



# A natural hybrid of *Viola* from Taiwan with cytological evidence

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**Abstract.** Although hybridization in the genus *Viola* L. (Violaceae) is common, there has been no previous report from Taiwan. This paper describes a new natural hybrid, putatively derived from *Viola adenothrix* Hayata ( $2n=24$ ) and *V. arcuata* Blume ( $2n=24$ ). This hybrid is not only morphologically intermediate between its putative parents, but also exhibits chromosomal aberrations in meiosis. At metaphase I, 24 univalents (sometimes 1-9 bivalents) are formed. Division is unequal, with lagging chromosomes. In addition, the pollen grains are sterile (0% in stainability, against >95% of both its parents) and reproduction is entirely asexual by vegetative propagation. The chromosomal behavior in meiosis suggests that the genomes of the two parental species are significantly different, although their chromosome numbers are the same.

**Key words:** Meiosis; Natural hybridization; Taiwan; *Viola*; *Viola adenothrix*; *Viola arcuata*; *V. adenothrix* × *V. arcuata*.

## Introduction

The genus *Viola* L., consists of about 400 species, mainly distributed in temperate regions throughout the world. The classification within the genus has long been difficult because of the extensive hybridization between species (Lin, 1950; Russell, 1954; Valentine, 1962). As early as 1904, Brainerd had reported eight hybrids. In 1924, he described no less than 73 hybrids belonging to the section *Nomimium* subsection *Boreali-Americanae*. Hashimoto (1967) and Hama (1975) listed 25 and 75 hybrids, respectively, from Japan. However, no hybrids have been reported from Taiwan up to this time.

The taxonomy of Taiwanese *Viola* is well understood; eighteen taxa belonging to eight groups have been recognized (Wang and Huang, 1990). In our past studies of the genus, many specimens were collected which appear to be natural hybrids based on their intermediate morphology and extremely low pollen stainability. One of them, collected from Lishan, Tai-

chung Hsien, is morphologically intermediate between *V. adenothrix* Hayata and *V. arcuata* Blume. Pollen grains are unstainable. And it is cytologically abnormal in meiosis. One population of the hybrid in a single restricted area has been found, and it is described here as a new natural hybrid.

## Materials and Methods

Flower buds to be examined for meiotic behavior were fixed in a 3:1 (v:v) mixture of absolute alcohol and acetic acid, stained by acetocarmine and squashed. Pollen stainability was tested by the Malachite green - acid fuchsin - orange G stain (Alexander, 1969). 500 grains per plant were examined.

## Results and Discussion

*Viola adenothrix* Hayata × *V. arcuata* Blume 梨山堇菜 (Fig. 1)

A perennial herb; rhizome erect to ascending, rather stout, bearing leaves, flowers, and usually stems

**Table 1.** Comparison of *V. adenothrix*, *V. arcuata* and their putative hybrid

Characters	<i>V. adenothrix</i>	Putative hybrid	<i>V. arcuata</i>
Stipule shape	Ovate to lanceolate	Ovato-lanceolate to lanceolate	Lanceolate
Apex	Acuminate	Acuminate	Acute
Margin	Fimbriate, laciniate	Sparsely laciniate or ciliate	Sparsely serrate
Size (mm)	3-16×2-6	10-12×1-2.5	6-10×1-2
Basal lobes of blade	Rounded	Intermediate	Broadly hastate, rather prominent
Vestiture	Hirsute	Hirsute	Glabrous or sparsely pubescent
Flower width (mm)	15-24	12-20	10-15
Pubescence of petals	Bearded on lateral petals	All bearded	Bearded on lateral petals
Basal petal shape	Truncate to emarginate	Emarginate	Emarginate
Capsules	Ellipsoid	Lacking	Ellipsoid
Pollen stainability	>95%	0%	>95%
Chromosome number	$n=12$	$2n=24$	$n=12$
Meiotic chromosome configurations	12 bivalents	0-9 bivalents & 6-24 univalents	12 bivalents

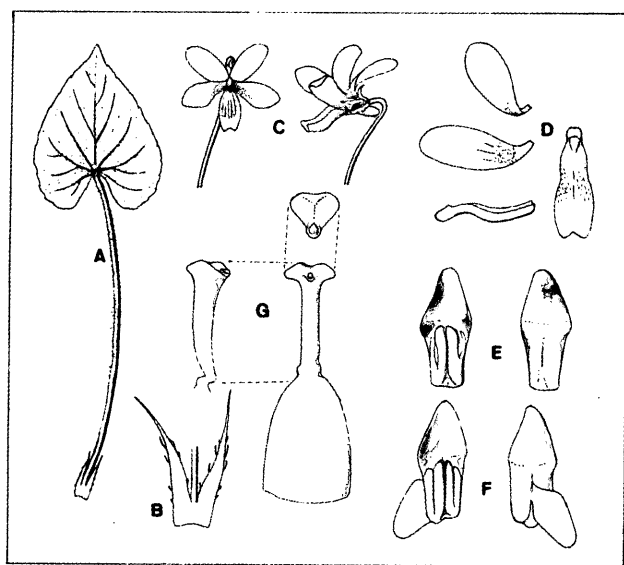


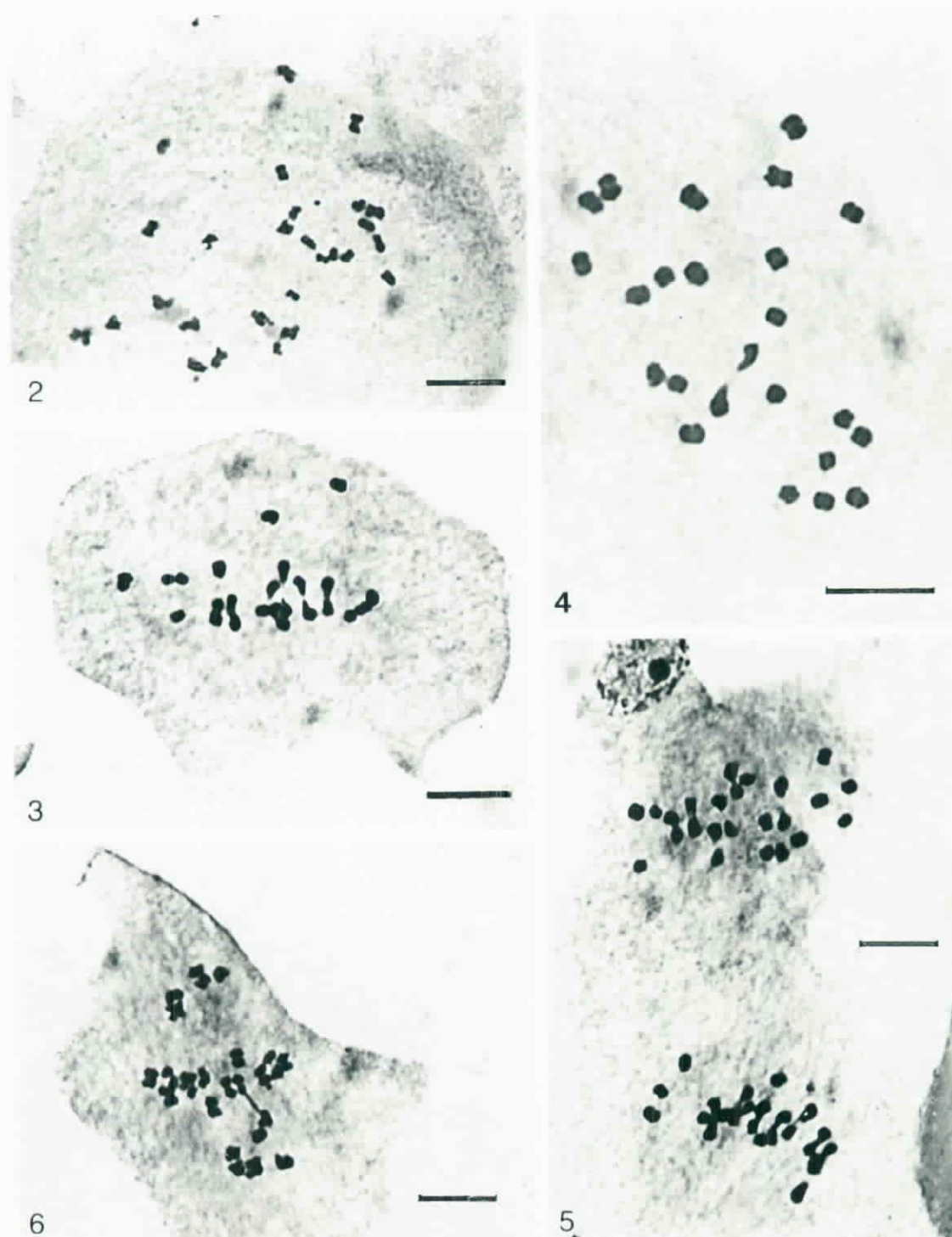
Fig. 1. *Viola adenothrix* Hayata  $\times$  *V. arcuata* Blume. A, Radical leaf; B, Stipules; C, Flowers, front view and side view; D, Upper, lateral and lower petals, from above to below, respectively; E, Upper stamens, adaxial view (left) and abaxial view (right); F, Lower stamens, adaxial view (left) and abaxial view (right); G, Ovary and style, ventral view, and part of style, side and front view.

at apex; stem up to 40 cm long, procumbent to ascending, sometimes rooting at nodes. Leaves 22-40 mm long, 20-35 mm wide, cordate to ovate-cordate, cordate at base with rounded basal lobes, acute at apex, crenate-serrate on margin, subdentate on lobes, usually

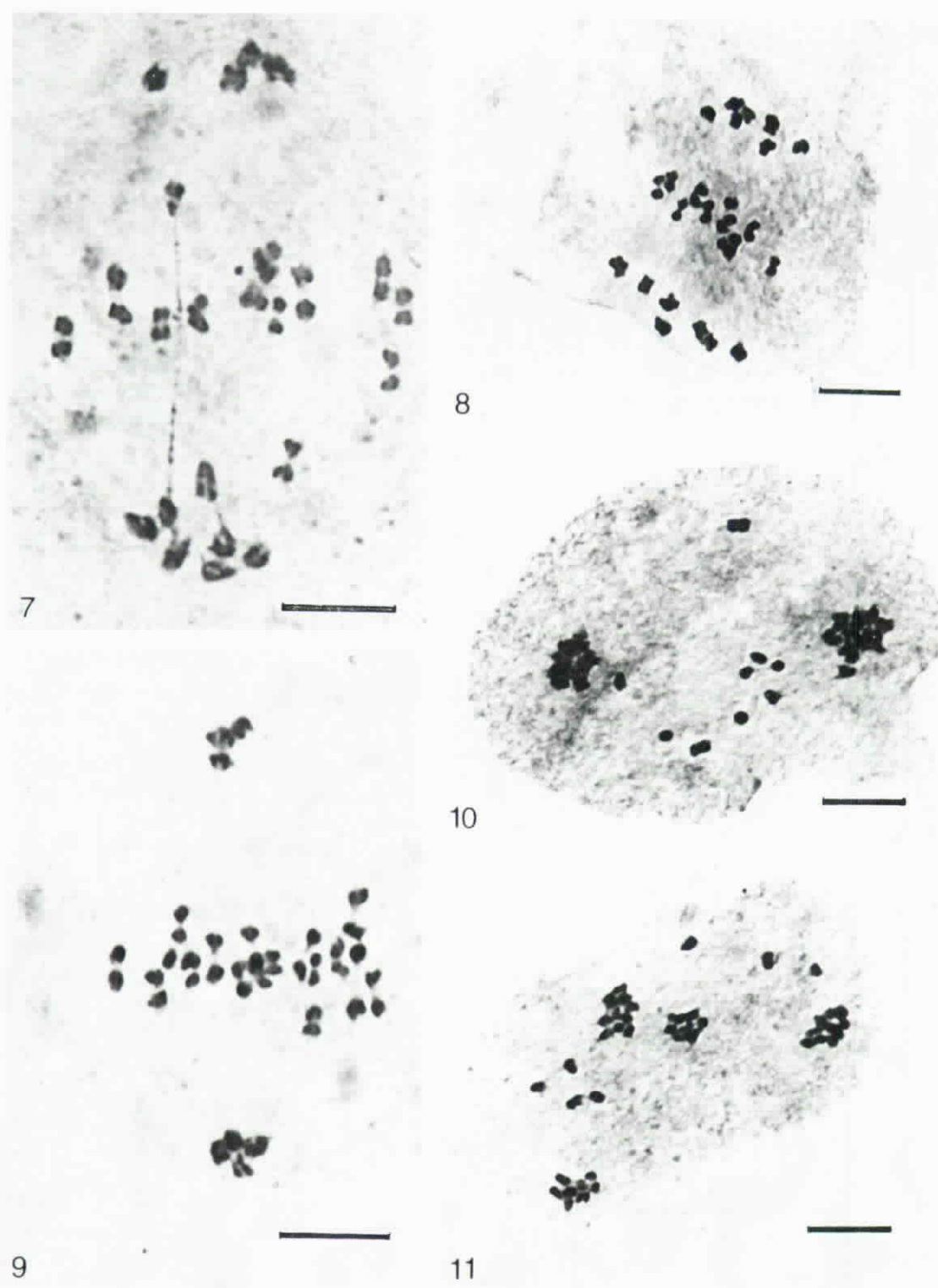
hirsute, rarely slightly pubescent, chartaceous; petioles 2-13 cm long, slender, wingless, hirsute. Stipules 10-12 mm long, 1-2 mm broad, ovato-lanceolate to lanceolate, acuminate at apex, sparsely fimbriate or ciliate on margin, glabrous. Flowers 12-20 mm across, pale purple to almost white with darker veins; peduncles 6-14 cm long, slender, usually exceeding leaves, glabrous or pilose. Sepals 3-7 mm long, ca. 1 mm broad, lanceolate, acute at apex, entire, sparsely pubescent; appendages ca. 0.5 mm long, rounded. Petals obovate to oblanceolate, all bearded, the basal one usually shorter than the others, emarginate at apex; spurs 1-2 mm long, exceeding calycine appendages, cylindrical, obtuse. Styles 1.4-1.6 mm long, geniculate at base, clavate distally, apex distinctly lobed on both sides, with anterior stigmatic beak. Capsules lacking. Chromosome number:  $n = 6-24I + 0-9II$ .

*Specimens examined:* Taichung Hsien, Lishan (梨山), ca. 1950m, J. C. Wang 3053 [Herbarium of Department of Biology, National Taiwan Normal University (TNU), TAI], 3477 (TAI, TNU), 3478 (TNU), 4132 (TAI, TNU).

The hybrid is morphologically intermediate between its putative parents (Table 1). However, it is more like *V. adenothrix* in vegetative morphology (leaf shape, vestiture of blades, and stipule shape) and more like *V. arcuata* in floral morphology. A single character distinguishes the hybrid from both its parents. The hybrid has all petals bearded. However, both its par-



Figs. 2-6. Chromosomal behavior in meiosis of *Viola adenothrix*  $\times$  *V. arcuata*. Fig. 2. Diakinesis, 24 univalents. Fig. 3. Metaphase I, 9 bivalents and 6 univalents. Fig. 4. Metaphase I, one bivalent and 22 univalents. Fig. 5. Metaphase I, 6 bivalents and 12 univalents (upper), 9 bivalents and 6 univalents (lower). Fig. 6. Anaphase I, showing lagging chromosome arranged in the equatorial plane. Scale bars equal 5  $\mu$ m.



Figs. 7-11. Chromosomal behavior in meiosis of *Viola adenothrix*  $\times$  *V. arcuata*. Figs. 7, 8. Anaphase I, showing lagging chromosomes. Figs. 9, 10. Telophase I, two groups and some lagging chromosomes. Fig. 11. Telophase II, four groups and 7 individual chromosomes. Scale bars equal 5  $\mu$ m.

ents have only the two lateral ones bearded.

Although the appearance of the novel morphological trait is unusual, it is not unique in *Viola*. A similar phenomenon is present in *V. betonicifolia* J. E. Sm. (syn. *V. caespitosa* D. Don). This had long been thought to be an allopolyploid ( $n=36$ ) derived from the hybridization between *V. inconspicua* Blume subsp. *nagasakiensis* (W. Becker) J. C. Wang & T. C. Huang (syn. *V. minor* (Makino) Makino,  $n=24$ ) or *V. mandshurica* W. Becker ( $n=24$ ), and *V. patrinii* A. P. DC ( $n=12$ ) or *V. yedoensis* Makino (probably a synonym of *V. confusa* Champ. ex Benth.,  $n=12$ ) (Hashimoto, 1967; Moore, 1963). This hybrid-derived species is characterized by having both the upper and the lateral petals bearded; nevertheless, none of its alleged parents have the upper ones bearded. Further study is necessary to explain the mechanisms of this phenomenon.

Both *V. adenotheix* and *V. arcuata* have 24 chromosomes which pair regularly to form 12 bivalents in meiosis (Wang, 1988). The hybrid was also found to have the same chromosome number, but its behavior in meiosis was irregular. At diakinesis and metaphase I, 24 univalents (or sometimes 1–9 bivalents) were formed (Figs. 2–5). Separation of chromosomes was irregular. Some chromosomes were arranged in the equatorial region and lagged until telophase (Figs. 6–10). When meiosis was nearly ended, four groups and several individual chromosomes were observed (Fig. 11).

Because of the irregular chromosomal behavior described above, the hybrid could not form viable pollen grains. This was confirmed by testing pollen-stainability. The pollen grains of the hybrid were almost unstained and morphologically abnormal, but those of both its parents were highly stainable ( $>95\%$ ). The hybrid does not produce capsules or seeds and is not capable of sexual reproduction. Instead, the creeping stems root at nodes, propagating the plants asexually.

The Taiwanese species of *Viola* were classified into eight groups comprising 18 taxa by Wang and Huang (1990). Although they may possess the same chromosome number, these groups are easily distinguished by their morphology (Wang, 1988). In our classification, *V. adenotheix* and *V. arcuata* belong to different groups, the *Serpentes* and the *Bilobatae* groups, respectively. However, both have the same chromosome number. Gershoy (1928, 1932, 1934) produced many artificial hybrids between North American species of *Viola*. He found that hybridization between

species of different subgroups in section *Nomimium* was possible, and he obtained viable but sterile  $F_1$  offspring from the hybridizations. Consequently, he suggested that these subgroups were phylogenetically rather distant. The relationships among the eight Taiwanese groups of *Viola* are still not clear because no thorough biosystematic studies have been conducted. The chromosomal behavior of *V. adenotheix*  $\times$  *V. arcuata* described above, in which few or no bivalents are formed at diakinesis or metaphase I, suggests that the genomes of the two parental species are significantly different. Their phylogenetic relationship is probably distant.

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## 台灣產堇菜屬之一天然雜交族群及其細胞學證據

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雜交現象普遍存在於堇菜屬植物，但在台灣迄未見此方面之記錄。本文首次報導台灣產堇菜屬之一天然雜交族群，根據外部形態及伴生之相關種類研判其親本為喜岩堇菜 (*Viola adenothrix*) 及如意草 (*Viola arcuata*)。此一天然雜交族群個體除形態特徵介於二推定親本之間外，其染色體在減數分裂時並呈現不正常之行爲，在第一次減數分裂中期僅形成少數二價體或全為單價體，隨後之分裂則有延遲及數目不等之不正常現象，故花粉敗育，無法行有性生殖，全賴無性生殖繁衍後代。由上述染色體之配對行為推測二親本雖具相同染色體數目，但染色體組已有顯著差異。