TYPES OF DICHOGAMY, BREEDING SYSTEMS AND POLLEN LIMITATION ON *Aeschynanthus pulcher* (Blume) G.Don. (Gesneriaceae)

Tipe dikogami, sistem penyerbukan, dan keterbatasan serbuk sari pada *Aeschynanthus pulcher* (Blume) G.Don. (Gesneriaceae)

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Abstrak

Observasi tentang tipe dikogami, sistem penyerbukan, dan keterbatasan serbuk sari telah dilakukan pada populasi *Aeschynanthus pulcher* di Kebun Raya Cibodas. Pengamatan fenologi bunga dilakukan untuk menentukan tipe dikogami. Dalam rangka menentukan sistem perkawinan dan keterbatasan serbuk sari, lima perlakuan penyerbukan telah dilakukan yaitu penyerbukan bebas sebagai kontrol, penyerbukan silang, penyerbukan sendiri, autogami, dan agamospermi. Hasil pengamatan menunjukkan bahwa dikogami pada *A. pulcher* adalah protandri, tidak sempurna dan durasi tampilan serbuk sari dan stigma reseptif masing-masing yaitu 3-9 hari dan 6-12 hari. *A. pulcher* merupakan tumbuhan yang dapat membuahi sendiri, sedangkan proses autogami dan agamospermi tidak terjadi. Sindrom keterbatasan serbuk sari pada populasi *A. pulcher* yang diamati diindikasikan terjadi. Derajat keterbatasan serbuk sari pada *A. pulcher* mencapai 0.79-0.80. Tiga faktor yang dapat menyebabkan keterbatasan serbuk sari adalah (1) kompetisi antar jenis tumbuhan yang berbunga bersamaan, (2) perilaku berbunga dalam satu periode yang sama, dan (3) kompetisi antara polinator dan pencuri nektar.

Kata kunci: *Aeschynanthus pulcher*, dikogami, sistem penyerbukan, keterbatasan serbuk sari, fenologi pembungaan.

INTRODUCTION

The genus *Aeschynanthus* Jack. (Gesneriaceae) has two major clades (strong possibility as subgenus), seven sections and approximately consists of 160 species (Mendum *et al.*, 2001; Denduangboripant *et al.*, 2001). The member of the genus distributes from Sri Lanka and Himalaya to Papua and Solomon Islands.

It is widely known that genus *Aeschynanthus* has dichogamous flowers (temporal separation of pollen presentation and stigma receptivity) (Backer and Bakhuizen, 1965; Middleton, 2007). Lloyd and Webb (1986) divide types of dichogamy based on five categories, i.e. (1) the order of presentation, (2) the floral unit involved (for diclinous species), (3) the degree of separation, (4) interval between presentation of pollen and stigmas, and (5) degree of flowering synchrony. Dichogamy on *Aeschynanthus* based on the order of presentation is protandrous (Docters van Leeuwen, 1937; Backer and Bakhuizen, 1965; Wang *et al.*, 1998; Middleton, 2007).

Dichogamy is related to breeding systems and pollination (Llyod and Webb, 1986; Bertin and Newman, 1993). It influences the sexual systems and selfing ability of flowering plant species. Routley and Husband (2003) stated that dichogamy may reduce pollen discounting (amount of pollen augmentation to self pollination) and possible to reduce total of pollen receipt by the stigmas (pollen limitation). Even though, there are lot of evidences that pollen limitation occurs on numerous flowering plant species, but there is less attention on its prevalence on dichogamous plant on recent review (Burd, 1994; Larson and Barrett, 2000; Knight *et al.*, 2005).

Aeschynanthus pulcher is distributed from Thailand, Malay Peninsula, Sumatra, Java, Borneo to Bali (Van Steenis, 1975; Middleton, 2007). *A. pulcher* is closely related to *A. radicans* Jack. Middleton (2007) distinguished *A. pulcher* and *A. radicans* based on presentation of hair on ovary and under leaf surface. Even though that characters are also noted by Backer and Bakhuizen (1965), but they assumed that *A. pulcher* is one of variation forms of *A. radicans*.

Aeschynanthus pulcher grows naturally as an epiphytic plant on tree in Cibodas Botanical Garden. The garden is located on northern slope of Gede-Pangrango Mountain at 1.300-1.425 m asl. Annual rainfall is about 3.380 mm per year.

The objectives of the study are to define types of dichogamy, breeding systems and degree of pollen limitation on *Aeshynanthus pulcher* in Cibodas Botanic Garden.

MATERIALS AND METHODS

The observation was conducted in Cibodas Botanic Garden from November 2007 to February 2008 and November 2008 to February 2009. *Aeschynanthus pulcher* at 8-10 host plant were observed to determine the flower development, breeding systems, and pollen limitation.

Sixty flowers of *A. pulcher* were tagged randomly, before anthesis at eight host plants. Total individuals of the species at host plant was counted. The day of anthesis of each flower was recorded and then harvested (0, 1, 3, 5, 7, and 10 days after anthesis). Some flowers were dropped before harvest time. The length of calyx, corolla, stamen (upper and lower part), gynoecium, and diameter of stigma of 6-10 flowers for each harvest time were measured. To determine flowering synchronization, the number of staminate and pistillate flowers at ten host plants were counted every week, from early to late flowering season in 2008-2009.

Five pollination treatments, i.e. open pollination, hand crossing, hand intrafloral selfing, autonomy, and agamospermy were set to determine the breeding systems. Flowers were covered with polyethylene before anthesis and after pollination on hand crossing, hand intrafloral selfing, and autonomy to prevent random pollen contamination. Emasculation was done for hand crossing and agamospermy before anthesis. The stigma was cutted before the anthesis on agamospermy to determine the existence of apomictic syndrome. Pollen limitation were counted based on fruit set initiated. Index of pollen limitation (L) follows Larson and Barrett (2000):

$$L = 1 - \left(\frac{Po}{Ps} \right)$$

Where *Po* is the percentage of fruit set on open pollinated control and *Ps* is the percentage of fruit set by artificial supplementation pollen. If L = 0, it indicates that there is no pollen limitation on the population.

RESULTS

Flower development

The development of flower are divided into 7 stages (Fig. 1). Stage A, flower buds (the small inflorescens hides inside the bracts before the calyx opened). Stage B, calyx stage (the calyx development periods from small calyx to mature size). Stage C, lipstick phase (periods of corolla development). Stage D, staminate phase (flower opens and the fresh stamens appear containing mature anther with mature pollen grain). Stage E, overlap phase (periods of anthesis with receptive stigma and fresh stamen). Stage F, pistillate phase (periods after filaments and corolla wilted). Stage G, fruit set (fruit development). The fruit matures after 3-6 months.

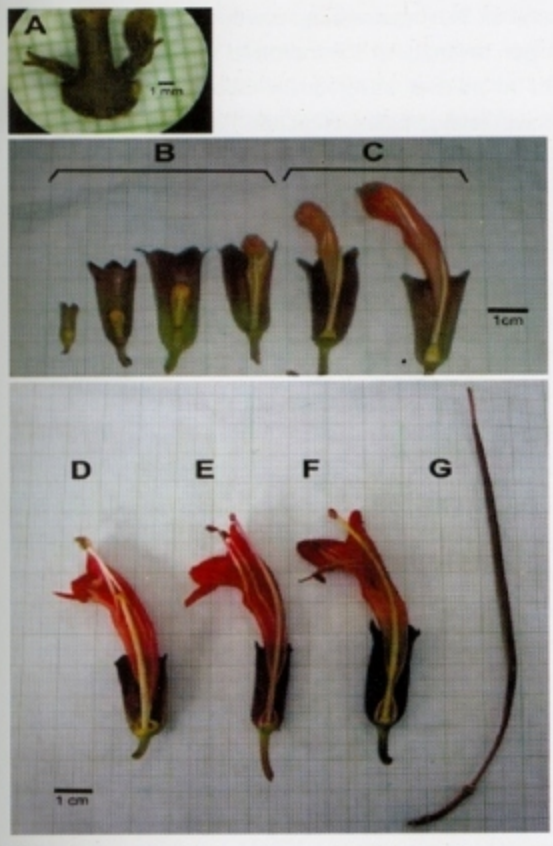

Figure 1. Longitudinal section of *Aeschynanthus pulcher* (Blume) G.Don flower on different development stage. (A) flowers bud phase, (B-C) un-opened flowers stage (B. calyx development phase and C. lipstick phase), (D-F) opened flower stage (D. staminate phase, E. overlap phase, and F. pistillate phase), and (G) fruit set.

There are differences among stages of flower development. Duration of staminate phase, overlap phase, and pistillate phase are 6.33±2.29 days (n=36), 3.27±2.08 days (n=18), and 6.16±3.36 days (n=18), respectively (means±SD).

During flower anthesis, not all part of the flowers are on maximum sizes, except the calyx and corolla. From anthesis up to 10 days after anthesis (DAA), the length of the calyx and the corolla is almost similar. The length of calyx and corolla were 2.57±0.32 cm and 6.18±0.36 cm, respectively (mean±SD) (n=45). While the length of stamen sligthly increase from the day of anthesis to 3 DAA and then shrivel. The mean length of the stament is 6.51±0.49 cm (upper part) and 6.23±0.44 cm (lower part) (n=23), (mean±SD). The gynoecium and the stigma were continue growing significantly after anthesis to 10 DAA (Fig. 2), and the distance between the stigma and the anther were decreased.

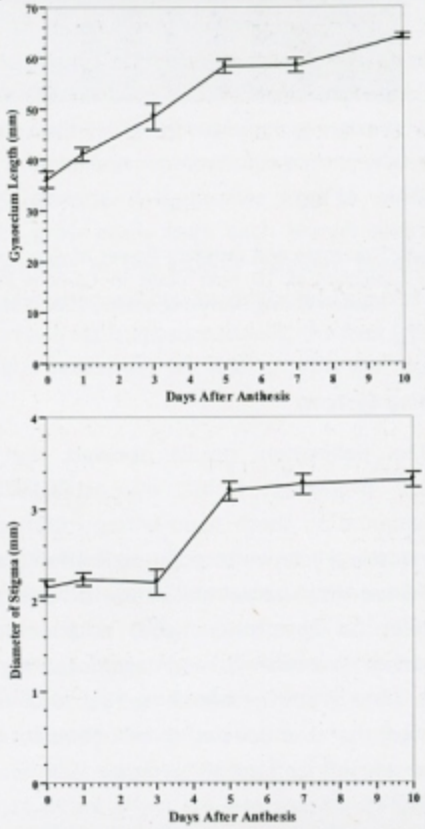

Figure 2. (A) The development of gynoecium of *A. pulcher* after anthesis. (B) The development of stigma (mean±1SE) of *A. pulcher* after anthesis. Bar (mean±1SE).

Flowering Synchronization

Based on weekly observation, the peak of flowering season of *A. pulcher* was at the end of January to early of February (Fig. 3). Both staminate and pistillate flowers of *A. pulcher* were found on all host plants and often on the similar branch. At the beginning flowering season, the staminate flowers were more dominant than pistillate flowers. While on the peak of flowering season, pistillate flowers were dominant.

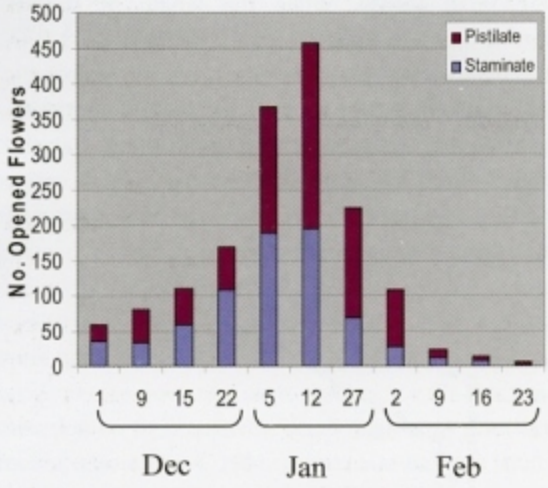

Figure 3. Staminate and pistillate flower of *Aeschynanthus pulcher* at 10 host plant in Cibodas Botanical Garden during flowering season 2008-2009.

Breeding Systems

The pollination results showed that more flowers produced fruits by artificial pollen supplementation (both hand-selfing intrafloral and hand crossing) relative to open pollination (Table 1). It indicated that natural pollination is relatively not effective. On the other hand, autonomous self pollination (autonomy) and agamospermy were failed. Data from intrafloral selfing and crossing indicated that the species is self compatible. The degree of self compatibility index (SCI) was high (1.20).

Table 1. Fruit sets and self compatibility index (SCI = percentage of selfing/percentage of crossing) of *A. pulcher* on five different pollination treatments.

Treatment	No. Flowers	Fruit Set	%	SCI
Control (open pollination)	40	7	17.5	
Hand-selfing intrafloral	20	20	100	1.2
Hand-crossing	24	20	83.3	
Agamospermy	20	0	0	
Autonomy	20	0	0	

Pollen Limitation

Artificial pollen supplementation could increase the percentage of fruit set to 79% (hand-crossing) and 82.5% (hand-selfing intrafloral) in comparison to open pollination. It is indicated that pollen limitation of *A. pulcher* observed in Cibodas Botanic Garden have found. Index of pollen limitation varied from 0.79 to 0.82.

DISCUSSION

The results of dichogamy observation on *A. pulcher* could be separated into four categories. First, the anther matures earlier than the stigma. It indicated that *A. pulcher* flower is protandrous as other species within the genus. Second, there are overlapped phases between staminate and pistillate phases. It is suggested that *A. pulcher* has incomplete temporal separation of two distinct phases. Duration of the overlapping phase took 1-6 days before the filament wilted. Third, the pollen and the receptive stigma were seen 3-9 days and 6-12 days after anthesis, respectively. Fourth, both of staminate flower and pistillate flower were found on each host plant and on each terminal branch (Fig. 3). It indicated that *A. pulcher* have asynchronous flowering type and also presented herchogamous flower syndrome (spatial separation on surface of pollen presentation and pollen receptive (Webb and Lloyd, 1986)). In the early stage of anthesis, the stigma is located deeply below the anthers. The

stigma was developed gradually until the position is above the anthers on the late of anthesis.

The results were shown that the degree of self compatibility index (Table 1) supported Burt and Woods (1975) (Middleton, 2007) that most of species of *Aeschynanthus* are self compatible. While, agamospermy and autogamy seems not to occur in natural habitat. Lloyd and Schoen (1992) separated autonomous self pollination (autogamy) into three modes. The modes are prior selfing (pollination before anthesis or before crossing available), competing selfing (pollination during anthesis or during crossing available), and delayed selfing (pollination at the end of anthesis or crossing not available). Prior selfing does not happen on *A. pulcher,* because it is dichogamy. Competing and delayed selfing are also hard to occur naturally. It is caused by design of flower morphology of *A. pulcher* that not allow the stigma and the anther splits (anther surface that contains pollen grain) to contact each others. Even on overlapping phase, position of stigma is behind of the anthers. Anomalous evident on congeners was reported in *Aeschynanthus horsfieldii*. The species cultivated in Cibodas Botanical Garden produces set fruit on the absence of pollinator. On the other hand, *A. pulcher* do not have abiotically pollinated syndrome. Based on the observation, the pollen grains are limited to discount until the filament wilted, if the flower are not visited by pollinators. It showed that both self and cross pollination on *A. pulcher* flowers are facilitated by pollinators.

In terms of plant-pollinator interaction, there are global trend that pollination are on crisis (Wilcock and Neiland, 2002; Kearns *et al.*, 1998). On the recent assessment, many plant species have failed on their sexual reproduction because limitation on pollen that they receipt (Vamosi *et al.*, 2006). This phenomenon is increased in rich diversity species of hotspots regions. One of the hotspots regions is the Sundaland (Sumatra, Java, Borneo, and Malay Peninsula), which has 15,000 endemic species (Myers *et al.*, 2000). On rich diversity species region, the existence of co-flowering species will reduce pollination success because it is increased the interspesific competition (Sargent and Ackerly, 2008; Chitka and Shurkens, 2001).

Other factors that influence pollen limitation on *A. pulcher* are the flowering behavior and pollinator-nectar robber competition. *A. pulcher* produces many flowers in one flowering season and almost all flowers are grown on dense group on each terminal branch. This condition will reduce pollination efficiency among the flowers because they are overlap and cover on each others (Dafni, 1994). On the other hand, McClure (1966) noted that flowers of *Aeschynanthus* sp. were visited by *Arachnothera* bird (spider hunter bird which belongs to family Nectariidae) and the nectar robber green leaf bird (*Chloropis* bird which belongs to family Aegthinidae). On the early and peak flowering seasons of *A. pulcher* in Cibodas Botanic Garden, many flowers were damaged by nectar robber. It suggested that there are competition between pollinator and nectar robber as noted by Irwin and Brody (2000).

The increasing degree on pollen limitation will lead to the increasing of the extinction risk of the flowering plant species (Vamosi *et al.*, 2006). However, some plant species have other strategies on their reproduction besides sexual reproduction to maintain their existence (compensatory traits) (Bond, 1994). In *A. pulcher*, compensatory traits would offer clonality process. It is possible since *A. pulcher* as creepers grow easily from each branch nodes that make contact to the growth medium. Eventhough, pollen limitation will reduce pollination success when flowers have set fruits successfully, the fruit (contains numerous seeds) will be able to grow and disperse widely.

CONCLUSION

Aeschynanthus pulcher flowers are protandrous, incomplete dichogamy, presented pollen on 3-6 days and the stigma receptive after 6-12 days, and asynchronous flowering. Pollination of the species are facilitated by pollinators. It is clear that the degree of pollen limitation index on *A. pulcher* is high. Three possible factors influence pollen limitation index, there are interspesific co-flowering competition, general flowering behaviour, and pollinator-nectar robber competition.

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