C. japonica* nor in *C. japonica* subsp. *rusticana,* and these were assumed to be anthocyanins of delphinidin derivatives. As Yokoi[10)] pointed out, they might contribute in breeding true blue coloured cultivars in the *Camellia.*

Furthermore, in most of the cultivars of *C. sasanqua,* spot 5 was a predominant pigment. Namely, 40 cultivars out of 42 cultivars examined had much higher amounts of spot 5 than of spot 1. And, as seen in Table 1, the ratio of spot 1 to spot 5 in pigment constitution was 0.24, the smallest value among the species examined here. Therefore, although *C. sasanqua* contained the pigments of lower Rf values as in the cases of *C. japonica* and *C. japonica* subsp. *rusticana,* it was assumed to be a group with spot 5 as a main pigment (Table 2).

Recently, Kumazawa *et al.*[4)] and Kumazawa (personal communication) suggested that certain red coloured cultivars of *C. sasanqua* found in Hirado Island originated from some stock plants of *C. vernalis* which had also originated in the same island from the interspecific cross between wild *sasanqua* and *japonica.* In other words, they, especially Kumazawa, had an idea that the pink to red flower colours found in garden forms of *C. sasanqua* are duly derived from *C. japonica* through the intermediary of *C. vernalis* and not from the genetic variation within wild forms of *C. sasanqua.*

If we accepted this unique idea, the following conclusion might be drawn from the present investigation. Namely, in the processes from natural crossing between wild *sasanqua* and *japonica,* through various *vernalis* (from tetraploid to higher ploidy) to garden *sasanqua,* the mechanisms of anthocyanin synthesis were modified from the production of spot 1 to that of spot 5. In other words, as compared to *C. japonica,* the white, wild form of *C. sasanqua* seemed to have latently a capacity for producing a more chemically complex pigment, because spot 5 is one of the complex derivatives of spot 1, cyanidin 3-monoglucoside. In 1974, Parks *et al.*[6)] also observed a situation similar to this on the synthesis of anthocyanins in the hybrid progenies between *C. saluenensis* and *C. japonica.*

4. *C. hiemalis*

Although there were only 5 cultivars of the *C. hiemalis* that we were able to analyze in the present study, the characteristics of their pigment constitutions were very similar to those of *C. sasanqua*. As shown in the appendix, Fig. 2 and Table 1, this species contained the pigments of lower Rf values, that is, spots 1 to 6, in quite a similar proportion to *C. sasanqua*. Furthermore, spot 5 was a predominant anthocyanin and the ratio of spot 1 to spot 5 was 0.25 which agreed with the value of 0.24 in *C. sasanqua*.

Up to now, there has been little evidence available concerning the origin of *C. hiemalis*. However, that the similarities to the cultivars of *C. sasanqua* on the standpoints of pigment constitution, as well as its chromosome number and morphological characteristics, indicates that *C. hiemalis* might be phylogenetically derived through some processes similar to those of the garden forms of *C. sasanqua*.

5. *C. vernalis*

Since Makino[5] had regarded *C. vernalis* as an interspecific hybrid between *C. japonica* and *C. sasanqua* on the basis of its morphological characteristics in 1918, about 30 cultivars of *C. vernalis* have been discovered in Japan mainly by Kirino[2,3] and Hakoda *et al.*[1] Recently, in Hirado Island some original stock plants of *C. vernalis* were found by Kumazawa *et al.*,[4] and the cytogenetic investigation of these stock plants was conducted by Uemoto *et al.*[9] In the studies on the cultivars of *C. vernalis*, many of these workers suggested that this species showed morphologically and cytologically intermediate characteristics between *C. japonica* and *C. sasanqua*.

In the previous experiment on the anthocyanin constitution of *C. vernalis* in Hirado Island, the authors[7] revealed that the same, intermediate situation could be applied to the pigment constitution. And this situation further substantiated in this present experiment, the more detailed analyses on *C. vernalis*, in which 14 more cultivars were added. As apparent from the appendix and Table 1, the anthocyanin constitution of this species was clearly intermediate between *C. japonica* and *C. sasanqua*. The same relationship was also found in the relative amounts of spot 1 and spot 5, as seen in Fig. 2.

On the other hand, the frequency of occurrence of cultivars containing both spot 2 and spot 6, *C. sasanqua*-specific anthocyanins, was comparatively fewer, while the relative amounts of these anthocyanins in *C. vernalis* were lower, than those of *C. sasanqua* and *C. hiemalis* (Appendix). Therefore, the gene involved in the syntheses of these pigments, the delphinidin derivatives, might be recessive, as opposed to the gene concerning the production of delphinidin in the other plants which usually behaves as dominant to the gene concerning the production of cyanidin derivatives.

In the previous experiment on *C. vernalis* of Hirado Island, the authors[7] also revealed that, as compared to the primary tetraploid hybrid, the secondary triploid *vernalis* more closely resembled *C. japonica* and the secondary pentaploid *vernalis* more closely resembled *C. sasanqua* in their anthocyanin constitution. However, in this present experiment, we could not disclose a clear-cut relationship of this sort, because fully detailed data on chromosome number and pedigree of the respective cultivars examined have been unavailable.

6. *C. wabisuke*

The constitution of anthocyanins in *C. wabisuke* was extremely diverse within the cultivars examined. As seen in the appendix and Fig. 3, however, it seemed that dividing this species into four groups might be possible. Namely, (1) group with pigments of lower Rf values specific to *C. japonica* ('Kochō-Wabisuke' and 'Beni-Wabisuke'), (2) that with pigments of higher Rf values specific to *C. saluenensis* ('Sukiya', 'Shōwa-Wabisuke' and 'Tarō-Kaja'), (3) that, in addition to the

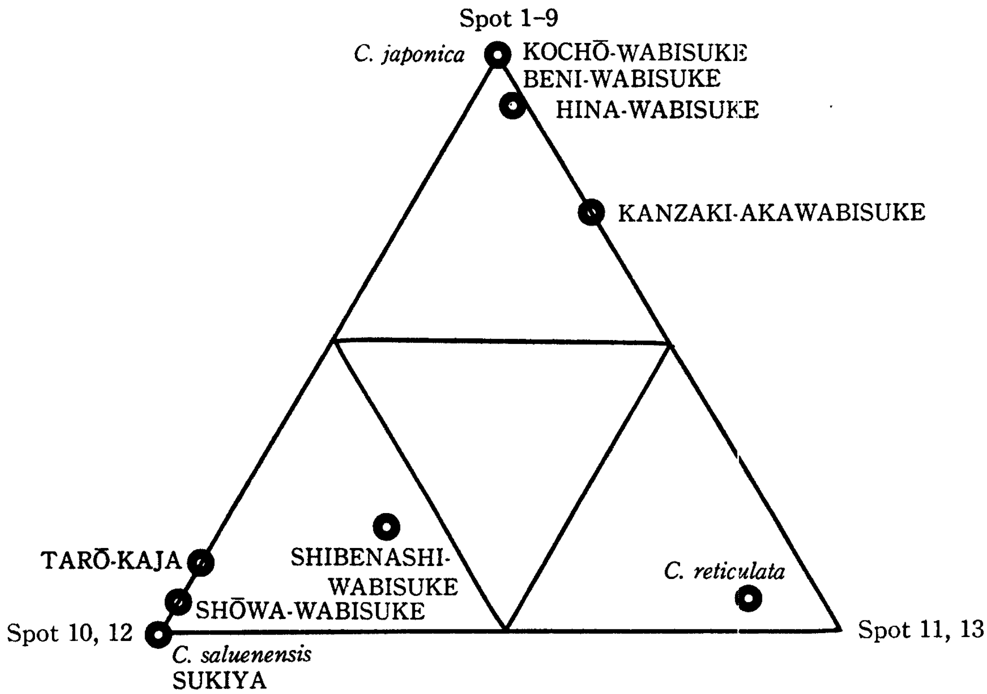

Fig. 3. The distribution of cultivars of *C. wabisuke* and allied species on the constitution of anthocyanins. Each corners of the triangle comprise 100% of each of the pigment-groups specific to *C. japonica* (spot 1–9), *C. saluenensis* (spot 10, 12) and *C. reticulata* (spot 11, 13), respectively.

pigments of lower Rf values, with pigments of higher Rf values specific to *C. reticulata* ('Hina-Wabisuke' and 'Kanzaki-Akawabisuke'), and (4) that, in addition to the pigments of lower Rf values, with pigments of higher Rf values specific to both *C. saluenensis* and *C. reticulata* ('Shibenashi-Wabisuke'), respectively. Thus, *C. wabisuke* contained cultivars with pigments of both lower Rf and higher Rf groups. Such a somewhat complicated situation as this suggests a phylogenetical diversity in this species, encompassing from the forms mainly derived from *C. japonica* or related species, through the forms mainly derived from *C. saluenensis* or their interspecific hybrids with lower Rf species, to the forms derived from some complicated participations of *C. reticulata* or related species on to such hybrid derivatives as *C. japonica-C. saluenensis*.

7. The accumulation of anthocyanins in petals

Generally, the deeper the colour of the petal, the more abundant the total amount of anthocyanin pigments in most of the ornamental plants. As indicated in the previous paper[7)], the same was the case in the cultivars of *C. japonica* in Hirado Island. However, the accumulation of the total amount of anthocyanins was controlled exclusively by the amount of spot 1 and not that of spot 5.

In Figs. 4 and 5, the interrelationship between the total amount of anthocyanin and the amount of spots 1 and 5 in the genus *Camellia* was presented. The amount of spot 1 both in *C. japonica* and *C. japonica* subsp. *rusticana* increased, but that of spot 5 did not increase, in accordance with the increasing of the total amount of anthocyanins. On the other hand, in *C. sasanqua* and *C. hiemalis* the amount of spot 5 increased, but that of spot 1 did not increase, in accordance with the increasing of the total amount of anthocyanins. In the cultivars of *C. vernalis,* however, both spot 1 and spot 5 increased equally in accordance with the increasing of the total amount of anthocyanins. Namely, the pattern of pigment-increments in *C. vernalis* was intermediate between that of *C. japonica* and *C. sasanqua*, which might be further evidence to support the hybridity of *C. vernalis* between *C. japonica* and *C. sasanqua*. In any case, there seemed to be two different factors participating in the pigment accumulation in the *Camellia*, one controlling the pigmentation of spot 1 in *C. japonica* etc., and the other controlling that of spot 5 in *C. sasanqua* etc., respectively.

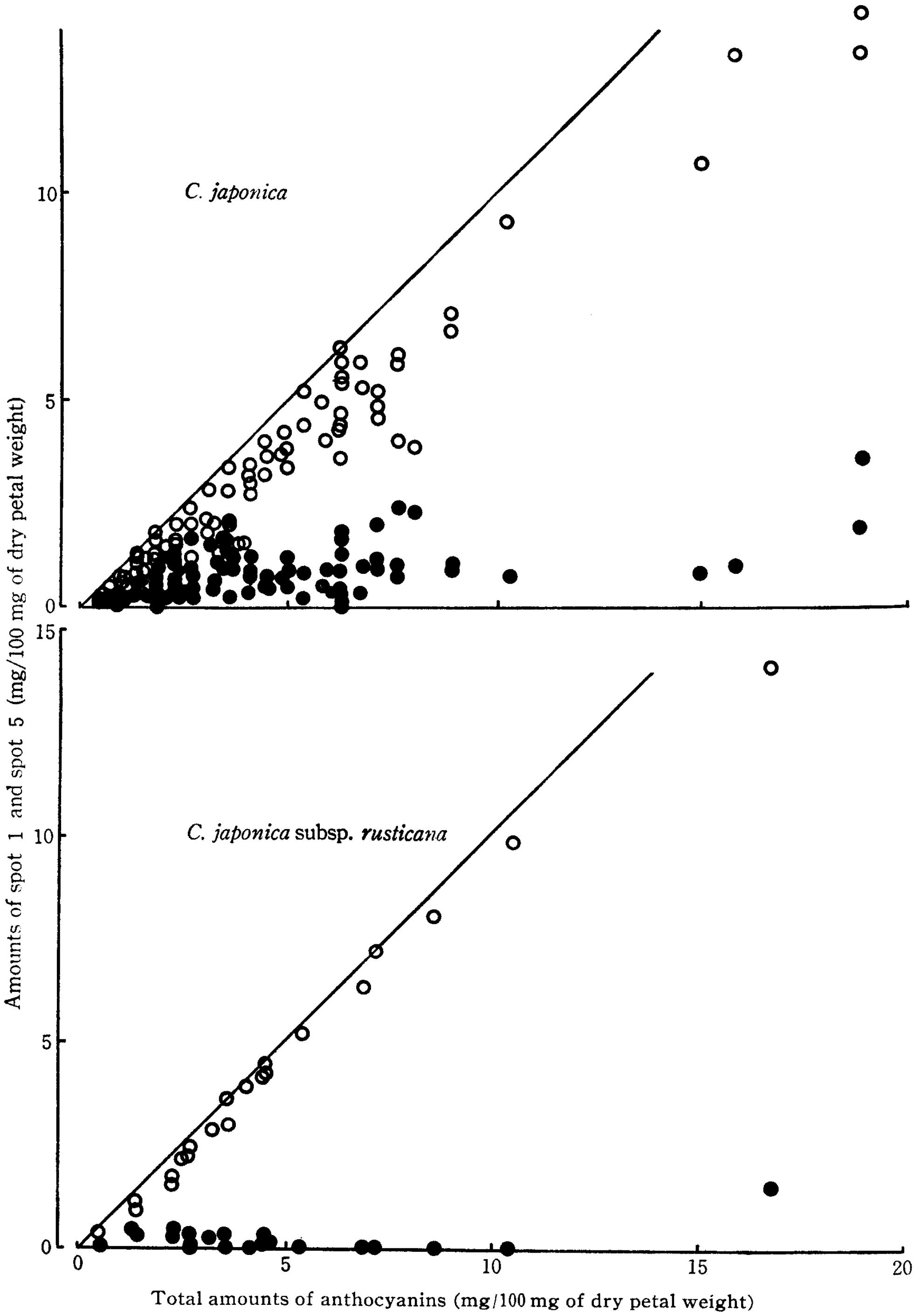

Fig. 4. Interrelationship between the total amount of anthocyanins and the amount of spot 1 (○) and spot 5 (●) in *C. japonica* and *C. japonica* subsp. *rusticana*. One dot represents one cultivar examined.

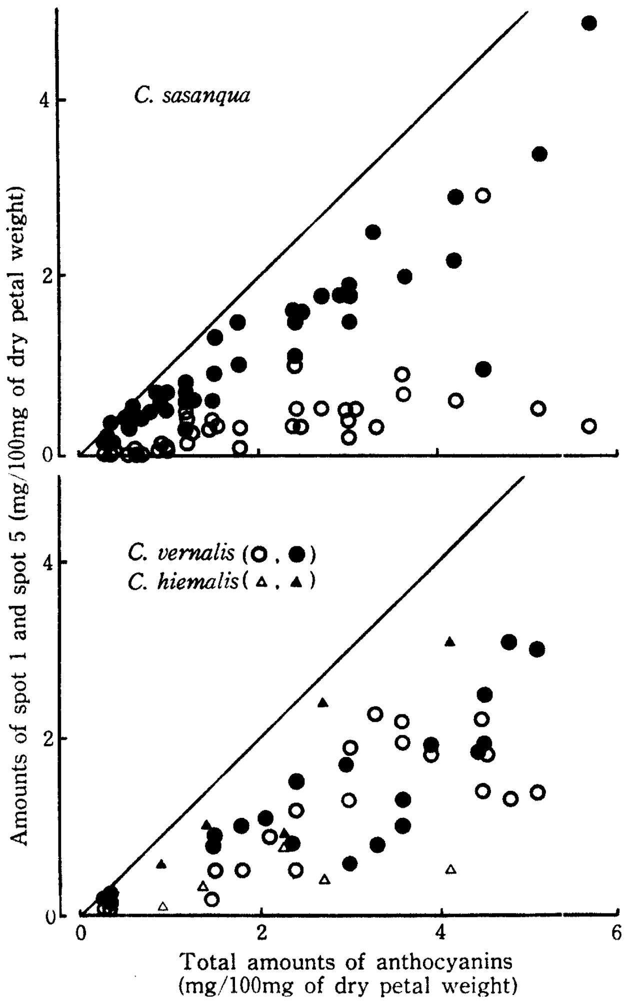

Fig. 5. Interrelationship between the total amount of anthocyanins and the amount of spot 1 (○, △) and spot 5 (●, ▲) in *C. sasanqua*, *C. vernalis* and *C. hiemalis*. One dot represents one cultivar examined.

Summary

The present investigation on the constitution of anthocyanins was conducted to clarify the origin and evolution of genus *Camellia*.

Fourteen kinds of anthocyanins were revealed to be present, and from its anthocyanin constitution the genus seemed to be divided into two groups, one with pigments of lower Rf values, and the other with those of higher Rf values. The former group contained *C. japonica*, *C. japonica* subsp. *rusticana*, *C. sasanqua*, *C. hiemalis* and *C. vernalis*, and the latter one contained *C. reticulata* and *C. saluenensis*. On the other hand, *C. wabisuke* contained cultivars with pigments of lower as well as of higher Rf values.

The major anthocyanin in *C. japonica* and *C. japonica* subsp. *rusticana* was spot 1 (cyanidin 3-monoglucoside), and the extent of variation in the anthocyanin constitution of the former was much more prominent than that of the latter, whereas the major anthocyanin in *C. sasanqua* and *C. hiemalis* was spot 5, one of the derivatives of spot 1, and in their anthocyanin constitution, both species were very similar.

On the other hand, the anthocyanin constitution of *C. vernalis* was intermediate between *C. japonica* and *C. sasanqua,* which again substantiated, as in the previous experiment, the hybrid origin of this species between the latter two species.

Furthermore, the cultivars of *C. wabisuke* displayed some extreme diversity in pigment constitution, which was interpreted as showing a phylogenetical diversity in this species, encompassing in scope the forms derived from *C. japonica,* through those from *C. saluenensis,* to those having some relation to *C. reticulata.*

Finally, in connection with the depth of flower colour, spot 1 determined the total amount of anthocyanins in *C. japonica* and *C. japonica* subsp. *rusticana,* whereas spot 5 determined in *C. sasanqua* and *C. hiemalis.* In this respect, *C. vernalis* was intermediate between the above two groups, in that both spots 1 and 5 increased equally in accordance with the increasing of the total amount of anthocyanins.

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Appendix; Constitution of anthocyanins in genus *Camellia*

Cultivar	Percentages of constituent anthocyanins														Total antho-cyanin*2
	1*1	2	3	4	5	6	7	8	9	10	11	12	13	14	
C. japonica															
TIFFANY	18			23	59										0.4
KIKUZUKI	23		6	16	55										2.2
KARIGINU	25			18	57										1.8
DAIKAGURA	27			29	35									9	3.6
cv. 1	27			30	43										0.4
AZUMA-SHIBORI	27			15	58										2.2
cv. 2	28		7	10	55										3.6
GUILIO NUCCIO	31			25	44										3.6
SHEBOSHUSHI	38				62										0.4
UMEGAKI	38			33	29										4.0
LINDSAY NEILL	38			26	36										1.3
SHISANTAIBAO	41			19	40										1.8
GUEST OF HONOR	41			19	40										1.3
KANYOTAI	41			+	59										2.7
BENI-OTOME	42		21		34										2.7
SHUN-SHOKKŌ	43				57										0.4
HIGURASHI	43			23	34										3.6
ZOUGONGSHENG	44			16	40										0.9
ŌNIJI	46			3	51										2.2
SHIKON-TSUBAKI	47			20	33		+	+							8.1
cv. 3	47			20	33										0.9
DEBUTANTE	47				53										0.4
DRAMA GIRL	48			32	20										2.2
NINA AVERY	48			29	23										0.4
HI-RENGE	49		10		41										2.2
HI-BOTAN	50			+	50										3.1
KUJAKU-TSUBAKI	52			15	30			3							7.6
NINNA-JI	53		29		18										0.9
ROSE DAWN	54		13		33										2.7
AKI-NO-YAMA	54		11		35										3.1
KINGYOBA-TSUBAKI	55		17		28										0.4
HIKARUGENJI	55		14		31										0.9
MURA-MUSUME	56			18	21						2		3		2.2
CHŪBU-TAIYŌ-NISHIKI	57		17		26										1.3
SHOKKŌ	57		10		28			5							6.3
MIKENJAKU	58		26		26										1.3
KIKU-SARASA	60		17		23										0.4
KIKU-TŌJI	61				39										3.1
AKASHIGATA	61		7		32										2.2

*1 Numbers used are corresponding to the spot numbers represented in Fig. 1.
*2 mg/100 mg of dry petal weight

Appendix; (Continued)

Cultivar	Percentages of constituent anthocyanins														Total antho-cyanin*2
	1*1	2	3	4	5	6	7	8	9	10	11	12	13	14	
SHUSU-GASANE	61			24	15										7.2
KUMASAKA	61			13	26										1.3
HARU-KAGURA	62			28	10										1.8
SHŌJHŌ	64			18	18										1.8
SAZANAMI	65			14	21										1.3
FUKURIN-BENTEN	67			14	19										2.2
GENJI-GURUMA	67		13		20										3.1
cv. 4	67			5	28										7.2
KAYOI-DORI	68				32										0.4
HIJIRIMEN	68		6		13			13							6.3
MASAYOSHI	68		10		17			1	4						4.0
HATSUSE-YAMA	69			10	21										0.9
IWANE-SHIBORI	69		12		19										4.9
AMA-NO-GAWA	69			12	19										2.2
PINK CHAPEAU	69			15	16										2.2
cv. 5	70			10	20										6.3
FUKU-TSUZUMI	70		17		13										1.3
DRIFTWOOD	70			12	18										1.8
KON-WABISUKE	71			7	19										18.9
HONGBAIGONG	71			14	15										4.5
ELIZABETH LE BAY	72		8		20										0.9
MON-JUSU	72			17	11										7.2
KURO-TSUBAKI	72				9		3	6	10						15.0
CM HOVEY	73				27										6.3
AMAGASHITA	73		10		17										4.0
EZO-NISHIKI	75				25										3.6
LADY MACON	75			+	25										0.9
SHICHIHENGE-TSUBAKI	76			14	10				+						18.9
HEART O'GOLD	76		11		13										4.9
APPOLLO	76		6		18										2.2
CHŪBU-SHIKAINAMI	76				24										4.9
ARAHŌSHI	76		8		16										2.7
YAMATO-NISHIKI	77			7	16										1.8
YAE-HIME	77			11	10			2							7.6
TSURIKAGARI	78		9		11			2							7.6
AMA-OBUNE	79				10		11								9.0
MOSHIO	79		6		15										6.7
UNRYŪ-TSUBAKI	79		6		12			3							4.5
OIMATSU	80		5		15										4.0
SHIN-TSUKASA	81				19										1.3
GENJI-SHIBORI	81		9		10										1.8

Appendix; (Continued)

Cultivar	Percentages of constituent anthocyanins														Total antho-cyanin*2
	1*1	2	3	4	5	6	7	8	9	10	11	12	13	14	
cv. 6	82		4		14										5.4
CHŌ-GA-SHIMA	83			8	9										5.8
KANSAI-KOKURYŪ	83		7		10										9.0
HIGO-KŌBAI	84			2	10		2	2							4.0
NISHIKI-GASANE	84		6		10										4.9
YURI-TSUBAKI	85		+	5	5			5							6.3
KŌ-ARAJISHI	85		5	4	6										15.7
FUIRI-KARAKO	87				13										2.2
YOSHI-TSUKASA	87		6		7		+	+							2.7
cv. 7 (SENKAKU Island)	88		+		12										4.5
KUMAGAI	88		7		5										6.3
KYŌ-NISHIKI	88				12										0.4
VICTOR EMMANUEL	88				12										3.1
seedling of MATSUKASA	88		4		4			4							6.7
cv. 8	90			3	7										10.3
KRAMER'S SUPREME	92			3	5										1.8
cv. 9	93		4		3										6.3
CHŌSEN-TSUBAKI	94				6										3.6
HINOMARU	96		+		4										5.4
NANBAN-KŌ	100														1.8
CHRISTMAS BEAUTY	100														6.3
C. japonica subsp. *rusticana*															
KOMATSU-HIME	100														7.2
HOSHI-HIME	100														3.6
SHIBORI-CHŌJI	97				3										4.5
AIAI-GASA	96								4						5.4
wild form	96								4						10.3
SENDAI-AKATSUBAKI	95								5						8.5
IWAI-NO-SAKAZUKI	95								5						4.0
RYŪGŪ	95								5						6.7
KASUGA-YAMA	93			3	1		+		+						4.5
cv. 1	93				7										3.1
cv. 2	93				7				+						4.5
NIIGATA	89								8		3				2.7
HANA-KANZASHI	88				12				+						2.7
SHIMA-CHIDORI	87		7		6										2.7
TACHIYAMA	85			6	9										16.6
HOSHI-GURUMA	84		8		8										3.6
OTOME-TSUBAKI	81				19										1.3
SENDAI-USUBENI	81								3		16				0.4
cv. 3	75		5		7						13		+		2.2
YUKI-KOMACHI	73		11	3	13										2.2

Appendix; (Continued)

Cultivar	Percentages of constituent anthocyanins														Total antho-cyanin*2
	1*1	2	3	4	5	6	7	8	9	10	11	12	13	14	
FUKURIN-IKKYU	64		15		21										1.3
C. sasanqua															
cv. 1	6			8	86	+									5.7
cv. 2	6		11		83	+									0.6
CHANSONETTE	2	+	14		82	2									1.8
MEIGETSU	14		7		79	+									0.9
MIKUNI-KŌ	8	+	13		75	4									3.3
MOMOYAMA	8		5	14	73	+									0.9
HŌREI	9		18		73	+									0.3
HIKARU-GENJI	10		19		71										0.6
SAOTOME	8	+	13		71	8									0.9
HITORI-SHIZUKA	11		9	9	69	2									1.2
ASAHI-NO-UMI	15	+	10		69	6									4.2
cv. 3	14	+	14		67	5									2.4
GOSHO-GURUMA	13		20		67										0.6
KŌ-OTOME	10	+	18		67	5									5.1
SHŌWA-NISHIKI	11		22		67	+									0.3
cv. 4	12	+	9	7	67	5									2.4
FUKU-WARAI	7	+	21		65	7									0.9
BENI-TSUKASA	20	+			65	15									2.7
SEKIYŌ	20	+		7	64	+									2.4
MIYAKO-NO-HARU	13	+	21		62	4									0.6
DAZZLER	12	5	11		62	10									3.0
cv. 5	19	+	14		61	6									1.2
IRIHI-NO-UMI	17	2	10	4	61	6									3.0
SAIKAI	18	+	17		60	5				+					3.6
cv. 6	13	+	20		60	7									0.9
cv. 7	7		25	8	60	+									3.0
cv. 8	12	+	19		59	10									0.6
cv. 9	13	+	23		58	7									0.9
ROSY MIST	19	+	12	4	58	7									1.5
HARUSAME-NISHIKI	13	+	26		57	4									0.6
TENNYO-NO-KAORI	19		15	10	56	+									1.5
cv. 11	24	+		12	55	9									3.6
EIKYŪ-SHIBORI	14	4	18		55	9									1.8
ŌMI-GOROMO	11	+	34		52	3									0.3
WAGŌJIN	30	+	14		51	5									1.2
cv. 12	11	3	18	11	50	7									0.9
HI-NO-KOROMO	18	5	16	5	49	7									3.0
WAKAKAEDE	16	7	18		48	11									1.2
NEGISHI-KŌ	14	+	24	7	48	7									0.3
HIRAN	43		4	6	47	+					+				2.4

Appendix; (Continued)

Cultivar	Percentages of constituent anthocyanins														Total antho-cyanin*2
	1*1	2	3	4	5	6	7	8	9	10	11	12	13	14	
SHICHIFUKUJIN	19	5	31	+	40	5									1.5
HI-NO-TSUKASA	64	+	8		22	6									4.5
IRO-MO-KA-MO	46		17	12	20										1.2
C. hiemalis															
SHISHI-GASHIRA	13		+		87										2.7
HI-OTOME	13		11		76	+									4.1
SHŌWA-NO-SAKAE	12		9	8	71										1.4
SHŌWA-NO-HOMARE	9	4	18	+	64	5									0.9
TACHI-KANTSUBAKI	36	4	7	8	38	7									2.3
C. vernalis															
cv. 1	21	+	10	5	64	+									2.4
ASAHI	27		9		64										0.3
KHŌREI	26		10		64										4.8
GAISEN	28	+	6	7	59										5.1
ŌMI-GOROMO	33		+	10	57										1.5
SAHO-HIME	43				57										3.0
HOSHI-HIME	32		+	12	56										4.5
BENI-SUZUME	15		16	13	56										1.5
HISADOMI	25			20	55										1.8
EGAO	31		+	15	54										0.3
EGAO-KURENAI	42			8	50										2.1
KOTOHIRA-BENI	40		5	5	50										—
SHOKKŌ-NISHIKI	46		5		49										3.9
KOKINRAN	35		15	6	44										0.3
SANDANKA	48		5	8	39										4.5
BŌKYO	40	3	8	6	39	4									4.5
RYŪKŌ	55			5	36										3.6
YŪGEN	54		3	6	35			2							16.0
SAYŌ-HIME	50		3	14	33										2.4
HOSHI-HIRYU	60	+	5	6	29										3.6
KARA-GOROMO	69			8	23										3.3
KAMAKURA-SHIBORI	64			16	20										3.0
C. wabisuke															
KOCHŌ-WABISUKE	76		16		8										1.8
BENI-WABISUKE	34			23	43										1.3
SUKIYA					+					29		71			0.8
SHŌWA-WABISUKE	8									30		62			0.8
TARŌ-KAJA	11				+					39		50			0.8
HINA-WABISUKE	15				76					1	7	1			1.8
SHIBENASHI-WABISUKE	9				9					14	12	45	11		2.2

Appendix; (Continued)

Cultivar	Percentages of constituent anthocyanins														Total antho-cyanin*2
	1*1	2	3	4	5	6	7	8	9	10	11	12	13	14	
KANZAKI-AKAWABISUKE	62				11						19		8		0.8
C. saluenensis															
wild form										35		65			0.9
C. reticulata															
CAPTAIN RAWES	1		2		2					6	37	7	45		3.6