Some Morphological and Pigmental Characteristics in *Rhododendron kaempferi* Planch., *R. kiusianum* Makino and *R. eriocarpum* Nakai in Southern Kyushu

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Summary

The variations in some morphological characteristics along with the pigmental ones of *Rhododendron kaempferi* Planch., *R. kiusianum* Makino and *R. eriocarpum* Nakai distributing in southern Kyushu, were investigated.

The populations of *R. kaempferi* in southern Kyushu showed much wider variations in flower-colour, flower-size and the occurrence of petal-blotch than those in northern Kyushu. Furthermore, in the former populations, anthocyanins of the delphinidin-series co-existed with the commonly occurring cyanidin-series, as compared with the latter whose anthocyanins were confined to the cyanidin-series. Hence, the southern populations of *R. kaempferi* seemed to have been resulted from the introgressions of other azaleas with anthocyanins of the delphinidin-series to the true forms.

Extremely narrow variations in the morphological and pigmental characteristics were shown in *R. kiusianum*, suggesting that populations distributing at and around the summit of volcanic mountain, Mt. Karakunidake, represent true species. On the other hand, population of *R. eriocarpum* at the summit of Mt. Kaimondake showed wider variations in flower-colour and the occurrence of flavonoid pigments than that of Mt. Ontake in Tokara-Archipelago. These variations seen in the former might be the result of the introgression of *R. kaempferi* occurring near the habitat of *R. eriocarpum* at the summit of Mt. Kaimondake.

In any case, the populations of *R. kaempferi* and *R. eriocarpum* in southern Kyushu exhibited wider variations and contained many peculiar individuals with respect to their floral characteristics. Therefore, we can easily adopt the more promising germplasms as the materials of future breeding among their natural variations.

Introduction

Japan is well known as a treasure house of the wild evergreen azaleas. Especially in Kyushu, wild evergreen azaleas classified as the series *Kaempferia* or allied series such as *Indica* and *Macrosepara* (7), occur widely and abundantly (8). Of these azaleas, *R. kiusianum* Makino, one of the most valuable species for ornamental use, distributes in the regions confined to the summits of high volcanic mountains, and *R. kaempferi* Planch., the

most commonly occurring azalea in our country, is found at the foot of these mountains and along the west coast of Kyushu. Furthermore, *R. eriocarpum* Nakai, the flower-colour of this azalea has exerted great influences on the purplish tone of *Satsuki* azalea (4), distributes at the summits of the mountains in Tokara-Archipelago and the most southern part of Satsuma-Peninsula.

Since 1981, the authors have investigated the geographical distribution of the wild evergreen azaleas in Kyushu, in connection with their morphological and pigmental characteristics, especially in southern Kyushu where they exhibit great variation (2).

The present investigation was conducted to ob-

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tain detailed figures concerning the variations in flower-colour, flower-size, the occurrence-manner of petal-blotch and flavonoid pigments of *R. kaempferi*, *R. kiusianum* and *R. eriocarpum*, in southern Kyushu. The results dealing with other azaleas occurring on the Kirishima and Takakuma mountain masses which were regarded as the result of natural hybridization between wild species, will be presented in a forthcoming paper.

Materials and Methods

1. Plant materials

The localities where the flowers of evergreen azaleas were collected and investigated are shown in Fig. 1. The materials used were *R. kaempferi* from Satsuma-Peninsula (alt. 100~150 m) and Ohsumi-Peninsula (alt. 50 m), *R. kiusianum* from Ebino-Plateau (alt. 1,100 m) and the summit of Mt. Karakunidade (alt. 1,700 m) and *R. eriocarpum* from the summits of Mt. Ontake (alt. 978 m) in Nakano-Shima and Mt. Kaimondake (alt. 922 m). In addition to these, *R. kaempferi* from Misumi Town (alt. 50 m) and Kikuchi City (alt. 150~200 m) were used in order to compare these two populations with those in southern Kyushu.

Fig. 1. Localities where the flowers of *R. kaempferi*, *R. kiusianum* and *R. eriocarpum* were investigated in Kyushu.

The number of individuals investigated for the phenotypic characteristics of petals was approximately 500 to 1,000 in each locality except for *R. kaempferi* from Misumi Town and Kikuchi City and *R. eriocarpum* from Mt. Kaimondake where nearly 100 individuals were sampled. For the pigmental characteristics of petals approximately 50 to 100 individuals were analyzed in each locality.

2. Investigation of phenotypic characteristics of petals

The colour of the flowers was recorded using the Royal Horticultural Society Colour Chart (RHSCC) and then, flower-diameter (mm) was measured followed by the assortment of flowers into three types according to the intensity of petal-blotch; prominent, slight or none.

3. Qualification and quantification of petal anthocyanins and flavonols

The detailed procedures used for pigment-extraction are shown in Fig. 2. Based on this method, no more than 10 mg of dried petal was needed for analysis. Using a system of the high performance liquid chromatography (HPLC), pigment-eluates were separated into the respective pigment-fractions, then they were identified by a

Fig. 2. Procedures for the extraction of pigments.

comparison with authentic specimens. The ratio of the amount of each pigment to the total amount was also estimated. The analytical conditions of an HPLC were as follows: Chromatograms were run on a CCPD-8000 HPLC-system (TOSHO) using a 4.6 mm (I.D.) $\times$ 25 cm column packed with Nucleosil 7C₁₈ (Macherey-Nagel) and a UV-8000 detector (TOSHO) set at 535 nm for anthocyanidins or 365 nm for flavonols. A flow-rate of 1.2 ml/min was maintained employing 50% MeOH containing 0.1% HClO₄ as an eluent.

Results and Discussion

Concerning flower-colour, the frequency distribution of individuals in the population of *R. kaempferi* based on the RHSCC presented in Fig. 3, showed that the variation found in the two southern populations (Satsuma- and Ohsumi-Peninsula) was somewhat wider than that found in the more northern ones (Misumi T. and Kikuchi

C). The former contained individuals with flower-colour ranging from vermillion through red to pink, whereas in the latter, the flowers were mainly red. Analogous situations to the above could be seen concerning flower-size and the presence of prominent blotches. Southern populations showed a much wider variation in flower-size, ranging from 26 to 60 mm in diameter, and contained nearly 10% of individuals with slight petal-blotch or without petal-blotch (Fig. 4).

As regards the petal-pigmentation, all individuals of all populations of *R. kaempferi* were flavonol free, and accordingly it was impossible for them to have contained 5-methylated flavonols (Fig. 5). On the other hand, as seen in Fig. 6, in petals of the two southern populations with RHSCC numbers above 49, anthocyanins of the delphinidin-series, the most important constituents modifying the flower-colour to a much bluer tone, co-existed with the cyanidin-series commonly found in *R. kaempferi*

Fig. 3. Frequency distribution of individuals concerning flower-colour in *R. kaempferi*, *R. kiusianum* and *R. eriocarpum*.

Fig. 4. Frequency distribution of individuals concerning flower-size and petal-blotch in *R. kaempferi*, *R. kiusianum* and *R. eriocarpum*. Open column, flowers with slight blotches or without blotches; filled column, flowers with prominent blotches.

and other azaleas (3, 5, 9). This may be one of the reasons why these southern populations showed a wider variation in flower-colour.

In general, the variation in morphological and pigmental characteristics in *R. kaempferi* is believed to be quite narrow, flower-colour being confined to vermilion or red, the flowers ranging in diameter from 40 to 50 mm with a prominent blotch (6), and having anthocyanins of the cyanidin-series alone and no flavonols. If we consider the typical *R. kaempferi* in Kyushu in the light of these concepts, populations in the northern localities which show such a narrow range of variation probably represent the true forms. On the other hand, populations in the southern Kyushu showing a wider range of variation might be the result of further introgressions of *R. kaempferi*, to the original

hybrid population derived from the cross between *R. kaempferi* and other species with anthocyanins of the delphinidin-series (5).

R. kiusianum showed extremely narrow variation in flower-colour. More than 90% of the individuals of both populations showed RHSCC numbers above 75, purple or violet shade (Fig. 3). This situation was also supported by their pigmental characteristics. In all individuals of both populations, 40 to 75% of the total anthocyanins were of the highly hydroxylated delphinidin-series, and 5-methylated flavonols made up 40% to nearly 90% of the total flavonols (Figs. 5 and 6). The occurrence of the latter pigments is also thought to be a factor contributing to the bluer tone of the flower (1).

Concerning flower-size and the presence of

Fig. 5. Frequency distribution of individuals concerning the occurrence of 5-methylated flavonols in *R. kaempferi*, *R. kiusianum* and *R. eriocarpum*.

prominent blotches, variation in both populations was quite narrow. The flowers ranged from 21 to 36 mm in diameter, and the prominent blotches occurred in less than 10% of the individuals (Fig. 4). Thus, the small flowers without prominent blotches were thought to be typical characteristics of *R. kiusianum*.

In view of their extremely narrow variations in the phenotypic and pigmental characteristics, and as their habitat in southern Kyushu is restricted to the high volcanic mountains, it seems likely that these populations also represent true forms.

As regard the flower-colour of *R. eriocarpum*, the variation found in the population of Mt. Kaimondake was wider than that found in the population of Mt. Ontake. The former population contained individuals with flower-colour ranging from red through pink to purple, whereas the latter contained those with flower-colour confined to purple or violet (Fig. 3). Analogous situations to the above could be seen concerning petal-pigmentation. As

compared with the former population which contained some individuals without the delphinidin-series or 5-methylated flavonols, in all individuals of the latter population, 22 to 70% of the total anthocyanins were the delphinidin-series, and 5-methylated flavonols amounted to 28 to 88% of the total flavonols (Figs. 5 and 6). This may be one of the reasons why the population of Mt. Kaimondake showed wider variation in flower-colour.

Concerning flower-size, individuals in both populations showed considerable intra-populational variations in diameter, ranging from 18 to 53 mm. However, concerning petal-blotch, two populations differed. In nearly 50% of individuals in the population of Mt. Ontake, the prominent blotches occurred, whereas those occurred in more than 75% of individuals in the population of Mt. Kaimondake (Fig. 4).

From the viewpoints of the narrow variation in the phenotypic and pigmental characteristics other than flower-size seen in the population of Mt. On-

Fig. 6. Frequency distribution of individuals concerning the occurrence of anthocyanins of the delphinidin-series in *R. kaempferi*, *R. kiusianum* and *R. eriocarpum*.

take, and as the habitat in Tokara-Archipelago is restricted to the active volcanic mountains, this population seems likely to be a true species. On the other hand, the wide variation in characteristics in the population of Mt. Kaimondake suggests that it has resulted from further introgressions of *R. kaempferi* to the true *R. eriocarpum* once distributed at the summit, as in the case of *R. kaempferi* in the southern Kyushu.

In any case, the populations of *R. kaempferi* and *R. eriocarpum* in southern Kyushu exhibited wider variations and contained many peculiar individuals with respect to their floral characteristics. Therefore, we can easily adopt the more promising germplasms as the materials of future breeding among their natural variations.

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九州南部のヤマツツジ、ミヤマキリシマおよびマルバサツキの形態と 色素分布の変異について

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摘 要

九州南部のヤマツツジ群落には花色が豊富なこと、花径に小輪から大輪までの幅があることおよびブロッチの無い花卉を持つ個体があることなど、九州中・北部の群落に比較して形態にかなりの変異が認められた。色素分布も、後者はシアニン系のみに限られるのに対し、前者はシアニン系にデルフィニン系の混在する個体が数多く見られることなど、変異に富んだ様相を示した。これらの変異は、九州南部の群落はヤマツツジにデルフィニン系色素を持つ他の種が浸透交雑したことによって成立した可能性があることを示唆している。これに対し、霧島山系韓国岳周辺のミヤマキリシマ群落の形態と色素分布の様相は変異にきわめて乏しかった。おそらく純粋種としてこの群落を捉えてよいであろう。

一方マルバサツキは、トカラ列島中之島御岳の群落で花径とブロッチの出現率を除いた諸形質に変異は認められなかったが、薩摩半島開聞岳の群落で花色に変異が認められた。色素的にも5-メチルフラボノールやデルフィニン系色素を含まない個体が見られ、この場合は山頂部にすでに侵入しているヤマツツジがマルバサツキとの交雑に与かり、群落の成立に濃い影を落としたと考えられる。

一般に異質の“germplasm”が出会うところでは、諸形質の変異は大幅に拡大されるが、九州南部のヤマツツジや開聞岳のマルバサツキがそうであろう。したがって将来これらの個体を育種材料として用いる場合には、本研究で認められた多くの変異の中から、より有用な個体を抽出することが肝要と思える。