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# Floricultural Traits and Transposable Elements in Morning Glories

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## Introduction

The Japanese morning glory (*Ipomoea nil* or *Pharbitis nil*), displaying blue flowers, is believed to be originated from southeast Asia and has an extensive history of genetic and physiological studies (Hagiwara, 1956; Iida et al., 1999; Imai, 1927; 1938; Vince-Prue and Gressel, 1985). The plant had been introduced into Japan from China in about 8th century as a medicinal herb, seeds of which were utilized as a laxative, and has become a traditional horticultural plant in Japan since around 17th century. A number of its spontaneous mutants related to the colors and shapes of the flowers and leaves have been isolated, and about 10% of these mutants carry mutable alleles conferring variegated phenotypes. Several lines of evidence indicate that an *En/Spm*-related transposable element *Tpn1* and its relatives, which we termed *Tpn1*-family elements, are major contributors to these spontaneous mutations (Iida et al., 1999). Indeed, we have succeeded to identify three of these mutable alleles for flower pigmentation, *flecked, speckled* and *purple-mutable (pr-m)*, which are caused by integration of *Tpn1*- related elements, *Tpn1*, *Tpn2* and *Tpn4*, respectively (Fukada- Tanaka et al., 2000; Iida et al., 1999). In addition, apparent stable mutation conferring white flowers is also due to insertion of another *Tpn1*-related element, *Tpn3* (Hoshino et al., 2000).

The common morning glory (*Ipomoea purpurea* or *Pharbitis purpurea*) bearing dark purple flowers is originated from central America. The plant was introduced to Europe probably in the 17th century, and cultivars with red and white flowers were already recorded in the late 18th century (Curtis, 1790). We were able to identify that the mutable *flaked* allele for flower variegation is caused by integration of an *Ac/Ds*-related transposable element, *Tip100* (Habu et al., 1998; Hoshino et al., 2000; Iida et al., 1999). Here we review our findings that transposable elements belonging to the *Ac/Ds* and *En/Spm* families have acted as major spontaneous mutagens in the Japanese and common morning glories and discuss transposon- promoted spontaneous mutations with regard to the generation of floriculturally important traits in these plants. We also briefly describe our efforts to identify a mutable allele for flower variegation in the morning glory (*Ipomoea tricolor*).

## Spontaneous mutations

### 1. The Japanese morning glory (*I. nil*)

#### (a) The mutable allele flecked

The mutable *flecked* mutant bearing white flowers with colored flecks and sectors (Figure 1a) is thought to have been isolated in the early 18th century in Japan. It was described by Gen-nai Hiraga (1763) in his famous Japanese natural history book, *Butsurui-hinshitsu*, and several paintings and wood block prints of variegated flowers of the mutant were made in the middle of the 18th century. Genetic properties of the *flecked* allele were studied by Imai (1931; 1934). We have shown that the mutable *flecked* allele occurs at one of the genes encoding dihydroflavonol 4-reductase, *DFR-B*, for anthocyanin biosynthesis and that the 6.4 kb transposable element *Tpn1* is inserted into the second intron 9 bp upstream of the third exon of the *DFR-B* gene (Hoshino et al., 1995; Inagaki et al., 1994; 1996; 1999). *Tpn1* was found to carry genomic DNA segment containing at least four exons encoding the HMG-box sequence, and spliced hybrid transcripts containing the *DFR-B* exon(s) and the *HMG* exons within *Tpn1* were detected in the flower buds of the mutable *flecked* line (Takahashi et al., 1999). These results strongly indicated that *Tpn1* is a non-autonomous element and that the excision of *Tpn1* from the *DFR-B* gene must be mediated by transposases of *Tpn1*-related autonomous element (Iida et al., 1999). It is known that the frequency and the timing of the flecking phenotype tend to be heritable by their progeny, although conversion of these phenotypes is sometimes observed. It is likely that the frequency and the timing of the flecking phenotype are mainly dependent on the activity of the Tpn1-related autonomous element (Iida and Hoshino, 2000; Iida et al., 1999).

#### (b) The mutable allele speckled

The mutable *speckled* line bears colorless (or pale yellow) flowers with fine and round colored spots distributed over the corolla (Figure 1b). The mutant is believed to appear in the early

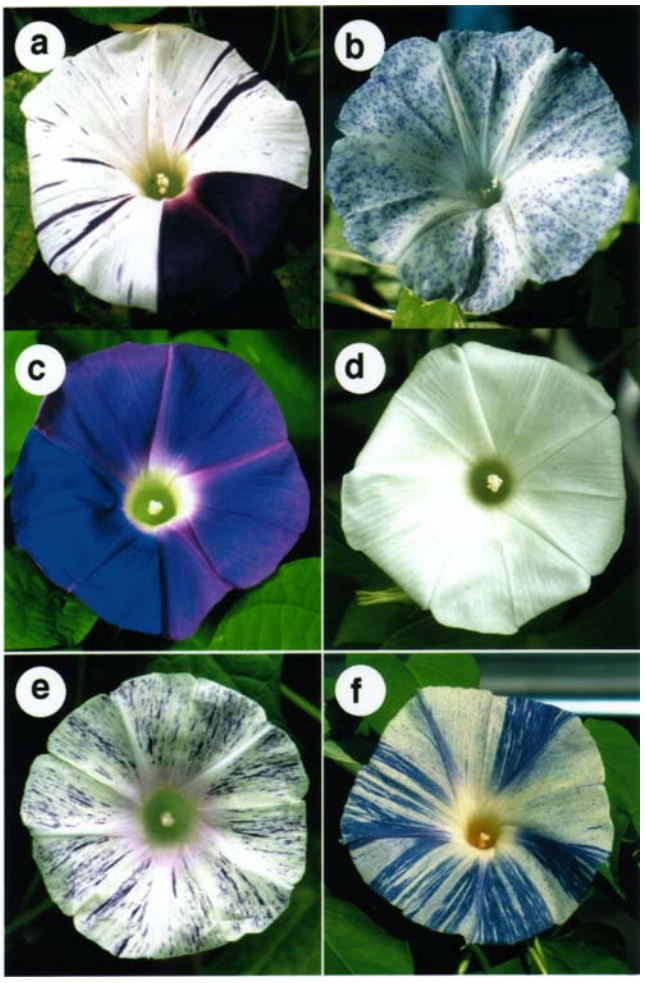


**Figure 1.** Flower variegation phenotypes.
(a) A *flecked* mutant in I. nil.
(b) A *speckled* mutant in I. nil.
(c) A *purple-mutable* mutant in I. nil.
(d) A white variant carrying the *flecked* allele in I. nil.
(e) A mutable *flaked* mutant in I. purpurea.
(f) A *maifs* mutant in I. tricolor.

19th century in Japan, and its genetic properties of the speckled mutation were studied by Imai (1931; 1934). Since the mutant occasionally produces flowers with colored sectors apparently due to somatic mutation, the mutable *speckled* allele is thought to be associated with a transposable element (Abe et al., 1997). We have shown that appearance of flower variegation in the *speckled* mutant was controlled not only by the recessive *speckled* allele but also by a dominant genetic element, *Speckled-activator*. We have further postulated that the putative transposable element in the *speckled* allele must be a non-autonomous element and that the dominant *Speckled-activator* be an autonomous element acting *in trans* on the non-autonomous element (Abe et al., 1997). Indeed, we have found that the *speckled* allele is the *CHI* gene for chalcone isomerase which contains a *Tpn1*-related element of 6.5 kb, termed *Tpn2* (Hoshino and Iida 1997; Iida et al., 1999). Since the structural characteristic of *Tpn2* is suggested to be a non-autonomous element, *Speckled-activator* is likely to be the autonomous element acting on both *Tpn1* and *Tpn2*.

### (c) The mutable allele *purple-mutable*

Both *flecked* and *speckled* alleles are caused by integration of non-autonomous elements belonging to the *Tpn1* family. These and other observations led us to consider that *Tpn1*-family elements have been a major contributor to the spontaneous mutagenesis processes for generation of floricultural traits in the Japanese morning glory (Hoshino et al., 2000; Iida and Hoshino, 2000; Iida et al., 1999). Bearing this concept in mind, we have developed a procedure, STDM (simplified transposon display method), to identify tagged genes by insertion of *Tpn1*-related elements (Fukada-Tanaka et al., in preparation), based on the previously developed an AMF [AFLP (amplified restriction fragment length polymorphism)-based mRNA fingerprinting] procedure (Habu et al., 1997). We have successfully applied STDM for identifying the mutable allele, *purple-mutable* (Imai, 1934), conferring purple flowers with blue sectors (Figure 1c). The Purple gene was found to encode a vacuolar $Na^+/H^+$ exchanger for increasing vacuolar pH responsible for blue flower coloration, and the purple-mutable allele is caused by insertion of a *Tpn1*-related element, *Tpn4* (Fukada-Tanaka et al., 2000).

### (d) Stabilization of unstable mutable alleles

Among selfed progeny of a mutable *flecked* line, we were able to obtain a white variant (Figure 1d), in which all the flowers bloomed were white (Iida et al., 1999). The phenotype of the plant is very similar to those of white variants described by Imai (1931). Some of the selfed progeny of our white variant bore only white flowers whereas others produced a few flecked flowers together with white flowers. In these white variant derivatives, the excision of *Tpn1* occured rarely (Hoshino, A. and S. Iida, unpublished). We are speculating that appearance of the white variant is probably due to epigenetic inactivation of the autonomous element (Iida et al., 1999; Iida and Hoshino, 2000). In accordance with this notion, we also found that the apparent stable *r-1* allele conferring white flowers is caused by insertion of a non-autonomous *Tpn1*-family element, *Tpn3*, into the *CHS-D* gene encoding a chalcone synthase for anthocyanin pigmentation (Hoshino and Iida, 1999; Hoshino et al., 2000).

Not only epigenetic inactivations but also genetic sequence alterations would certainly cause stabilization of unstable mutable alleles generated by integration of the *Tpn1*-family elements. A mutation in a floral homeotic gene *duplicated* was found to be caused by a *Tpn1*-related non-autonomous element, *Tpn-botan*, and a subsequent deletion within the duplicate gene resulted in the stable mutant phenotype (Nitasaka, 1997). Like other plant transposable elements (Kunze et al., 1997), *Tpn1*-related elements generate characteristic small DNA rearrangements called footprints upon excision (Inagaki et al., 1994; 1996). It is thus conceivable that such footprints formed within

exon sequences would also generate stable mutant alleles.

### 2. The common morning glory (*I. purpurea*)

The mutable allele *flaked*

The mutable *flaked* lines of the common morning glory bear white flowers with colored flakes and sectors (Figure 1e). It is the best studied mutation of the plant (Barker, 1917; Clegg and Durbin, 2000; Epperson and Clegg 1992; Habu et al., 1998; Hisatomi et al., 1997; Hoshino et al., 2000; Iida et al., 1999; Imai and Tabuchi, 1935; Kasahara, 1956), and earlier illustrations of variegated flowers characteristic of the mutant appeared in England (Sims, 1814) and in Japan (Naganuma, 1903). We have identified that the *flaked* allele is caused by integration of a transposable element *Tip100* into the *CHS-D* genes for flower pigmentation and that *Tip100* belongs to the *Ac/Ds* family (Fukada-Tanaka, 1997; Habu et al., 1997; 1998). It is known that the timing and frequency of the flower variegation in the *flaked* lines are generally heritable by their progeny. Interestingly, the timing and frequency of the flower variegation may vary in different lines, even though they contain the identical *flaked* allele (Habu et al., 1998). Kasahara (1956) postulated that the timing and the frequency of the variegation are determined by the state of the activity of another genetic element *modulator* (originally termed *mutator*; see Habu et al., 1998). Since preliminary results indicated that *Tip100* is an autonomous element active in transgenic tobacco plants (Ishikawa et al., 1999), it is highly interesting to elucidate the molecular nature of modulator.

### 3. The morning glory (*I. tricolor*)

Like *I. Purpurea*, the morning glory (*Ipomoea tricolor*) is also originated from central America. Its wild-type cultivar called Heavenly Blue displays blue flowers while a mutant cultivar Flying Saucers bears white flowers with blue spots and sectors (Yoneda and Takenaka, 1981; Figure 1f). We tentatively named this apparent mutable allele *maifs* (mutable allele in Flying Saucers) in *I. tricolor*. Preliminary Northern blot analysis using the cDNAs for anthocyanin biosynthesis genes as probes revealed that the expression of the *DFR* gene in 'Flying Saucer' is drastically reduced, compared with the expression in 'Heavenly Blue'. DNA rearrangements were also detected in the DFR gene region of 'Flying Saucers' (Choi, J.D., A. Hoshino, and S. Iida, unpublished). It remains to be seen whether the mutable *maifs* allele is also caused by insertion of a transposable element or not.

### Floricultural traits and transposable elements

We showed that *En/Spm*-related transposable elements belonging to the *Tpn1*-family are major contributors to spontaneous mutations in *I. nil* whereas an *Ac/Ds*-related transposable element, *Tip100* acts as a spontaneous mutagen in *I. purpurea*. For generation of floriculturally important traits, it is clear that insertion of these transposable elements into genes associated with such traits must be an essential step. However, it is human beings who have played more important roles in selecting mutants, in preserving plants with interesting traits and in further isolating stable derivatives from the unstable traits caused by integration of transposable elements. Such breeding efforts are clearly seen in the processes to convert *I. nil* from of a medicinal herb into a sophisticated floricultural plant in the 18th and 19th centuries by esthetic fanciers under repeated booms in cultivation of the mutants in Japan (Iida and Hoshino, 2000; Iida et al., 1999). Not only the genetic processes such as transposon-mediated DNA rearrangements including excision and deletion but also epigenetic gene silencing and repression of the autonomous element must be involving in stabilization of the unstable mutable alleles generated by transposon insertions.

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