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Differences in ploidy levels of inter-specific hybrids obtained by reciprocal crosses between *Primula sieboldii* and *P. kisoana*

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Abstract Differences in ploidy level were found in inter-specific hybrids obtained by reciprocal crosses between *Primula sieboldii* and *P. kisoana*. When *P. sieboldii* was used as the maternal parent, the inter-specific hybrids were triploids; when *P. kisoana* was the maternal parent, the inter-specific hybrids were diploids. The possibility of diploid female gamete formation in *P. sieboldii* is discussed as a causal factor in the production of triploids occasionally found in crosses between diploids of this species.

Key words Inter-specific hybrid · *Primula sieboldii* · *Primula kisoana* · Triploid hybrid · Unreduced female gamete

Introduction

Primula is one of the largest and most widespread plant genus present in the moist and cool regions of the Northern Hemisphere, and approximately 425 species classified into 37 sections have been categorized (Richards 1993). Most of the *Primula* species have beautiful attractive flowers, and some species such as *P. malacoides* Franch., *P. obconica* Hance and *P.×polyantha* are now ranked among the most economically important ornamental plants. In Japan, *P. sieboldii* Morr., which is classified into Sect. Cortusoides, Subsect. Cortusoides (Richards 1993), is an important ornamental species, with approximately 300 cultivars having been produced since Edo Era (about 300 years ago) (Torii 1985).

In the genus *Primula*, inter-specific hybrids have been produced between species belonging to the same sections; i.e. *P.×kewensis* W. Wats., a natural hybrid between *P. verticillata* Forsk. and *P. floribunda* Wall. in Sect. Sphondylia, hybrids between *P. japonica* A. Gr. and *P. burmanica* in Sect. Proliferae and those between *P. veris* L. and *P. vulgaris* Huds. in Sect. Primula. However, there has been no report on the production of hybrids in the Sect. Cortusoides (Richards 1993).

In the present paper, we demonstrate the production of inter-specific hybrids between *P. sieboldii* and *P. kisoana*, which is another important ornamental species found in Sect. Cortusoides, Subsect. Geranioides (Richards 1993), and show that the hybrids obtained from crosses using *P. sieboldii* as the maternal parent were totally triploid.

Materials and methods

Plant materials

Three lines/cultivars of *P. kisoana* Miq. and 12 cultivars of *P. sieboldii* Morr. were used in the present study. The lines/cultivars of *P. kisoana* Miq. were purchased from a local nursery in late March, just before bolting. The cultivars of *P. sieboldii* used had been maintained outside the glasshouse as potted plants at the Laboratory of Plant Cell Technology, Faculty of Horticulture, Chiba University. After bolting, the plants used for crossing were transferred into the glasshouse. Types of heterostyly (pin or thrum) of these lines/cultivars are shown in Table 1.

Crossing method

In total, 34 crosses, mostly including compatible combinations between pin and thrum or between thrum and pin, were conducted between *P. sieboldii* and *P. kisoana*. In these inter-specific crosses, *P. sieboldii* was used the maternal parent in 16 cross combinations and *P. kisoana* in 18 combinations (Tables 2 and 3). Two to three days before blooming, the flowers were emasculated after excision of the petals and covered with paraffin paper bags. Two to three days after emasculation, the flowers were pollinated with pollen of the partner species. Pollinated flowers were again covered with the bags.

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Table 1 List of cultivars and strains used for the inter-specific reciprocal cross between *P. sieboldii* Morr. and *P. kisoana* Miq. and their type of heterostyly

Name of cultivar or strain	Type of heterostyly
P. sieboldii	
Akatonbo	thrum
Ayanami	pin
Ginsekai	pin
Hinotsukasa	pin
Kourohou	pin
Kurumajiro	pin
Mihonokoji	pin
Miyuki	pin
Momijibashi	thrum
Ooasahi	pin
Setsugekka	thrum
Tagonoura	pin
Youdainoyume	pin
Zendaimimon	thrum
P. kisoana	
Iyobeni	thrum
Shirobana-Shikoku	thrum
Shikoku W1	pin

Establishment of seedlings in vitro

Immature ovaries were collected 4–5 weeks after pollination in the cross between *P. sieboldii* (female) and *P. kisoana* (male), and 6–7 weeks after pollination in the cross between *P. kisoana* (female) and *P. sieboldii* (male), and the number of seeds, including those only partially developed, was counted for each pollinated flower. The seeds were then surface-sterilized for 10 min with a sodium hypochlorite solution (1% available chlorine) containing a drop of Tween 20, followed by two rinses with sterile distilled water. After surface sterilization, the placenta with ovules was excised from an ovary and put on 0.2% (w/v) gellan gum-solidified half-strength MS medium (Murashige and Skoog 1962) (1/2MS) containing 5% (w/v) sucrose and 100 mg/l gibberellic acid (GA_3). Seedlings that germinated normally were transferred to half-strength MS medium with 0.2% (w/v) gellan gum and 3% (w/v) sucrose. Following two or three sub-cultures on the same medium, the plants with more than five leaves and well-developed roots were acclimatized. The seedlings that did not show vigorous growth were transferred onto 0.2% (w/v) gellan gum-solidified MS medium supplemented with 3% (w/v) sucrose, 0.2 mg/l 1-naphthaleneacetic acid (NAA) and 1 mg/l 6-benzylaminopurine (BA) to induce calli and regenerate plants. The regenerated plantlets were also acclimatized and transferred to the glasshouse.

DNA extraction and random amplified polymorphic DNA (RAPD) analysis

DNAs of the parents and the plants derived from inter-specific crosses were extracted by the CTAB method (Murray and Thompson 1987) for confirmation of the hybridity by RAPD analysis. Tth DNA polymerase chain reaction system (Toyobo Co) was used for the RAPD analysis, in which 25 µl of reaction solution containing 1 ng of total plant DNA was subjected to the analysis according to the manufacturer's protocol. The primer used was OPK19 (5′-CACAGGCGGA-3′) in Operon random 10-mer primers (Operon Co). DNA fragments were amplified by 40 repeating cycles of the following thermal treatments: 94°C for 1 min, 41°C for 1 min and 72°C for 1 min, in a program temperature control system PC 700 (Astec Co). Electrophoresis of the amplified DNAs was conducted on a 5% acrylamide gel in a TBE buffer system. Digested øx174/*Hae*III was used as the size marker.

Determination of ploidy level

The ploidy level of the plants obtained from inter-specific crosses was analyzed by flowcytometry using CA-2 (Partec, Münster, Germany). Leaf segments 1 cm^2 were chopped with a razor blade in 3 ml DAPI solution [0.1 *M* TRIS-HCl, pH 7.5, containing 2 mg/l DAPI (4′,6-diamidino-2-phenylindole dihydrochloride), 0.1% (v/v) Triton X-100 and 2 m*M* $MgCl_2$]. Diploid cv. Miyuki, triploid cv. Shiratama and tetraploid cv. Yamato-Kamikaze of *P. sieboldii* were used as the standards for each ploidy level (Yamaguchi 1973).

Counting chromosome numbers

Root tips were pre-treated with 0.001% (w/v) colchicine solution for 4 h at room temperature, then fixed with ethanol-acetic acid (3:1 v/v). The fixed root tips were macerated with enzyme solution containing 4.0% (w/v) Cellulase Onozuka RS (Yakult Pharmaceutical Ind. Co, Japan), 1.0% (w/v) Pectolyase Y-23 (Seishin, Japan), 0.3% (w/v) Macerozyme R10 (Yakult Pharmaceutical Ind. Co, Japan) and 1 m*M* EDTA, which was adjusted to pH 4.2 according to the method of Fukui and Iijima (1991). The dispersed root-tip cells were stained with a drop of aceto-carmine.

Results

Crossability

The mean number of seeds obtained per placenta ranged between 2 and 65 in the crosses where *P. sieboldii* was used as the maternal parent (Table 2). In the inter-specific crosses between pin-type cultivars, *P. sieboldii* cv. Kourohou and *P. kisoana* cv. Shikoku W1, the mean number of seeds per placenta was almost the same as that in the other crosses between pin and thrum or vice versa, but the mean number of seeds in the pin-pin cross between *P. sieboldii* cv. Ooasahi and *P. kisoana* cv. Shikoku W1 was smaller than in another pin-pin cross, that between *P. sieboldii* cv. Kourohou and *P. kisoana* cv. Shikoku W1 (Table 2). When pin-type cultivars of *P. sieboldii* were used as maternal plants, most crosses produced sterile seeds, such as those with a seed coat only. However, fertile seeds were obtained from 5 crosses, and these germinated successfully in *in vitro* culture (Table 2). The seedlings derived from the cross between *P. sieboldii* cv. Kourohou (pin) and *P. kisoana* cv. Shikoku W1 (pin) were albino and stopped growth without proliferation of true leaves. However, these albino seedlings turned green, callused and ultimately regenerated some shoots after being transferred onto the medium for callus induction and shoot regeneration.

In most of the crosses with *P. kisoana* as maternal parent, many seeds were produced 60 days after pollination (Table 3). However seed germination was only observed in 5 combinations using *P. kisoana* cv. Shirobana-Shikoku as maternal parent. The seedlings thus obtained showed poor growth due to inhibited root growth during the initial stage of development. Two months after germination, some of the seedlings started to grow vigorously.

Table 2 Seed production and germination in the inter-specific crosses between *P. sieboldii* Morr. and *P. kisoana* Miq.

Female	Male	Number of flowers pollinated	Number of flowers producing seeds	Total no. of seeds (a)	Number of germinated seeds (b)	Percentage of germinable seeds (b/a ×100)
Ginsekai (p)[a]	Iyobeni (t)	7	4	187	0	0
Ginsekai (p)[a]	Shirobana-Shikoku (t)	6	5	244	0	0
Hinotukasa (p)	Iyobeni (t)	1	1	50	0	0
Hinotukasa (p)	Shirobana-Shikoku (t)	4	4	155	0	0
Kourohou (p)	Iyobeni (t)	5	4	163	0	0
Kourohou (p)	Shirobana-Shikoku (t)	3	3	195	0	0
Kourohou (p)	Shikoku W1 (p)	1	1	47	3	6.4
Miyuki (p)	Iyobeni (t)	4	4	158	42	26.6
Miyuki (p)	Shirobana-Shikoku (t)	3	3	81	12	14.8
Mihonokoji (p)	Iyobeni (t)	2	2	114	4	3.5
Mihonokoji (p)	Shirobana-Shikoku (t)	2	2	93	0	0
Momijibashi (t)	Shikoku W1 (p)	1	1	8	0	0
Ooasahi (p)	Iyobeni (t)	1	1	2	0	0
Ooasahi (p)	Shikoku W1 (p)	2	2	22	2	9.1
Setugekka (t)	Shikoku W1 (p)	1	1	28	0	0
Tagonoura (p)	Iyobeni (t)	1	1	11	0	0

[a] p, pin type; t, thrum type

Table 3 Seed production and germination in the inter-specific crosses between *P. kisoana* Morr. and *P. sieboldii* Miq.

Female	Male	Number of flowers pollinated	Number of flowers producing seeds	Total no. of seeds (a)	Number of germinated seeds (b)	Percentage of germinable seeds (b/a ×100)
Shirobana-Shikoku (t)[a]	Ayanami (p)	6	4	386	1	0.3
Shirobana-Shikoku (t)	Hinotsukasa (p)	1	1	90	2	2.2
Shirobana-Shikoku (t)	Kurumajiro (p)	2	2	121	0	0
Shirobana-Shikoku (t)	Mihonokoji (p)	2	2	43	0	0
Shirobana-Shikoku (t)	Ooasahi (p)	7	7	513	13	2.5
Shirobana-Shikoku (t)	Tagonoura (p)	3	3	122	3	2.5
Shirobana-Shikoku (t)	Youdainoyume (p)	6	6	285	19	6.7
Iyobeni (t)	Ayanami (p)	4	4	178	0	0
Iyobeni (t)	Ginsekai (p)	3	1	66	0	0
Iyobeni (t)	Hinotsukasa (p)	1	0	0	0	0
Iyobeni (t)	Kourohou (p)	1	1	46	0	0
Iyobeni (t)	Mihonokoji (p)	2	2	72	0	0
Iyobeni (t)	Mijuki (p)	1	1	30	0	0
Iyobeni (t)	Setsugekka (t)	1	1	51	0	0
Iyobeni (t)	Youdainoyume (p)	2	2	76	0	0
Shikoku W1 (p)	Akatonbo (t)	1	1	79	0	0
Shikoku W1 (p)	Momijibashi (t)	1	1	46	0	0
Shikoku W1 (p)	Zendaimimon (t)	2	2	67	0	0

[a] p, pin type; t, thrum type

Hybridity analysis

RAPD analysis was performed to detect specific markers for both parents in the putative hybrids. Fragments of 310–603 bp were obtained using the OPK-19 primer; these differed in size between *P. sieboldii* and *P. kisoana*, with the *P. kisoana*-specific fragment being smaller than that of *P. sieboldii* (Fig. 1). Inter-specific hybrids had the common bands specific for both parents (Fig. 1). In *P. kisoana* cv. Iyobeni, two RAPD bands were obtained as a major small-sized band; these corresponded to one of cv. Shirobana-Shikoku and a minor large one also specific to this particular cultivar. Those bands segregated independently in the inter-specific hybrids (Fig. 1).

All of the plants obtained from the cross using *P. sieboldii* cvs. Miyuki and Ooasahi as maternal parents had species-specific bands from both parents, with the exception of 1 plant; these plants were confirmed to be inter-specific hybrids. However, plants obtained from the crosses using *P. sieboldii* cvs. Kourohou and Mihonokoji as maternal parents had only the band that was specific to *P. sieboldii*, and the hybridity of the plants was not confirmed.

Table 4 Ploidy level of progenies of inter-specific cross between *P. sieboldii* and *P. kisoana* as determined by flowcytometric analysis

Cross		Number of progenies	Diploid	Triploid
P. sieboldii/*P. kisoana*				
Miyuki	Iyobeni	42	1[a]	41
Miyuki	Shirobana-Shikoku	11	0	11
Ooasahi	Shikoku W1	2	0	2
Kourohou	Shikoku W1	3	3[a]	0
Mihonokoji	Iyobeni	4	4[a]	0
P. kisoana/*P. sieboldii*				
Shirobana-Shikoku	Ooasahi	12	12	0
Shirobana-Shikoku	Youdainoyume	18	18	0
Shirobana-Shikoku	Tagonoura	3	3	0
Shirobana-Shikoku	Hinotsukasa	2	2	0

[a] No evidence of hybridity by RAPD analysis

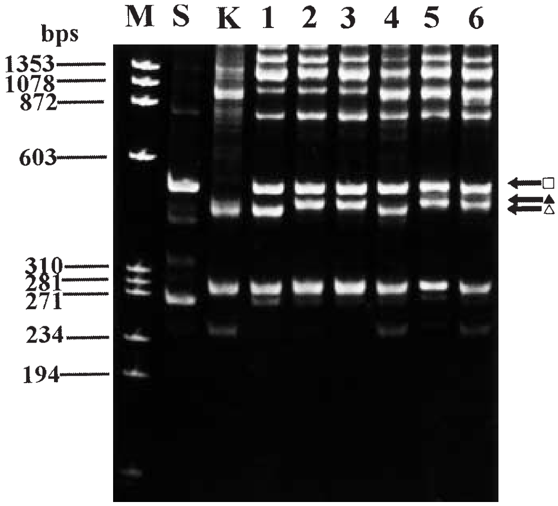

Fig. 1 PCR analysis on the hybridity of the progenies of the inter-specific cross between *P. sieboldii* and *P. kisoana*. *M* ϕX174/*Hae*III digestion size marker, *S P. sieboldii*, *K P. kisoana*, *lanes 1–6* progenies obtained from the cross between *P. sieboldii* cv. Miyuki as maternal parent and *P. kisoana* cv. Iyobeni as pollen parent. □ Band specific to *P. sieboldii*, △ major band specific to *P. kisoana* cv. Iyobeni, ▲ minor band specific to *P. kisoana* cv. Iyobeni

Analysis of ploidy level

The results of the flowcytometric analysis of ploidy level revealed that plants produced from crosses between *P. kisoana* and *P. sieboldii* were all diploids, whereas those produced from the crosses between *P. sieboldii* and *P. kisoana* were mostly triploids (Fig. 2, Table 4). All of the triploid plants produced from crosses between *P. sieboldii* and *P. kisoana* coincided with those previously confirmed to be the inter-specific hybrids by RAPD analysis, whereas all of the plants which could not be confirmed with respect to their hybridity were diploid.

The results of the flowcytometric analysis were also confirmed by counting the chromosome numbers in root-tip cells of the parental plants and those derived from inter-specific crosses. Both of the parental species showed the same diploid chromosome numbers (2n=24), as expected (Fig. 3A, B). Among the progenies obtained by

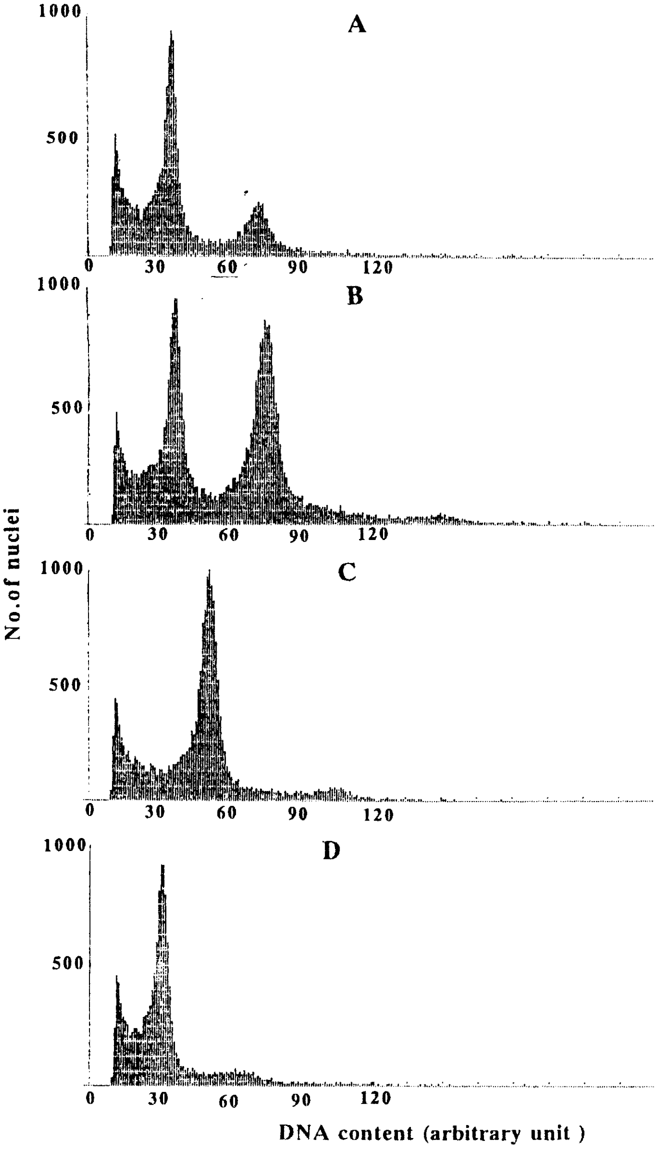

Fig. 2 Flowcytometric profiles of **A** *P. kisoana* 2n=24 (diploid), **B** diploid (*left peak*, 2n=2x=24) and tetraploid (*right peak*, 2n=4x=48) cultivars of *P. sieboldii*, *C* triploid hybrid obtained from inter-specific cross between *P. sieboldii* cv. Miyuki and *P. kisoana* cv. Iyobeni, **D** diploid hybrid obtained from inter-specific cross between *P. kisoana* cv. Shirobana-Shikoku and *P. sieboldii* cv. Ooasahi

inter-specific crosses, those plants derived from the crosses between *P. kisoana* and *P. sieboldii* with diploid peaks in the flowcytometric analysis had 24 chromosomes (2n=2x) (Fig. 3C), whereas those produced by crosses between *P. sieboldii* and *P. kisoana* with triploid peaks had 36 chromosomes (2n=3x) (Fig. 3D).

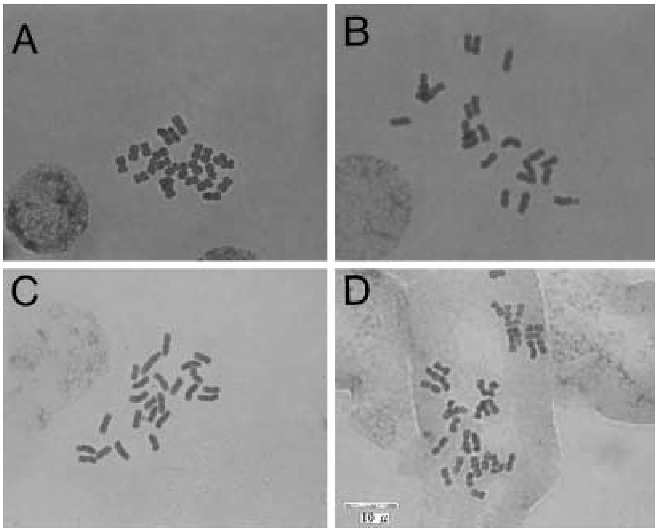

Fig. 3A–D Somatic chromosomes of the inter-specific hybrid between *P. sieboldii* and *P. kisoana* **A** *P. sieboldii* 2n=24, **B** *P. kisoana* 2n=24, **C** diploid (2n=24) hybrid using *P. kisoana* as a maternal parent, **D** triploid (2n=3x=36) hybrid using *P. sieboldii* as a maternal parent

Flower trait in hybrid plants

Several of the hybrid plants obtained were successfully acclimatized and grown in the glasshouse. They bloomed 20 months after transfer to the glasshouse (Fig. 4A). The leaf shape of the hybrids was intermediate between that of both parents, irrespective of the difference in ploidy level, diploid vs. triploid (Fig. 4B). However, flower shape and color varied among the hybrids. One diploid hybrid derived from the cross between *P. kisoana* cv. Shirobana-Shikoku (thrum type with white petal color and yellow eye) and *P. sieboldii* cv. Ooasahi (pin type with red petal color, serrated petal and white eye) had a thrum-type flower with intermediate petal color and

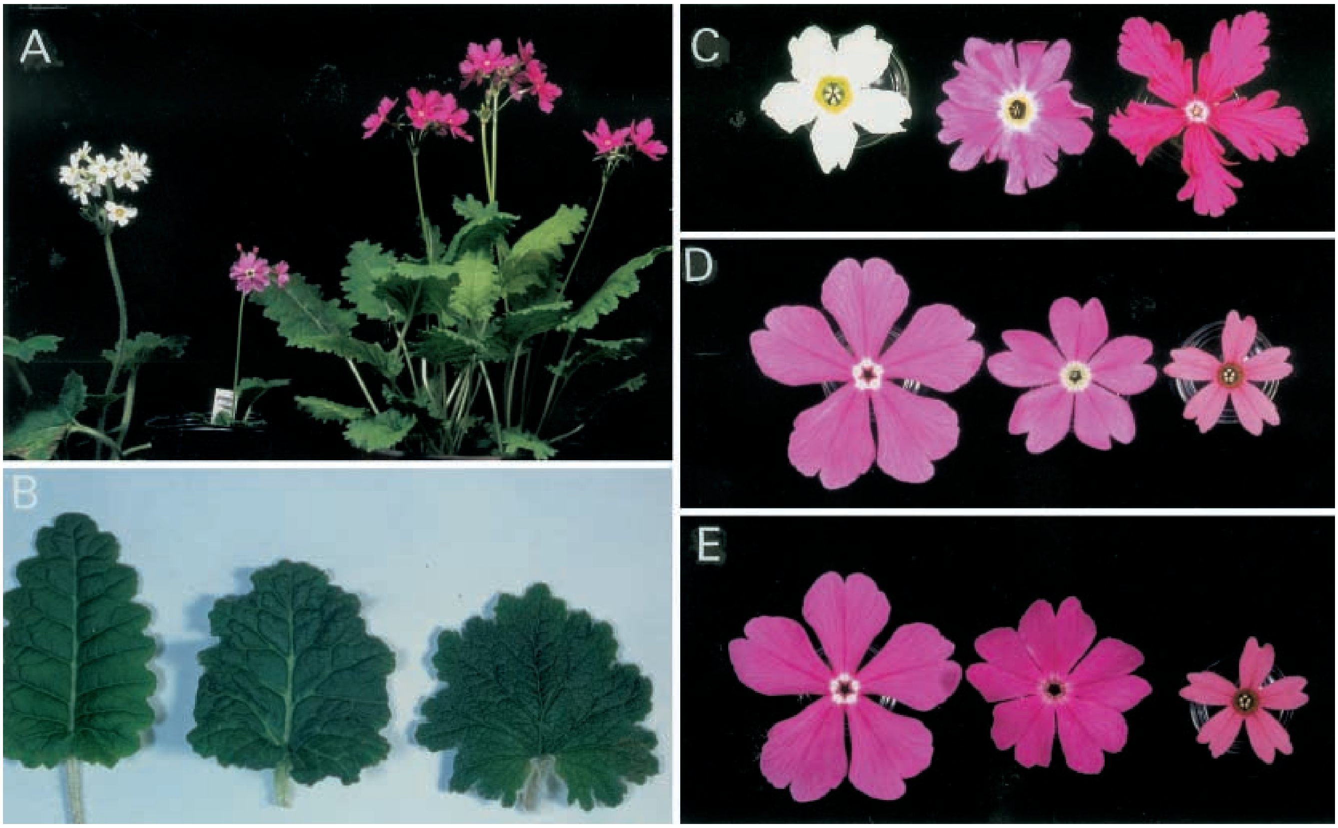

Fig. 4A–E Characteristics of the hybrids obtained by reciprocal crosses between *P. kisoana* and *P. sieboldii*. **A** Flowering plants of *P. kisoana* cv. Shirobana-Shikoku (*left*) used as a maternal parent, inter-specific diploid hybrid (*center*) and *P. sieboldii* cv. Ooasahi (*right*) used as a pollen parent, **B** leaf shape of *P. sieboldii* cv. Miyuki (*left*) used as a maternal parent, inter-specific triploid hybrid (*center*) and *P. kisoana* cv. Shirobana-Shikoku (*right*) used as a pollen parent, **C** flower shape of *P. kisoana* cv. Shirobana-Shikoku (*left*) used as a maternal parent, inter-specific diploid hybrid with pale yellow eye (*center*) and *P. sieboldii* cv. Ooasahi (*right*) used as a pollen parent, **D** flower shape of *P. sieboldii* cv. Miyuki (*left*) used as a maternal parent, inter-specific triploid hybrid with pale-yellow eye and *P. kisoana* cv. Iyobeni (*right*) used as a pollen parent, **E** flower shape of *P. sieboldii* cv. Miyuki (*left*) used as a maternal parent, inter-specific triploid hybrid without yellow eye (center) and *P. kisoana* cv. Iyobeni (*right*) used as a pollen parent shape and a double colored eye in which the inside was yellow and the outside was white (Fig. 4C). Another hybrid derived from the same cross combination had a pin-type flower with wide, purple-red petals and a double-colored eye, and produced more than one flower stalk from each shoot, which is not a character of *P. sieboldii*. Other diploid hybrids derived from the cross between *P. kisoana* cv. Shirobana-Shikoku and *P. sieboldii* cv. Tagonoura (pin type with white flower color) had thrum-type white flowers with a yellow eye. The flower petal shape of these triploid hybrids was intermediate between that of the parents with some variations. Although flower color was close to the maternal color, two types of eye color were found in these triploid hybrids; one had double eyes in which the inside was pale yellow and the outside was white (Fig. 4D), and the other had only traces of a white eye or no white eye at all (Fig. 4E). All of the hybrids had non-dehiscent anthers without normal pollen.

Discussion

In the present study, 12 out of 34 cross combinations in the reciprocal inter-specific cross between *P. sieboldii* and *P. kisoana* successfully yielded progeny plants. In the crosses where *P. kisoana* was used as maternal parent, only cv. Shirobana-Shikoku produced hybrid plants and others failed to produce germinable seeds (Tables 3, 4), suggesting that genetic background of the maternal parent might affect the formation of hybrid plants. A similar phenomenon was previously reported in wheat-barley inter-specific hybridization, where crossability was greatly affected by the specific combination of genetic background of parental lines/cultivars (Taketa et al. 1996).

In the legitimate crosses (pin-thrum) where *P. sieboldii* was used as a maternal parent, most combinations were compatible in terms of seed production, but only 4 out of 14 crosses with seed production showed seed germination. Among the 4 successful crosses, those using *P. sieboldii* cv. Miyuki as maternal parent gave the highest yields of germinable seeds (Table 2). The illegitimate pin-pin cross combinations using *P. sieboldii* cv. Kourohou (pin) and cv. Ooasahi (pin) as female parents also showed some possibility of producing some germinable seeds (Table 2). In our preliminary study on this inter-specific hybridization, the illegitimate cross combinations between thrum-type cultivars never yielded seeds. Moreover, our previous study on *P. sieboldii* showed that heteromorphic self-incompatibility is more strongly conserved in thrum-thrum cross combinations than pin-pin combinations and that some of the latter combinations yielded germinable seeds (unpublished results). Therefore, it is likely that the heteromorphic self-incompatibility system was commonly preserved in these two *Primula* species and that the mode of the incompatibility may depend on the genetic background of each cultivar.

The fact that plants derived from the crosses between *P. kisoana* and *P. sieboldii* were all true-diploid hybrids (Table 4) indicates that hybrid dysgenesis did not occur between the genomes of *P. sieboldii* and *P. kisoana* in the maternal background of *P. kisoana*. When *P. sieboldii* was used as maternal parent, the progenies obtained were mostly triploids with true hybridity, whereas no evidence for hybridity was obtained in the remaining diploid progenies. These results may suggest that embryos of the true-diploid hybrids failed to develop by embryo abortion and/or failure of endosperm development. In inter-specific crosses between *P. elatior* and *P. veris*, the successful development of hybrid embryos was also affected by the species used as the maternal parent, hybrid embryos were obtained only when *P. veris* was used as the maternal parent (Woodel 1960).

Triploid hybrid production was previously observed in inter-specific crosses in which *P. japonica* was used as a maternal parent (Ernst 1950). In this study, species used as the male parent affected the nature of the progenies; triploid true-hybrids were produced when *P. japonica* was pollinated with *P. burmanica*, whereas maternal progenies were produced when the former was pollinated with *P. cockburniana*, *P. chungensis* and *P. purverulenta* (Ernst 1950). In the present study, however, genotypes of *P. sieboldii* used as the female parent affected the nature and ploidy level of the progenies. Cultivars Kourohou and Mihonokoji produced diploid maternal-type progenies, whereas cvs. Ooasahi and Miyuki produced triploid true-hybrids when they were used as female parents. These results might suggest that some of the egg cells in these cultivars were diploid.

Takamura and Miyajima (1996) reported that only tetraploid plants were produced in reciprocal crosses between diploid and tetraploid cultivars of *Cyclamen persicum* irregardless of the cross direction. Based on these results, they postulated that diploid egg cells and pollen formation in the diploid cultivar resulted in the formation of tetraploid plants. The formation of triploid hybrids in the present study might have been induced by the fertilization of unreduced diploid egg cells of *P. sieboldii* with normal pollen of *P. kisoana*, since the pollen size of *P. kisoana* was not variable but uniform and no polyploids were obtained in the crosses between cultivars/strains of *P. kisoana*. Even if it were possible that rare diploid pollen of *P. kisoana* had the chance to contribute to the formation of triploid hybrids, it is difficult to explain why so many triploid hybrids were produced when *P. sieboldii* cv. Miyuki was used as the maternal parent.

There are several possible explanations for the formation of maternal-type progenies in the crosses using *P. sieboldii* cv. Mihonokoji or cv. Kourohou as a maternal parent: (1) parthenogenesis and chromosome doubling of haploid egg cells, which might be stimulated by inter-specific pollination; (2) parthenogenesis of putative diploid egg cells; (3) diploid hybrid zygote formation followed by total elimination of pollen-derived chromosomes and then haploid genome doubling; (4) triploid hybrid formation between the diploid egg cell of *P.*

sieboldii and haploid pollen of *P. kisoana*, followed by the total elimination of pollen-derived chromosomes. At present, it is still unclear which possibility is correct, but the formation of both triploid hybrid and diploid maternal-type progenies suggests that diploid egg cell formation occurred at least in *P. sieboldii*.

Hermsen (1984) described diploid gamete formation, which occurs as a result of first-division restitution (FDR) or second-division restitution (SDR) in meiosis. Gamete genotypes resulting from FDR should be identical to the parent genotype, while those resulting from SDR should segregate into fixed diploid homo genotypes that are different from the parental genotype even though crossing-over does not occur at the chromosome pairing stage. If *P. sieboldii*-type maternal progenies were produced by SDR, they would be considered to be doubled haploids and cultivars such as Mihonokoji and Kourohou could be utilized to produce doubled-haploid lines by pollinating with *P. kisoana* because the genome of the latter might be eliminated after fertilization. Since it is difficult to produce genetically pure lines by recurrent self crosses in most *Primula* species because of their heteromorphic self-incompatibility, the method could be efficiently used for the breeding of *P. sieboldii*.

The hybrids derived from the cross between *P. kisoana* cv. Shirobana-Shikoku (white flower) and a white-flowered cultivar of *P. sieboldii*, Tagonoura, had thrum-type flowers with white petals and a yellow eye, suggesting that alleles for white petal color in *P. sieboldii* and *P. kisoana* might have been induced by a mutation on the same gene. However, the gene for red flower color in *P. sieboldii* might be incompletely dominant over the gene for white flower color in *P. kisoana* because the flower of the hybrid between *P. kisoana* cv. Shirobana-Shikoku and *P. sieboldii* cv. Ooasahi (red flower) was salmon pink in color, which did not resemble neither parents.

This is the first report on the production of inter-specific hybrids in Sect. Cortusoides, achieved by the combination between Subsect. Cortusoides and Subsect. Geranioides. Although these inter-specific hybrids can not be directly used for further breeding due to hybrid sterility, amphidiploid or amphitriploid production by artificial chromosome doubling may be useful in recovering the fertility of these hybrids and they could then be used for breeding new cultivars in *P. sieboldii*, such as a yellow-flowered cultivar by introducing the yellow eye trait of *P. kisoana*. Until now, proteins or genes associated with heteromorphic incompatibility have not been identified. Diploid thrum-type hybrids, such as those obtained from the cross between *P. kisoana* cv. Shirobana-Shikoku (thrum) and *P. sieboldii* cv. Tagonoura (pin), are expected to have dominant the S-gene of *P. kisoana*. Amphidiploid production of the hybrids could be efficiently used to produce *P. sieboldii* plants with a thrum-associated chromosome of *P. kisoana*.

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