

Full Length Research Paper

Floral syndrome and breeding system of *Senna (Cassia) corymbosa*

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***Senna (Cassia) corymbosa* is an ornamental plant with asymmetric flower in which petals and stamens are also involved in floral asymmetry. The pollen number of abaxial lateral stamen (AL), abaxial median stamen (AM) and middle stamen (MI) are descended in sequence. In field, the insects of visiting flowers are available and pollinators are essential to the pollination success of *S. corymbosa*. Bombidae was presumably the effective pollinators by buzzing pollination and wasp may be the potential pollinators. Pollen number and germination rate per type of stamen experiments supported the hypothesis of “division-of-labour” among stamens by Darwin. Both AL, AM and MI may afford food to visiting insects, while long stamens (including AL and AM) function as the “pollinating” stamens and the brownish yellow is presumably the effective color attractants to pollinators.**

Key words: *Senna*, pollen, pollination, breeding system.

INTRODUCTION

Species of *Senna* were formerly included among the approximately 600 species of *Cassia* (Irwin and Turner, 1960). Subsequent taxonomic treatments subdivided this large genus into the smaller *Cassia*, *Chamaecrista* and *Senna* (Irwin and Barneby, 1982). The genus *Senna* worldwide comprises approximately 350 species of shrubs and herbs (Williams, 1998; Randell and Barlow, 1998). 80% of species of *Senna* occur on the American continent and some are found in tropical Africa and Australia, only a few species are distributed in southeastern Asia (Marazzi et al., 2006).

Senna corymbosa is an ornamental plant, which is a deciduous to semi-evergreen shrub to about 3 m, in the genus *Senna*. Its flower has three different types of stamens (that is, abaxial lateral stamen, abaxial median stamen and four middle stamens, hereafter AL, AM and MI). *S. corymbosa* can be grown in temperate climates as it is somewhat frost-hardy. It blooms from late summer through winter in frost free areas. Okogun et al. (2000)

studied the nitrogen contribution of *S. corymbosa* to alley cropped maize and the seed oils of *S. corymbosa* were analyzed by Hosamani and Sattigeri (2003). Yin et al. (2006) studied the relationship of karyotype analysis and modulate of *S. corymbosa*. The main aspects of seed ontogeny in *S. corymbosa* were studied by Rodriguez Pontes (2007). But the breeding system character was not described in detail. This study was set up to: (1) obtain the floral quantitative characters of *S. corymbosa*; (2) monitor the functional significance of style morphology and different stamens and discuss whether it support the hypotheses that, a ‘division-of-labour’ among stamens, with some stamens providing pollen as food, others providing pollen for fertilization; (3) observation of the behavior of visiting insects and which are potential pollinators? (4) discuss the pollination biology of the species.

MATERIALS AND METHODS

Study species and sites

This study was carried out in Anhui Province, China in 2008 and 2009. *S. corymbosa* used for this study were from herbarium specimens or from living materials in five populations in Anhui

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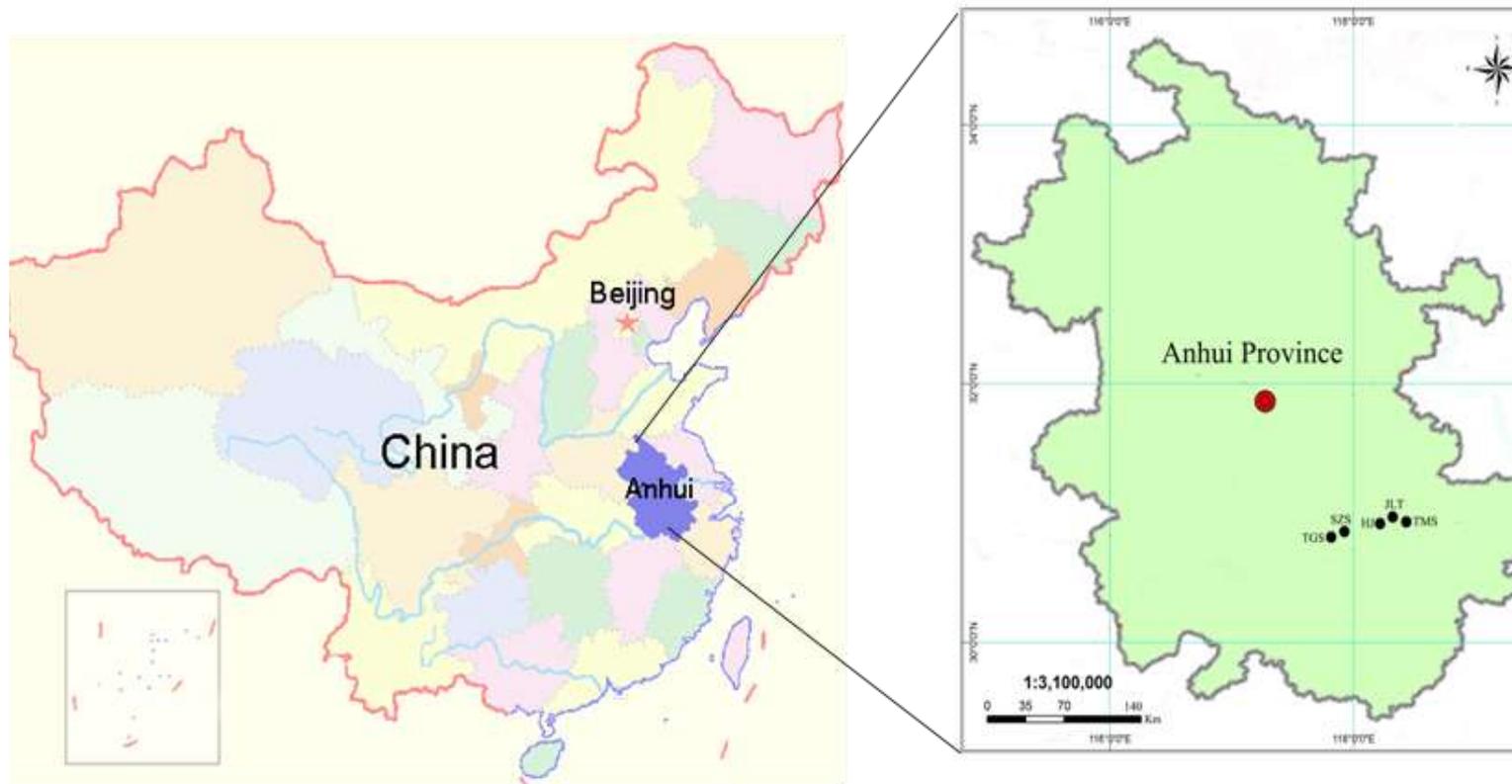


Figure 1. The position of study populations of *S. corymbosa* in Anhui Province, China (●: City; ▲: Population. SZS = Shi Zi Shan; TGS = Tong Guan Shan; JLT = Jiu Lian Tang; HJ =Hua Jin; TMS= Tian Men Shan).

Province (Figure 1). All vouchers were deposited at the Herbarium of Anhui Normal University, China.

Floral morphology

The data of floral morphology traits were from three study populations on October 2009, from which 30 flowers were taken from different individuals at random. We measured the height of stigma, three different types of stamens (AL, AM and MI), length and width of upper and lower petal, then

took the mean values, including 30 replicates. All floral measurements were made to 0.01 mm using digital calipers.

SEM observation of pollen

For SEM, pollen grains treated with the standard acetolysis method (Erdtman, 1960), were placed on double stick tape. Observations and photographs were taken with a Hitachi S4800 scanning electron microscope (Magnification ranges

from 30x to 800,000x).
Pollen number and size

Twenty (20) flowers that will open soon were taken at random at Wuhu Population. Different anthers (including AL, AM and MI) from each bud was carefully dissected and then crushed in a calibrated Eppendorf transferpettor. The solution (1:3 mixture of lactic acid and glycerin) was added to the Eppendorf transferpettor up to 1 ml. The suspension was stirred with a vortex mixer for 60 s in order to obtain a uniform pollen suspension. Five samples of 10 µl were

taken from this pollen suspension and all pollen grains were counted under 100x magnification. The total number of pollen grains in the five 10 µl subsamples was used to estimate the total number of grains in 1 ml, which would represent the number of pollen grains per flower (Vidal et al., 2006; Chen, 2009). Pollen sizes selected at random were measured with a micrometric ocular from 20 pollen grains from AM, AL and MI, respectively.

Rate of pollen germination

Pollen grains isolated from AL, AM and MI of *S. corymbosa* were immediately germinated *in vitro* in a culture medium containing 5% (w/v) sucrose, 0.01% (w/v) H₃BO₃, 0.01% (w/v) CaCl₂, 0.01% (w/v) KH₂PO₄, at 20°C and the pH was adjusted to 7.0. They were then observed under a microscope and the number of germinating and unchanged pollen grains was counted (only those pollen tubes with their length longer than the diameter of pollen grain were considered germinated) in 12 to 24 h. For each treatment, we selected five fields of vision and repeated it five times.

Fruit set and seed set

To test whether insects are essential for pollination of *S. corymbosa*, we enclosed five inflorescences on each of the five shrubs in JLT and Huajin populations in bags made of fine nylon mesh with openings of 0.30 to 0.33 mm to exclude pollinators, respectively.

Fruit set was observed from five study sites. Thirty (30) plants from each site were marked. The number of flowers, inflorescences and pods per flowering branch were recorded. The number of developing pods per number of impact flowers was gotten by counting 30 marked inflorescences. At the same time, we got the percentage of pods in inflorescences per flowering branch in 30 flowering branches.

To test the seed set of *S. corymbosa*, we recorded the seeds from 20 fruits under the treatment populations and the average number of seed per flower was gotten from the full developed pods in the experimental populations.

Observations and collection of flower visitors

Flower visitors were observed during peak blooming in JLT population on October 16 to 18, 2008 and in HJ population on October 20 to 22, 2009, but only species that came into contact with the stigma or stamens were considered potential pollinators. We conducted observations for six succeeding days for a total of 90 h from 08:00 until 18:00.

The visitation frequencies of different visitors to flowers were also recorded. In order to test the efficiency of transportation of pollen, visitors were captured with a handnet and brought to laboratory to be examined with the light microscope for the presence of pollen on their bodies. Voucher specimen of insect visitors have been deposited in the herbarium of Anhui Normal University, China. All statistical analysis was conducted using SPSS 11.5 software.

RESULTS

Floral characters

S. corymbosa had dark-green, blue-toned even-pinnately compound leaf with 4 or 6 leaflets, at the base of which grows extrafloral nectaries (Figure 2a). The flower is com-

posed of five yellow calyces which were different in size and three upper petals and two lower petals (Figure 2a, b). The three abaxial stamens often form two sets. The two lateral abaxial stamens belong to the inner androecial whorl, while the median abaxial stamen belongs to the outer one. The two lateral stamens in *S. corymbosa* have stout filaments with large, curved anthers, while the third centric stamen had a much smaller anther, but slightly longer filament. At the center of the flower, there are four short stamens with erect filaments and anthers. Both sets of stamens produce long globose tricolpate pollen grains (Figure 2c). The three adaxial stamens (staminodes) are sterile and flattened, producing no pollen. Moreover, the anthers of different types of stamens show different colors: yellow for the staminodes and brownish yellow for the other types. The sickle-shaped and curved pistil with pore on its tip emerges between the bases of AL and above AM. The length of pistil, in every case is longer than that of AL ($P < 0.05$), which is longer than that of AM ($P < 0.05$) (Figure 2d). All of the floral quantitative traits are given in Table 1.

Pollen number and size

The number of pollen grains from different sets stamens of the investigated species is compared in Table 2. Pollen quantity varied among stamen types. In all species investigated, the anther of the single AL stamen contains more pollen grains than the anther of the AM stamen followed by MI stamen ($F_{(AL, AM)} = 65.5$, $P < 0.001$; $F_{(AL, MI)} = 286.1$, $P < 0.001$; $F_{(AM, MI)} = 98.5$, $P < 0.001$). Moreover, the two long stamens (AL) contain many more pollen in total than the four short ones (MI) in *S. corymbosa* ($F_{(long, short)} = 24.5$, $P < 0.001$). We found no differences in structure or size of pollen grains from AM, AL and MI.

Pollen germination rate

Experimental results showed that, pollen germination rate from AL, AM and MI are 85.8 ± 6.36 , 82.5 ± 7.78 and $60.9 \pm 13.80\%$, respectively. Pollen germination rate from AL and AM had no difference ($F_{(AL, AM)} = 65.5$, $P > 0.05$), but both are higher than that from MI ($F_{(AL, MI)} = 54.0$, $P < 0.05$; $F_{(AM, MI)} = 37.3$, $P < 0.05$).

Fruit and seed set

The fruit set of *S. corymbosa* was always low (Table 3). In cultivation population, some fruit set have significant difference and some have no difference. When all flowers in bagged inflorescences had abscised, no fruit had developed from bagged flowers in two study populations. We found that not all inflorescence have pod but always

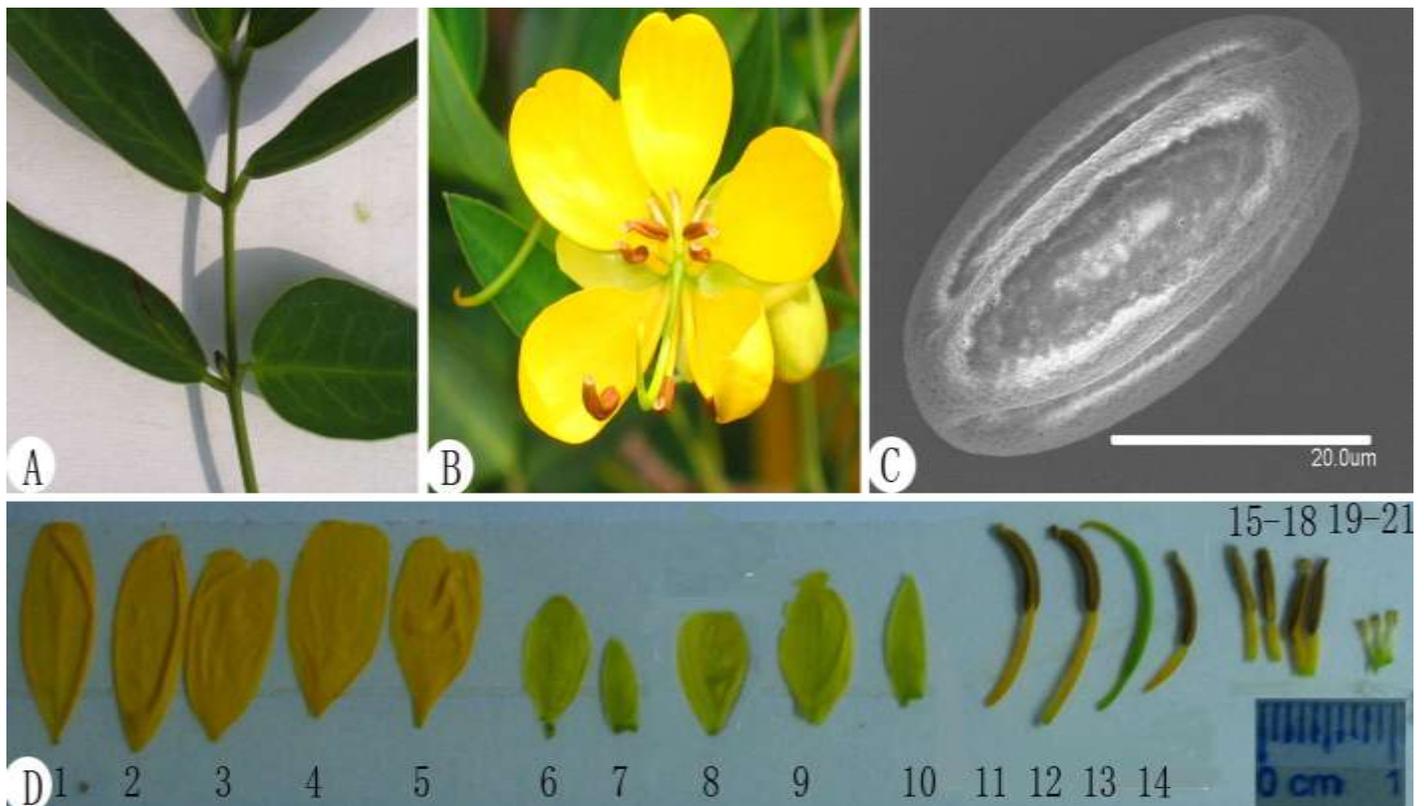


Figure 2. Leaf and flower of *S. corymbosa* (A: leaf; B: flower; C: pollen; D: 1-2: UP; 3-5: LP; 6-10: CA; 11-12: AL; 13: P; 14: AM; 15-18: MI; 19-21: AT).

one or no more than two pods are presented on the pollinated inflorescence. The fruit set of inflorescence to flower branch have no significant difference except in JLT population. At the same time, each fruit was evenly distributed on the branch. Comparatively, the seed set of *S. corymbosa* in the study populations is high (Table 2).

Behavior of flower visitors

During the 90 h field observations, we collected at least 10 species of flower visitors at the 2 study sites; their visiting frequencies are listed in Table 4. Some of the visitors are shown in Figure 3a to g. Individuals of *Apis*, Bombidae, Lycaenidae, *Pieris rapae* were the most common visitors and were observed in the study populations and the individuals of wasp was found in JLT and Huajin populations, while other insects were occasional visitors (Table 4).

During the visiting process, we found that individuals of *Apis* sp. visited not only MI, but also AL and AM as seen in Figure 3a, b, but its body was not sufficiently large to contact the tip of long anthers and stigma, at the same time, its vibration force is too weak to buzz the anthers. The Bombidae usually hovers momentarily over the flower and

forage pollen for a few seconds (usually 3 to 10 s) (Figure 3c), the vibration force by the wings is so strong that it is easy to vibrate the pollen from the anther and we found the pollens on the stigma when Bombidae visit a flower only one time. As for wasp, when foraging the pollen from MI, we found that the apical pore of AM, AL and the curved style are very easy to touch the dorsal side of the insect during visit.

Individuals of *P. rapae* and Lycaenidae were the frequent visitors of *S. corymbosa*, but we seldom found pollen adhered to its bodies. For the individuals of Mantidae, they mainly crawl between the leaf and inflorescence and we could not find the behavior of preying on pollen. Ants mainly explored extrafloral nectaries and flowers, but they were seldom found visiting AL and AM. When explored flowers, they were mainly for the pollen that remained on the petals due to previous visits of other buzzing visitors that can extract pollen and sometimes it nibble the petals of *S. corymbosa*. Some other insects also visited the flowers of *S. corymbosa*; their visiting frequencies were not high and regular.

DISCUSSION

According to Marazzi et al. (2006), species of *Senna* are

Table 1. Floral characters of *S. corymbosa*.

Item	Parameter	Value
UP	Length (mm)	11.53±0.83
	Width (mm)	7.49±1.05
LP	Length (mm)	13.77±1.32
	Width (mm)	6.54±0.74
G	Length (mm)	14.69±0.88
AL	Anther (mm)	7.81±0.63
	Filament (mm)	6.11±0.41
	In sum (mm)	13.92±0.99
	Pollen number	46953±9081.5
	Pollen size	39.7±2.35
AM	Anther (mm)	5.95±0.63
	Filament (mm)	6.12±0.49
	In sum (mm)	12.07±1.02
	Pollen number	33100±6560.9
	Pollen size	40.7±2.53
MI	Anther (mm)	4.50±0.59
	Filament	2.11±0.18
	In sum (mm)	6.61±0.67
	Pollen number	19700±3048.6
	Pollen size	39.5±1.96
ST	Anther (mm)	1.95±0.21
	Filament (mm)	0.97±0.13
	In sum (mm)	2.92±0.30
	Pollen number	0
	Pollen size	/
CA	Length (mm)	4.8-10
	Width (mm)	2.2-6.9

UP, Upper petal; LP, lower petal; G, pistil; AL, abaxial lateral stamen; AM, abaxial median stamen; MI, set of four middle stamens; ST, set of three abaxial staminodes; CA, calyx.

Table 2. Origin of some samples and vouchers.

Number	Collector	Locality	Sampling time	Origin
2008091201	Chen Ming-Lin	Shi Zi Shan, Anhui Province	September 12, 2008	ANUB
2008091207	Chen Ming-Lin	Tong Guan Shan, Anhui Province	September 12, 2008	ANUB
2009090715	Chen Ming-Lin	Hua Jin, Anhui Province	September 7, 2009	ANUB
2009090724	Chen Ming-Lin	Hua Jin, Anhui Province	September 7, 2009	ANUB
2009101305	You Ya-Li	Jiu Lian Tang, Anhui Province	October 13, 2009	ANUB
2009101315	You Ya-Li	Jiu Lian Tang, Anhui Province	October 13, 2009	ANUB
2009101526	Chen Ming-Lin	Tian Men Shan, Anhui Province	October 15, 2009	ANUB

Table 3. Fruit set of *S. corymbosa* in study populations.

Treatment	Population				
	SZS	TGS	JLT	HJ	TMS
Bagged inflorescences (%)	-	-	0	0	-
Fruit set of flower per flowering branch (%)	2.8±0.34 ^a	5.2±0.34 ^b	3.9±0.34 ^b	8.2±0.34 ^c	7.9±0.34 ^c
Fruit set of inflorescence per flowering branch (%)	33.4±7.12 ^a	32.5±7.85 ^a	28.6±5.47 ^b	35.2±6.84 ^a	32.1±5.94 ^a
Seed set per pod (%)	78.4±7.90 ^a	79.9±7.00 ^a	85.2±7.94 ^b	76.6±9.14 ^a	87.2±6.91 ^b

Means within a row followed by different superscripts are different at $P = 0.05$.

Table 4. Visitors to *S. corymbosa* flowers and the presence of pollen grains on their bodies.

Number	Taxa	Order	Frequency	Visited stamen
1	<i>Apis</i> sp.	Hymenoptera	+++	AL and AM
2	Bombidae	Hymenoptera	++	AL and AM
3	Wasp	Hymenoptera	++	AL and AM
4	Lycaenidae	Lepidoptera	++++	MI
5	<i>Pieris rapae</i>	Lepidoptera	+++	AL and AM
6	Mantidae	Mantodea	+	Not AL and AM
7	Formicidae	Hymenoptera	+++	Not AL and AM
8	Chrysomelidae	Coleoptera	++	MI
9	Muscidae	Diptera	+	MI
10	Curculionidae	Coleoptera	+	MI

++++: very frequently; +++: often; ++: occasionally; +: seldom.

categorized into three types: (1) Monosymmetric flower; (2) asymmetric flower in which only the gynoecium is involved in the floral asymmetry; (3) asymmetric flower in which also petals and stamens are involved in the floral asymmetry. As stated earlier, *S. corymbosa* belongs to asymmetric flower with extrafloral nectaries.

About 20,000 species of flowering plants offer only pollen as a reward for their pollinators (Luo et al., 2008). A widespread character of such pollen-offering flowers is stamen dimorphism, involving two sets of stamens that differ in position, size, colour and/or shape (Buchmann, 1983). Darwin was the first to hypothesize that a 'division-of-labour' among stamens, with some stamens providing pollen as food while others provides pollen for fertilization (Luo et al., 2008). Some studies demonstrated that, one set stamens always act as the "feeding" stamens, the others act as the "pollinating" stamens in *Senna* (Luo et al., 2009) which was supported by the pollen germination rate of *S. corymbosa* in this study for higher pollen germination rate from AL and AM than from MI and pollen germination rate from AL is highest, which indicates that the stigma of *S. corymbosa* is easier to receipt pollen from AL. On the other way, pollen number from AL, AM and MI is large, but the pollen number from AL, AM and MI are descended in sequence. The more pollen in AL mainly functions as pollen donator to ensure the success of reproduction. Owing to the limitation of resources, the

flower tend to form more stamen and less pollen number, which may attract visiting insects and provide food to them, to facilitate the insect pollination. As for the function significance of why pollen numbers of AM are less than that of AL is still unclear. Apart from the difference of pollen number and germination rate, we could not find colour differences among AL, AM and MI. Moreover, we observed that some insects visit not only yellow AL and AM, but also MI for foraging pollen, but never a insect visit yellow anthers of staminodes; so AL, AM and MI may function as the "feeding" stamen and the brownish yellow is presumably the effective color attractants to pollinators. Despite the fact that extrafloral nectaries grows on the base of leaflets, the flowers have no nectar. So pollen was the main reward to insects and coloration of corolla and anthers are primary attractants.

Many previous studies on fruit set are always limited in a florescence (Ge et al., 1999; Mohoro, 2002), both fruit set florescence and flower branch were counted in this study. In some species, the lower fruit set is because of pollen limitation, which is for the lack of a pollen vector or the lack of a pollen supply (Byers, 1995; Ushimaru and kikuzawa, 1999) and buzz-pollinated plants are especially sensitive to pollen limitation (Kawai and Kudo, 2009). But in this experiment, in spite of having enough pollen number and visiting insects, the fruit set is still very low, which indicates that pollen did not always succeed in

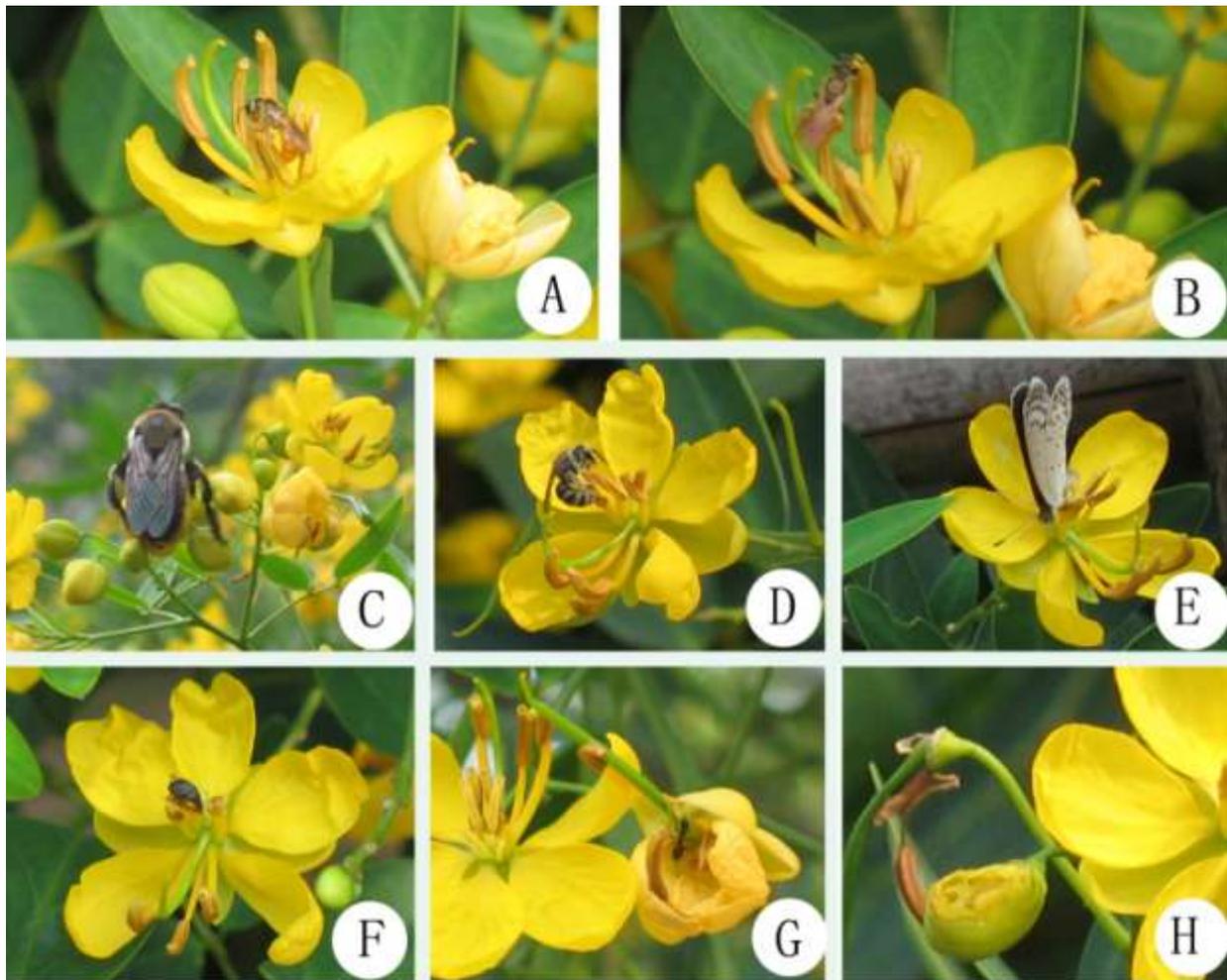


Figure 3. Visiting insects on flowers *S. corymbosa* (A-B: *Apis* sp.; C: Bombidae; D: Wasp; E: Lycaenidae; F: Chrysomelidae; G: Formicidae; H: destroyed flower).

fertilizing the ovules or that a large proportion of pistils with fertilized ovules were aborted by the plant. Fewer flowers often attract fewer pollinators, which results in reduced seed set as a consequence of pollen limitation. We found many barren hermaphrodite flowers in *S. corymbosa* in our observation populations, which indicated that they mainly attract insects to ensure the success of reproduction. Moreover, observation results showed that the fruit were evenly presented on the branch and fruit number in each florescence were no more than two, which indicated that the fruit set were influenced not only outside ecological factors, but also internal cause formed during long time evolution and the factor that no one top inflorescence fruit also confirm it. Once a flower was pollinated, its seed set was high, which can ensure the progeny heredity.

Despite the pistil length is longer than that of AL, the pistil is more curved than that of AL, such that the height of pistil is almost as high as that of AL. So, it reduces

interference and facilitates the pollination from AL. The stigma is so small that it is not easy to receive precise pollen, but the pollen is so much that pollen cloud is produced at the vibration by the buzz insects. The curved sickle style may facilitate the pollination by the contraction between the stigma and the abdomen of visiting insect which is formed by the results of co-evolution between plants and animals. Although, it has specialized pollination system, it does not depend on a single pollinator species for pollination.

For many plant species, we will need to know more about pollinator behavior and the pollen-flow dynamics that occur when pollinators visit, in order to gauge the importance of pollination biology and mating system (Duncan et al., 2004). Based on the results of bagged experiments, pollinators are essential for pollination for that no fruit had developed from bagged flowers. 6 to 8% of angiosperms have poricidal anthers (Kawai and Kudo, 2009). Insects cannot collect pollen by the usual method

of brushing and grooming, but by vibrating the anthers in these plants. This unique form of pollination is called buzz pollination (Dukas, 1990; Kawai and Kudo, 2009). In this study, although, individuals of *Apis* visits both AL and AM, their body is so small that it is difficult for them to transmit the pollen grains on their back to the stigma, so they presumably act as pollen predator. Bombidae is a big, hairy, black and yellow bee whose size can range from 1.5 to 2.8 cm. It is a very important and beneficial insect that pollinate plants and flowers. Its most active season being autumn, was consistent with the peak blooming period of *S. corymbosa*. We found Bombidae sp. in five study populations. During the process of visiting flower, its vibration force by the wings is so strong that it is easy to vibrate the pollen from the poricidal anther, so we speculate that the individuals of Bombidae are presumably the effective pollinators. The body of wasp is large enough to contact the stigma, at the same time; the vibration force of it can vibrate anthers to eject pollen cloud, so this species may be the potential pollinators to *S. corymbosa*, the potential pollination role of the species may be due to the limited numbers of Bombidae. For animal-pollinated plants, when pollinator visits are not enough, for example, removal of most pollen by a single pollinator contributes to successful pollen transport, as seen in orchids that provide a pollinium to the first visitor to each flower (Nilsson, 1992). And the compact pollen organized in masses (pollen cloud in this study) may lower investment in pollen production, which facilitated the reproduction of the species. Although, *S. corymbosa* has specialized pollination system, it does not depend on a single pollinator species for pollination. As a generalist-pollinated species, it may be prone to suffer reproductive failure, because of being swamped by pollen from other plant species, which accounted for the low fruit set of flower of *S. corymbosa*.

Insects such as *P. rapae*, Chrysomelidae, etc. are often visit flower, but their wings is too smooth to stick the pollen of *S. corymbosa* and their small bodies nearly have no chance to contact the stigma. Thus, they are not considered as effective pollinators. When ants explore extrafloral nectaries odor which was increased, it facilitates attraction of the other insects to pollinate *S. corymbosa*. On the other hand, too much ants may destroy the unmaturing buds, but it also becomes the prey of individuals of Mantidae, to form stable protooperational interspecific relationship. And other insects such as Chrysomelidae are not considered as effective pollinators.

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