FLOWERING PHENOLOGY AND FLORAL BEHAVIOR OF *Scutellaria discolor* Colebr. AND *S. slametensis* Sudarmono & B.J. Conn (Lamiaceae)

[Fenologi dan Perilaku Pembungaan pada *Scutellaria discolor* Colebr. dan *S. Slametensis* Sudarmono & B.J. Conn (Lamiaceae)]

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**ABSTRAK**

Kata kunci: kasmogami, kleistogami, perilaku bunga, fenologi pembungaan, *Scutellaria discolor*, *S. slametensis*

**ABSTRACT**
The flowering phenology and floral behavior of *Scutellaria discolor* and *S. slametensis* were investigated. *S. discolor* is distributed over a wider range of habitats but *S. slametensis* is distributed over a restricted area on Mt. Slamet. Observations of flowering phenology and floral behavior were almost the same and pollination of cleistogamous flowers did not occur in both species. Based on our observations on floral and pollinator behavior, we consider both *S. discolor* and *S. slametensis* to have chasmo- and cleistogamous flowers.

Keywords: chasmogamy, cleistogamy, floral behavior, flowering phenology, *Scutellaria discolor*, *S. slametensis*

**INTRODUCTION**
Flowers are the functional units of plants for sexual reproduction. Pollen acts as the floral attractant for reproduction in the male phase of maturation while nectar serves to attract pollinators, which touch pollen that then attaches to the stigma in the female phase. Wind can also move small pollen which might increase genetic diversity by outcrossing or, if within flowers, ensure the production of offspring by self-pollination.

In several species, flowers are dichogamous, which is commonly attributed to selection to avoid self-fertilization (Bertin, 1993; Sudarmono and Okada, 2003). On the other hand, in the female phase, on the first day of anthesis, the stamens release pollen and recurve downward. At that moment the style is newly opened and maintains the status of a stigma in the female phase, and is receptive. In protandrous species with dichogamous flowers, dichogamy may have played a key role in the evolution of protandry or protogyne as a barrier to self-fertilization (Navarro, 1997). However, protandry is common in flowers of members of the Lamiaceae (Owens and Ubera-Jiménez, 1992) and is an effective barrier to self-fertilization in *Lavandula stoechas* L. (Munoz and Devesa, 1987). In protandry, however, self-fertilization is not precluded (Ubera and Valdés, 1983) although the flowers that cease to be attractive to pollinators, shortly following pollination, tend to be from families that are known mainly to comprise species in which flower longevity, petal colour, or flower closure is sensitive to exogenous ethylene (van Doorn, 1997).

Claßen-Bockhoff (2007) predicted that bilabiate blossoms characteristic of the Lamiaceae allow for nototribic (dorsal) pollen deposition. Walker and Sytsma (2007) investigated in *Salvia*, the largest group in the Lamiaceae (Claßen-Bockhoff et al., 2004), that parallel evolution was characterized by the well-known staminal lever mechanism in which the bee has to push back the movable upper lip, a staminal lever, to gain access to nectar; as a result it is loaded with pollen on its forehead.

Many factors are known to affect genetic diversity within species, and parameters in genetic variation such as interbreeding between populations,
allele heterozygosity, genetic diversity, genetic differentiation and gene flow can provide information on gene mutation, genetic drift, bottle-necks, and even the endangered status of a population (Hamrick et al., 1979), among which breeding systems and distribution patterns are the most important (Olmstead, 1990). Therefore, analysis of breeding systems is an important step to understand evolutionary processes (Grant 1981) and for conservation purposes (Proctor et al., 1996). It is expected that xenogamous species maintain high genetic diversity within populations, while cleistogamous (CL) species consist of genetically uniform individuals (Loveless and Hamrick, 1984; Hamrick and Godt, 1990). It has been considered that in this type of floral dimorphism outcrossing with other plants via chasmogamous (CH) flowers could ensure seed set by selfing in CL flowers (Culley and Klooster, 2007). As a multiple reproductive strategy, dimorphic CL species produce both closed (CL) and open (CH) flowers on the same or on different individuals. A study by Pitts-Singer (2000) showed that visits by bees did not benefit cleistogamy in Scutellaria floridana Chapman. Conversely, Sun (1999) inferred that the CL mating system in Scutellaria indica is effective and that the population is genetically structured. If a xenogamous species contains very low genetic diversity, some abnormal events have to have occurred in the history of the species (Sun, 1999).

Scutellaria (Lamiaceae) is the largest genus of the family with about 360 species (Huang, 1994; Paton, 1990; Paton in Harley et al., 2004). The genus is widespread, subcosmopolitan, but poorly represented in moist tropical lowlands. There are currently four known species in Indonesia, namely, S. discolor Colebr., S. indica L., S. javanica Jungh. and S. slametensis Sudarmono & B.J. Conn (Backer and Bakhuizen van den Brink Jr 1965; Keng 1978; Steenis 1972; Sudarmono and Conn 2010). The Indonesian species are all members of the subgenus Scutellaria sect. Scutellaria (Paton, 1990) and are informally classified (Conn, 2009) personal communication) into three species-groups namely: ‘S. discolor’ (only including S. discolor); ‘S. humilis’ (including S. javanica); ‘S. violacea’ (including S. indica). The ‘species-group’ of S. slametensis is unclear.

The objective of the present study was (1) to observe flowering phenology and floral behavior of S. discolor and S. slametensis and (2) to analyze pollinator behavior on CL flowers of both Scutellaria species.

MATERIALS AND METHODS

Sample collection

Samples of S. slametensis were collected from areas of Mt. Slamet at different altitudes, namely: (i) from the southern part of Mt. Slamet, on the Baturraden climbing track occur between elevations of 1,390 and 2,215 m, (ii) from the western part of the mountain along Kaligua and Brebes (on Paron Block, a border area of the southern and western part of Forest Holding Unity Division (BKPH) on the top of Mt. Slamet) route in the PTPI IX National Tea Plantation between elevation of 1,802 m and 1,980 m. Samples of S. discolor were randomly collected from the Camping Ground and Telaga Sunyi of Baturraden, Mt. Slamet at elevations less than 800 m. Populations consisted of ~100-500 individuals depending on the location or topography. Fifteen individuals were collected, 10 of which were used in treatments, and 5 were used in case treatment individuals withered or died. Collections of all populations were cultivated at Kebun Raya Bogor. They were planted in plastic bags in a screening house with an average temperature of 23.6°C at 08:00 am and 31.4°C at 01:00 pm as well as 305.54 mm of rainfall per month from January to November 2008.

Flowering phenology

Observations of flowering phenology were carried out using 10 individual of S. discolor and 20 individual of S. slametensis flowers randomly selected per location. A total of 30 flowers were observed every week.

Floral behavior

The floral behavior of S. discolor and S. slametensis was observed using 30 flowers of 6 individuals cultivated in Bogor Botanical Gardens. S. discolor and S. slametensis flowers produced a closed corolla (true cleistogamy) or facultative cleistogamy (semi-opened corolla). At first, all flower buds were numbered. The condition of petals, stamens and style was observed every two hours from 5 am to 7 pm daily from June 25 to November 30, 2008.
Receptivity of the stigmatic area was examined as follows. Flower buds were bagged. Pollen grains (approx. 10-20) from 30 different randomly selected individuals were dusted on the stigma. After 4 hrs of hand-pollination, flowers or flower buds were fixed with FAA (fixation acetic acid). They were then soaked in 8 N NaOH for about 8 hrs and stained with 0.1% aniline blue solution in 0.1 N K_2PO_4 and observed with a fluorescence microscope (Martin, 1958).

**Crossing tests**

To analyze *Scutellaria* breeding systems the following six treatments were applied: T1) control: flowers were left in natural conditions; T2) bagging: flower buds were bagged with a 30 mm diameter nylon mesh bag (EW-06630-88 Nylon Spectra/Mese® USA) to exclude insect visitors and wind pollination (pollen grains of *S. discolor* and *S. slametensis* were about 40-42 mm in diameter); T3) emasculation: before anthesis all stamens were emasculated and bagged. Fourth and fifth treatments were carried out to examine self compatibility; T4) self pollination: after emasculation the stigmatic area was dusted with pollen grains from the same individual; T5) cross-pollination 1: after emasculation the stigmatic area was dusted with pollen grains from a different individual in the same population; T6) cross-pollination 2: after emasculation the stigmatic area was dusted with pollen grains from a different individual in the same population; T6) cross-pollination 2: after emasculation the stigmatic area was dusted with pollen grains from an individual of a different population (populations from the southern and western parts of Mt. Slamet for *S. slametensis*; Camping ground and Telaga Sunyi population for *S. discolor*). After one month the fruits (4 fruits/flower; total = 120 fruits) were harvested from T1-T6. Seed set was estimated from maturation of seed by a change in the color from green to dark brown; each flower contained 4 seeds for each treatment.

**RESULTS**

**Flowering phenology**

Flowering phenology of *S. discolor* and *S. slametensis* was observed from June to November in 2008. The flowering peak of most populations studied was in August (63.8%). The mean maximum daily temperature in August was about 32.1°C (Botanical Garden Documents, 2008). Flower visitors between 07.00 am and 17.00 pm were honey bees, namely *Thyrea japonicus, Ceratina flavipes* and *Apis indica japonica*, all Apidae, and some butterflies. *S. discolor* displayed flowers for a shorter period (2 months) than *S. slametensis* (6 months). The flowering peak of *S. discolor* was in September - October.

**Floral behavior**

Anthesis started asynchronously in individual *S. discolor* and *S. slametensis* flowers between 06.00 am and 05.00 pm. After flowers opened petals remained continuously open for 5-6 days, then withered or senesced (Fig. 1). On the first and second days of anthesis, stamens successively released pollen while the stigmatic area remained closed. On the third day, stamens recurved downward, and at the same time the stigmatic area was newly opened, doing so until the sixth day. During this 6-day period, the stigmatic area was receptive since pollen grains dusted on the opened stigmatic area could successfully germinate (Fig. 2). Since the stamens and stigmatic area mature at different times (i.e. between the first day at 06:00 am to the third day at 06:00 am), autogamy is prevented. Based on this evidence, *S. discolor* and *S. slametensis* are protandrous and allogamous, respectively.

**Crossing tests**

Crossing tests were carried out to examine the breeding system (e.g. self-compatibility, apogamy, etc.) of *S. discolor* and *S. slametensis* (Table 1). T1 (control) resulted in 33.3% ± 0.13% (± standard error) and 20% ± 0.15% seed set (*S. slametensis* and *S. discolor*, respectively). T2 (bagged) formed 36.7% ± 0.15% seed set (*S. slametensis* and 23.3% ± 0.10% seed set (*S. discolor*) but T3 (emasculations) formed no seeds, which indicated no possibility of autogamy and agamospermy in both *S. discolor* and *S. slametensis*. T4 (self-pollination) resulted in 43.3% ± 0.16% seed set (*S. slametensis*) and 66.7% ± 0.15% seed set (*S. discolor*). T5 (cross-pollination 1) resulted in 23.3% ± 0.16% and no seed set for *S. slametensis* and *S. discolor*, respectively. T6 (cross-pollination 2) resulted in no seed set. The low seed set by the cross-pollination test showed that *S. discolor* and *S. slametensis* were not complete but mostly self-compatible (Fig. 2).

**DISCUSSION**

Previous studies indicated that reproductive
traits have diverged variously even among closely related species within a taxonomic group (Ohashi, 2002; Claßen-Bockhoff et al., 2003). Cooley and Klooster (2007) reported that most Scutellaria species with several varieties demonstrated self-pollination. Sun (1999) reported that Scutellaria indica showed both chasmogamy (CH) and cleistogamy (CL) and that there were 19 times more CL flowers than CH flowers indicating a much greater success of self fertilization. Cooley and Klooster (2007) renamed this category dimorphism instead of true CL or facultative CL to emphasize the production of different floral forms. Scutellaria CL played a major role as key structures in evolution (Sun, 1999). The present study clearly indicated that S. discolor and S. slametensis were protandrous and mostly self-compatible, just as other Scutellaria species such as S. indica (Sun, 1999). S. discolor and S. slametensis had high fruit set when pollinated within a flower (Table 1; Fig. 2). Flowering in both species was asynchronous, i.e., the order of flowering within a population proceeded differently from each other. One of the effective pollinators of S. discolor and S. slametensis was honey-bees judging from field observations. The long-tongued bees visited to collect nectar and then they buzzed the corolla with their heads, in a process termed "floral sonication" or "buzz pollination" (King and Buchmann, 2003). Bumble bees vibrate their flight muscle to make a sound at a certain frequency that causes anthers to release their pollen. Although carpenter bees are also capable of sonicating flowers, we saw them "robbing flowers", i.e. stealing nectar by cutting into the base of the tube of the flowers. With this behavior, these bees do not touch the sexual parts of the flower and do not pollinate them. Thus the floral rewards are taken, but the bees' visits do not benefit the plant. Pitts-Singer (2000) predicted that bees i.e. leafcutter bees (Family Megachilidae) and sweet bees (Family Halictidae) are important pollinators of Scutellaria floridana or Florida Skullcap. At the peak of anthesis of S. discolor and S. slametensis (in the dry season) pollinator activities were sufficient, while in the rainy season (November) their activities were reduced due to wet weather. Bertin (1993) explained that monoecy and dichogamy are most likely uncommon among self-incompatible species because physiological barriers prevent self fertilization in such species. This statement supports results of the crossing test for S. slametensis that indicated self incompatibility (Table 1). Conversely, S. discolor was absolutely selfing. This result indicates that floral traits are not uniform in S. slametensis and S. discolor and that some S. slametensis flowers show facultative CL judging from observation. Perhaps self-pollination in S. discolor acts as an effective reproductive isolation system. This assumption is supported by a case of Ranunculus cantoniensis investigated by Okada and Kubo (1999) in which this species originated as hybrid offspring of R. silerifolius and R. chinesis. While S. slametensis results in both self- and cross-pollination seed set (43.3 and 23.3%, respectively: Table 1), it is perhaps between- and within-population variation in corolla shape that shows that S. discolor and S. slametensis are morphologically distinct and do not integrate (see Fig. 3), except for places where they co-existed on Mt. Slamet (Sudarmono and Conn, 2010). Ford and Johnson (2008) concluded that long-tubed species have the highest diversity of floral visitors; their nectar rewards are greater than those of species with a short corolla tube, yet are accessible due to a broad corolla mouth. S. slametensis with a long corolla tube have exposed anthers (Fig. 3) and produce pollen and thus also receive visits by pollen-collecting bees.

I conclude that protandrous S. discolor and S. slametensis were permitted both self pollination within flowers, namely, geitonogamous pollination. But cross pollination on S. discolor acts as an effective reproductive isolation system. This manuscript was supported by a case of Ranunculus cantoniensis investigated by Okada and Kubo (1999) in which this species originated as hybrid offspring of R. silerifolius and R. chinesis. While S. slametensis results in both self- and cross-pollination seed set (43.3 and 23.3%, respectively: Table 1), it is perhaps between- and within-population variation in corolla shape that shows that S. discolor and S. slametensis are morphologically distinct and do not integrate (see Fig. 3), except for places where they co-existed on Mt. Slamet (Sudarmono and Conn, 2010). Ford and Johnson (2008) concluded that long-tubed species have the highest diversity of floral visitors; their nectar rewards are greater than those of species with a short corolla tube, yet are accessible due to a broad corolla mouth. S. slametensis with a long corolla tube have exposed anthers (Fig. 3) and produce pollen and thus also receive visits by pollen-collecting bees.

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REFERENCES


Martin FW. 1958. Staining and observing pollen tubes in the style by mean of fluorescence. Stain Technology 34, 125.


Pitts-Singer TL. 2000. Scutellaria floridana Chapman or Floridiana Skullcap, Family Labiatae. USDA Forest Service Technology Publishing R8TP Vols. 1 and 2, Atlanta, USA.


Ubera JL and B Valdés. 1983. Revisión del género Nepeta (Labiatae) en la Pen-sinsula Ibérica e Islas Baleares. Lagascania 12, 3-8.

Table 1. Results of crossing tests in *Scutellaria slametensis* and *S. discolor*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of samples</th>
<th>Seed set (%)</th>
<th>Mean ± Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Scutellaria slametensis</td>
<td>S. discolor</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>33.3 ± 0.13</td>
<td>20 ± 0.15</td>
</tr>
<tr>
<td>Bagging</td>
<td>30</td>
<td>36.7 ± 0.15</td>
<td>23.3 ± 0.10</td>
</tr>
<tr>
<td>Emasculation</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Self-pollination</td>
<td>30</td>
<td>43.3 ± 0.16</td>
<td>66.7 ± 0.15</td>
</tr>
<tr>
<td>Cross-pollination 1</td>
<td>30</td>
<td>23.3 ± 0.16</td>
<td>0</td>
</tr>
<tr>
<td>Cross-pollination 2</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

A. *Scutellaria slametensis*

B. *S. discolor*

0 12 24 36 48 60 72 84 96 108 120 132 144

Time (hours)

Fig. 1. Floral behavior of *Scutellaria slametensis* (A) and *S. discolor* (B). 0 at X axis: 0 a.m. At the first day of anthesis. There is possible deviation in the start/finishing time of petal movement, pollen exclusion and/or stigma receptivity among individual flowers.

![Floral behavior](image)

**Figure 2.** Flourescence (A) and contrast (B) microphotographs of selfing germinated pollen grains on the stigmatic area of *Scutellaria slametensis*. pg = pollen grain, pt = pollen tube, bar = 100 μm.
Figure 3. Inflorescences of *Scutellaria slametensis* (A) and *S. discolor* (B). uc = the upper lip of the corolla withered and closed; ct = corolla tube.