Floral biology of *Bidens pilosa* var. *radiata*, an invasive plant in Taiwan

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**ABSTRACT.** Invasion of exotic plants is threatening global biodiversity. *Bidens pilosa* L. var. *radiata*, a member of Asteraceae, has successfully invaded Taiwan after being introduced to the island. Knowledge of its reproduction is necessary in developing effective ways to control the spread of this plant. The objective of this study was to investigate the floral biology including: (1) floral structure, (2) the process of secondary pollen presentation, and (3) pollen/ovule ratio (P/O ratio) of this invasive plant. Plants were grown in pots from seeds and placed at the experimental farm of the National Taiwan University. Besides describing floral morphology, number of disk florets per capitulum, number of pollen grains per floret, and P/O ratio were quantified. Furthermore, we defined six floral stages according to anther tube and style morphology to investigate the process of pollen presentation and assessed pollen viability and stigma receptivity. Significant differences were found in the number of disk florets per capitulum (range 19-61, average 44.1) and the number of pollen grains in each floret (range 6,556-11,378) among individuals. Secondary pollen presentation was observed as the growing style brushes and pumps pollen grains out of the anther tube. Incomplete protandry was found from the observation of the flowering process and the tests of pollen viability and stigmatic receptivity. High P/O ratio (means ± s.e.: 8,827 ± 464) was measured which suggested that *B. pilosa* var. *radiata* might be obligately xenogamous.

**Keywords:** Autogamy; *Bidens pilosa* var. *radiata*; Capitulum; Pollen/ovule ratio (P/O ratio); Protandry; Secondary pollen presentation; Stigmatic papillae; Sweeping hairs; Xenogamy.

**INTRODUCTION**

Invasion of exotic species is a global phenomenon which often has negative environmental and economic impact on the region being invaded (Mooney and Hobbs, 2000; Pimentel et al., 2000). Therefore, invasion by non-indigenous species has been recognized as one of the most serious threats to global biodiversity (Begon et al., 1996; Vitousek et al., 1997; Shortt and Vamosi, 2012). Reducing population size of invasive species and preventing their spread are critically important in controlling their expansion. Reproduction is necessary for plants to increase population size and to spread into other habitats (Richardson, 2004). Through increasing our understanding of invasive plant reproduction, we will have a better chance to find the most effective way(s) to control them and reduce their environmental threat.

*Bidens pilosa* L. var. *radiata* Sch. Bip., an annual or perennial herb belonging to Asteraceae, was first collected and documented in 1842 (Peng et al., 1998) and recorded in Taiwan in 1909 (Wu et al., 2010). After being introduced into Taiwan (probably from North America), it is distributed from low to mid elevations throughout the island (Peng et al., 1998) and has become one of the twenty most noxious invasive plants in Taiwan (Chiang et al., 2003). One of the fundamental questions about invasive plants is what makes them invasive (Sutherland, 2004). Reproduction plays an important role in establishing a new colonizing population (Baker, 1955). Plants with efficient reproductive strategies could potentially have a better chance to increase population size and distribution range than those without (Xiao et al., 2011). *Bidens pilosa* var. *radiata* was found to be able to reproduce vegetatively (Hsu, 2006; Huang, 2008), which might partially contribute to its success in dominating colonized habitats.

In addition to vegetative reproduction, *B. pilosa* var. *radiata* also produces achenes on its flower heads by sexual reproduction which could increase genetic diversity. To our knowledge, sexual reproduction and floral biology of *B. pilosa* var. *radiata* in Taiwan have not been studied. A detailed study of the functional morphology of the flowers of this invasive plant would provide clues regarding its
mode of sexual reproduction.

Secondary pollen presentation, the relocation of pollen grains from anthers onto another flower organs as pollen presenting organ for pollination, is found in five monocotyledon and 20 dicotyledon families of angiosperms (Howell et al., 1993). It is a typical characteristic of Asteraceae. The apical portion of its style may be externally covered with sterile hairs (called sweeping hairs) which often participate in the pollen presentation process (Ladd, 1994). Three types of secondary pollen presentation have been described in this family, which are correlated with the arrangement of the sweeping hairs (Leins and Erbar, 1990; Erbar and Leins, 1995). Species with hairs only at the tip of the style operated the pump mechanism, those with hairs reaching below the branches of the style performed the brush mechanism, and those with hairs clothing only part of the externals of the style branches conducted an intermediate mechanism. Most species with secondary pollen presentation were also protandrous to avoid self-pollination (Howell et al., 1993; Yeo, 1993; Ladd, 1994; Shivanna, 2003). Therefore, the stylar morphology, pollen viability and stigma receptivity of Bidens pilosa var. radiata were studied to better understand the pollen presentation mechanism and to test the hypothesis whether this taxon is protandrous.

Although outcrossing can increase genetic heterogeneity, it also reduces the reproduction assurance. Because their pollen need to be transferred to other individuals for fertilization, xenogamous (cross-pollinating) plants often invest more resources to male versus female than autogamous (self-pollinating) ones, like producing more pollen grains, to increase reproduction assurance. Consequently, xenogamous species often have higher pollen/ovule ratio (P/O ratio) than autogamous species (Richards, 1997; Shivanna, 2003). Cruden (1977) found significant difference in P/O ratio between xenogamous and autogamous species, and suggested that the P/O ratio of flowering plants could reflect their breeding system (Cruden, 1977; Richards, 1997; Wang and Hu, 2011). Thus, in this study, we also investigated the P/O ratio of the plant to gain a prediction about the possible breeding system of this invasive plant.

This study aims to understand floral biology of B. pilosa L. var. radiata, including (1) floret morphology, (2) the process of secondary pollen presentation and (3) the P/O ratio.

MATERIALS AND METHODS

Plant materials

Plant materials were planted from seeds, which were collected from central Taiwan (23°26′ N, 120°36′ E, 500 m a.s.l.). Plants were grown in 4 L pots (18.5 cm × 19.5 cm) and placed at an experimental farm of the National Taiwan University (25°00′ N, 121°32′ E, 15 m a.s.l.). When the plants started flowering, about two to three months after planting, we observed the flowering process and analyzed the adult flowers.

Floral structure

The inflorescence of B. pilosa var. radiata is a capitulum composed of two types of sessile florets. Five to eight white ligulate florets (sterile, 0.5-1.5 cm long) surround disk florets arising at the same level on a flattened axis, and the whole is surrounded by involucre bracts.

The size of fresh pollen grains was measured with a light microscope equipped with a calibrated ocular micrometer. To examine the detailed morphology of pollen grains, florets were removed from capitula and put in a dry cabinet (SBM-B1B, Bossmen, Taipei, Taiwan) more than 72 hrs, and then pollen grains were spread on metal stubs with double-sided adhesive tape and sputter-coated with gold by a gold-particle coating machine (E101, Hitachi, Tokyo, Japan). The resulting materials were then observed with an SEM (inspect S, FEI, Brno, Czech).

To prevent the dehydration of the styles, a different procedure was used for preparation. Fresh styles were collected, mounted directly on a metal stub with double-sided adhesive tape and observed with a SEM with cryoholder facilitates (TM 1000, Hitachi, Tokyo, Japan) (Tang et al., 2012).

Floral stage and lifespan

Bidens pilosa var. radiata is a hermaphrodite and its yellow disk florets are all monochilous. Six floral stages of a disk floret (A, B, C, D, E, and F) were defined based on the morphology and behavior of anther tube and style (Figure 3). At stage A, corolla was closed. At stage B, corolla opened, anther tube raised and part of pollen grains were presented at top of anther tube. At stage C, the style elongated and brushed and pumped pollen grains out of anther tube. At stage D, style branches separated and exposed the stigmatic areas. At stage E, style branches were bent downward. Flower was withered at stage F. The durations of each floral stage and total floral lifespan were monitored daily on a capitulum (marked before anthesis) until the last floret of the capitulum senesce.

Stigma receptivity and pollen viability

At each floral stage, styles were removed from florets and put on a slide for the detection of stigmata receptivity. A droplet of the test solution, mixture of one 15 × 15 mm peroxtesmo Ko peroxidase test paper (Macherey-Nagel, Dueren, Germany) with 1 ml distilled water, was applied onto the stigma (style branches). The stigma was considered receptive if it turned dark (Dafni and Maués, 1998).

At each floral stage, pollen grains were collected from anther tube, spread on a slide, stained by fluorescein diacetate (FDA) solution, and finally examined under a fluorescence microscope (Nikon, Tokyo, Japan). Viable pollen grains would show bright fluorescence when excited by blue illumination (filter set: B-2A excitation filter 450-490 nm and DM 510 dichroic mirror; Nikon, Tokyo, Japan). We counted 200 pollen grains of each sample and determined the pollen viability (= viable pollen number/200). The average of three florets taken from 3 different capitula...
of the same individual was used to represent the value of that individual, and five individuals were sampled.

**Number of pollen grains and pollen/ovule ratio**

To estimate the number of pollen grains and P/O ratio, pollen grains of a single tubular floret was counted with a counter chamber under a microscope. Pollen numbers of two florets from one individual were counted and means were calculated to represent the pollen number of this individual. In total, 20 florets from 10 individuals were counted. The difference of number of pollen grains among 10 individuals was analyzed with ANOVA (general linear model procedure of SAS, release 9.1, SAS Inst. Inc.).

**RESULTS**

**Number of disk florets/capitulum**

Among the 58 capitula counted, most (50) capitula had 35 to 55 disk florets, only 5 capitula had disk florets less than 35 and 3 capitula had disk florets more than 55 (Figure 1). The mean florets number of each capitulum was 44.1 ± 1.0 (mean ± s.e., n = 58), and there was significant difference in the number of disk florets per capitulum among the ten individuals ($F_{9, 48} = 2.11, p = 0.047$).

**Floral morphology**

The dissection of a disk floret of *B. pilosa* var. *radiata* is shown in Figure 2A. A disk floret was composed of five joined petals (forming a tubular corolla, Co), five stamens with coherent brown anthers (forming an anther tube) and separated white filaments (Figure 2B1, 2B2), a long style with two branches at the apex ascending from the center of the anther tube and an inferior ovary.

Before disk floret anthesis, the anthers dehisce and release of pollen grains into the anther tube (Figure 2B1). The pollen grains (Figure 2C), diameter of 32.1 ± 0.5 µm (with spinule) or 27.9 ± 0.8 µm (without spinule), were echinate globular with tricolporate. After the style grew out of the anther tube, only a few pollen grains would be left in the anther tube (Figure 2B2).

Style tip was covered by two kinds of microstructure (Figure 2D). The tip and abaxial surface of the style branches were covered by longer brushing hairs (or sweeping hairs) (Figure 2D) while the adaxial surface (the stigmatic area) was occupied with smaller and shorter papillae (Figure 2D).

**Flowering process and floral stage**

Flowering of *B. pilosa* var. *radiata* occurred continually in the field. A capitulum of *B. pilosa* var. *radiata* began anthesis with maturation of sterile ligulate florets. Following up, disk florets opened sequentially, about one whorl per day, from periphery to the centre of a capitulum. The flowering duration from the first disk floret to the final one was approximately four to six days.
According to the flowering process of the floret, six floral stages (A, B, C, D, E, and F) was defined according to the morphology of the floret, and it extended for about 6 days from flower bud to flower withered (Figure 3). In general, the disk floret started anthesis with the opening of the corolla before 8:00 am. Within 24 hours before anthesis, the corolla was closed and its tip turned yellow. The disc florets with this appearance were defined as at stage A (Figure 3A). Following stage A, the corolla tube elongated, the tip of the corolla tube separated into five lobes, and the anther tube (with its tip still closed) grew out of corolla (stage B, Figure 3B). About 16 hours after stage B (00:00 a.m. on day 2), the style elongated through the anther tube, when was defined as stage C (Figure 3C). The growing style brushed and pumped the pollen grains out of the anther tube (the process of secondary pollen presentation). Thus, at stage C the pollen grains were exposed out for pollination. After protruding through the anther tube, the style gave off two branches at the apex and then exposed the stigma, this stage was defined as stage D (Figure 3D). Stage D usually happens around 8:00 am. About 24 hours later (08:00 a.m. on day 4), the style branches bended downward, and the stigma was at the highest position of the floret, the floret was defined as at stage E (Figure 3E). Finally, at stage F (on day 5 and day 6), anthers and style branches were dehydrated, withered, and shrank back (Figure 3F).

**Stigma receptivity and pollen viability**

Results from the peroxidase test revealed that the stigma surface occupied with papillae was the site for the reception of pollen grains and where were not receptive (Figure 4A, 4B) when the style branches were still in the anther tube, i.e. at stage A and B. Surprisingly, although the stigma had not been exposed at stage C, it already appeared receptive (Figure 4C). The stigma remained receptive after being exposed, at stage D and E (Figures 4D and 4E). Accordingly, the stigma was exposed and receptive for about 2 days.

The change of pollen viability was very different from that of stigma receptivity. Before florets flowering, at stage A, 77.2 ± 2.1% (mean ± s.e., n = 5) pollen grains were viable (Figure 5). After florets open, the percentage of viable pollen decreased gradually from stage B to stage F. At stage F, only 3.5 ± 1.8% pollen grains were viable.

**Number of pollen grains and pollen/ovule ratio**

Number of pollen grains of a single tubular floret was 8,827 ± 464 (mean ± s.e., n = 10). Significant difference
was found in the number of pollen grains in each floret (range 6,556–11,378) among the 10 individuals ($F_{9,10} = 6.78, p = 0.003$). Since there is only one ovule in each floret, the average number of the pollen grain per floret can also be used to represent the P/O ratio.

**DISCUSSION**

*Bidens pilosa var. radiata* has invaded Taiwan and is threatening local biodiversity (Chiang et al., 2003; Wu et al., 2004; Hsu, 2006; Huang, 2008; Wu et al., 2010). The growth and physiology of the plant have been studied (Hsu, 2006; Huang, 2008; Hsu and Kao, 2009) while its sexual reproduction is nearly unknown. According to our observations, the invasive plant produces flowers and sets fruits in field year-round. Thus, sexual reproduction is expected to play an important role in spreading of the plant. This study provides some basic information on floral biology of this invasive plant.

All taxa in the Asteraceae were reported having pollen presenters (Ladd, 1994). Consistent with Ladd’s report, secondary pollen presentation was also observed in *B. pilosa var. radiata* (Figure 3). Secondary pollen presentation has been suggested as a mechanism enhancing the efficiency and accuracy of pollen exportation and/or pollen reception (Yeo, 1993). According to which organ is presenting pollen grains, whether pollen is exposed or concealed within a structure, and how the pollen loaded onto the presenters, nine different secondary pollen presentation types were identified in 16 angiosperm families (Howell et al., 1993). In Asteraceae, the tip or the abaxial surface of the style branches are the pollen presenters (Howell et al., 1993). In Asteraceae, the P/O ratio ranged from 262 to 12,890 in facultative xenogamy, facultative autogamy to cleistogamy. In Asteraceae, the P/O ratio ranged from 262 to 12,890 in facultative xenogamy, facultative autogamy, obligate autogamy to cleistogamy. It was found that P/O ratio was highest (2,108–20,035, n = 25) in obligately xenogamous species and decreased from obligate xenogamy, facultative xenogamy, facultative autogamy, obligate autogamy to cleistogamy.

**Figure 5.** Mean pollen viability (mean ± s.e., n = 5) of *Bidens pilosa var. radiata* at six floral stages (as defined in Figure 3).

Mechanisms were divided into pumping, brushing, and a combination of both types (Leins and Erbar, 1990; Yeo, 1993; Leins and Erbar, 2006). In *B. pilosa var. radiata*, the tip and the abaxial surface of the style branches were covered by the sweeping hairs (Figure 2D). Based on Leins and Erbar’s (1990, 2006) classification, the pollen presentation mechanism of *B. pilosa var. radiata* combined a pump and a brush mechanism which is a typical feature in the Asterales complex (Leins and Erbar, 1990; Yeo, 1993; Leins and Erbar, 2006).

Dichogamous protandry, male matures before female, is common in hermaphroditic floret in the Asteraceae (Faegri and Pijl, 1979; Cerana, 2004). If the flower is protandrous, secondary pollen presentation would promote xenogamy (Howell et al., 1993; Yeo, 1993; Ladd, 1994; Shivanna, 2003). According to our observation of the flowering process of the plant (Figure 3), the pollen grains were presented out for pollination at stage C (referred as functional male phase). Though the stigma was already receptive at stage C (Figure 4C), however, it was not exposed until stage D (referred as functional female phase). Because its male phase (stage C) appeared prior to the female phase, the hermaphroditic floret of *B. pilosa var. radiata* could be considered as protandrous. However, further assessments revealed that there was some overlap between functional male and functional female phase. Though pollen viability decreased gradually from stage A to stage F (Figure 5), there were about 20% of pollen grains which remained viable at stage D during the presence of the functional female phase. Thus, protandry was incomplete in this plant. A similar situation was also found in *Mikania micrantha* (Hong et al., 2008), another invasive weed belonging to the Asteraceae.

Cruden (1977) measured P/O ratio of many taxa with different breeding systems to investigate the relationship between P/O ratio of these plants and their breeding system. It was found that P/O ratio was highest (2,108–195,525, n = 25) in obligately xenogamous species and decreased from obligate xenogamy, facultative xenogamy, facultative autogamy, obligate autogamy to cleistogamy.

The size (range: 20–40 μm) and the morphology of the pollen grains (with many spinules, xeromorph) are adapted for adherence to insects vectors (Wodehouse, 1935). It was reported that *B. pilosa var. radiata* could not produce seeds in an insect excluded greenhouse (Hsu, 2006). Thus, it is highly possible that *B. pilosa var.*
radiata is self-sterile or self-incompatible and depends on animals for cross-pollination.

Autogamous (self-pollinating) plants can produce offspring and establish a new population from a single individual, thus have reproductive assurance. On the contrary, xenogamous (cross-pollinating) plants need another individual plant and vectors for pollination. Thus, the autogamous plants are expected to have higher propagation rates than xenogamous ones in many closely related taxa (Erbar and Langlotz, 2005). However, xenogamous plants can increase genetic heterogeneous, thus are favored in heterogeneous and variable environments. It was reported that B. pilosa var. radiata could reproduce asexually by clonal growth (Hsu, 2006; Huang, 2008). Thus, the combination traits of vegetative reproduction and xenogamy might contribute to the widespread distribution of this invasive plant in Taiwan.

In conclusion, Bidens pilosa var. radiata has the mechanism of secondary pollen presentation. The growing style brushes and pumps the pollen grains out of the anther tube. Results from the observation of the flowering process, the test of stigma receptivity and the measurement of pollen viability revealed that B. pilosa var. radiata is incomplete protandrous. The high P/O ratio found in this plant suggested that B. pilosa var. radiata might be obligately xenogamous.

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**臺灣入侵植物大花咸豐草花部生物學之研究**

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外來植物入侵對全球生物多樣性造成嚴重的威脅。大花咸豐草被引入臺灣後，已成為嚴重的入侵植物之一。了解入侵植物的生殖生物學可以幫助我們有效控制其擴散。本研究主要在探討大花咸豐草的花部生物學，包含其 (1) 花部形態，(2) 次級花粉現象，(3) 花粉 / 胚珠比。將採自島南的大花咸豐草種子種植在盆子中並放置於台大實驗農場；待植物開花後，觀察和分析其特性。觀察發現大花咸豐草的一個頭狀花序上，有 5-8 個不孕的舌狀花和許多可孕的管狀花；除了觀察管狀花、花藥筒、花柱與花粉的形態，並定量一個頭狀花序上管狀花的數量、一個管狀花中花粉的數量與花粉 / 胚珠比；又根據花藥筒與花柱的形態將小花綿綿過程分為六個階段，並分別檢驗這六個階段的花粉與柱頭活性。結果發現：一個頭狀花上管狀花的數量 (19-61, 平均 44.1) 與一個管狀花所含花粉數量 (6,556 – 11,378) 在個體間有顯著差異。大花咸豐草的次級花粉呈現機制是由花柱伸長將花粉推出花藥筒。花粉與柱頭活性測定結果顯示，雖然雌性功能成熟期較雄性成熟期早，但兩者並未完全分開，故為不完全雄先熟。又根據花粉 / 胚珠比 (8,827 ± 464) 的結果推測大花咸豐草的有性繁殖方式可能是完全異交。

**關鍵詞：**自交；大花咸豐草；頭狀花序；花粉 / 胚珠比；雄先熟；次級花粉呈現；柱頭乳突；掃粉毛；異交。