

Segregation of Plants with Undeveloped Anthers among Hybrids Derived from the Seed Parent, 'Kiyomi' (*Citrus unshiu* × *C. sinensis*)

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Summary

One of the male sterile traits in citrus is the interruption of anther development. It has been known that hybrids with undeveloped anthers segregate among hybrids with satsuma mandarin. In this study, the mode of inheritance of undeveloped anthers was studied using 'Kiyomi' (*C. unshiu* × *C. sinensis*) and 20 citrus cultivars and strains. 'Kiyomi' is a superior commercial variety with cytoplasm identical to the satsuma mandarin. Although undeveloped anthers in citrus are designated as cytoplasmic-genic male sterility, it was possible to study the nuclear genes only by using 'Kiyomi' as a seed parent in all cross combinations.

When three strains that are progenies of 'Kiyomi' were backcrossed with 'Kiyomi,' the ratios of undeveloped anthers to developed anthers in their progenies were 1 : 7 or 1 : 3, indicating that the undeveloped anther is related to three pairs of homomeric nuclear genes *AD1*, *AD2*, and *AD3*. The genotype of 'Kiyomi' with an undeveloped anther is supposed to be *ad1ad1ad2ad2ad3ad3*, according to this hypothesis, which is supported by the segregation ratios in the progenies from 12 other cross combinations. However, when 'Kiyomi' was crossed with three satsuma mandarins, most of the progenies produced undeveloped anthers which reveal that this anomaly in citrus is controlled by more than three homomeric genes. Because satsuma mandarin and 'Kiyomi' have accumulated these genes, these cultivars are very important for the breeding of seedless citrus.

Key Words: citrus, cytoplasmic-genic male sterility, seedless plants, undeveloped anther.

Introduction

Seedlessness is one of the most important breeding objectives in citrus. One approach to create seedless plants is to use male sterility and high parthenocarpy. In citrus, there are several kinds of male sterility, e.g. undeveloped anthers, lack of pollen or its infertility. These traits which have been studied for a long time have revealed that pollen fertility differs greatly among cultivars and pollen mother cells of male sterile cultivars degenerate in an early stage of anther development (Nakamura, 1943; Iwamasa, 1966). One male sterile phenotype, designated as undeveloped anther, has string-shaped anthers that lack pollen. Undeveloped anthers result when cultivars that have the cytoplasm of satsuma mandarin and 'Encore' (*C. nobilis* × *C. deliciosa*) are used as seed parents (Iwamasa, 1966; Okudai et al., 1982; Vardi and Spiegel-Roy, 1988; Yoshida, 1982; Yoshida and Hanaori, 1990; Yamamoto et al., 1992a, b). When other cultivars are used as seed parents, progenies seldom have undeveloped anthers (Yoshida and Hanaori, 1990). Therefore, the heritability of undeveloped anthers

is designated as cytoplasmic-genic male sterility to which satsuma mandarin and 'Encore' cytoplasm are related. Nesumi et al. (1997) observed a degree of anther development, anther color, and pollen fertility in a reciprocal cross between 'Imamura unshiu' (*C. unshiu*) and 'Hayasaki' (*C. grandis*). They concluded that the cytoplasm of satsuma mandarin influences anther development and pollen production. Yamamoto et al. (1992a) who investigated the segregation of hybrids with undeveloped anthers in 18 cross combinations found that the incidence of undeveloped anthers is controlled by two homomeric recessive genes and that male sterile plants have homozygous recessive genes for sterility.

We crossed 'Kiyomi' as a seed parent with 11 cultivars and seven strains to investigate the inheritance system of nuclear genes for male sterility in plants with satsuma mandarin cytoplasm. 'Kiyomi,' which has a cytoplasm identical to satsuma mandarin, manifests undeveloped anthers, monoembryony, high parthenocarpy, and good quality. Therefore, 'Kiyomi' was used as a seed parent. In this paper, we observed the degree of anther development of 'Kiyomi' progenies to reveal the mode of inheritance for undeveloped anthers.

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Materials and Methods

Table 1 shows 14 cultivars and seven strains used in this study. Since 'Kiyomi' is a hybrid from 'Miyagawa wase' (*C. unshiu*) × 'Trovita' orange (*C. sinensis*), its cytoplasm is satsuma mandarin. It was used as a seed parent in 18 cross combinations except for two combinations to characterize the genotypes of its parents. Thirteen cultivars and seven strains were used as pollen parents to prove the mode of inheritance for nuclear genes that cause undeveloped anther in progenies having a cytoplasm of satsuma mandarin. Okitsu No.41, Okitsu No.45, and E-647, which are hybrids of 'Kiyomi', were backcrossed as pollen parents to 'Kiyomi'. Okitsu No.46, A-7 and Okitsu No.14 have the cytoplasm of satsuma mandarin. Eight cultivars except for 'Trovita' orange and Okitsu No.26 are unrelated to satsuma mandarin. Other three satsuma mandarins except for 'Jutaro unshiu' were used to backcross to 'Kiyomi'. 'Trovita' orange, 'Aoshima unshiu' and 'Jutaro unshiu' were crossed to estimate the genotypes of parents of 'Kiyomi'. All F_1 progenies and their parents were observed for the degree of anther development by analyzing flower buds immediately before anthesis. Five flower buds from each F_1 progeny and 18 to 100 buds from their parents were observed. The degree of anther

development was classified into five categories (Fig. 1). Category 1 shows string-shaped anthers with undeveloped pollen sacs. Category 3 shows anthers with incomplete distal pollen sacs. Category 2 is intermediate between categories 1 and 3. In category 5, anthers have sufficiently developed pollen sacs. The degree of anther development in category 4 is intermediate between categories 3 and 5. All parents with different degrees of anther development were classified into three groups: undeveloped anther (UNA: category 1), incomplete anther (IA: most anthers belong to categories 2 or 3), and complete anther (CA: categories 4 and 5). The IA and CA plant groups were combined to form one group IA+CA to simplify the genetic analysis. Hence, the mode of inheritance for undeveloped anthers was analyzed by using two plant groups, UNA and IA+CA. We decided to include IA plants with CA plants because IA plants such as Okitsu No.46 and satsuma mandarin can be used as pollen parents.

Results and Discussion

Anther development in the parents

The degree of anther development in the parents is

Table 1. Cultivars and strains used as parents.

Parent name	Taxa or cross combination
'Kiyomi'	'Miyagawa wase' × <i>C. sinensis</i> cv. Trovita
Okitsu No. 41 ^z	'Kiyomi' × 'Wilking'
Okitsu No. 45 ^z	'Kiyomi' × 'Wilking'
E-647 ^z	'Kiyomi' × 'Osceola' ^y
Okitsu No. 46 ^z	'Sweet spring' ^y × <i>C. sinensis</i> cv. Trovita
A-7 ^z	'Sweet spring' ^y × <i>C. sinensis</i> cv. Trovita
Okitsu No. 14 ^z	'Miyagawa wase' × <i>C. clementina</i>
'Wilking'	<i>C. nobilis</i> × <i>C. deliciosa</i>
'Nova'	<i>C. clementina</i> × 'Orlando' ^y
'Murcott'	Parentage unknown
'Hira kishu'	<i>C. kinokuni</i>
'Mukaku kishu'	<i>C. kinokuni</i>
'Kousyun ponkan'	<i>C. reticulata</i>
'Fukuhara'orange	<i>C. sinensis</i>
'Hamlin'orange	<i>C. sinensis</i>
Okitsu No. 26 ^z	<i>C. hassaku</i> × <i>C. tangerina</i> cv. Dancy
'Aoshima unshiu'	<i>C. unshiu</i>
'Trovita'orange	<i>C. sinensis</i>
'Jutaro unshiu'	<i>C. unshiu</i>
'Miyagawa wase'	<i>C. unshiu</i>
'Dobashibeni unshiu'	<i>C. unshiu</i>

^zThese strains were produced by the National Institute of Fruit Tree Science.

^y'Orlando': *C. paradisi* cv. Duncun × *C. tangerina* cv. Dancy;
'Sweet spring': *C. unshiu* cv. Ueda unshiu × *C. hassaku*;
'Osceola': *C. clementina* × 'Orlando'.

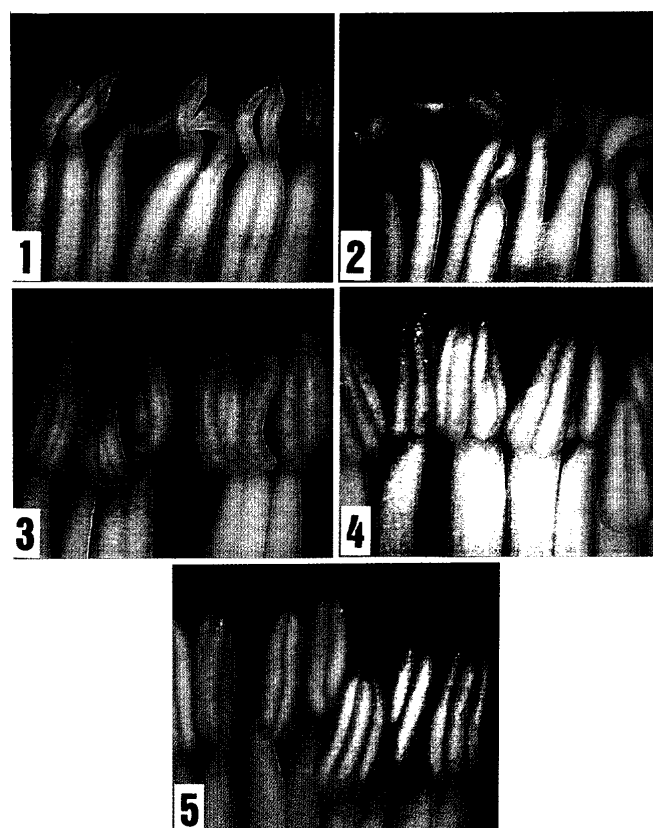


Fig. 1. The categories of anther development. 1: String-shaped anthers with undeveloped pollen sacs, 'Kiyomi'. 2: Intermediate between categories 1 and 3, Okitsu No.46. 3: Anthers with incomplete distal pollen sacs, 'Aoshima unshiu'. 4: The degree of anther development intermediate between categories 3 and 5, 'Kousyun ponkan'. 5: Anthers with sufficiently developed pollen sacs, Okitsu No.26. Magnification for all categories is × 30.

shown in Table 2. All anthers of ‘Kiyomi’ were in category 1 and, therefore, designated as UNA. Four satsuma mandarins, ‘Miyagawa wase’, ‘Dobashibeni unshiu’, ‘Aoshima unshiu’, and ‘Jutaro unshiu’, six strains: Okitsu No.46, A-7, Okitsu No.14, E-647, Okitsu No.41, and Okitsu No.45, and two cultivars, ‘Wilking’ and ‘Nova’, were assigned as IA because most of their anthers were in categories 2 or 3. Eight

other cultivars and strain were in categories 4 to 5, so that they were designated as CA. The degree of anther development was a continuous gradation among plants that were classified in the same anther group. Although anther development of Okitsu No.46 is inferior to that of A-7, these strains are hybrids of ‘Sweet spring’ × ‘Trovita’ orange. Based on their anther morphology, they are categorized as IA. The anther development in the four satsuma mandarins is distinguishable from each other. ‘Miyagawa wase’ exhibits more incomplete anthers than the three other satsuma mandarins. Although these cultivars originated through bud mutation, their fruits mature at different times and have different peel colors. Our results reveal that their degree of anther development also varied.

BC₁ progenies of ‘Kiyomi’

Okitsu No.41 and Okitsu No.45 are hybrids of ‘Kiyomi’ × ‘Wilking’, whereas E-647 is a hybrid from ‘Kiyomi’ × ‘Osceola’ (Table 1). The backcrosses between ‘Kiyomi’ and these three strains, which are categorized as IA, segregated UNA progenies (Table 3). The ratio of UNA to IA+CA in their progenies except for E-647 is 1 : 7. In the progenies derived from E-647, the ratio was closer to 1 : 3 than to 1 : 7. Nesumi et al. (1997) proved that anther development and pollen production were influenced by the cytoplasm of seed parents. Since ‘Kiyomi’ with satsuma mandarin cytoplasm was the only seed parent in this study, we have only to consider the influence of nuclear genes with the exception of cytoplasmic genes. Yamamoto et al. (1992b) suggested that undeveloped anther plants have one or two pairs of homozygous recessive nuclear genes for anther development. If one or two pairs of nuclear genes control anther development, the ratios of UNA to IA+CA in the above backcrosses of ‘Kiyomi’ should be 1 : 1 or 1 : 3. However, the ratios do not approximate 1 : 1 and 1 : 3 in the progenies derived from Okitsu No.41 and Okitsu No. 45 but rather 1 : 7. We propose that anther development is controlled by three pairs of homomeric nuclear genes *AD1*, *AD2*, and *AD3*. If dominant genes affect developing anthers, the genotype

Table 2. The degree of anther development based on the morphological observation of anther in seed and pollen parents.

Parent name	No. of flower observed in indicated category of anther development ^z					Total	Degree of anther development ^y
	1	2	3	4	5		
‘Kiyomi’	100					100	UNA
Okitsu No. 46	23	56				79	IA
‘Miyagawa wase’	12	82				94	IA
‘Dobashibeni unshiu’		67	27			94	IA
A-7		31	60			91	IA
‘Aoshima unshiu’		11	89			100	IA
‘Jutaro unshiu’			20			20	IA
‘Wilking’			20			20	IA
‘Nova’			20			20	IA
Okitsu No. 14			20			20	IA
E-647			18			18	IA
Okitsu No. 41			19	1		20	IA
Okitsu No. 45			11	9		20	IA
‘Hira kishu’				20		20	CA
‘Fukuhara’orange				20		20	CA
‘Trovita’orange				18		18	CA
‘Kousyun ponkan’				19	1	20	CA
‘Murcott’				18	2	20	CA
‘Hamlin’orange				13	7	20	CA
‘Mukaku kishu’				9	11	20	CA
Okitsu No. 26				8	12	20	CA

^zThe category 1 to 5 are the same in Fig. 1.

^yUNA: undeveloped anther; IA: incomplete anther; CA: complete anther.

Table 3. The segregation of UNA plants in the BC₁ progenies of ‘Kiyomi’.

Pollen parent	No. of seedlings observed	Segregation of seedlings ^z			% of UNA plants	Estimated genotype	Expected ratio	X ²	P
		UNA	IA	CA			UNA: (IA+CA)		
						(ad1ad1ad2ad2ad3ad3) ^y			
Okitsu No. 41	133	20	30	83	15.0	AD1ad1AD2ad2AD3ad3	1:7	0.783	0.376
Okitsu No. 45	35	3	7	25	8.6	AD1ad1AD2ad2AD3ad3	1:7	0.494	0.482
E-647	41	8	4	29	19.5	AD1ad1AD2ad2ad3ad3, AD1ad1ad2ad2AD3ad3, ad1ad1AD2ad2AD3ad3	1:3	0.659	0.417

^zUNA includes the category 1 shown in Fig. 1; IA the category 2 and 3; and CA the category 4 and 5.

^yThe formula in parenthesis represents the genotype of ‘Kiyomi’. Dominant genes influence developing anthers.

of 'Kiyomi' is estimated to be *ad1ad1ad2ad2ad3ad3*. If the genotype of Okitsu No.41 and Okitsu No.45 is *AD1ad1AD2ad2AD3ad3*, the ratio of UNA to IA+CA is 1 : 7 (Table 3). In this case, the genotype of E-647 is *AD1ad1AD2ad2ad3ad3*, *AD1ad1ad2ad2AD3ad3* or *ad1ad1AD2ad2AD3ad3*. This assumption would fit the phenotype of Okitsu No.41, Okitsu No.45, and E-647 (Table 2).

Progenies of 'Kiyomi' crossed with strains with satsuma mandarin cytoplasm

When the pollen parents, Okitsu No.46, A-7, and Okitsu No.14, all having satsuma mandarin cytoplasm, were hybridized with 'Kiyomi', UNA plants segregated in progenies of Okitsu No.46 and A-7, but not among progenies of Okitsu No.14 (Table 4). Progenies of Okitsu No.46 segregated into UNA to IA+CA in a ratio of 1 : 1, whereas those of A-7 fitted a 1 : 3 ratio. Therefore, the genotype of Okitsu No.46 was proposed to be *AD1ad1ad2ad2ad3ad3*, *ad1ad1AD2ad2ad3ad3*, or *ad1ad1ad2ad2AD3ad3*, that of A-7 was considered to be *AD1ad1AD2ad2ad3ad3*, *AD1ad1ad2ad2AD3ad3*, or *ad1ad1AD2ad2AD3ad3*. Because no UNA plants appeared among progenies of Okitsu No.14, we suppose that at least one of the *AD* loci has a homozygous dominant gene (Table 4).

Progenies of 'Kiyomi' crossed with varieties unrelated with satsuma mandarin

When eight cultivars and one strain were crossed to 'Kiyomi', the ratios of UNA plants in their progenies differed among the cross combinations (Table 5). When 'Wilking', 'Nova', 'Murcott', 'Hira kishu', 'Mukaku kishu', and 'Kousyun ponkan' were crossed with 'Kiyomi', UNA plants were not obtained. These results led us to suppose that these six cultivars have dominant genes for three *AD* loci and that at least one of these loci

is homozygous. Although we did not obtain UNA plants in the progeny of 'Kiyomi' × 'Murcott', Yamamoto et al. (1992a) reported that UNA plants were obtained in the progeny of 'Encore' ('King' × 'Willow leaf') × 'Murcott'. As 'King' and 'Encore' cytoplasm are identical to that of satsuma mandarin, according to mitochondrial DNA and chloroplast DNA analysis (Yamamoto et al., 1993; Yamamoto and Kobayashi, 1996; Omura et al., 1998), the difference between the two cross combinations might be caused by a nuclear genome. But when 'Fukuhara' orange, 'Hamlin' orange, and Okitsu No.26 were used as pollen parents, some UNA plants segregated among the progenies. Although no genetic analysis could be made in 'Kiyomi' × 'Hamlin' orange because of only a few progenies, the ratio of UNA to IA+CA was 1 : 7 in the progeny of 'Kiyomi' × 'Fukuhara' orange. Progenies of Okitsu No.26 segregated into a ratio of 1 : 3 or 1 : 7. According to a hypothesis proposed by Yamamoto et al. (1992b), the ratio of UNA to IA+CA in the backcross is supposed to be 1 : 1 or 1 : 3 but the above progenies do not fit the ratios. Our results support our assumption, so that we estimated that the genotype of 'Fukuhara' orange would be *AD1ad1AD2ad2AD3ad3*. Moreover, it was confirmed that this cross combination such as 'Kiyomi' × sweet orange would yield UNA plants.

The genotypes of parents of 'Kiyomi'

To determine the genotype of 'Kiyomi', 'Aoshima unshiu' and 'Jutaro unshiu' were crossed with 'Trovita' orange (Table 6). These cultivars and 'Miyagawa wase', which is seed parent of 'Kiyomi', possess the satsuma mandarin cytoplasm. Analysis of pollen fertility and anther development distinguished hybrids from nucellar seedlings. The use of CAPS (Cleaved Amplified Polymorphic Sequences) markers (Omura et al., 2000) allowed us to isolate hybrids from nucellar seedlings.

Table 4. The segregation of UNA plants in the progenies of 'Kiyomi' crossed with three strains derived from satsuma mandarin.

Pollen parent	No. of seedlings observed	Segregation of seedlings ^z			% of UNA plants	Estimated genotype	Expected ratio UNA: (IA+CA)	X ²	P
		UNA	IA	CA					
Okitsu No. 46	28	12	4	12	42.9	(<i>ad1ad1ad2ad2ad3ad3</i>) ^y <i>AD1ad1ad2ad2ad3ad3</i> , <i>ad1ad1AD2ad2ad3ad3</i> , <i>ad1ad1ad2ad2AD3ad3</i>	1:1	0.571	0.450
A-7	15	3	2	10	20.0	<i>AD1ad1AD2ad2ad3ad3</i> , <i>AD1ad1ad2ad2AD3ad3</i> , <i>ad1ad1AD2ad2AD3ad3</i>	1:3	0.200	0.655
Okitsu No. 14	20	0	8	12	0.0	<i>AD1-AD2-AD3</i> ^x	0:1	---	---

^zUNA includes the category 1 shown in Fig. 1; IA the category 2 and 3; and CA the category 4 and 5.

^yThe formula in parenthesis represents the genotype of 'Kiyomi'.

^xAt least one of three pairs of homomeric genes was homozygous dominant, i. e., *AD1AD1AD2AD2AD3AD3*, *AD1ad1AD2AD2AD3AD3*, *AD1AD1AD2AD2AD3ad3*, *AD1AD1AD2ad2AD3AD3*, *AD1AD1AD2ad2AD3ad3*, *AD1ad1AD2AD2AD3ad3* and *AD1ad1AD2ad2AD3AD3*.

Table 5. The segregation of UNA plants in the progenies of ‘Kiyomi’ crossed with nine parents unrelated to satsuma mandarin.

Pollen parent	No. of seedlings observed	Segregation of seedlings ^z			% of UNA plants	Estimated genotype	Expected ratio	X ²	P
		UNA	IA	CA			UNA: (IA+CA)		
						(ad1ad1ad2ad2ad3ad3) ^y			
‘Wilking’	20	0	1	19	0	AD1–AD2–AD3– ^x	0:1	---	---
‘Nova’	31	0	4	27	0	AD1–AD2–AD3– ^x	0:1	---	---
‘Murcott’	33	0	3	30	0	AD1–AD2–AD3– ^x	0:1	---	---
‘Hira kishu’	8	0	0	8	0	AD1–AD2–AD3– ^x	0:1	---	---
‘Mukaku kishu’	45	0	5	40	0	AD1–AD2–AD3– ^x	0:1	---	---
‘Kousyun ponkan’	49	0	1	48	0	AD1–AD2–AD3– ^x	0:1	---	---
‘Fukuhara’orange	15	2	0	13	13.3	Ad1ad1AD2ad2AD3ad3	1:7	0.010	0.922
‘Hamlin’orange	4	1	0	3	25.0	-----	---	---	---
Okitsu No. 26	22	4	2	16	18.2	AD1ad1AD2ad2ad3ad3, AD1ad1ad2ad2AD3ad3, ad1ad1AD2ad2AD3ad3 AD1ad1AD2ad2AD3ad3	1:3 1:7	0.545 0.649	0.460 0.420

^zUNA includes the category 1 shown in Fig. 1; IA the category 2 and 3; and CA the category 4 and 5.^yThe formula in parenthesis represents the genotype of ‘Kiyomi’.^xAt least one of three pairs of homomeric genes was homozygous dominant, i. e., AD1AD1AD2AD2AD3AD3, AD1ad1AD2AD2AD3AD3, AD1AD1AD2AD2AD3ad3, AD1AD1AD2ad2AD3AD3, AD1AD1AD2ad2AD3ad3, AD1ad1AD2AD2AD3ad3 and AD1ad1AD2ad2AD3AD3.**Table 6.** The segregation of UNA plants in the progenies of satsuma mandarin crossed with ‘Trovita’ orange.

Cross combination ^z	No. of seedlings observed	Segregation of seedlings ^y			% of UNA plants	Estimated genotype		Expected ratio	X ²	P
		UNA	IA	CA		Satsuma mandarin	‘Trovita’orange	UNA: (IA+CA)		
‘Aoshima unshiu’(2–3) × ‘Trovita’orange(4)	7	0	2	5	0.0	-----	-----	---	---	---
‘Jutaro unshiu’(3) × ‘Trovita’orange(4)	39	4	4	31	10.3	-----	-----	---	---	---
Total	46	4	6	36	8.7	AD1ad1ad2ad2ad3ad3, AD1ad1AD2ad2ad3ad3, AD1ad1ad2ad2AD3ad3	AD1ad1AD2ad2AD3ad3	1:15	0.470	0.493

^zThe figures in parenthesis represent the category of anther development.^yUNA includes the category 1 shown in Fig. 1; IA the category 2 and 3; and CA the category 4 and 5.

Some progenies of ‘Jutaro unshiu’ × ‘Trovita’ orange segregated into UNA plants. In this study, the number of cross combinations between satsuma mandarin and sweet orange and that of the hybrid seedlings were not sufficient to estimate the genotype of ‘Miyagawa wase’ and ‘Trovita’ orange. However, since ‘Aoshima unshiu’ and ‘Jutaro unshiu’ have mostly category 3 anthers (Table 2), the AD loci apparently did not mutate among these satsuma mandarins. Therefore, the genetic analysis on satsuma mandarin × ‘Trovita’ orange was performed by combining cross seedlings to form one group in these two combinations. In this case, the ratio of UNA to IA+CA is 1 : 15 (Table 6). The genotype of satsuma mandarin is postulated to be *AD1ad1ad2ad2ad3ad3*,

ad1ad1AD2ad2ad3ad3 or *ad1ad1ad2ad2AD3ad3* and that of ‘Trovita’ orange *AD1ad1AD2ad2AD3ad3*. The genotype of ‘Trovita’ orange is identical with that of ‘Fukuhara’ orange so that their phenotype is similar. However, since the number of observed seedlings and that of cross combinations are statistically insufficient, more data on progenies of satsuma mandarin × sweet orange are needed.

Progenies of ‘Kiyomi’ crossed with satsuma mandarins

When ‘Miyagawa wase’, ‘Aoshima unshiu’, and ‘Dobashibeni unshiu’ were crossed with ‘Kiyomi’, more than 84% were UNA plants, whereas none were CA plants (Table 7). The results lead us to believe that

Table 7. The segregation of UNA plants in the progenies of 'Kiyomi' crossed with three satsuma mandarins.

Pollen parent ^z	No. of seedlings observed	Segregation of seedlings ^y			% of UNA plants	Expected ratio UNA: (IA+CA)	X ²	P
		UNA	IA	CA				
'Dobashibeni unshiu'(2-3)	19	16	3	0	84.2	---	---	---
'Aoshima unshiu'(2-3)	48	47	1	0	97.9	---	---	---
Total	67	63	4	0	94.0	15:1	0.009	0.925
'Miyagawa wase'(1-2)	71	71	0	0	100	---	---	---

^zThe figures in parenthesis represent the category of anther development.

^yUNA includes the category 1 shown in Fig. 1; IA the category 2 and 3; and CA the category 4 and 5.

satsuma mandarin accumulated many nuclear genes for anther development. Although the number of cross combinations between 'Kiyomi' and satsuma mandarin are insufficient to make genetic analysis, anther developments of 'Dobashibeni unshiu' and 'Aoshima unshiu' place them in the same group. When the hybrids were composed to form one group within these combinations, UNA: IA+CA ratio was 15 : 1 (Table 7). This finding is inexplicable on the basis of three pairs of homomeric nuclear genes, *AD1*, *AD2*, and *AD3*. Thus the mode of inheritance for an undeveloped anther is complicated, so that more than these three genes are associated with this trait. As most progenies of 'Kiyomi' crossed with satsuma mandarin were UNA plants and these cross combinations yield few IA+CA plants, we suppose that anther development is also controlled by some homomeric nuclear genes (*IAD*) which interrupt anther development. It is considered that the anther development is regulated by an additive effect or synergism with the dominant *IAD* genes. 'Miyagawa wase' has more incomplete anthers than the other satsuma mandarins (Table 2) and all progenies from 'Kiyomi' × 'Miyagawa wase' were UNA plants. On the other hand, the progenies of 'Kiyomi' crossed with 'Dobashibeni unshiu' or 'Aoshima unshiu' segregated not only UNA plants but also IA plants. The progenies of 'Kiyomi' × 'Miyagawa wase' became poor development anthers by virtue of accumulating more dominant *IAD* genes than other satsuma mandarins. In conclusion, the mode of inheritance for undeveloped anthers in progenies with satsuma mandarin is complicated and requires many more progeny testing using 'Kiyomi', satsuma mandarin and their derivatives.

This paper reports the heredity of undeveloped anthers in 18 cross combinations using only 'Kiyomi' as a seed parent. While undeveloped anther is designated as cytoplasmic-genic male sterility, our data did not reveal whether cytoplasm, with the exception of that of satsuma mandarin, determine male sterility. Although the degree of anther development is a continuous variation, we grouped the mode of heredity into two phenotypes, UNA and IA+CA, for simplification of genetic analysis. Consequently, it is quite possible that we failed

to notice some factors that might have had a small effect on the results of the study. Thus, a more detailed analysis such as molecular biological methods would also be required in addition to the conventional analysis. In the breeding to obtain seedless varieties, UNA plants such as 'Kiyomi' and IA plants such as satsuma mandarin are often used as seed parents. Because the heritability of undeveloped anther is yet unclear, their selection often relied on experience. Nevertheless, our results reveal that satsuma mandarin and 'Kiyomi' have accumulated a peculiar variety of genes for the dystrophy of the male organ and, therefore, are important for breeding of UNA seedless plants with high parthenocarpy. When 'Kiyomi', satsuma mandarin and their progenies are used as cross parents, it will be possible to effectively obtain seedless plants.

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‘清見’(*Citrus unshiu* × *C. sinensis*)を種子親とした雑種におけるやく発育不全個体の分離

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摘 要

カンキツにおける雄性不稔性の一つであるやくの発育不全に関する遺伝様式を明らかにするために、‘清見’を種子親に用いた18組合せの雑種集団を供試した。やくの発育不全は細胞質・核遺伝子型雄性不稔であるが、本報告ではウンシュウミカン型細胞質を持つ‘清見’のみを種子親として用いているので、ウンシュウミカン型細胞質に対する核遺伝子の作用についてのみ考察を行った。

‘清見’の戻し交雑3組合せを用いた分離調査を行った結果、やくの発育不全と発育やくの比は1:7あるいは1:3に適合した。この結果より、やくの発育不全は3対の等価同義遺伝子により支配されており、3対の遺伝子を劣性

ホモで持つ場合にやくの発育不全が生じると仮定した。この仮説は、ウンシュウミカン由来系統を用いた3組合せ、ウンシュウミカンに由来しない品種・系統を用いた9組合せの計12組合せの分離調査により得られた分離比からも裏付けられた。しかし、ウンシュウミカン3品種を用いた組合せにおける分離様式は他の品種・系統の分離様式と大きく異なり、ほとんどの後代はやくの発育不全個体であった。前述の仮説ではこの現象に対する説明ができないことから、さらに多くの遺伝子の関与が示唆された。また、ウンシュウミカンや‘清見’はこれらの遺伝子を多数蓄積していることが明らかとなった。