Morphological Observation on Floral Variations of the Genus Cuscuta in Taiwan

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ABSTRACT: The variations in floral structure of *Cuscuta* in Taiwan were studied with special reference to *C. campestris*. The variations of the floral structure were described and illustrated by using light and scanning electron microscopy. The variations including the following: (1) the absence of floral organs; (2) the abnormal fusion of floral organs; (3) petaloid stamens; (4) the lack of distinctive anther lobes; (5) the unusual protrusions on ovaries; and (6) the extrusion of ovule on ovary surfaces. The variations occur in early developmental stages when the primordia of floral organ were initiated. The findings that the abnormal position of the ovule and the lateral fusion of the scale with the filament or the petal in the species of *Cuscuta* are first time reported here.

KEY WORDS: Abnormal flowers, Cuscuta, Cuscuta campestris, morphological variation.

INTRODUCTION

The dodders, genus *Cuscuta*, are all parasitic vines of the family Cuscutaceae, has 170 species, widely distributed in temperate and tropical regions. Since the flowers of *Cuscuta* are very small and the floral organs are not easily seen with the naked eye, the flowers must be dissected and examined with care when studying them. Gandhi and Thomas (1983) found some variations in the floral structure from herbarium specimen of four species, *Cuscuta campestris* Yunck., *C. gronovii* Willd., *C. pentagona* Engelm. and *C. polygonorum* Engelm., noting that variations were mostly generally the absence of stamens or their sterility.

Variations in the flowers of different species were also observed in a long-term study of the genus *Cuscuta* in Taiwan from 1999-2004 (Liao, 2004). Many abnormal flowers were especially found on *C. campestris*, collected from the population in a medicinal garden at Long-Chang, Taitung County in 1999.

We document and present the variations in the floral structure of *Cuscuta* in Taiwan by using light and scanning electron microscopy with special reference to *C. campestris*.

MATERIALS AND METHODS

Collection of materials

In Taiwan, *Cuscuta chinensis* Lam. and *C. campestris* bloom nearly all year round, while *C. japonica* Choisy var. *formosana* (Hayata) Yunck. and *C. japonica* Choisy. var. *japonica* bloom from October to February. We have collected numerous flowers of *Cuscuta* sp. from

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1999-2004 at about 200 sites in Taiwan, however we have only found the abnormal flowers in 4 sites (Table 1). In which, we had collected many abnormal flowers (about 70%) in *C. campestris* from January 1999 at a medical garden. We have recollected flowers from the same site in July 1999 and January 2000 respectively, however we couldn't find abnormal flower anymore.

All abnormal flower samples were freshly collected during their flowering season for further studies. (Table 1).

Taxon	Collection site and number	No. of abnormal flower detected
Cuscuta campestris	Long-Chang, Taitung County (22°26'N 121°16'E) Liao F010	361
C. chinensis	Wangan, Penghu County $(23^{\circ}22^{\circ}N, 110^{\circ}30^{\circ}E)$ Lizo E013	1
C. japonica var. formosana	Zhangshan, Kaohsiung County	1
C. japonica var. japonica	(23°14 N, 120°49 E) Liao F014 Shenmutsun, Nantou County (23°31'N, 120°51'E) Liao F015	2

Table 1. Samples of abnormal flowers freshly collected from 200 sites during 1999-2004.

Preparations for scanning electron microscopy (SEM) and light microscopy (LM)

Flower buds and flowers were fixed in FAA (formalin : acetic acid : 50% ethanol = 1:1:18) for 12-24 hr and then stocked in 70% alcohol. For SEM, samples were dissected under a Zeiss dissecting microscope, critical point dried with CO_2 , mounted onto aluminum stubs, coated with gold palladium, and then examined and imaged using a Hitachi H2500 scanning electron microscope at 15 kV. For LM, samples were gradually dehydrated in an acetone series, slowly infiltrated with and embedded in Spurr epoxy resin, and then thick-sectioned (500 nm) on an ultramicrotome using a diamond knife. The sections were stained with toluidine blue O, and examined and imaged with a Zeiss compound microscope.

RESULTS AND DISCUSSION

Variations in the floral structure of *Cuscuta* were observed and described as follows:

1. Cuscuta chinensis Lam.

Cuscuta chinensis was growing on *Ipomoea pes-caprae* (L.) R. Br. A single flower specimen (Liao F013) had four normal spoon-shape scales, and one abnormal, bifid fimbriate scale (Fig. 1A).

2. Cuscuta japonica Choisy var. formosana (Hayata) Yunck.

Cuscuta japonica var. *formosana* was growing on *Ficus microcarpa* L. A single flower specimen (Liao F014) had four normal anthers with equal, regular lobes, and one abnormal anther with unequal, irregular lobes (Fig. 1B).

3. Cuscuta japonica Choisy var. japonica

Cuscuta japonica var. *japonica* was growing on *Boehmeria densiflora* Hook. & Arn. One specimen (Liao F015) has a single flower with unequal stigma lobes (Fig. 1C), and another flower also with unequal stigma lobes, meanwhile each lobe with many irregular small-lobes, and a flattened style (Fig. 1D).



Fig. 1. Scanning electron micrographs of dissected flowers. A: Abnormal flower of *Cuscuta chinensis* shows one normal scale and one abnormal bifid scale (arrows). B: Abnormal flower of *C. japonica* var. *formosana* shows the abnormal anther with irregular lobes (L). C & D: Abnormal flowers of *C. japonica* var. *japonica*. C: The abnormal stigma with unequal lobes. D: The abnormal pistil had flat style (S) and unequal lobed stigma, which had many irregular small-lobes (arrow). E & F: Normal flowers of *C. campestris*. E: Part of mature flower shows petal (P), stamen (A) and scales (S). F: The mature pistil shows stigma (G), style (S) and ovary (O). Scale bars = $300 \mu m$.

4. Cuscuta campestris Yunck.

The population of *C. campestris*, growing on plants of *Artemisia indica* Willd., *Kalanchoe gastonis-bonnieri* Raym.-Hamet & H. Perrier, *Paederia foetida* L., and *Vernonia cinerea* (L.) Less. at Long-Chang, had both normal flowers and abnormal flowers in the same population (Liao F010).

Normal flower of *C. campestris* revealed the characteristic calyx lobes 4-5; corolla short, campanulate, 4 or 5-lobed, lobes broadly triangular, apex acute, spreading horizontally, tips inflexed; scales spoon-shape, exceeding bases of filaments, long fimbriae, often exserted; stamens exserted, anthers ovoid, 2 lobes (Fig. 1E); ovary ellipsoid; styles 2, slender; stigmas capitate (Fig. 1F).

The abnormal flowers markedly differed in several aspects when large samples were examined. Some anthers were absent (Fig. 2A), some scales were reduced by various degrees and others were totally absent (Fig. 2B). The absence of floral organs was visible at an early stage of flower development (Figs. 2C & D). Several flowers were without petals and had filaments fused with scales (Fig. 2E). Some flowers had various kinds of fusion of petals, stamens and scales (Figs. 2F-H); in addition, several flowers had petaloid stamens (Fig. 2I).



Fig. 2. Scanning electron micrographs of dissected abnormal flowers of *Cuscuta campestris*. A: Part of a mature flower show the loss of stamens (arrows). B: A mature flower with the pistil removed to show various degree of reduction of the scales (arrows). C & D: Early development of abnormal flower. C: The loss of one stamen initial (arrow) locate between two petal initials (P). D: The loss of floral organ initials (arrow, include two stamens and one petal) locate between two petal initials (P). E: Part of a mature flower show the loss of petals, whereas the filament was fused with the scale (arrows). F: Part of a mature flower show the lateral fusion of the petal with the scale (arrows). G: Part of a mature flower show the fusion of petal, stamen and scale on one side (arrow) and the lateral fusion of petal with scale on the other side (arrowhead). H: Part of a mature flower show the fusion of petal and stamen (arrow), and the lateral fusion of petal with scale (arrows). J: Part of a mature flower show the loss of connective and a large slit was visible on the anther sacs (arrowhead). Scale bars = 300 μ m (Figs. A, B, E-I), 100 μ m (Figs. C, D, J).

One flower had a stamen without well development of the connective (Fig. 2J). Some flowers had a lobed ovary and some had irregular projection on the ovary (Figs. 3A & B). Furthermore, some flowers had the ovules exposed on the ovary surface (Fig. 3C). Compared with the normal ovary development (Fig. 3D), the development of the four ovules was not



Fig. 3. Scanning electron and light micrographs of pistils of *Cuscuta campestris*. A: A mature pistil shows the lobed ovary (O) and unequal length of the style (S). B: A mature pistil shows the irregular projections on the ovary (arrow). C: A mature pistil shows the extrusion of ovules (V) on the ovary (O), two ovules were removed (arrows) to reveal the reduced style (asterisk). D: Early development of normal flower shows four developing ovules (V), noted that two carpels (C) were enclosing at this time (arrows). E: Early development of abnormal flower shows four developing ovules (V), noted that the ovule were not enclosed by the carpels (C) at this stage (arrows). F: Longitudinal section of mature ovary shows one of the ovule (arrowhead) extruded from the placenta with elongated funicule (arrows). Scale bars = 300 µm (Figs. A-C, F), 100 µm (Figs. D & E).

synchronized, and the ovules grew faster than the two carpels (Fig. 3E). Hence, the ovules were not enclosed by the ovary wall when the two carpels gradually fused to form the ovary. The extruded ovule had a long funiculus (Fig. 3F).

In the small amount of herbarium specimen, Gandhi and Thomas (1983) found variations in the floral structure of one flower of *Cuscuta campestris*, some flowers of three specimens of *C. gronovii*, some flowers of one specimen of *C. pentagona* and one flower of *C.*

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polygonorum respectively. They noted that the variations observed were mostly the absence of stamens or their sterility. However, in our study, other variations were also found, including the following: (1) the absence of floral organs such as stamens, scales and petals; (2) the abnormal fusion of floral organs such as filaments with scales, petals with scales, stamens with petals, and some caused the separation of petals; (3) petaloid stamens; (4) the lack of distinctive anther lobes; (5) unusual protrusions on ovaries; and (6) the extrusion of ovules on ovary surfaces.

Some taxonomical studies included *Cuscuta* within Convolvulaceae, while others segregated the genus in its own family Cuscutaceae, based on its parasitic habit, and a corolla usually with 5 fimbriate scales. These scales, diagnostic characters for identification, have also been named corolla appendages, corolla scales, infrastaminal scales, antestaminal scales, staminal scales or filament scales in different publications (Yuncker, 1932; Lawrence, 1951; Gandhi and Thomas, 1983; Beliz, 1986; Parker and Riches, 1993; Press and Graves, 1995; Bhattacharyya and Johri, 1998; García, 2001). Watson and Dallwitz (1992) interpreted scales alternating with stamens as staminodes. However Prenner *et al.* (2002) described them as secondary nectar receptacles in flower of *Cuscuta reflexa*. Although some studies described and illustrated the floral development and morphology in *C. campestris* (misidentified as *C. australis* R. Br. by Kuoh and Liao, 1993) and in *C. reflexa* Roxb. (Prenner *et al.*, 2002), the origin and the homology of the scales are still not clear.

Additionally, Gandhi and Thomas (1983) noted that scales occurred with stamens despite the sterility of the latter. They pointed that a stamen was lost in the development stage when its scale was lost. However, this is not the case in our results. We found that the scales can be developed very well even though the stamens were absent (Figs. 2A & 2F) and in abnormal flower a scale maybe fused with the stamen or the petal. Normally developed flowers of *C. reflexa* (Prenner *et al.*, 2002) have long corolla tube, while the flowers of *C. campestris* (Liao *et al.*, 2000) were sympetalous, even though there was no distinct corolla tube. Interestingly, fusion of floral organs (Figs. 2F-H) would cause the petals to separate in abnormal flowers, altering the corolla from sympetalous to choripetalous.

Cuscuta campestris, a holoparasitic flowering plant with special floral organs and diverse hosts, is easier propagated by ramets, can be grown on several tested plants of different families, and it's floral organs are liable to variation, will be a suitable model plant for studying parasitic plant biology in many aspects.

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台灣產菟絲子屬植物花部變異之形態觀察

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摘 要

本文以光學顯微鏡及掃描式電子顯微鏡觀察台灣的菟絲子屬 (Cuscuta) 植物花部 構造的變異,特別關注於平原菟絲子的不正常花。花部變異包括:(1)花部的缺失;(2) 花部不正常的癒合,有些導致花瓣的分離;(3)雄蕊花瓣化;(4)花藥隔的缺失;(5)子房 的突出構造;(6)子房上裸露的胚珠。花部的變異在發育早期各花部始原形成時即發生。 胚珠位置的不正常、鱗片可能與花絲或花瓣側面癒合成一體,這些變異現象為首次發現 於菟絲子屬植物。

關鍵詞:不正常花、菟絲子屬、平原菟絲子、形態變異。

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