

Review

Heredity of flake- and stripe-variegated traits and their introduction into Japanese day-neutral winter-flowering sweet pea (*Lathyrus odoratus* L.) cultivars

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Sweet pea (*Lathyrus odoratus* L.) is a major cut flower in Japan, generally grown in greenhouses in winter to spring. The wild-type sweet pea is a long-day summer-flowering plant. The day-neutral winter-flowering ability, which allows cut-flower production in Japan, is a recessive phenotype that emerged by spontaneous mutation. Although Japanese winter-flowering cultivars and additionally spring-flowering cultivars, which have semi-long-day flowering ability generated by crossing the winter- and summer-flowering cultivars, have superior phenotypes for cut flowers, they have limited variation in color and fragrance. In particular, variegated phenotypes do not appear in modern winter- and spring-flowering cultivars, only in summer-flowering cultivars. We try to expand the phenotypic diversity of Japanese cut flower cultivars. In the processes, we introduced the variegated phenotypes by crossing with summer-flowering cultivars, and succeeded in breeding some excellent cultivars. During breeding, we analyzed the segregation ratios and revealed the heredity of the phenotypes. Here we review the heredity of these variegated phenotypes and winter-flowering phenotypes and their related genes. We also describe how we introduced the trait into winter-flowering cultivars, tracing their pedigrees to show both phenotypes and genotypes of the progeny at each generation. This knowledge is useful for the efficient breeding of new variegated cultivars.

Key Words: breeding, cut flower, epistatic suppression, segregation ratio, two-locus control.

Production of sweet pea

Sweet pea (*Lathyrus odoratus* L.; Fabaceae) is an annual climbing plant that originated in Sicily, Italy. Today, sweet pea is a favorite ornamental plant worldwide owing to its wide range of petal colors and sweet fragrance. It is favored as both a garden plant and a cut flower. Sweet peas for cut flower usage are produced mainly in Japan (with the greatest production amounts), the Netherlands, the UK, and Australia (Inoue *et al.* 2000, Inoue 2007, Parsons 2011, Rice 2002). Sweet pea accounts for 1.47% of all ornamental plants in trading volume and ranks 11th among cut flowers in trading value in Japan in 2012 (Japan Flower Promotion Center Foundation, personal communication). Among cut flowers, whose supplies are occupied by domestic products in Japan, it ranks third in wholesale volume following *Gerbera* and *Limonium* (MAFF 2010). It is presumed that

so far sweet pea ranks first in handling exports of cut flower from Japan and the quality is considered high around the world (Naniwa Flower Auction Co. Ltd., personal communication).

Flowering characteristics of Japanese cultivars

Sweet pea cultivars are classified as winter-flowering (day-neutral, early flowering), spring-flowering (semi-long-day, mid flowering), or summer-flowering (long-day, late flowering, requiring >15 h day-length). Flowering of all types is promoted by cold storage of germinated seeds (Ross and Murfet 1985). It is thought that summer-flowering is original, winter-flowering is a recessive phenotype that emerged by spontaneous mutation, and spring-flowering resulted from crossing of winter- and summer-flowering plants (Little and Kantor 1941).

The summer-flowering cultivars are popular in the USA, UK and other Europe area, Australia, and New Zealand for garden planting, and cultivars with a wide range of flower color and fragrance have been bred. In contrast, the winter- and spring-flowering cultivars are grown for cut flower

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usage in Japan. Winter in some parts of Japan is suitable for their production, being warm enough and with enough day-length and daylight.

Unlike the summer-flowering cultivars, the Japanese winter- and spring-flowering cultivars have numerous flowers per peduncle when grown in greenhouses in winter season, making them suitable as cut flowers (Inoue 2007, Inoue *et al.* 2001b). Their long, thick peduncles are an additional superior trait for cut-flower usage. However, they have less variation in flower color and fragrance than summer-flowering cultivars (Inoue *et al.* 2001a). Frequent crossing among winter- and spring-flowering cultivars (Doi and Kohno 1996, Nakamura *et al.* 2006a, 2006b, Yagishita and Yamamoto 2004, Yamamoto 1994) may explain the lesser flower color and fragrance variation of these cultivars, and prompted us to try crossing with summer-flowering cultivars to generate winter- and spring-flowering cultivars with new these traits.

Reason for analysis of heredity of variegated phenotypes

Variegated sweet pea flowers show either the flake-variegated phenotype, in which narrow streaks appear on both sides of the petals (Fig. 1A), or the stripe-variegated phenotype, in which small spots appear only on the abaxial side of petals with a colored edge (Fig. 1B). Both phenotypes occur in summer-flowering cultivars. Among winter-flowering cultivars, some variegated forms were recorded by Beal (1912), and a flake-variegated cultivar, ‘Cristina’, was listed in the Sakata Seed catalog from 1999 until 2004 (Sakata Seed Co., Yokohama, Japan). Even so, winter-flowering cultivars with these variegated traits are rare, and it seems difficult to find such modern cut flower cultivars in Japan.

To expand the diversity of Japanese cut flower cultivars, we introduced these phenotypes into winter- and spring-flowering cultivars by cross-breeding with summer-flowering cultivars, and succeeded in breeding some excellent cultivars. In this review, we discuss the heredity of the variegated phenotypes and their related genes in the breeding processes based on the segregation ratios in our breeding process. We also describe how we introduced the trait into the winter-flowering cultivars, showing both phenotypes and genotypes of progeny at each generation. We

expect that this knowledge will improve the efficiency of breeding by enabling planning of population sizes for selection of the phenotypes and prediction of generation times for fixation of the traits.

Basic information for genetic analysis of sweet pea

It is thought that for a century after the discovery of sweet pea in Sicily by Francesco Cupani in 1695, new forms were derived mainly from spontaneous mutations. Serious cross-breeding began in the 1870s in Europe, in the 1890s in California, USA, and in the 1980s in Japan (Inoue 2007, Inoue *et al.* 2000, Rice 2002).

Reginald Punnett, a British geneticist, elucidated the heredity of some sweet pea phenotypes, including pollen form, flower color, and variegation, during the 1920s to the 1940s (Punnett 1923, 1936, 1940). We use Punnett’s genetic model to discuss the heredity of our target phenotypes in modern cultivars. As sweet pea is autogamous, we presumed that the genotypes of all fixed cultivars and lines are homozygous.

The white phenotype is determined by complementary genes (Bateson *et al.* 1904). Punnett (1923) designated the alleles “*R*-white” and “*C*-white”, which are recessive alleles of the *R* and *C* loci, respectively. In this review, we designate them “*r*” and “*c*”. Plants homozygous for either *r* or *c* are white; otherwise, they are colored (Punnett 1923). If two white parents generate colored F₁ progeny, the parents’ genotypes are *ccRR* and *CCrr*. We observed this outcome in our studies using Japanese cultivars (Table 1). The segregation ratio of F₂ plants generated by inbreeding of colored F₁ plants generated by crossing of ‘Robe Décolleté’ and ‘Diana White’ was self-colored: white = 87:65, not significantly different from the theoretical segregation ratio of 9:7 (χ^2

Table 1. Flower color phenotypes of F₁ plants obtained by crossing of two white parents

Cross combination	Self-colored	White
Easter Parade × Diana White	8	0
Diana White × Robe Décolleté	64	0
Robe Décolleté × Diana White	52	0
Diana White × Shirayuki-hime	0	14
White Queen × Diana White	12	0
Robe Décolleté × Easter Parade	0	6

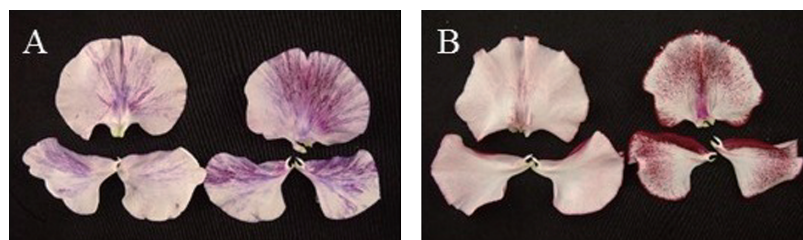


Fig. 1. Flower-variegation phenotypes of sweet pea. (A) Flake-variegated petals. (B) Stripe-variegated petals. In each photograph, the flower on the left presents the adaxial side of the petals, and that on the right presents the abaxial side.

= 0.060, $P = 0.8063$). These results indicate the occurrence of both dominant and recessive alleles at the C and R loci in Japanese cultivars.

As described in the next section, a semi-dominant allele of the C locus, called G_1 , is involved in the expression of the flake-variegated phenotype. We identified the genotypes of the C and R loci of ‘Diana White’ as $ccRR$. Those of ‘Easter Parade’, ‘Robe Décolleté’, and ‘White Queen’ were deduced to be $CCrr$ from results that the phenotype of F_1 progeny of their crosses with ‘Diana White’ was self-colored (Table 1). They were confirmed by the result that the phenotype of F_1 progeny of their crosses with ‘Robe Décolleté’ and ‘Easter Parade’ was white. The genotype of ‘Shirayukihime’ might be $ccRR$ or $ccrr$, which could be clarified based on phenotype of F_1 plants obtained by crossing with ‘Easter Parade’, ‘Robe Décolleté’, or ‘White Queen’.

Genotypes underlying flake-variegated phenotype

Punnett (1936) reported that the flake-variegated phenotype was mediated by alleles of a principal gene (G_1 , self-color; G_1' , flake-variegated; and g_1 , white) and a color density gene (D_3 , dense; d_3 , sparse). The flake-variegated phenotype is controlled by these two loci, with allelic dominance relationships of $G_1 > G_1' > g_1$ and $D_3 > d_3$. The self-colored phenotype has a genotype of $G_1_ _ _$ or $G_1'D_3D_3$, the white phenotype has $g_1g_1_ _ _$, and the flake-variegated phenotype has $G_1'G_1'd_3$ or $G_1'g_1d_3$ (Fig. 2). Punnett (1940) identified a fourth allele, G_1'' , encoding the flake-variegated phenotype as $G_1'G_1''_ _ _$ or $G_1''G_1''_ _ _$. In Punnett’s hypothesis, g_1 is identical to c/C -white.

The modern winter- and spring-flowering cultivars have self-colored or white phenotypes; the flake-variegated phenotype seems not to arise by crossing among them. We supposed that both spring- and winter-flowering cultivars lack the same gene concerned with expression of the phenotype, so that we used both cultivars to analyze genotype underly-

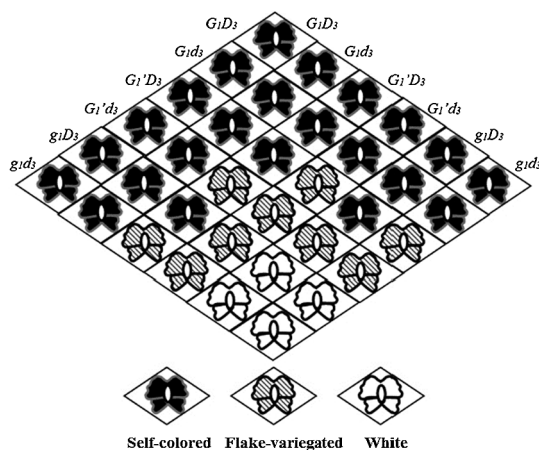


Fig. 2. Relationship between genotype and flake-variegated phenotype based on Punnett’s hypothesis. G_1 , G_1' , and g_1 alleles underlie the flake-variegated phenotype. D_3 and d_3 alleles modify color density.

ing the phenotype. At first, we eliminated the possibility of the occurrence of G_1'' in them, and implies that those cultivars lack G_1' and/or d_3 as well.

All flake-variegated plants used for crossing in our breeding were ‘America’ or its descendants. Punnett (1936) classified types of flake variegation density as light, medium, or dark, and later he added a type of very light (Punnett, 1940). We referred pictures and description about the classification in these reports and classified ‘America’ as light or very light type cultivar. Density is determined by the D_3 allele: the light and very light traits are expressed in a d_3d_3 homozygous plant. Therefore, the genotypes of ‘America’ or its descendants must be $G_1'G_1'd_3d_3$ or $G_1''G_1''d_3d_3$.

All F_1 plants obtained by crossing of spring-flowering self-colored ‘Royal Crimson’, ‘Royal Salmon’, or ‘Royal Navy Blue’ with flake-variegated ‘St-Pink’ or ‘Splash Red’, which are descendants of ‘America’ (Fig. 5), had the flake-variegated phenotype (Table 2), so their genotype was $G_1'G_1'd_3$, $G_1'G_1''d_3$, or $G_1''G_1''d_3$. Because the parental self-colored plants lack G_1'' , the genotype of the F_1 plants is $G_1'G_1'd_3$ or $G_1'G_1''d_3$. This implies that self-colored ‘Royal Crimson’, ‘Royal Salmon’, and ‘Royal Navy Blue’ have G_1' , and their genotype must be $G_1'G_1D_3D_3$, confirming that the modern cut flower cultivars lack d .

All F_1 plants obtained by crossing of winter-flowering self-colored ‘Shonan Orion’ with the flake-variegated ‘03-34’, which is a descendant of ‘America’ assigned as (i) in Fig. 5, had the self-colored phenotype (Table 2). Because the parental self-colored plants lack d_3 and G_1'' , the genotype of the F_1 plants was $G_1G_1D_3d_3$ or $G_1G_1''D_3d_3$. This implies that self-colored ‘Shonan Orion’ has G_1 , and the genotype must be $G_1G_1D_3D_3$. We conclude that winter- and spring-flowering cultivars have G_1 , G_1' , g_1 , and D_3 but not G_1'' or d_3 . As sweet pea is autogamous, the genotype of winter- and spring-flowering cultivars is $G_1G_1D_3D_3$, $G_1'G_1'D_3D_3$, or $g_1g_1D_3D_3$.

F_2 plants obtained by selfing the flake-variegated F_1 plants segregated into self-colored and flake-variegated phenotypes (Table 2). The segregation ratio of the F_2 progeny was not significantly different from the theoretical ratio supposing that the genotype of ‘America’ was $G_1'G_1'd_3d_3$. Therefore, the genotype of ‘America’ is $G_1'G_1'd_3d_3$. We clarified that the introduction of d_3 is essential for the introduction of the flake-variegated phenotype into the winter- and spring-flowering cultivars from ‘America’ and its descendants.

In crossing of white cultivars with flake-variegated cultivars also, the F_1 phenotype depends on the white parent. If the genotype of the white parent was $g_1g_1D_3D_3RR$ (ccD_3D_3RR), that of all F_1 progeny was $G_1'g_1D_3d_3RR$ and the phenotype was flake-variegated, as seen in crossing between ‘Diana White’ and ‘America’ (Table 3). If the genotype of the white parent was $G_1G_1D_3D_3rr$ (CCD_3D_3rr), that of all F_1 progeny was $G_1G_1'D_3d_3Rr$ and the phenotype was self-colored, as seen in crossing between ‘Easter Parade’ and ‘America’. In both cases, actual segregation ratios of the F_2 population did not differ significantly from the theoretical

Table 2. Segregation in F₁ and F₂ plants of self-colored × flake-variegated cultivars

Cross combination	Generation	Phenotype		Theoretical segregation ratio		χ^2	<i>P</i>
		Self-colored	Flake-variegated	Self-colored	Flake-variegated		
Shonan Orion (Self-colored) × 03-34 ^c (Flake-variegated)	F ₁	4	0	1	0	2.053	0.1519
	F ₂	13	6	13 3	3 1		
St-Pink (Flake-variegated) × Royal Crimson (Self-colored)	F ₁	0	8	0	1	0.556	0.4561
	F ₂	5	10	1	15		
St-Pink (Flake-variegated) × Royal Salmon (Self-colored)	F ₁	0	5	0	1	3.000	0.0833
	F ₂	7	9	1	15		
Splash Red (Flake-variegated) × Royal Navy Blue (Self-colored)	F ₁	0	8	0	1	2.000	0.1573
	F ₂	1	6	1	3		

^a The genotype of flake-variegated parent is assumed to be $G_1'G_1'd_3d_3$.

^b The genotype of flake-variegated parent is assumed to be $G_1''G_1''d_3d_3$.

^c 03-34 is a descendant of ‘America’ assigned as (i) in Fig. 5.

Table 3. Segregation in F₁ and F₂ plants of white cultivars × flake-variegated ‘America’

Cross combination	Generation	Phenotype			Theoretical segregation ratio			χ^2	<i>P</i>
		Self-colored	White	Flake-variegated	Self-colored	White	Flake-variegated		
Diana White × America	F ₁	0	0	4	0	0	1	0.296	0.8623
	F ₂	1	1	4	3	4	9		
Easter Parade × America	F ₁	10	0	0	1	0	0	2.917	0.2325
	F ₂	20	9	1	39	16	9		

ratio, but we could not obtain enough plants, so additional analysis is needed. We presume the additional occurrence of white cultivars whose genotype is $G_1'G_1'D_3D_3rr$, and which, when crossed with flake-variegated cultivars, produce F₁ progeny with the $G_1'G_1'D_3d_3Rr$ genotype and the flake-variegated phenotype.

Genotypes underlying stripe-variegated phenotype

Little information about the heredity of the stripe-variegated phenotype had been published before our report (Yagishita *et al.* 2013). All F₁ plants obtained by crossing of self-colored cultivars with stripe-variegated cultivars had the self-colored phenotype. F₂ progeny of the F₁ plants were segregated into self-colored and stripe-variegated phenotypes at a ratio of *ca.* 3:1. These results indicate that the stripe-variegated phenotype is regulated by a single recessive gene, which we named *v*. This phenotype has never been found in winter- and spring-flowering cultivars, so their genotype is *VV*.

We supposed that expression of the stripe-variegated phenotype is epistatically suppressed by the white phenotype, and that the *C* and *R* loci equally and recessively regulate expression of the white phenotype; that is, plants with the *cc*__ or __*rr* genotype are white regardless of the genotype of the *V* locus (Yagishita *et al.* 2013). Under that sup-

position, the genotype of stripe-variegated cultivars is *RRCCvv*, that of self-colored cultivars is *RRCCVV*, and that of white cultivars is *RRccVV* or *rrCCVV*. Therefore, all F₁ plants obtained by crossing of white cultivars with stripe-variegated cultivars would be self-colored, and the theoretical segregation ratio of F₂ progeny is self-colored:white:stripe-variegated = 9:4:3, where each case that genotype of white cultivar is *RRccVV* or *rrCCVV* shows equivalent relationship (Fig. 3). Actual segregation ratios did not differ significantly from the theoretical ratio (Table 4). Therefore, we conclude that the stripe-variegated phenotype is regulated by a single recessive allele, *v*, and expression of this phenotype is epistatically and recessively suppressed by either of the *C* and *R* loci.

Heredity of winter-flowering phenotype

Ross and Murfet (1985) designated the alleles underlying flowering habit as *Dn^h* (summer-flowering) and *dn* (winter-flowering). We verified the heredity of the winter-flowering phenotype of current Japanese cultivars on this basis.

All F₁ plants obtained by crossing of summer-flowering cultivars with winter-flowering cultivars were summer-flowering (Yagishita *et al.* 2013). The flowering phenotypes of the F₂ population were estimated from the position of the flower initiation node: flowering at a low node indicated

Breeding variegated sweet pea

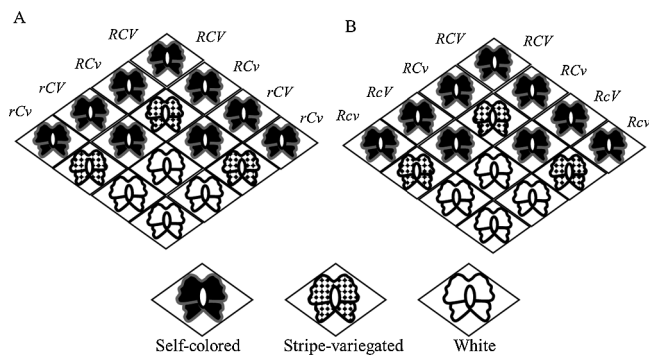


Fig. 3. Relationship between genotype and stripe-variegated phenotype. *V* locus regulates spray-variegated expression. *C* and *R* loci regulate pigmentation. Figure A presents a case where pigmentation is regulated by the *R* locus and both parents are *CC* and Figure B presents *vice versa*. Each case shows equivalent relationship.

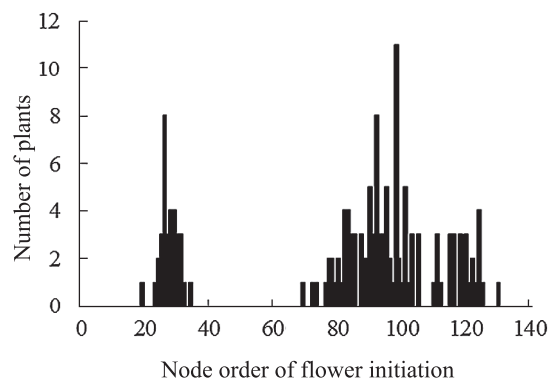


Fig. 4. Frequency distribution of the position of the flower initiation node of F_2 plants derived from a cross of winter-flowering line '99-41-8' \times summer-flowering cultivar 'Maggie May' ($n = 174$). Seeds were sown on 8 August 2001. There were 34 plants with a low node (20–35), 117 with a high node (70–131), and 23 unclear.

Table 4. Segregation in F_2 plants of white 'Easter Parade' \times stripe-variegated 'Lilac Ripple' (Yagishita *et al.* 2013)

Number of plants	Flowering habit	Phenotype			Theoretical segregation ratio			χ^2	<i>P</i>	
		Subtotal	Self-colored	White	Stripe-variegated	Self-colored	White			Stripe-variegated
104	Summer-flowering	79	43	19	17	27	12	9	2.279	0.809
	Winter-flowering	25	11	9	5	9	4	3		

winter-flowering and flowering at a high node indicated summer-flowering. The segregation ratio of the F_2 population was summer-flowering:winter-flowering = 3:1 (Fig. 4). This confirms that the winter-flowering phenotype of current Japanese cultivars is regulated by a single recessive gene, *dn*.

We analyzed the F_2 populations obtained by crossing of stripe-variegated summer-flowering cultivars with white winter-flowering cultivars (Yagishita *et al.* 2013). The segregation ratios of flowering phenotype corresponded to the theoretical ratio. Moreover, in each flowering phenotype group, the actual segregation ratio of self-colored:white:stripe-variegated phenotypes corresponded to the theoretical ratio. Our data therefore indicate that the flowering phenotype and the stripe-variegated phenotype are independently inherited (Table 4).

Breeding of winter-flowering flake-variegated cultivars

For breeding winter-flowering flake-variegated cultivars, the *dn**dnRRG*₁'*G*₁'*d*₃*d*₃ genotype must be fixed by the following steps: (1) Introduction of *d*₃ by crossing with summer-flowering flake-variegated cultivars. (2) Exclusion of *G*₁ by selecting flake-variegated phenotype. (3) Exclusion of *g*₁/*c* and *r* (white) by confirming that all inbred plants are not white. (4) Exclusion of *D*₃ by confirming that all inbred plants are flake-variegated. (5) Exclusion of *Dn*^{*h*} by selecting winter-flowering phenotype. These steps fix the *RRG*₁'*G*₁'*d*₃*d*₃ genotype in the generation in which all inbred

plants have a flake-variegated phenotype, and the *dn**dn* genotype in the generation with a winter-flowering phenotype.

In our actual breeding, *G*₁'₁, *d*₃, *R*, and *Dn*^{*h*} were introduced into the winter-flowering white 'Easter Parade', whose genotype is *dn**dnrrG*₁'*G*₁'*D*₃*D*₃, by crossing with the summer-flowering flake-variegated 'America', whose genotype is *Dn*^{*h*}*Dn*^{*h*}*RRG*₁'*G*₁'*d*₃*d*₃ (Fig. 5). All F_1 plants (*a*) were summer-flowering and self-colored, with the *Dn*^{*h*}*dnRrG*₁'*G*₁'*D*₃*d*₃ genotype. In the F_2 population (*b–d*), flowering habit and flower color were segregated. All F_3 plants (*e, f*) obtained by selfing F_2 (*c*), which is summer-flowering and self-colored, were flake-variegated (*e*) or self-colored (*f*), but not white. This indicates that *r* was excluded in the F_2 generation (*c*). All F_4 plants (*g*) obtained by selfing F_3 (*e*) were winter-flowering and flake-variegated. This indicates that *G*₁, *D*₃, and *Dn*^{*h*} were excluded, and the genotype was possibly fixed as *dn**dnRRG*₁'*G*₁'*d*₃*d*₃ at the F_3 generation (*e*).

To improve properties for cut flower usage and to diversify the coloration of the variegation, we crossed F_3 (*e*) with winter-flowering self-colored 'Diana', whose genotype is *dn**dnRRG*₁'*G*₁'*D*₃*D*₃. We then inbred the descendants (Fig. 5). All F_3 plants (*i*) obtained by selfing F_2 (*h*) were winter-flowering and flake-variegated. This indicates that genotype was possibly fixed as *dn**dnRRG*₁'*G*₁'*d*₃*d*₃ at the F_2 generation (*h*). We continued fixing the color and properties for cut flower usage, and selected 'Splash Red' (Fig. 6A) and 'St-Pink' from inbred descendants of F_2 plant (*h*).

We also crossed a F_4 plant (*i*) fixed as *dn**dnRRG*₁'*G*₁'*d*₃*d*₃

with winter-flowering self-colored ‘Shonan Orion’, whose genotype is $dndnRRG_1G_1D_3D_3$ (Fig. 5). We inbred the descendants and all F_4 plants (k) were winter-flowering and flake-variegated. This indicates that genotype was fixed as

$dndnRRG_1'G_1'd_3d_3$ at the F_3 plant (j). We selected two winter-flowering flake-variegated cultivars, ‘Splash Blue’ and ‘Splash Purple’ (Fig. 6B, 6C), from inbred descendants of (j).

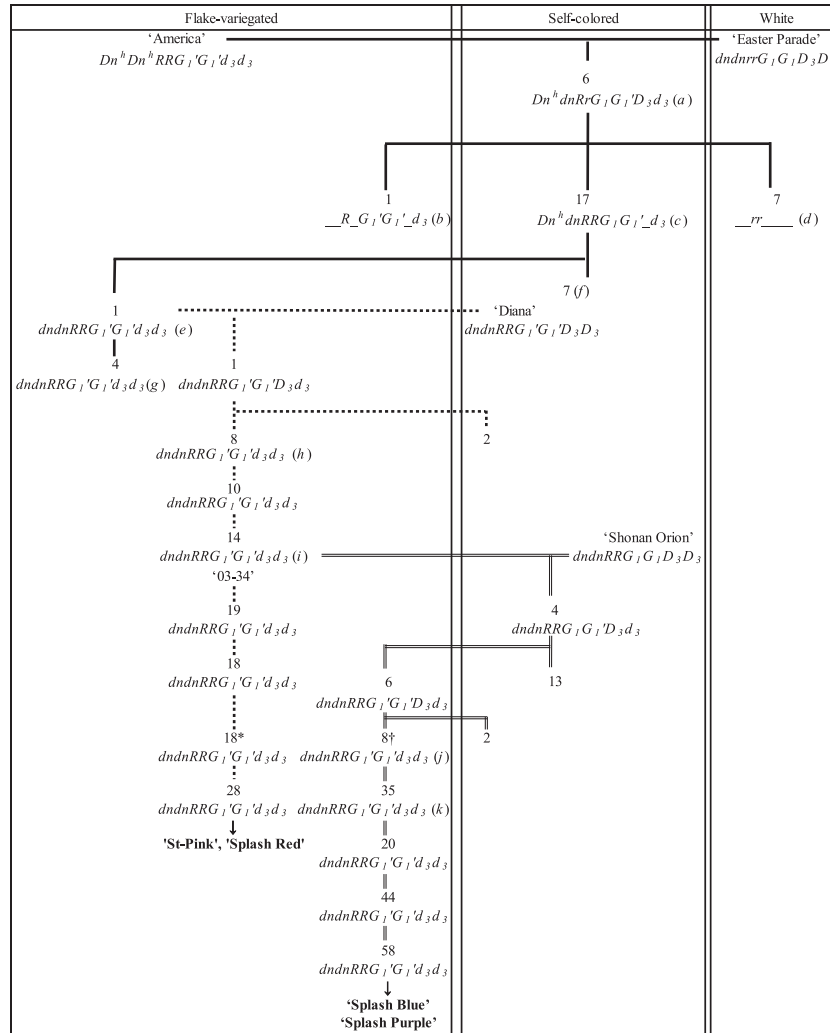


Fig. 5. Pedigree of winter-flowering flake-variegated cultivars bred in our study leading to ‘Splash Red’, ‘Splash Blue’ and ‘Splash Purple’. Numbers of plants in each generation are presented. Generations marked by letters are discussed in the text. Genotypes were assumed from phenotypes in the current generation or segregation of phenotypes in the next generation. Phenotypes coincided with genotypes of all plants except one indicated by *, where one self-colored plant appeared, we presume owing to a spontaneous mutation. †: Among eight plants, only one plant was used for self-crossing.



Fig. 6. Winter-flowering flake-variegated cultivars bred at the Kanagawa Agricultural Technology Center: (A) ‘Splash Red’, (B) ‘Splash Blue’, (C) ‘Splash Purple’.

Breeding of winter-flowering stripe-variegated cultivars

For breeding winter-flowering stripe-variegated cultivars, the *dndnRRCCvv* genotype must be fixed by the following steps: (1) Introduction of *v* and *R* and/or *C* by crossing with

stripe-variegated cultivars. (2) Exclusion of *V* by selecting stripe-variegated phenotype. (3) Exclusion of *r* and/or *c* by confirming that all inbred plants are not white. (4) Exclusion of *Dn^h* by selecting winter-flowering phenotype. These steps fix the *RRCCvv* genotype in the generation in which all inbred plants have a stripe-variegated phenotype, and the

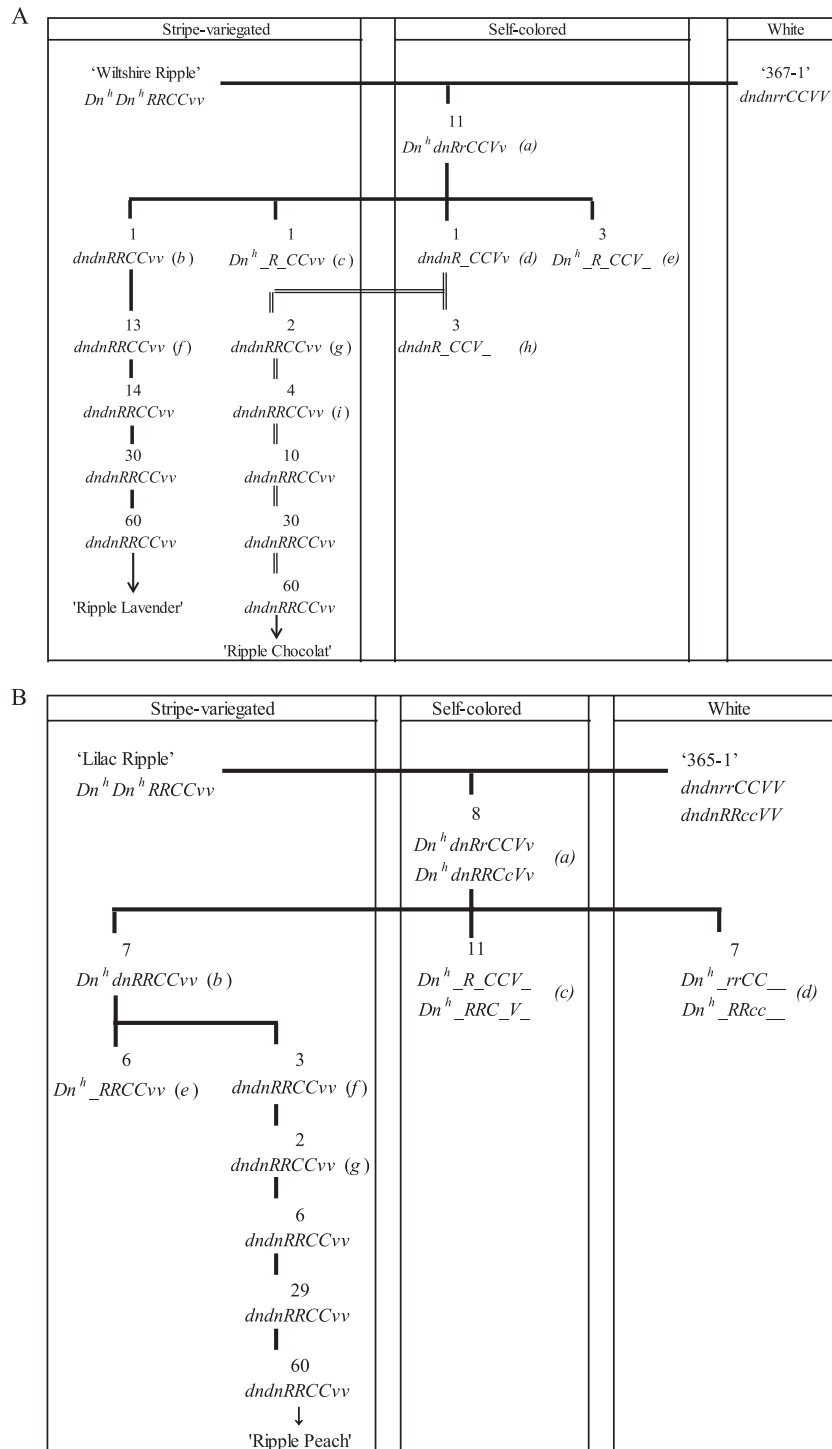


Fig. 7. Pedigree of winter-flowering stripe-variegated cultivars bred in our study leading to 'Ripple Lavender' and 'Ripple Chocolat' (A) and to 'Ripple Peach' (B). Numbers of plants in each generation are presented. Generations marked by letters are discussed in the text. Genotypes were assumed from phenotypes in the current generation or segregation of phenotypes in the next generation.



Fig. 8. Winter-flowering stripe-variegated cultivars bred at the Kanagawa Agricultural Technology Center: (A) ‘Ripple Lavender’, (B) ‘Ripple Chocolat’, (C) ‘Ripple Peach’.

dndn genotype in the generation with a winter-flowering phenotype.

In our actual breeding, *v*, *R* or *C*, and *Dn^h* were introduced into the winter-flowering white ‘367-1, whose genotype is *dndnrrCCVV*, by crossing with the summer-flowering stripe-variegated ‘Wiltshire Ripple’, whose genotype is *Dn^hDn^hRRCCvv*; ‘367-1’ is a F₃ plant obtained by crossing of white ‘Easter Parade’ and self-colored ‘Diana’, so that its genotypes of *R* and *C* loci must be *rrCC* being the same as those of ‘Easter Parade’ (Fig. 7A). All F₁ plants (*a*) were summer-flowering and self-colored, with the *Dn^hdnRrCCVv* genotype. In the F₂ population (*b–e*), flowering habit and flower pattern phenotypes were segregated. F₃ plants (*f*) obtained by selfing a winter-flowering stripe-variegated F₂ plant (*b*) were all winter flowering and stripe-variegated. This indicates that *r*, *V*, and *Dn^h* were excluded, and the genotype was fixed as *dndnRRCCvv* in the F₂ generation (*b*). The other F₃ plants (*g*, *h*) obtained by selfing a winter-flowering self-colored F₂ plant (*d*) were all winter-flowering, indicating that *Dn^h* was excluded, but they were segregated into stripe-variegated (*g*) and self-colored (*h*). All F₄ plants (*i*) obtained by selfing a stripe-variegated F₃ plant (*g*) were stripe-variegated. This indicates that *r* and *V* were excluded, and the genotype was fixed as *dndnRRCCvv* in the F₃ generation (*g*). After fixing the winter-flowering and stripe-variegation phenotypes, we continued fixing the color and properties for cut flower usage, and selected two winter-flowering stripe-variegated cultivars, ‘Ripple Lavender’ and ‘Ripple Chocolat’ (Fig. 8A, 8B), in inbred descendants of (*b*) and (*g*), respectively.

We also crossed the winter-flowering white ‘365-1’, which is a tall type line selected from mutants of a winter-flowering dwarf type cultivar ‘View’, with the summer-flowering stripe-variegated ‘Lilac Ripple’, whose genotype is *Dn^hDn^hRRCCvv* (Fig. 7B). The genotype of ‘365-1’ is *dndnrrCCVV* or *dndnRRccVV*. All F₁ plants (*a*) were summer-flowering and self-colored, with the *Dn^hdnRrCCVv* or *Dn^hdnRRcCvV* genotype. All F₂ plants (*b–d*) were summer-flowering, but they segregated into stripe-variegated (*b*), self-colored (*c*), and white (*d*). F₃ plants (*e*, *f*) obtained by selfing stripe-variegated F₂ (*b*) were all stripe-variegated. This indicates that *r* or *c* and *V* were excluded at the F₂ gen-

eration (*b*). F₃ plants segregated into summer-flowering (*e*) and winter-flowering (*f*), and F₄ plants (*g*) obtained by selfing (*f*) were winter-flowering and stripe-variegated. This indicates that *Dn^h* was excluded, and the genotype was fixed as *dndnRRCCvv* at the F₃ generation (*f*). We continued fixing the color and properties for cut flower usage and selected ‘Ripple Peach’ in inbred descendants of (*f*) (Fig. 8C).

Conclusion

By revealing the dominance, independence, and epistatic effects involved in the expression of the variegation phenotypes, we identified how to fix winter-flowering and variegated phenotype. Recessive phenotypes are fixed in the generation in which the phenotype appears, and dominant phenotypes are fixed in the generation when all inbred lines show them.

Sweet pea cultivation requires much labor and sparse planting density because of large climbing extent and indeterminate flowering properties of the plants (Inoue 2007). These factors limit the population size for breeding. Furthermore, sweet pea plants are prone to setting few seeds due to climate condition, plant form, etc. Therefore, efficient breeding based on planning of the optimum population size for selection and the number of crosses, which are calculated from the properties of the genes underlying the objective phenotype, is especially important. Our findings will contribute to sweet pea breeding.

Information on the heredity of many traits of sweet pea is scattered, because many cultivars have been bred by individual breeders. The information should be gathered by public organizations as it is important for the selection of breeding parents. We hope that accumulating and sharing information on the phenotypes and genotypes of cultivars will contribute to the construction of a more precise theory for breeding of sweet pea.

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