Cytogenetic Studies on the Origin of *Camellia* × *vernalis*

IV. Introgressive Hybridization of *C. sasanqua* and *C. japonica*

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Summary

The chromosome numbers of the natural seedlings of *Camellia* × *vernalis*, cv. 'Gaisen' (tetraploid, $n=15$) and cv. 'Bokyo' (pentaploid) were determined and the origin of red pigmentation in *C. sasanqua* is discussed. The seedlings of *C. sasanqua* × *vernalis* cv. 'Gaisen' were triploid, tetraploid and pentaploid. When *C. sasanqua* was abundant around the parent tree, pentaploid seedlings were more numerous than triploid ones. The pentaploid seedlings were considered to be backcrosses with *C. sasanqua* (hexaploid), and the triploid to be backcrosses with *C. japonica* (diploid). The chromosome numbers of the seedlings of *C. sasanqua* × *vernalis* cv. 'Bokyo', a pentaploid, ranged from $2n=79$ to 86, suggesting that only backcrosses to *C. sasanqua* are occurring. If *C. sasanqua* × *vernalis* hybrids typically only backcross with the *C. sasanqua* parent, and some fertility is maintained in the interspecific backcrosses with *C. sasanqua*, then this backcrossing system becomes a mechanism for the flow of *C. japonica* genes into the species *C. sasanqua*. Thus, the red flowers of *C. sasanqua* are thought to have originated from repetitive backcrossing (introgression) with *C. sasanqua* × *vernalis*.

Introduction

In the first paper(23) in this series, 'Gaisen', a tetraploid ($2n=60$) cultivar of *Camellia* × *vernalis*, was postulated to be a primary hybrid between *C. sasanqua* (hexaploid) and *C. japonica* (diploid). Then in the second paper (21), the hybrid species *C. sasanqua* × *vernalis* was reported to include triploid, tetraploid and pentaploid individuals. The triploids and pentaploids were postulated to be backcrosses between 'Gaisen' type tetraploids and *C. japonica* and *C. sasanqua*, respectively.

While the flowers of wild forms of *C. sasanqua* are white or white with a faint pink margin, cultivated forms of this species are often brightly colored with red anthocyanin pigmentation. Pinkish-white flower forms are occasionally found in wild population. Recently, Kondo *et al.*(9) found a pinkish-white flower form of *C. sasanqua* on Shikoku Island, and Parks (personal communication) found a *C. sasanqua* in a wild population near Kurume with pale pink flowers. *C. sasanqua* may in-

clude a great range of variation in wild forms as well as *C. japonica*(24, 25). However, wild *C. sasanqua* forms with deep anthocyanin pigmentation have never been found, and foliage size and thickness and the petal width of the cultivars of *C. sasanqua* (including white ones) show some morphological similarities with *C. japonica*. Also segregation can typically be observed in the seedlings of cultivars of *C. sasanqua*. Parks *et al.*(14) suspected that *C. japonica* is the most probable source of this anthocyanin flower pigmentation in *C. sasanqua* despite the sterility barrier, since both species have a long history of cultivation in Japan and since *C. japonica*, *C. sasanqua* and *C. sasanqua* × *vernalis* have the same cyanidin glycosides.

The term, "introgressive hybridization" was defined as the process of the gradual infiltration of the germplasm of one species into that of another by Anderson(5), and in his monograph, he discussed its ecological and genetic bases, and its roles in finite populations and evolution. Since then, various genera of higher plants have been investigated for chemotaxonomical evidences of introgressive

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hybridization(4, 7, 8, 10, 11, 12, 14, 15, 16, 19, 22); however, it has proved very difficult to document introgression in the large number of plant groups where it has been postulated.

The present investigation was done to determine the origin of triploid and pentaploid cultivars of *C. × vernalis* and to investigate the hypotheses of the origin of red pigmentation in the flowers of *C. sasanqua* by Parks *et al.*(14).

Materials and Methods

The seeds used in this study were collected from the two very old trees of *C. × vernalis* cv. 'Gaisen' and the original tree of cv. 'Bokyo' in private gardens on Hirado Island, Japan. Root tips were taken from the seedlings, treated in 8-hydroxyquinoline for five hours and rinsed in tap water. Immediately, or after storage in Newcomer solution, the root tips were hydrolyzed in 1 N HCl for 15 min and stained in basic Fuchsin. Slides made by the

squash method were frozen and then the cover slip was removed. Finally, the preparations were restained with Giemsa's solution(21).

Results and Discussion

Twenty-one open-pollinated seedlings of cv. 'Gaisen' (tetraploid) and twenty-three open-pollinated seedlings of 'Bokyo' (pentaploid) were grown from the seeds obtained from the stock trees of *C. × vernalis* on Hirado Island. Though the cultivars of *C. × vernalis* are usually very infertile, these trees are both very large and heavily flowering and set some viable seeds.

Note from the data presented in Table 1 that

Table 1. Somatic chromosome numbers of the seedlings of *Camellia × vernalis* cv. 'Gaisen' (V1).

Ploidy	3×	4×	5×	>5×
Garden 1	3	1	3	1
Garden 2	1	5	6	1
Total	4	6	9	2

Figs. 1—3. Somatic chromosomes in root-tip cells of the open-pollinated seedlings of *G. × vernalis* cv. 'Gaisen'.

Fig. 1. ($\times 1795$), $2n=45$, Fig. 2. ($\times 1190$), $2n=60$, Fig. 3. ($\times 1795$), $2n=75$

Fig. 4. Somatic chromosomes in root-tip cells of the open-pollinated seedlings of *C. × vernalis* cv. 'Bokyo' ($\times 1795$), $2n=82$.

Fig. 5. Vegetation map of garden 1. J: *Camellia japonica*, JO: cv. Hirado-Oyabu, S: *C. sasanqua*, V: *C. vernalis*, V1: cv. 'Gaisen' (seed parent), V2: cv. 'Sayohime', T: *C. sinensis*, H: residence.

the somatic chromosome numbers of the open-pollinated seedlings of *C. $\times$ vernalis* cv. 'Gaisen' were triploid ($2n=45$, Fig. 1), tetraploid ($2n=60$, Fig. 2), and pentaploid ($2n=75$, Fig. 3). The variable ploidy levels of the seedlings are unusual but can be explained by the strong self-incompatibility of the *C. $\times$ vernalis* parent (20) and the presence of compatible pollen from the parent species, *C. sasanqua* (hexaploid) and *C. japonica* (diploid).

Vegetation maps of the private gardens from which the seeds of *C. $\times$ vernalis* cv. 'Gaisen' were obtained are shown in Figs. 5 and 6. There are forty-five species of trees, nine shrubs, eight climbing plants and thirty-three herbs in the two gardens, including three species of the genus *Camellia*, *C. japonica*, *C.*

sasanqua and *C. sinensis*. Around both parent stocks of *C. $\times$ vernalis* (V1), *C. sasanqua* (S) was abundant. However, the occurrence of *C. japonica* (J) was different in the two gardens. In garden 1, a few large trees of *C. japonica* were observed, but in garden 2, the only reproductive *C. japonica* was a small hedge which flowered sparingly and was located 30 m from the parent stock tree of *C. $\times$ vernalis* cv. 'Gaisen'. Furthermore, on Hirado Island the blooming period of *C. $\times$ vernalis* cv. 'Gaisen' (November to February) partly overlaps both *C. sasanqua* (October to December) and *C. japonica* (December to April). Three open-pollinated pentaploid seedlings were obtained from the first garden, and six from the second garden, while three open-pollinated triploid

Fig. 6. Vegetation map of garden 2. See Fig. 5 for an explanation of the abbreviations used here.

seedlings were obtained from the first garden and one from the second garden (see Table 1). Seedlings from 'Gaisen' type tetraploid cultivars of *C. × vernalis* are usually either triploid or pentaploid, depending upon whether the parent tree is surrounded by *C. sasanqua* or *C. japonica*. Thus, the pentaploid and triploid seedlings were likely formed by natural backcrosses with *C. sasanqua* and *C. japonica*.

Six tetraploids also appeared in the seedlings from the two stock trees (Table 1) and were considered to be self-pollinated despite the known self-incompatibility of *C. × vernalis* cv. 'Gaisen'(20). These rare events of self-pollination are detectable because the two parent trees are so large and produce so many flowers. Although about 1/3 of the seedlings produced

were derived from self-pollination, it should be noted that only twenty-one seedlings were produced from thousands of flowers. Therefore, the actual percentage of self-pollination must not be calculated among twenty-one seedlings but among thousands of open-pollinated flowers. These results suggest that the triploid, tetraploid and some pentaploid cultivars of *C. × vernalis* could all be produced by the 'Gaisen' type tetraploid as was postulated in the previous paper(21).

In general, interspecific hybrids are rarely found in natural populations(5); however, *C. × vernalis* cv. 'Gaisen' produced seedlings with different ploidy levels which were considered to be hybrids with *C. sasanqua* or *C. japonica*. This result is possible because cv. 'Gaisen' has

Table 2. Somatic chromosome numbers of the seedlings of *C. × vernalis* cv. 'Bokyo' ($2n=75$).

Chromosome number ($2n$)	79	80	81	82	83	84	85	86
Number of seedling	2	3	2	2	4	5	4	1

not only self-incompatibility but also cross-compatibility with both putative parent species (unpublished results).

Somatic chromosome numbers of the open-pollinated seedlings of the pentaploid *C. × vernalis* cv. 'Bokyo' are shown in Table 2. They are all aneuploid ranging in number from 79 to 86. Large trees of *C. japonica* were abundant near the stock tree of *C. × vernalis* cv. 'Bokyo' and also large trees of *C. sasanqua* were planted in the neighboring gardens. The blooming season of *C. × vernalis* cv. 'Bokyo' was from November to December so that seasonal isolation between *C. × vernalis* cv. 'Bokyo' and *C. japonica* was stronger than that between *C. × vernalis* cv. 'Gaisen' and *C. japonica*. Further, the chromosome numbers of the seedlings ($2n=79-86$, Fig. 4) of *C. × vernalis* cv. 'Bokyo' are intermediate between *C. × vernalis* cv. 'Bokyo' ($2n=75$) and *C. sasanqua* ($2n=90$), which suggests that the pollen parent of the seedlings was *C. sasanqua*. It is postulated that 45 chromosomes of the seedlings originated from *C. sasanqua* while the other 34-41 chromosomes were from *C. × vernalis* cv. 'Bokyo'.

Thus, once the primary hybrid (the 'Gaisen'

type tetraploid *C. × vernalis*) is backcrossed with *C. sasanqua*, the backcross generation (cv. 'Bokyo', pentaploid of *C. × vernalis*) no longer crosses with *C. japonica*. The pentaploid cultivars of *C. × vernalis* crossed only with the putative recurrent parent species, *C. sasanqua*. It is suggested that this trend is strengthened in advanced backcross generations by the inherent cross-incompatibility between *C. japonica* and *C. sasanqua*.

Although several reports are available on chromosomal translocation(1) and the aneuploid individuals(2,6) in species of *Camellia*, there are no reports of entirely aneuploid progeny being produced. The aneuploid gamete originated from the seed parent tree ($n=75/2$). In anisoploid plants, egg cells have much greater chances of enacting fertilizations than pollen grains and the higher the ploidy levels are, the more fertile the plants are. This is the reason why so many aneuploid individuals were produced in the seedlings of *C. × vernalis* cv. 'Bokyo'. Pollen fertility of *C. × vernalis* cv. 'Bokyo', however, is rather low (62.5%) and some giant grains were observed, suggesting the presence of orthoploid gametes in pollen grains such as $2n=30, 45, 60$ or 75 . The authors postulated the presence of unreduced pollen grains in 1980 and Ackerman and Kondo(3) also found relationships between pollen size and chromosome number. Therefore, it is anticipated that orthoploid seedlings, such as hexaploid, may be produced directly

Fig. 7. Schematic diagram of probable origins of red pigmentation in *Camellia sasanqua*.

by reciprocal crosses (*C. sasanqua* × *C. × vernalis* cv. 'Bokyo').

From these results, the origin of triploid and pentaploid cultivars of *C. × vernalis* and also the red pigmentation of *C. sasanqua* is postulated and shown in Root 1 of Fig. 7. It is postulated that the red flower of *C. sasanqua* originated from repetitive backcrossing with *C. × vernalis* and the gene for red pigmentation in *C. japonica* was introgressed into *C. sasanqua* in this manner. Parks *et al.* found cyanidin 3-monoglucoside both in *C. japonica* and in red flowers of *C. sasanqua* and suggested it was transferred to *C. sasanqua* by introgression from *C. japonica*. Sakata *et al.* (17, 18) found four common cyanidin glucosides in *C. japonica*, *C. sasanqua*, *C. hiemalis* and *C. × vernalis* including cv. 'Gaisen' and cv. 'Bokyo'. Yokoi (26) and Sakata *et al.* (18), however, pointed out the presence of different anthocyanins, delphinidin derivatives, in most of *C. sasanqua*, some of *C. hiemalis*, and cv. 'Bokyo' among twenty-two *C. × vernalis* cultivars despite the absence of them in *C. japonica*. This delphinidin pigmentation of the flower may originate from the faint pink margin of *C. sasanqua* sometimes found in wild forms or be formed by bizarre recombinations in back-cross generations (5).

There are two other possible routes by which red color may have been introduced into *C. sasanqua*, and the second possibility involves *C. hiemalis* (Root 2, see Fig. 7). *C. hiemalis* seems to be involved in the origin of the double-flowered trait as well as red color in cultivars of *C. sasanqua*. On Hirado Island where *C. × vernalis* including cv. 'Gaisen', cv. 'Bokyo' and triploid cultivars are thought to have originated, only single flowers were found among hundreds of old red *C. sasanqua* trees. No double flower cultivars were found in the offspring from *C. × vernalis* cv. 'Gaisen' and wild forms of *C. sasanqua*. *C. hiemalis* has high cross-compatibility with *C. sasanqua* and some double flowers have been found in its seedlings (13). Although *C. hiemalis* is a cultivated variant of *C. sasanqua* of unknown origin, intermediate cultivars are known which range in morphology from typical *C. hiemalis* to typical *C. sasanqua*. The original cultivar of *C. hiemalis*, 'Shishigashira (=Kantsubaki)'

has a double red flower with thicker and wider leaves than *C. sasanqua* and blooms in the winter season. The origin of *C. hiemalis* is still unknown but it seems to be related to *C. × vernalis* on the basis of morphological characteristics and blooming season. The oldest tree of *C. hiemalis* is planted in the central part of Honshu Island, Japan. It has produced a great variety of seedlings around the stock tree and some of them are generally considered to be typical *C. sasanqua*.

The cultivar 'Asahi' which has been considered to be *C. × vernalis* is hexaploid and is cross-compatible with *C. sasanqua*. Its breeder said that this cultivar is a seedling of triploid *C. × vernalis* cv. 'Kyonishiki'. In Hakoda's collection of seedlings from triploid cultivars of *C. × vernalis* there are some hexaploids. 'Camellia hiemalis' may also be a polyploidized seedling of triploid *C. × vernalis* (Root 3, see Fig. 7).

Between *C. sasanqua* and *C. japonica*, seasonal, ecological and genetic isolation work as strong barriers to hybridization. However, once the primary hybrids are produced, introgression of the genes of *C. japonica* to *C. sasanqua* seems to occur easily. On the other hand, the introgression of the genes of *C. sasanqua* to *C. japonica* seems to be blocked by the hybrid sterility of triploid cultivars. Parks *et al.* (16) surveyed for the presence of the eugenol glycoside, sasanquin, and two flavonoid sulfates, camellin I and camellin II, and presented evidence which supports the idea that occasional events of hybridization between *C. japonica* and *C. sasanqua* have produced hybrids that backcross to *C. sasanqua* and have introgressed the cultivated forms of *C. sasanqua*.

Since the flowers of wild types of *C. sasanqua* are white or white with pink margins, selection of a red-colored mutation in the flowers of *C. sasanqua* from a wild forest had been considered to be a reasonable explanation of the presence of red color in the flowers of cultivated *C. sasanqua*. However the author doubts this hypothesis because the foliage size and thickness, and the petal width of the cultivars of *C. sasanqua*, including white ones, seem to be intermediate between those of *C. japonica* and wild *C. sasanqua* and segregations

were observed in the seedlings of cultivars of *C. sasanqua*.

From the present investigation, the hypotheses of the origin of triploid and pentaploid cultivars of *C. × vernalis* (21, 23) and the origin of red pigmentation in the flowers of *C. sasanqua* (14) were supported strongly.

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ハルサザンカの起原に関する細胞遺伝学的研究

IV. サザンカおよびヤブツバキ間の浸透交雑

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摘 要

この研究では、4倍体ハルサザンカ‘凱旋’および5倍体ハルサザンカ‘望郷’の実生群の染色体を調べることで、前報までに推定したハルサザンカ品種群の成立過程の実証的裏付けを得るとともに、紅花サザンカの起原についても推定した。‘凱旋’の実生は、親株附近の植生により、3倍体、4倍体および5倍体に分離した。親株の回りにヤブツバキが多い時には3倍体に、サザンカが多い時には5倍体に実生の倍数性が傾いたことから、3倍体および5倍体の実生は、それぞれヤブツバキ

およびサザンカが交雑して生じたものと考えられた。一方、‘望郷’の実生の染色体数は、すべて異数体で、 $2n=79$ から 86 の間にあり、これは、サザンカの花粉がかかったためと考えられた。このことは、一度サザンカが戻し交雑をすると、その次の世代からも同じ親と主に戻し交雑を繰り返すことを暗示し、紅花サザンカの成立起原は、ハルサザンカを経由してヤブツバキの Anthocyanin 遺伝子がサザンカに浸透したものと推定した。