

Allelic Differences at Two Loci Govern Different Mechanisms of Intraflower Self-Pollination in Self-Pollinating Strains of Periwinkle

R. N. KULKARNI, Y. SREEVALLI, AND K. BASKARAN

From the Central Institute of Medicinal and Aromatic Plants, Field Station, Allalandra, Bangalore 560 065, India.

Address correspondence to R. N. Kulkarni at the address above, or e-mail: krnpbg@yahoo.co.in.

Abstract

Periwinkle [*Catharanthus roseus* (L.) G. Don], an ornamental and medicinal plant, is a self-compatible, insect-pollinated plant species in which intraflower self-pollination does not occur because of spatial separation of the stigma and anthers. Recently three self-pollinating strains—MJ, VI, and OR—were identified. Self-pollination in these strains was found to be brought about by continuous increase in gynoecium length from anthesis to self-pollination, in contrast to non-self-pollinating strains, in which the stigma remained below the base of the anthers from anthesis to flower drop. Self-pollination in these strains was found to be controlled by duplicate, recessive genes. Self-pollination in strains MJ and VI was brought about by an increase in gynoecium length resulting from an increase in the length of the ovary, while in the strain OR, the increase in gynoecium length was because of an increase in the length of the style from anthesis to self-pollination. The three strains were intercrossed to determine the relationship between genes governing self-pollination in these strains. The F_1 plants and all plants of the F_2 generation of the cross MJ \times VI exhibited self-pollination that was brought about by an increase in the length of the ovary, indicating that the same genes were involved in these two strains. The F_1 plants of crosses OR \times MJ and OR \times VI, exhibited self-pollination that was brought about by an increase in the length of the ovary, indicating that self-pollination brought about by an increase in the length of the ovary was dominant over self-pollination brought about by an increase in the length of the style. In the F_2 and backcross [(OR \times MJ) \times OR and (OR \times VI) \times OR] generations, both self-pollinating and non-self-pollinating plants were observed. The ratio of plants with self-pollination brought about by an increase in the length of the ovary, non-self-pollinating plants, and plants with self-pollination brought about by an increase in the length of the style in the F_2 and backcross generations fit 9:6:1 and 1:2:1 ratios, respectively. All plants of the backcrosses [(OR \times MJ) \times MJ and (OR \times VI) \times VI] exhibited self-pollination brought about by an increase in the length of the ovary. The results thus supported the earlier finding that self-pollination in the studied strains was controlled by duplicate, recessive genes and suggested that three alleles at two loci determine the occurrence or nonoccurrence of intraflower self-pollination in periwinkle.

Periwinkle [*Catharanthus roseus* (L.) G. Don], an ever-blooming, perennial, tropical plant, derives its economic importance from the anticancer (vincristine and vinblastine) and antihypertension (ajmalicine) alkaloids present in its leaves and roots, respectively. The small amounts of these alkaloids in the plant and their high value have led to extensive work on the production of these alkaloids through cell and tissue culture, and chemical synthesis (Moreno et al. 1995). Further, periwinkle has been proposed as a model medicinal plant for genomics and proteomics (Jacobs et al. 2000). It is also grown as an ornamental plant because of its variously colored flowers and ever-blooming nature. In spite of its both ornamental and medicinal importance,

very little work has been done on the genetics or breeding of this plant.

Darwin and Delpino reported long ago that intraflower self-pollination does not occur in periwinkle and pollination is provided by insects (Knuth 1909). The corolla of the normal periwinkle flower is salver-shaped and the stamens are located in the throat of the corolla tube. The stigmatic head, with a sticky secretion and a brush of hairs, is below the anthers and takes up all the pollen as it is shed. The receptive portion is, however, at the base of the stigmatic head and thus intraflower self-pollination is excluded. Pollination occurs through nectar-seeking insects which effect pollination by depositing pollen collected during their visits to

various plants (Knuth 1909; Rendle 1971). Until recently, however, periwinkle had been considered as a self-pollinating species. Studies have now confirmed that intraflower self-pollination normally does not occur in periwinkle and pollination is brought about mainly by butterflies (Kulkarni 1999; Sreevalli et al. 2000). Geitonogamy (fertilization between different flowers of the same plant) and phenotypic assortative mating for flower color brought about by pollinating butterflies and the existence of self-pollinating strains were suggested as possible reasons for considering periwinkle as a self-pollinated species in early genetic studies (Kulkarni 1999). Three self-pollinating strains were identified recently and the mechanism of intraflower self-pollination in these self-pollinating strains and its mode of inheritance were reported (Kulkarni et al. 2001).

Intraflower self-pollination in these three self-pollinating strains was found to be brought about by the continued increase in the length of the gynoecium beyond the base of the anthers, resulting in self-pollination, in contrast to the stigma remaining below the base of the anthers until flower drop in normal non-self-pollinating strains. This mechanism of self-pollination in the three self-pollinating strains was found to be controlled by duplicate, recessive genes (Kulkarni et al. 2001). However, whether the same or different genes were involved in self-pollination in these three strains was not determined. In two of these three self-pollinating strains, a continued increase in the length of the gynoecium responsible for intraflower self-pollination was found to be because of an increase in the length of the ovary, while in the third it was because of an increase in the length of the style. The relationship between genes governing these two types of mechanisms responsible for self-pollination and whether increase in the length of the gynoecium in these strains occurred until stigma maturation, pollination, or flower drop were studied.

Materials and Methods

The material studied included three self-pollinating strains of periwinkle—MJ, VI, and OR. These strains were procured from a local dealer in horticultural plants. Self-pollination and fruit set in these strains occurred in the absence of pollinators when plants were grown in a greenhouse.

Gynoecium, Style, and Ovary Lengths, and the Height of Anthers in the Corolla Tube

Ovary, style (including stigma), and gynoecium lengths, and the height of anther base in the corolla tube in these strains were measured from anthesis to 2 days after anthesis to determine whether the growth of gynoecium that was responsible for intraflower self-pollination in the self-pollinating strains was brought about by an increase in the length of the ovary or style, or both.

Stigma Maturity

Maturity of the stigma was determined by pollinating emasculated flowers with the pollen from the same plant.

The flowers were emasculated 1 day before anthesis and pollinated on the same day, or 1, 2, or 3 days after emasculat

Effect of Emasculat

Flowers were emasculated 1 day before anthesis and lengths of the gynoecium, ovary, and style were measured until 3 days after emasculat to determine whether the increase in the length of the gynoecium occurred until stigma maturation or flower drop.

Effect of Pollinat

To determine whether the increase in the length of the gynoecium occurred until pollinat, flowers were emasculated 1 day before anthesis and pollinat on the same day, or 1, 2, or 3 days after emasculat. The length of the gynoecium before and after pollinat was measured.

In all instances, observations were recorded on 30 flowers.

Relationship Between Genes Governing Self-Pollinat

The three strains were intercrossed to determine whether the same or different genes governed the mechanism responsible for self-pollinat in these strains. Plants of the F₁, F₂, and backcross generations were raised in a greenhouse and scored for the occurrence of self-pollinat (as inferred from the formation of fruits and seeds). In the strain OR, fruit growth could be seen only 3–4 days after shedding of the corolla (Figure 1). In this strain, an increase in the length of the gynoecium responsible for self-pollinat was brought about by an increase in the length of the style. Plants of segregating generations showing fruit formation only after shedding of the corolla, as in the strain OR, were scored as exhibiting an S-type mechanism of self-pollinat. In strains MJ and VI, an increase in the length of the gynoecium responsible for self-pollinat was because of an increase in the length of the ovary, and fruit growth could be observed within the corolla tube. The corolla and the corolla tube remained adhered to the fruit almost until complete development of the fruit (Figure 2). Plants of segregating generations showing fruit formation with corolla tubes adhering to the fruit, as in strains MJ and VI, were scored as exhibiting an O-type mechanism of self-pollinat. Gynoecium, ovary, and style lengths of flowers of 10 random plants from each of the three types of plants (self-pollinat O type, self-pollinat S type, and non-self-pollinat) of the F₂ generation of the crosses OR × MJ and OR × VI were measured on the day of anthesis and 2 days later.

Results

Gynoecium, Style, and Ovary Lengths, and the Height of the Anthers in the Corolla Tube

On the day of anthesis, the gynoecium length and the height of the anthers in the corolla tube ranged from 22.7 mm to



Figure 1. Fruit formation in the strain OR after shedding of the corolla.

24.0 mm and from 21.8 mm to 23.3 mm, respectively, in the studied strains (Table 1). The height of the stigma in the corolla tube was 0.5 to 0.9 mm above the base of the anthers on the day of anthesis, while it was 1.4 to 2.0 mm above the base of the anthers on the day of self-pollination in the three studied self-pollinating strains (Table 1). The increase in the gynoecium length from anthesis to the day of self-pollination was mainly because of an increase in the length of the style in the strain OR (Table 1), while it was due to an increase in the length of the ovary in strains MJ and VI (Table 1).

Stigma Maturity

In all three strains, the stigma was receptive from 1 day before anthesis to 2 days after anthesis and 100% fruit set was obtained on all 4 days of pollination (i.e., -1, 0, 1, and 2 days after anthesis). Thus the stigma was receptive at all stages of gynoecium growth in the corolla tube.

Effect of Emasculation on Gynoecium Length Increase

Flowers were emasculated 1 day before anthesis and gynoecium lengths were measured on different days after emasculation. For all three strains, gynoecium lengths of emasculated flowers were greater than those of nonemasculated flowers, on all 3 days after emasculation (Table 2). Among the three strains, the differences between the length of the gynoecium of emasculated and nonemasculated flowers was largest in the strain OR.

Effect of Pollination on Gynoecium Length Increase

The effect of pollination was studied by emasculating the flowers 1 day before anthesis and pollinating them on



Figure 2. Fruit formation with corolla tubes adhering to the fruits in the strain, MJ.

different days after emasculation. In the strain OR, no increase in the length of the gynoecium was observed after pollination, irrespective of the day of pollination. The styles withered and dropped 3 to 4 days after pollination, as in normal nonemasculated flowers. In strains MJ and VI, however, the gynoecium continued to grow after pollination, as in the nonemasculated flowers of these strains.

Relationship Between Genes Governing Self-Pollination

The F_1 plants of all three crosses—OR \times MJ, OR \times VI, and MJ \times VI—showed the occurrence of intraflower self-pollination, suggesting that the same genes might be responsible for intraflower self-pollination. However, self-pollination in the F_1 plants of these crosses was of the O type (i.e., an increase in the length of the ovary was responsible for self-pollination similar to strains MJ and VI) (Table 1). This suggested that the O-type mechanism of self-pollination was dominant over the S-type mechanism of self-pollination, where an increase in the length of the style was responsible for self-pollination (as in strain OR). The O-type mechanism operating in the F_1 plants was also indicated by the corolla tube adhering to the fruit wall on fruits of these plants.

In the F_2 generations of OR \times MJ and OR \times VI, both self-pollinating and non-self-pollinating plants were observed. The self-pollinating plants could be further differentiated into O and S types. Observations recorded on gynoecium, style, and ovary lengths of three kinds of plants (self-pollinating O type, self-pollinating S type, and non-self-pollinating) of the F_2 generation confirmed that self-pollination in the O- and S-type self-pollinating plants was brought about by an increase in the length of the ovary and

Table 1. Mean (\pm standard error) values of some floral traits of self-pollinating strains OR, MJ, and VI of periwinkle and their F₁s^a

Trait	OR	MJ	VI	F ₁ (OR \times MJ)	F ₁ (OR \times VI)
Gynoecium, style, and ovary lengths (mm) on the day of anthesis					
Gynoecium	22.7 \pm 0.1	22.7 \pm 0.1	24.0 \pm 0.2	24.2 \pm 0.3	24.3 \pm 0.2
Style	20.7 \pm 0.1	20.0 \pm 0.1	21.1 \pm 0.2	21.5 \pm 0.3	21.4 \pm 0.2
Ovary	2.0 \pm 0.0	2.7 \pm 0.0	2.9 \pm 0.0	2.7 \pm 0.0	2.9 \pm 0.0
Height of anther base (mm) in corolla tube on the day of anthesis					
	22.2 \pm 0.1	21.8 \pm 0.1	23.3 \pm 0.2	23.3 \pm 0.3	23.5 \pm 0.1
Height of stigma (mm) above the anther base on the day of anthesis					
	0.5 \pm 0.0	0.9 \pm 0.0	0.7 \pm 0.1	0.9 \pm 0.0	0.8 \pm 0.1
Mean gynoecium, style and ovary lengths (mm) on the day of self-pollination					
Gynoecium	23.8 \pm 0.1 (1.1) ^b	23.3 \pm 0.2 (0.6)	25.9 \pm 0.2 (1.9)	25.0 \pm 0.1 (0.8)	25.9 \pm 0.2 (1.6)
Style	21.8 \pm 0.1 (1.1)	20.0 \pm 0.2 (0.0)	21.2 \pm 0.2 (0.1)	21.5 \pm 0.1 (0.0)	21.4 \pm 0.2 (0.0)
Ovary	2.0 \pm 0.0 (0.0)	3.3 \pm 0.0 (0.6)	4.7 \pm 0.0 (1.8)	3.5 \pm 0.0 (0.8)	4.5 \pm 0.0 (1.6)
Mean height of anther base (mm) in the corolla tube on the day of self-pollination					
	22.4 \pm 0.1	21.9 \pm 0.1	23.9 \pm 0.9	23.4 \pm 0.2	23.6 \pm 0.1
Mean height of the stigma (mm) above anther base on the day of self-pollination					
	1.4 \pm 0.1 (0.9)	1.4 \pm 0.1 (0.5)	2.0 \pm 0.1 (1.3)	1.6 \pm 0.1 (0.7)	2.3 \pm 0.1 (1.5)

^a Observations were not recorded for F₁ plants of the cross MJ \times VI since in both strains, self-pollination was brought about by an increase in the length of the ovary, which was clearly evident from the corolla tubes adhering to the fruits on plants of these strains and their F₁s.

^b Values in parentheses show increases in the length/height as compared with their respective values on the day of anthesis.

style, respectively, while in the non-self-pollinating plants, there was no increase in the length of the gynoecium (relative to the height of the anther base) from anthesis to flower drop (Table 3). The observed ratio of O (self-pollinating) to non-self-pollinating to S (self-pollinating) plants fit a ratio of 9:6:1 (Table 4). The ratio of these three kinds of plants in backcrosses, (OR \times MJ) \times OR, and (OR \times VI) \times OR fit a ratio of 1:2:1 (Table 4). All plants of backcrosses, F₁ \times MJ, and F₁ \times VI were self-pollinating and exhibited the O-type self-pollinating mechanism. All plants of the F₂ generation of the cross MJ \times VI exhibited the O-type self-pollination mechanism, like the parental plants.

Discussion

In two of the three self-pollinating strains used in the present study, an increase in the gynoecium length that resulted in self-pollination occurred because of an increase in the length of the ovary, while in one of them it occurred because of an increase in the length of the style. In non-self-pollinating plants, the stigma remains below the base of the anthers until flower drop (Kulkarni et al. 2001). Thus this variation in the floral structure of self-pollinating and non-self-pollinating strains of periwinkle resembles heteromorphy (the coexistence of two or three genetically controlled hermaphrodite floral types in a population). Heteromorphy is detected in several families and genera (see Richards 1997). Although heteromorphism may be expressed in a variety of morphological features, it is most clearly, but not necessarily manifested as heterostyly (i.e., the coexistence of genetically controlled hermaphrodite floral types with different style lengths, and usually with reciprocal

anther positions) (Frankel and Galun 1977). Most heterostylous species are self-incompatible, but self-compatible heterostylous species are also found (Richards 1997). In periwinkle, the normal non-self-pollinating plants are self-compatible.

Generally heterostyly is closely associated with perennial, often herbaceous plants with long, fused corolla tubes. Although the floral structure in periwinkle suggests the possible existence of heteromorphy, no heteromorphy has been reported. Further, heteromorphy is evident from the gynoecium lengths of self- and non-self-pollinating strains of periwinkle, but there is no heteromorphy for anther position. According to Al Wadi and Richards (1993), anther position monomorphy and self-compatibility may represent an intermediate condition in the evolution of full distyly.

Richards (1997) listed eight species of self-compatible diploid species of *Primula* L. with various degrees of heteromorphy. In *Primula floribunda* Wall., low-altitude populations are distylous and show entomophily (pollination by insects), while those at higher altitudes are homostylous and show automatic self-pollination. At lower altitudes where insect activity is more prevalent, reciprocal herkogamy (separation of anthers and stigma in space within a flower in such a way that self-pollination cannot occur in the absence of an insect visit) has been favored, while at higher altitudes, absence of insects has led to selection for automatic self-pollination. Periwinkle is a tropical plant and has been classified as an insect-pollinated species (Knuth 1909). However, in genetic studies, this plant has been treated as a self-pollinated species and some of these have been carried out in temperate countries. Apart from the reasons given earlier (Kulkarni 1999; Kulkarni et al. 2001), it is possible that

Table 2. Effect of emasculation on the lengths of the ovary, style, and gynoecium (in millimeters) in self-pollinating strains OR, MJ, and VI of periwinkle

	OR			MJ			VI		
	Ovary	Style	Gynoecium	Ovary	Style	Gynoecium	Ovary	Style	Gynoecium
Emasculated flowers ^a									
Days after anthesis									
-1	2.0 ± 0.0	20.7 ± 0.0	22.7 ± 0.1	2.5 ± 0.0	20.0 ± 0.2	22.5 ± 0.2	2.9 ± 0.0	21.1 ± 0.2	24.0 ± 0.2
0	2.0 ± 0.0	22.1 ± 0.3	24.1 ± 0.3	2.7 ± 0.0	20.0 ± 0.5	22.7 ± 0.5	3.1 ± 0.0	21.1 ± 0.2	24.2 ± 0.2
1	2.0 ± 0.0	23.2 ± 0.3	25.2 ± 0.3	3.1 ± 0.0	20.0 ± 0.3	23.1 ± 0.3	4.2 ± 0.1	21.1 ± 0.2	25.3 ± 0.3
2	2.0 ± 0.0	23.6 ± 0.3	25.6 ± 0.3	3.8 ± 0.0	20.1 ± 0.4	23.9 ± 0.4	5.3 ± 0.0	21.1 ± 0.3	26.4 ± 0.3
Nonemasculated flowers									
Days after anthesis									
-1	2.0 ± 0.0	20.7 ± 0.0	22.7 ± 0.1	2.5 ± 0.0	20.0 ± 0.2	22.5 ± 0.2	2.9 ± 0.0	21.1 ± 0.2	24.0 ± 0.2
0	2.0 ± 0.0	20.7 ± 0.3	22.7 ± 0.3	2.5 ± 0.0	20.0 ± 0.1	22.5 ± 0.1	2.9 ± 0.0	21.1 ± 0.2	24.0 ± 0.2
1	2.0 ± 0.0	21.6 ± 0.1	23.6 ± 0.1	2.8 ± 0.0	20.0 ± 0.1	22.8 ± 0.1	3.8 ± 0.0	21.1 ± 0.2	24.9 ± 0.2
2	2.0 ± 0.0	21.8 ± 0.1	23.8 ± 0.2	3.4 ± 0.0	20.0 ± 0.2	23.4 ± 0.2	4.7 ± 0.0	21.2 ± 0.2	25.9 ± 0.2

^a Flowers were emasculated 1 day before anthesis.

strains used in these studies may have originated in these regions due to selection for autogamy under low-temperature conditions and may have evolved as self-pollinated strains. The strains used in our studies may have been procured from these regions by the dealer in horticultural plants from whom they were obtained by us. Alternatively, these strains could have been bred for automatic self-pollination to facilitate seed production and varietal maintenance. Seed

production in normal strains of periwinkle depends on pollinating butterflies which produce high levels of cross-pollination and whose prevalence is seasonal (Kulkarni 1999). Seed collection is further rendered difficult by seed shedding due to fruit shattering.

In *Amsinckia*, all species are self-compatible and distyly is controlled by a single locus with two alleles, *S* and *s* (Ganders 1975). However, according to Richards (1997), heterostyly in

Table 3. Mean (± standard error) values of some floral traits of three phenotypic classes of the F₂ generation of OR × MJ and OR × VI of periwinkle

Trait	Phenotypic classes		
	F ₂ (O type) ^a	F ₂ (NS type)	F ₂ ('S' type)
Gynoecium, style and ovary lengths (mm) on the day of anthesis			
Gynoecium	24.7 ± 0.1	24.4 ± 0.2	23.4 ± 0.1
Style	21.8 ± 0.1	21.8 ± 0.2	21.4 ± 0.1
Ovary	2.9 ± 0.0	2.6 ± 0.0	2.0 ± 0.0
Height of anther base (mm) in corolla tube on the day of anthesis			
	23.8 ± 0.1	23.7 ± 0.2	22.7 ± 0.1
Height of stigma (mm) above the anther base on the day of anthesis			
	0.9 ± 0.0	0.7 ± 0.1	0.7 ± 0.1
Mean gynoecium, style and ovary lengths (mm) on the day of self-pollination ^b			
Gynoecium	26.1 ± 0.1 (1.4) ^c	24.6 ± 0.2 (0.2)	24.2 ± 0.1 (0.8)
Style	21.8 ± 0.1 (0.0)	21.8 ± 0.2 (0.0)	22.2 ± 0.1 (0.8)
Ovary	4.3 ± 0.0 (1.4)	2.8 ± 0.0 (0.2)	2.0 ± 0.0 (0.0)
Mean height of anther base (mm) in the corolla tube on the day of self-pollination			
	23.9 ± 0.1	23.9 ± 0.2	22.7 ± 0.2
Mean height of the stigma (mm) above anther base on the day of self-pollination			
	2.2 ± 0.1 (1.3)	0.7 ± 0.1 (0.0)	1.5 ± 0.1 (0.8)

^a O type = plants exhibiting self-pollination because of an increase in gynoecium length occurring because of an increase in ovary length. S type = plants exhibiting self-pollination because of an increase in gynoecium length occurring because of an increase in style length. NS type = non-self-pollinating plants.

^b Observations on non-self-pollinating plants were recorded 2 days after anthesis, as self-pollination generally occurred 2 days after anthesis in parental strains and in O- and S-type self-pollinating plants of the F₂ generation.

^c Values in parentheses show increases in length/height as compared with their respective values on the day of anthesis.

Table 4. Segregation in the F₂ and backcross generations of crosses between self-pollinating strains, MJ and VI, both with O-type mechanism of self-pollination, and strain OR, with the S-type mechanism of self-pollination

Generations	Number of plants Phenotype			Total	Expected ratio	$\sum\chi^2$	P
	O ^o type	NS type	S type				
F ₂							
OR × MJ	132	73	14	219	9:6:1	1.642	.440
OR × VI	37	34	3	74	9:6:1	2.446	.294
Total	169	107	17	293	9:6:1	0.275	.871
Backcross							
(OR × MJ) × OR	8	14	10	32	1:2:1	0.750	.687
(OR × VI) × OR	6	8	4	18	1:2:1	0.666	.717
Total	14	22	14	50	1:2:1	0.720	.698

^a O type = plants exhibiting self-pollination because of an increase in gynoecium length occurring because of an increase in ovary length. S type = plants exhibiting self-pollination because of an increase in gynoecium length occurring because of an increase in the style length. NS type = non-self-pollinating plants.

Amsinckia is probably under polygenic control. In *Mimulus ringens* L., a bumblebee-pollinated perennial herb with a mixed mating system, outcrossing rate and anther-stigma separation (the difference between pistil length and stamen height) were found to be positively correlated (0.68) with broad-sense heritabilities of 0.37 and 0.88, respectively (Karron et al. 1997). Similarly, anther-stigma separation and autogamy in a population of *Mimulus guttatus* DC had broad-sense heritabilities of 0.72 and 0.45, respectively; however, the correlation between these traits was low and nonsignificant (Carr and Fenster 1994). Tristyly (a heteromorphic heterostylus condition in which short-styled, mid-styled, and long-styled morphs with reciprocal anther positions coexist) in *Lytbrum junceum* Bank and Sol., is controlled by epistatic interaction between two independent genes *L* and *M*, each with two alleles (Frankel and Galun 1977). In *Primula* L., an extensively studied heterostyly system, the heterostyly supergene *S* consists of six closely linked loci—*G*, *S*, *I*, *Ip*, *P*, and *A*—that control style length, stigmatic surface, stylar incompatibility, pollen incompatibility, pollen size, and anther height, respectively (de Nettancourt 2001). It is responsible for only about 0.005 of the length of the *S* chromosome, and therefore recombinants within the supergene appear to be sufficiently unusual (Richards 1997).

Moore and Lewis (1965) reported that an increase in style length and the timing of stigma maturation differentiated selfers from nonselfers in *Clarkia xantiana* Gray. Early maturation of the stigma, such that it is receptive when it is level with the dehiscing anthers, brought about self-pollination in self-pollinating types. Segregation for selfing and nonselfing in the F₂ generation of crosses involving selfers and nonselfers showed a close fit to a monohybrid ratio, with selfing being recessive to nonselfing. However, style length and time of stigma maturation showed continuous variation without separation into any discrete classes. In periwinkle, autogamy brought about an increase in the length of the gynoecium in self-pollinating strains was found to be controlled by duplicate genes, which were recessive to the wild type (i.e., nonautogamy) (Kulkarni et al. 2001). The results of the present study revealed that two different alleles at each of these two loci controlled style and ovary length,

and alleles governing ovary growth were dominant over those governing style growth. Thus three alleles at two loci appear to control autogamy/allogamy in periwinkle. Gene symbols SP₁, SP₂, sp^o₁, sp^o₂, sp^s₁, and sp^s₂ are proposed for genes governing the absence of self-pollination (SP₁, SP₂), self-pollination brought about by increase in ovary length (sp^o₁, sp^o₂), and style length (sp^s₁ and sp^s₂), respectively. Although a vast literature exists on allelic genes governing self-incompatibility/compatibility in plants (Frankel and Galun 1977; de Nettancourt 2001), there seems to be no report on similar alleles promoting self-pollination in cross-pollinating plant species.

Measurements made of ovary, style, and gynoecium lengths of F₁ and three groups of F₂ plants (namely O type, non-self-pollinating, and S type) revealed that an increase in ovary length was responsible for an increase in the gynoecium length in the F₁ and the O-type plants of the F₂ generation, while an increase in style length was responsible for an increase in gynoecium length in the S-type plants of the F₂ generation. There was no increase in gynoecium length after anthesis in the non-self-pollinating group of plants of the F₂ generation. The allelic relationship between genes governing ovary and style growth, and that self-pollination was governed by duplicate genes, was also evident from the expression of one of the parental phenotypes (self-pollination occurring because of an increase in the length of the ovary) in the F₁ generation and 9:6:1 and 1:2:1 ratios observed in the F₂ and test-cross generations, respectively.

In *C. xantiana* Gray, automatic self-pollination was found to be controlled by a single recessive gene. However, self-pollinating plants differed from non-self-pollinating plants for two seemingly different traits, shorter style and early maturing stigma. Since only a single gene differentiated selfers from nonselfers, Moore and Lewis (1965) proposed that the same gene may determine both stigma maturation and style elongation since they are developmentally related. However, in the present study, gynoecium length continued to increase until pollination, although the stigma was receptive 1 day before anthesis. Thus a single trait, an increase in gynoecium length, was governed by two genes. The self-pollinating strains of periwinkle, MJ and VI, on the

one hand, and OR, on the other, may have originated from non-self-pollinating strains through independent mutations at different sites in these two genes, leading to self-pollination occurring because of an increase in the length of the ovary and style, respectively.

According to Stebbins (1957), self-pollinating races or species occur widely throughout the angiosperms, and there seems little doubt that they have been derived from outcrossing relatives. Self-pollinating plants of periwinkle have, however, not become common in nature, despite their reproductive advantage, due to nondependence on pollinators as compared with normal non-self-pollinating plants. In the studied self-pollinating strains, anther dehiscence and self-pollination occurred only 1 to 2 days after anthesis (Kulkarni et al. 2001). Therefore intraflower self-pollination occurring in these strains does not completely exclude outcrossing in the presence of pollinators leading to varying levels of cross-pollination. Genetic control of self-pollination by duplicate, recessive genes and the need for self-pollination would further reduce the frequency of self-pollinating plants in the progeny of self-pollinating plants. The self-pollinating strains were found to be highly susceptible to dieback, a devastating disease of periwinkle in the rainy season (Kulkarni and Baskaran 2003; Kulkarni and Ravindra 1988). Self-pollinating plants can be identified only in the absence of pollinators. All these reasons seem to explain why self-pollinating plants have not been observed commonly in natural populations. However, genetic control of the kind of pollination (self or cross) in periwinkle provides opportunities for the development of both pureline and hybrid cultivars, depending on the nature of the gene action governing the traits of interest.

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Corresponding Editor: J. Perry Gustafson