Cross Incompatibility between *Rhododendron* sect. *Tsutsusi* Species and *Rhododendron japonicum* (A. Gray) J. V. Suringar f. *flavum* Nakai

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Summary

The nature of cross incompatibility between evergreen azaleas (*Rhododendron* section *Tsutsusi*) × *R. japonicum* (A. Gray) J. V. Suringar f. *flavum* Nakai, on intersubgeneric crossings, was examined using 22 plants of 13 species in section *Tsutsusi*. Pre-fertilization barriers were: 1) the arrest of pollen tube growth and 2) the failure of pollen tubes to penetrate into the ovules, whereas the post-fertilization ones were: 1) the failure of fertilized ovules to develop, 2) lack of seed germination, and 3) death of young seedlings. In 22 cross combinations, the number of pre-, post-, and both pre- and post-fertilization barriers that played the principal roles in cross incompatibility were fifteen, six, and one, respectively. Thus, we conclude that the variation in the propensity for the cross incompatibility existed in this intersubgeneric hybridization, and that it differed among individuals rather than among species.

Key Words: cross incompatibility, evergreen azalea, fertilization barrier, intersubgeneric hybridization, *Rhododendron japonicum*.

Introduction

Evergreen azaleas, *Rhododendron* subgenus *Tsutsusi* section *Tsutsusi*, do not have a pure yellow-flowered species but only a few cultivars with pale yellowish-white flowers. Commercial azalea growers have long wanted yellow-flowered evergreen azaleas, such as those of deciduous azaleas, *R. japonicum* (A. Gray) J. V. Suringar f. *flavum* Nakai, belonging to subgen. *Pentanthera* sect. *Pentanthera*, was generally used as a parent to produce the yellow-flowered evergreen azaleas. Intersubgeneric crosses between evergreen azaleas and *R. japonicum* f. *flavum* were described during the Taisho era in Japan (Miyazawa, 1922), but there appeared to be reproductive barriers. When evergreen azaleas were used as a pollen parent, pollen tube growth was inhibited in the style (Creech, 1955; Ureshino et al., 2000), whereas when used as a seed parent, chlorophyll defects in cotyledons of seedlings were detected (Akabane et al., 1971; Noguchi, 1932). Occasionally, viable seedlings were obtained when evergreen azaleas were used as the seed parent (e.g. *R. obtusum* (Lindley) Planch. (Noguchi, 1932), hybrids of *R. kiusianum* Makino × *R. indicum* (L.) Sweet and *R. kiusianum* (Akabane, 1993), and *R. nakaharai* Hayata (Kurashige and Akabane, 2001)). These reports show a possibility

that the crosses using evergreen azaleas as the maternal parent have a variation in the degree of hybridization. However, there is little research employing many evergreen azalea species and many surveys disclosing nonviable hybrids. In this study, we examined pre-fertilization barriers (gametic isolation) and post-fertilization barriers (hybrid inviability) between on evergreen azalea species × *R. japonicum* f. *flavum*; we describe the nature of intersubgeneric incompatibility between them more precisely.

Materials and Methods

Twenty two plants of 13 species belonging to sect. *Tsutsusi*, grown in the glasshouse, were used as seed parents; *R. japonicum* f. *flavum* accession no. 27026136, grown under open-air was used as the pollen parent. As a control, outcrossing was carried out using another *R. japonicum* f. *flavum* plant (accession no. 27026142) as the seed parent because *R. japonicum* is a cross-pollinating (self-incompatible) species (Goto and Nishimura, 1971). These plants were collected as cuttings or seeds from their natural habitats (Table 1), and were preserved at Department of Vegetable and Flower Research, National Agricultural Research Center for Kyushu Okinawa Region. Anthers, collected from flower buds at the beginning of anthesis, were put in 1.5 ml sample tubes and preserved in a desiccator at room temperature in the dark from the middle of April to early June. Flower buds were emasculated at the beginning of anthesis, and the stigma, covered with mucus, was hand-pollinated.

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Table 1. Codes and sources of *Rhododendron* species used as parents in this study.

Species	Code	Locality (pref.)	Flower color
<i>R. kaempferi</i> Planch.	KAE1	unknown (Miyagi)	white
	KAE2	Mts. Minou (Fukuoka)	white
	KAE3	Mt. Hosshin (Fukuoka)	red
	KAE4	unknown	red
<i>R. kiusianum</i> Makino	KIU1	Houbaru (Nagasaki)	white
	KIU2	Mt. Kuroiwa (Oita)	white
	KIU3	Mt. Yufu (Oita)	white with pink flecks
<i>R. sataense</i> Nakai	SAT1	Sakurajima (Kagoshima)	white
	SAT2	Takatouge (Kagoshima)	white
<i>R. tosaense</i> Makino	TOS1	unknown (Ehime)	white
<i>R. simsii</i> Planch.	SIM1	Is. Amami (Kagoshima)	red
<i>R. serpyllifolium</i> (A. Gray). Miq.	SER1	Gobo (Wakayama)	pale purple
<i>R. macrosepalum</i> Maxim.	MAC1	Gobo (Wakayama)	pink
	MAC2	Gobo (Wakayama)	pink
	MAC3	Kyoto (Kyoto)	lilac-pink
<i>R. ripense</i> Makino	RIP1	Simanto-gawa (Kochi)	white
<i>R. yedoense</i> Maxim. var. <i>poukhanense</i> (H. Lévl.) Nakai	YED1	Is. Tsushima (Nagasaki)	purple
<i>R. scabrum</i> G. Don	SCA1	Higashison (Okinawa)	deep red
<i>R. indicum</i> (L.) Sweet	IND1	Koza-gawa (Wakayama)	deep red
<i>R. eriocarpum</i> (Hayata) Nakai	ERI1	Is. Yakushima (Kagoshima)	white
	ERI2	Is. Yakushima (Kagoshima)	white
<i>R. tschonoskii</i> Maxim.	TSC1	Mt. Akagi (Gunma)	white
<i>R. japonicum</i> (A. Gray) J. V. Suringar f. <i>flavum</i> Nakai	—	Seidagawa (Oita)	yellow

Pollen tube growth after pollination was studied by examining the number of pollen tubes in the styles and those penetrated into the ovules. Ten days after pollination, three pistils were collected from each seed parent, fixed in FAA (formalin: 70% ethanol: acetic acid = 1:18:1 (v/v/v)) for 24 hr, after which they were transferred to 70% ethanol and stored at room temperature until observation. The fixed pistils, were soaked in 1N NaOH at 38°C for 24 hr, washed three times in distilled water, and stained with a 0.1% solution of aniline blue dye dissolved in 0.1N K₃PO₄ for 7 hr. After the styles and ovaries were separated, the latter were cut lengthwise in half and two or three carpels in each half ovary picked out by tweezers. After the styles and carpels were placed on a microscope slide and squashed by gentle pressure on a coverslip, the preparations were examined by a Nikon ECLIPSE E400 microscope with a high-pressure mercury vapor lamp and a combined filter (Nikon EX450-490, BA520, DM505). The distinction between fertilized and unfertilized ovules was made according to Williams et al. (1982).

Capsules, collected in October from each cross were counted and dried artificially. The number of seeds per capsule was counted for five capsules per cross combination. In November, the seeds were sown on seedling beds that consisted of Kanuma volcanic sands in plastic flat (51 × 36 cm) and covered with sphagnum moss layer. The flats were placed in a glasshouse that reduced photosynthetic photon flux density (PPFD) by 15% and

under intermittent mist. Germinating seeds were counted once a week from December to February and the color of cotyledons noted. On March, the seedling beds were transferred to another glasshouse where PPFD was reduced by 23%. Two years after sowing, the number of viable seedlings for each cross was counted and the hybridity of the seedlings determined by the existence of pubescence in leaves. The presence of leaf hairs was used to separate seedlings of *R. japonicum* from non-hybrid ones.

A parameter estimate population was generated, using the JMP software (SAS Institute Inc.), to examine the effect sizes of each character to the number of viable seedlings per pollinated flower.

Results and Discussion

Pollen tetrads germinated on the stigma and the pollen tubes penetrated the upper style in all crosses, except in RIP1 (Codes for the sect. Tsutsusi plants given in Table 1); the number of pollen tubes present in the lower portion of the style was more than 100; the largest count was 555.0 in the cross using ERI2 (Table 2). In the RIP1, pollen tube numbers were extremely low, which suggests that most of them aborted in the stylar canal. The number of ovules per ovary varied from 118.3 (TSC1) to 1854.3 (SCA1) (Table 2). These numbers differed among individual plants significantly (F ratio = 56.14, $P < 0.001$); therefore, the number of pollen tubes present in the lower style is not sufficient to assess the

Table 2. Pollen tube growth, ovule numbers per ovary and fertilized ovule rate in crosses between section *Tsutsusi* species and *Rhododendron japonicum* f. *flavum*.

Seed parent	Number of pollen tubes in lower style	Number of ovules per ovary	Pollen tubes/ovules (%)	Ovules penetrated by pollen tubes (%)
KAE1	348.7	593.3	58.8	4.6
KAE2	367.0	965.3	38.0	13.1
KAE3	355.0	797.3	44.5	15.0
KAE4	293.0	919.3	31.9	12.6
KIU1	216.7	453.0	47.8	3.7
KIU2	156.0	355.0	43.9	7.3
KIU3	230.0	434.3	53.0	22.3
SAT1	266.3	825.0	32.3	0.9
SAT2	263.0	969.0	27.1	6.1
TOS1	135.5	419.7	32.3	5.6
SIM1	379.5	815.0	46.6	32.8
SER1	129.0	202.7	63.6	15.8
MAC1	209.3	964.7	21.7	6.6
MAC2	348.0	1067.3	32.6	10.3
MAC3	398.0	736.3	54.1	56.5
RIP1	6.0	1406.3	0.4	0.3
YED1	310.0	587.0	52.8	42.9
SCA1	463.0	1854.3	25.0	9.9
IND1	323.5	865.3	33.5	15.3
ERI1	450.5	1153.0	39.1	18.6
ERI2	555.0	1436.7	38.6	39.1
TSC1	239.5	118.3	202.5	18.4
<i>R. japonicum</i> f. <i>flavum</i> ^z	358.3	569.0	63.0	32.1

^z Cross pollination.

limit of the pollen tubes' penetration into the ovules of the maternal parents. We expressed this result by using pollen tubes/ ovules (P/O) derived as a percentage of the pollen tube numbers in the lower style divided by the ovule numbers per ovary. P/O ranged from 0.4% (RIP1) to 63.6% (SER1) and 202.5% (TSC1) (Table 2), to show that the number of pollen tubes reaching in the lower style was less than the number of ovules available for fertilization except for the cross using TSC1. This result indicates that the pollen tube behavior in the style is one cause of pre-fertilization barrier.

As for fertilized ovules, tips of pollen tubes passed through micropyles and traveled straight to the filiform apparatus of embryo sacs 10 days after pollination. The rate at which pollen tubes penetrated the micropyle varied from 0.3% (RIP1) to 56.5% (MAC3) and was 32.1% in the control (Table 2). Except for ERI2, MAC3, RIP1, SIM1, and YED1, this value was less than half the P/O, indicating that the failure of pollen tubes to penetrate into the ovules is another pre-fertilization barrier.

The crosses, using RIP1 and SER1, set no capsule, whereas 20 other cross combinations produced capsules; capsule sets exceeded 80% (Table 3). In SER1, the ovule growth may have stopped completely after fertilization because some crosses with fewer micropylar

penetrations compared with those involving SER1, had higher percentages of capsule set. The number of seeds per capsule varied from 4.8 (KAE1) to 333.4 (ERI2) and 204.8 in the control (Table 3). Creech (1955) noted that intersectional crosses ranged from complete failure to set seed to fully developed capsules with or without viable seed. In KAE1, KAE3, KIU1, KIU2, MAC2, SAT1, TOS1, and TSC1, the capsule sets were high, although they contained a small number of seeds indicating that capsule set is not a suitable character for evaluation of the cross incompatibility in our hybridization. The potentiality of forming seeds per capsule depended on the number of ovules per capsule that differed among female parents (Table 2). Thus, we express this by using seeds/ ovules (S/O) derived as the percentage of the mature seed numbers per capsule divided by the ovule numbers per ovary to reconcile the development of ovules after fertilization. Except for crosses using RIP1 and SER1, S/O ranged from 0.7% (MAC2) to 44.5% (YED1) and 36.0% in the control (Table 3). S/O in the control closely approximated the percentage of ovules that the pollen tubes penetrated, while crosses using ERI1, ERI2, IND1, KAE1, KAE2, KAE3, KIU2, MAC1, MAC2, MAC3, and SCA1 had relatively low values of S/O, compared with the rate of

Table 3. Capsule set and number of seeds produced in crosses between section *Tsutsusi* species and *Rhododendron japonicum* f. *flavum*.

Seed parent	Number of pollinated flowers	Number of capsules	Capsule set (%)	Number of mature seeds per capsule	Seeds/ovules (%)
KAE1	10	8	80.0	4.8	0.8
KAE2	11	11	100	50.2	5.2
KAE3	10	10	100	16.4	2.1
KAE4	10	9	90.0	178.2	19.4
KIU1	12	10	83.3	17.6	3.9
KIU2	13	12	92.3	16.0	4.5
KIU3	10	10	100	116.0	26.7
SAT1	10	10	100	17.0	2.1
SAT2	10	10	100	58.4	6.0
TOS1	13	12	92.3	24.4	5.8
SIM1	12	12	100	219.2	26.9
SER1	11	0	0	–	0
MAC1	15	13	86.7	38.2	4.0
MAC2	10	10	100	7.4	0.7
MAC3	10	10	100	29.8	4.0
RIP1	12	0	0	–	0
YED1	10	10	100	261.0	44.5
SCA1	10	9	90.0	121.6	6.6
IND1	10	10	100	91.6	10.6
ERI1	10	10	100	144.5	12.5
ERI2	10	10	100	333.4	23.2
TSC1	11	9	81.8	23.4	19.8
<i>R. japonicum</i> f. <i>flavum</i> ^z	10	10	100	204.8	36.0

^z Cross pollination.

ovules penetrated by the pollen tubes. This suggests that a limited number of fertilized ovules continued to grow until they reached maturity. Thus, the failure of fertilized ovules to develop is one cause of post-fertilization barrier.

Of the mature seeds obtained from 20 crosses that were sown, the percentage germination varied from 0% (KAE3, MAC2, and TSC1) to 86.6% (MAC3) and 88.1% in the control (Table 4); the mean was less than 50%, which adds the lack of germination as a post-fertilization barrier.

Color variation of the cotyledons in hybrid seedlings was attributed by Noguchi (1932) to incomplete development of normal green pigment, and a phenomenon observed in intersectional hybrids between *R. japonicum* and the species in sect. *Tsutsusi*. In our study, cotyledon color segregated into green, yellow-green, pale-green, yellow, white, and variegated (Table 4). Yellow-green cotyledons were not recorded by Noguchi (1932) and Ureshino et al. (1998), but were present in progenies of our crosses. With respect to the variegated cotyledons, some color combinations which consisted of green, yellow-green, pale-green, and white were detected, but were not recorded in detail. Seedlings, exhibiting pale-green, yellow, and white cotyledons, did not unfold

initial pair of foliage leaves, died before the first summer. The percentage of green seedlings varied from 0% (KAE1, KIU1, MAC1, SAT2, SIM1, and SCA1) to 69.2% (SAT1) and 98.6% in the control (Table 4). All crosses had fewer green seedlings than the control; the rate of pale-green, yellow, and white seedlings being relatively high except in crosses using KIU2, MAC1, and MAC3. Akabane (1971) and Sakai et al. (2001) reported that by using tetraploid cultivars as the seed parent, the appearance of albino seedlings could be avoided, thus, increasing the percentage of green seedlings. The evergreen azaleas examined in this study were all diploid plants based on chromosome counts or flow cytometric analysis (data not shown). Hence, a relatively high percentage of the green seedlings in our crosses (using KIU3 and SAT1) was unrelated to the ploidy level of the maternal plants. The majority of the seedlings with green and yellow-green cotyledons were dwarfed; such seedlings died during the second growing season.

Out of 17 crosses in which the resulting seeds germinated, nine had no surviving seedling two years after sowing. For the remainder, the percentage of viable seedling numbers/ the germinating seedling numbers varied from 0.7% (SCA1) to 71.3% (MAC3) and 100%

Table 4. Germination rate, color of cotyledons and rate of viable seedlings in crosses between section *Tsutsusi* species and *R. japonicum* f. *flavum*.

Seed parent	Number of seeds sowed	Germination rate (%)	Color of cotyledons ^z					Viable seedlings ^y / seeds germinated (%)	Number of viable seedlings per flower pollinated ^y
			G	YG	PG	Y+W	V		
KAE1	19	10.5	0	0	50.0	50.0	0	0	0
KAE2	198	20.2	35.0	12.5	50.0	2.5	0	0	0
KAE3	135	0	–	–	–	–	–	–	0
KAE4	891	7.9	1.4	1.4	78.6	18.6	0	0	0
KIU1	88	45.5	0	2.5	52.5	45.0	0	0	0
KIU2	80	28.8	21.7	60.9	0	13.0	4.3	0	0
KIU3	580	59.1	67.1	2.0	4.1	17.5	9.3	19.0	13.0
SAT1	85	30.6	69.2	0	11.5	19.2	0	46.2	2.4
SAT2	292	16.8	0	0	46.9	53.1	0	0	0
TOS1	122	13.1	6.2	50.0	0	43.8	0	0	0
SIM1	472	34.5	0	1.2	90.2	8.6	0	0	0
MAC1	191	0.5	0	100.0	0	0	0	100.0	0.2
MAC2	37	0	–	–	–	–	–	–	0
MAC3	149	86.6	20.2	75.2	4.7	0	0	71.3	18.4
YED1	251	68.1	4.1	5.3	77.2	12.9	0.6	0	0
SCA1	608	25.2	0	43.8	54.2	2.0	0	0.7	0.2
IND1	458	15.7	16.7	34.7	31.9	16.7	0	6.9	1.0
ERI1	578	40.8	28.8	29.7	25.4	16.1	0	19.1	11.3
ERI2	308	23.7	15.1	27.4	34.3	23.3	0	13.7	10.8
TSC1	117	0	–	–	–	–	–	–	0
<i>R. japonicum</i> f. <i>flavum</i> ^x	402	88.1	98.6	1.4	0	0	0	94.9	168.0

^z Classification of color of cotyledons: G, green (141B); YG, yellow – green (149B); PG, pale – green (149D); Y, yellow (4D); W, white (155B); and V, variegated. () are the Royal Horticultural Society colour chart number.

^y Two years after sowing.

^x Cross pollination.

Table 5. Parameter estimates population of the characters acted as pre- and post-fertilization barriers and their sizes of effects to the viable seedling numbers per pollinated flower in the crosses between section *Tsutsusi* species and *Rhododendron japonicum* f. *flavum*.

Term	Original estimate	Orthogonal coded estimate	Orthogonal t test	Size of effects ^z (%)
Pollen tubes/ ovules	0.002	–0.470	–0.58NS ^y	6.2
% of ovules penetrated by pollen tubes	0.177	3.493	4.32***	45.9
Seeds/ ovules	–0.122	0.936	1.16NS	12.3
Germination rate	0.092	1.727	2.14*	22.7
Viable seedlings/ seeds germinated	0.043	0.992	1.23NS	13.0

^z Composition of each absolute value of orthogonal coded estimates relative to the sum of the absolute values.

^y *, ***, and NS indicate significance at $P=0.05$, 0.001 , and non-significance, respectively.

(MAC1, one plant), and the control with 94.9% (Table 4). Eventually, the number of viable seedlings per pollinated flower ranged between 0 to 18.4 (MAC3), while that of the control was 168.0 (Table 4). These results suggest that seedling death, including the chlorophyll defect, is another post-fertilization barrier. From these results, two pre- and three post-fertilization barriers were found in this intersubgeneric crosses. Our analysis of the effect sizes contributing to the viable

seedling numbers reveal that the rate of ovule penetration by the pollen tubes played the most important role (45.9%) and the germination rate, the second (22.7%) (Table 5).

For cross incompatibility, the level of stylar rejection of pollen, the lack of pollen tube penetration into the ovules, and the inability of ovules to develop into seeds are the strong fertilization barriers. These barriers, however, appeared in the control cross; P/O, the rate of

Table 6. Indices of three characters acted as the cause of pre- and post-fertilization barriers in crosses between section *Tsutsusi* species and *Rhododendron japonicum* f. *flavum*.

Seed parent	Index of pollen tubes/ ovules	Index of ovules penetrated by pollen tubes	Index of seeds/ ovules
KAE1	0.93	0.14	0.02
KAE2	0.60	0.41	0.14
KAE3	0.71	0.47	0.06
KAE4	0.51	0.39	0.54
KIU1	0.76	0.12	0.11
KIU2	0.70	0.23	0.13
KIU3	0.84	0.70	0.74
SAT1	0.51	0.03	0.06
SAT2	0.43	0.19	0.17
TOS1	0.51	0.17	0.16
SIM1	0.74	1.02	0.75
SER1	1.01	0.49	0
MAC1	0.34	0.21	0.11
MAC2	0.52	0.32	0.02
MAC3	0.86	1.76	0.11
RIP1	0.01	0.01	0
YED1	0.84	1.34	1.24
SCA1	0.40	0.31	0.18
IND1	0.53	0.48	0.29
ERI1	0.62	0.58	0.35
ERI2	0.61	1.22	0.64
TSC1	3.21	0.57	0.55

Index number expressed the relative value in contrast to the control value.

ovules penetrated by the pollen tubes, and S/O were 63.0%, 32.1%, and 36.0%, respectively (Table 2 and 3). For evaluating cross incompatibility, individual values of these three characters on each cross were converted into the index numbers (the relative value in contrast to the control value) (Table 6); and the index number below 0.5 indicate that it was the principal factor governing cross incompatibility. Except in crosses using ERI1, ERI2, KIU3, MAC3, SIM1, TSC1, and YED1, 15 crosses showed that the index of ovules penetrated by the pollen tubes was below 0.5, indicating that the pre-fertilization barriers were the principal factor of cross incompatibility. Especially in crosses using MAC1, RIP1, SAT2, and SCA1, the direct factor would be the arrest of pollen tube growth since the index of P/O was below 0.5.

Concerning the S/O, it was reported to be less than 33% in woody plants (Wiens, 1984), indicating that our control cross had a normal value. Among the remaining seven crosses for which the pre-fertilization barriers were not the principal factor of cross incompatibility, the crosses using ERI1 and MAC3 had on the index of S/O which is below 0.5, suggesting that the inhibition of

ovule development after fertilization was the main factor of cross incompatibility. Additionally in using ERI1 the index of the ovules penetrated by the pollen tubes was not high so that the fertilization failure (pre-fertilization barrier) and subsequent zygotic failure are considered the two main factors of cross incompatibility. Similarly, the poor germination and the viable seedling rates (Table 4), especially among crosses using ERI2, KIU3, SIM1, TSC1, and YED1, are the main factors of cross incompatibility.

We, therefore, conclude that the variation in the propensity for cross incompatibility exists in the inter-subgeneric hybridization between evergreen azaleas and *R. japonicum* f. *flavum*, and this variation differs among individuals rather than among species. Consequently, the crossing ability of evergreen azaleas to *R. japonicum* f. *flavum* must be evaluated for each individual evergreen ones.

In the crossing ability, Sax (1930) stated that the genus *Rhododendron* crosses between different subgenera and sections were usually difficult or impossible to make. In our crosses, although many crosses did not produce hybrid plants, more than 10 vigorous seedlings per pollinated flower were obtained from the crosses using ERI1, ERI2, KIU3, and MAC3. Donnelly and Clark (1962) reported that it was possible to increase the percentage of fertilization by choosing the right parental combination in *Vicia*. Thus, it is expected to get many viable seedlings from this intersubgeneric hybridization if the right maternal plants can be chosen among evergreen azaleas.

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ツツジ亜属ツツジ節種とレンゲツツジ亜属レンゲツツジ節キレンゲツツジとの交雑不和合性

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摘 要

常緑性ツツジ (*Rhododendron* section *Tsutsusi*) × キレンゲツツジ (*R. japonicum* (A. Gray) J. V. Suringar f. *flavum* Nakai) における交雑不和合性の要因を明らかにするため、常緑性ツツジ 13 種 22 個体を用いて亜属間交雑を行った。受精前の交雑阻害として、花粉管の伸長停止および花粉管の胚珠への貫入失敗が、また、受精後の交雑阻害として、胚珠の発育停止、発芽不能および夭折が認められた。実施した 22

交雑中、受精前、受精後、およびその両方の交雑阻害が主な不和合性の要因である組合せ数は、それぞれ 15、6 および 1 あった。したがって、常緑性ツツジ × キレンゲツツジにおける交雑不和合性の要因には変異があり、またその変異は種間というよりはむしろ個体レベルで異なると考えられた。

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