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Pollination biology of *Caesalpinia decapetala* (Leguminosae) in Korea

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Morphological changes of flowers and insect visitors were observed to investigate pollinator of *Caesalpinia decapetala*. The flowers of *C. decapetala* are protandrous. Functionally, the flower is changed from male to female. As a male, pollen grain is released after anther dehisced while style is immature. After completed pollen grain release, the style starts to lengthen. It helps the stigma to easily touch the carpenter bee's thorax covered with pollen grain. At this time, flower functions as a female. The majority of taxa and individuals observed were Hymenoptera. The most frequent visitor was the *Xylocopa appendiculata circumvolans*, carpenter bee. Carpenter bees exhibited only typical pollinator behavior among flower visitors, with touching reproductive organs and seeking nectar at the same time. The pollination behavior is as follows. Soon after carpenter bees perceived guide mark, they foraged rightward and grasped style and stamens with legs and they inserted proboscis into standard petal to seek nectar. With this behavior, the pollen grains of the male flower transfer to the ventral thorax of the carpenter bee. As the carpenter bee moves to another female flower, the deposited pollen grains are delivered to the stigma.

Keywords : *Caesalpinia decapetala*, pollination, *Xylocopa appendiculata circumvolans*

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Introduction

Insects and other animal pollinators obtain food from the flowers they visit, usually in the form of pollen grain or nectar. This is one of a mutually beneficial relationship, the plants obtaining, in return, the services of the pollinators in carrying pollen grain from one flower to another. The interrelations between the adaptations of insect-pollinated flowers and those of the specialized flower-visiting insects are a classic instance of co-evolution (Michael *et al*., 1996).

It is in the Hymenoptera that we see the greatest variety of specialist adaptations, perhaps predictably since they are then grouped most intimately and completely associated with flowers (Michener, 1979; Roubik, 1989). Precise structural adaptation is shown by bees of several families in relation to certain plant groups, e.g., a brush tongue type with hooked hairs and a rake on the foremetatarsi is found in certain species in several families and appears to be associated specifically with visiting flowers of the borage family (Boraginaceae). In a detailed study on *Rediviva* bees, which collect oil as a food source from *Diascia* species in South Africa, Steiner and Whitehead (1990) showed that the length of the spur in which the oil was secreted by *Diascia* varied between species and between races of one species, and that the length of the forelegs of bees, used to collect the oil, varied precisely with it. Some very long proboscis associated with particular flowers, are recorded from bees, as in the moths (Roubik, 1989).

Caesalpinia is known to specialize a pollinator using a flower structure. In Mexico, butterflies were identified to be the primary pollinators of *Caesalpinia pulcherrima* and the pollen grain is carried primarily on their wings. Of the numerous species that visit the flowers, members of Papilionidae are the most important pollinators (Cruden *et al*., 1979). In Argentina, the pollination of *Caesalpinia gilliesii*, a legume species with long, brush-type flowers, was studied. Successful contact with anthers while drinking nectar depended upon hawkmoth tongue length. Long-tongued hawkmoths carried *C. gilliesii* pollen grain more frequently than short-tongued individuals (More *et al*., 2006).

Korean *Caesalpinia decapetala* has yellow petals and flower shape is zygomorphic with deep nectary gland

and a red guide mark on standard petals. Because of these characteristics, flowers of *C. decapetala* are presumed to be preferred by bees, but there is still no scientific evidence. The aim of this paper is to discover visitors of *C. decapetala* and to identify pollinators which get food from the plant and serve pollination to the plant, and to investigate specialized interrelationships between insect and flower.

Materials and Methods

Field observations of *C. decapetala* conducted in Giryong-ri, Youngwang-gun, Jeollanamdo Province from May 9-May 30 2009 were subjected to investigated changes of morphological structure and insect visitors of five plants. The study site was near foot of a Ongnyeobong peak and nearby houses and farmland. The site remained sunny all day because it was situated to the south. There were no nectary plants around target populations.

Principal features of the flowers and inflorescences, such as flower shape, especially style and stamen, were examined. For the period of flowering season, morphological changes divided into three stages were observed by measuring style and stamen length. The pre-anthesis stage was named A1; flowers which came out but the anther was not matured. Anthesis stage was named A2; flowers whose pollen grains were releasing from anther. The post-anthesis stage was named A3; flowers whose completed pollen grain releasing and no pollen grain remains on anther surface. Measurements were conducted in mid-May 2009. Stigma height (style length from stigma to ovary) and anther height (stamen length from anther to basal stamen) were measured with 30 flowers at each three stage (A1, A2, A3) by digital calipers (Digimatic, Mitutoyo Corporation) and looked for average values. A inflorescence from each of the five plants was selected and five inflorescences were used to observe visiting frequency and insect behaviors.

To investigate insect visitors of flowers for day time, observations were conducted at the site on the 15th, 18th, 19th, 23th and 24th of May 2009 during the flowering season. Field observations were made between 09:00 and 16:00 h. All kinds of insect visitors and frequency of visit are recorded and looked for average values of five times. Visit-frequency counted the number of insects which visited inflorescene during observations. To investigate insect visitors of flowers for night time, observations were conducted at the site on the 23th, 24th of May 2009 during flowering season between 20:00 and 23:00 h (Fig. 1B). To identify insect visitors and find attachment site of pollen grain, all kinds of insects were collected with sweep net and stored in a paper bag. All insect visitors were identified by illustrated guide to Korean fauna and flora and Insect's life in Korea. To investigate whether *C. decapetala* flowers were pollinator dependent or self-pollinated, five inflorescences (N = 5 plants) were selected randomly at the site. 163 flowers of five inflorescences were wrapped with nylon bags which prevented natural pollination for a month from May 1-May 30 2009 (Fig. 1A).

Results

Breeding system

163 flowers of five inflorescences were wrapped with nylon bags which prevented foraging insects that did not produce fruit. 1,340 flowers of 39 inflorescences which were open to nature produced 38 legumes, statistically 2.84% fruit set. These results show *C. decapetala* is considered insect-pollinated, not self-pollinated.



Fig. 1. A. inflorescences of *C. decapetala* wrapped with nylon bags on May. B. leaves are folded down at night.

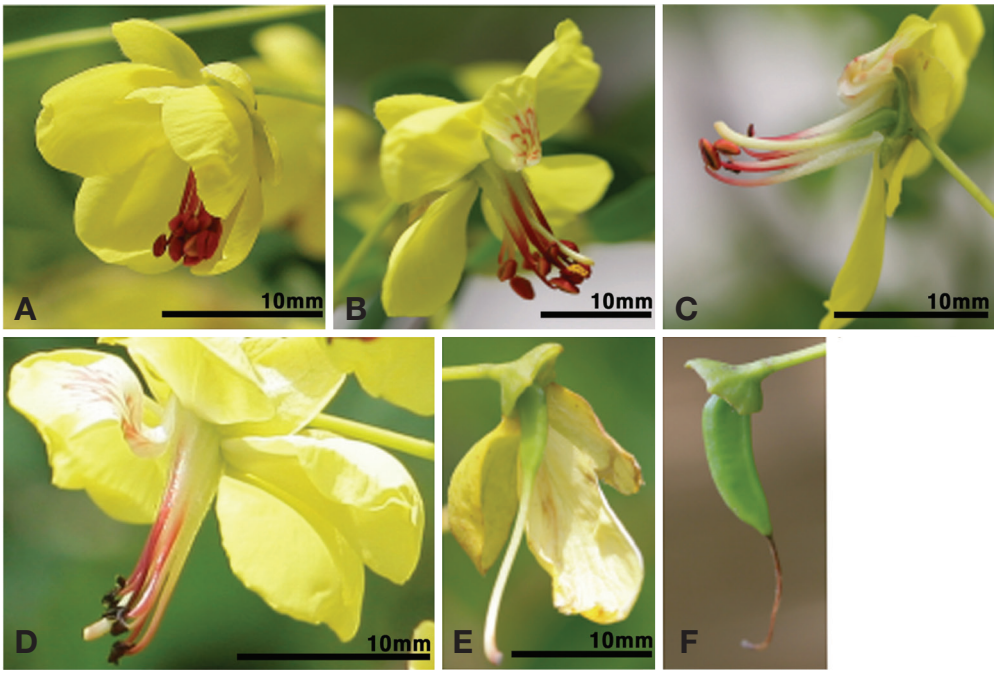


Fig. 2. Functional morphology of *C. decapetala*. A. pre anthesis: A1. B. anthesis: A2. C. cross section of flower. D. post anthesis: A3. E. the ovary swelled after pollination. F. legume is growing.

Functional morphology of flowers

The zygomorphic corolla consists of five free petals such as a standard, two keels and two wings. Guide mark in standard petal plays a role as leading insect visiting. Pistil has a white carpel surrounded by ten stamens. The length of each stamen is different. Basal parts of nine stamens are fused. Nectary gland of *C. decapetala* was deep and concealed (Fig. 2C). When anther started to mature, morphological changes begin. Each stage of the morphological changes was observed by A1, A2 and A3. As of A1 stage, standard petal was folded, and the guide mark could not be observed at front (Fig. 2A). The length of style was 12.4 mm which was 0.9 mm longer than stamens, 11.5 mm. Pollen grain was not released because the anthers did not dehisce. Nectar was not observed in this stage. During stage A2, the standard petal was unfolded and guide mark was visible at the front (Fig. 2B). The stigma height was 13.7 mm and anther height was 12.1 mm. The stigma height was 1.6 mm longer than anther one. The anthers dehisced and released pollen grains. The nectar could be observed by the naked eye. During A3 stage, guide mark was observed at front. The stigma height was 15.7 mm and that was 3.7 mm longer than anther height, 12 mm. The gap between anther height and stigma height was remarkable (Fig. 2D). There was no pollen grain released because all anthers were dehisced. Also nectar could be observed in basal part of plant. The anther height was 11.5 mm at the A1 stage,

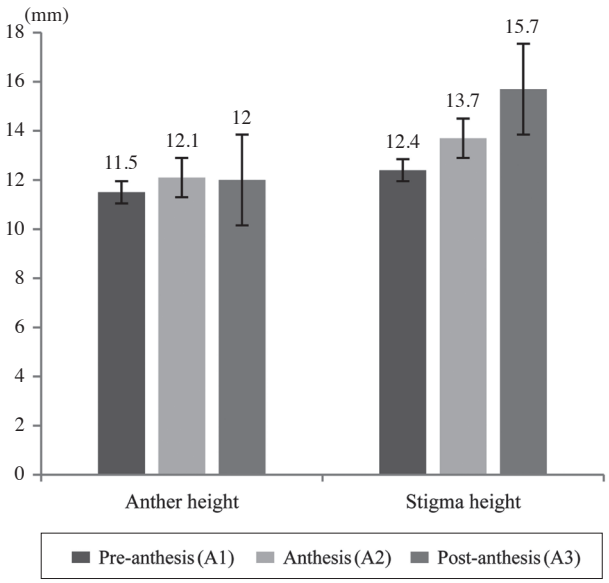


Fig. 3. The anther height and stigma height of *C. decapetala* during pre-anthesis (A1), anthesis (A2), and post-anthesis (A3).

12.1 mm at A2 stage which was 0.6 mm longer comparatively A1 stage (Fig. 3). The anther height of A3 grew less than 12.0 mm, 0.1 mm shorter comparatively A2. The shrinkage results because stamens started to dry after dehiscence. The stigma height was 12.4 mm at A1 stage, 13.7 mm at A2, and 15.7 mm at A3 (Fig. 3). Con-

Table 1. Species composition, behavior, body length, visit number (N) and pollen grain attachment site of visitors to *C. decapetala* flower.

Species	Behaviors of flower visitors						N	Pollen grain attachment	Body length (mm)
	Using habitat	Feeding pollen grain	Collecting pollen grain	Feeding nectar	Insertion of proboscis	Touching stigmas and anthers			
Hemiptera									
Reduviidae									
Nysius plebejus	○						1	–	3.5
Coleoptera									
Oedemeridae									
Oedemeronia lucidicollis				○			1	–	
Cerambycidae									
Anastrangalia sequensi				○			2	–	8
Leptura arcuata				○			1	–	12
Diptera									
Syrphidae									
Metasyrphus corolla		○					2	Mouth	8
Tachinidae									
Gymnosoma rotundatum	○						1	–	5
Hymenoptera									
Halictidae									
(Unidentified)			○				1	–	
Apidae									
Bombus ardens ardens			○				4	Ventral side	14
Megachile japonica			○				3	Ventral side	12
X. appendiculata circumvolans				○	○	○	25	Ventral thorax	22
Lepidoptera									
Papilionidae									
Papilio bianor				○	○		2	–	20
Lycaenidae									
Celastrina argiolus				○	○		3	–	18

sequently, the stigma height was the longest at A3. A2 flowers function as a male which released pollen grains after anther maturation. A3 flowers function as a female. When the stamens started to wilt after releasing all pollen grains, the style grew longest. After A3 stage, the dried stamens fell down and style which became pollinated developed into legumes (Fig. 2E, F).

Flower visitors and their behaviors

For the day time observation, twelve species, nine families and five orders were observed. The visitors were four species of Hymenoptera, three species of Coleoptera, two species of Diptera, a species of Hemiptera and two species of Lepidoptera (Table 1). No insect-visitor to flowers was observed at night. Flower-visiting behaviors differed distinctly from Hymenoptera Diptera, Coleoptera, and Lepidoptera. Members of Hemiptera and Coleoptera were also observed visiting flowers of *C. decapetala*, although they did not exhibit typical pollinator behavior, with most individuals merely used as a habitat (Table 1). Coleopteran visitors such as *Oedemeronia lucidicollis*, *Leptura arcuata* and *Anastrangalia sequensi* sucked nectar by piercing a hole in nectary gland of basal part without contacting anthers and stigma (Fig. 4F). Dipteran insects exhibited inappropriate behavior for pollination. Behaviors of Dipteran visitors were different with two species. *Gymnosoma rotundatum* visited flower to lay eggs on *Nysius plebejus* and *Metasyrphus corollae* consumed pollen grain (Fig. 4A). Nectar seeking behavior with a proboscis was observed by Lepidoptera such as *Papilio bianor* and *Celastrina argiolus* without touching reproductive organs. Soon after foraging the nectary gland located in basal part of flower, they sucked nectar in a reverse position (Fig. 4C). The field observations revealed that the most frequent insect visitors were Hymenopterans. Three species of medium and large-bodied bee were found : *B. ardens ardens*, *M. japonica*, and *X. appendiculata circumvolans*. Two hymenopteran species such as *B. ardens ardens* and *M. japonica* were only gathering pollen grain, not feeding on nectar. They collected pollen grain with middle legs and stuck them to pollen basket on tibia of hind legs (Fig. 4D, E). During this collecting process, the pollen grains were scattered to the ventral side of bees entirely. In contrast, *X. appendiculata circumvolans*, carpenter bee was seeking nectar. Soon after carpenter bees perceived guide mark on the standard petal, they foraged rightward and

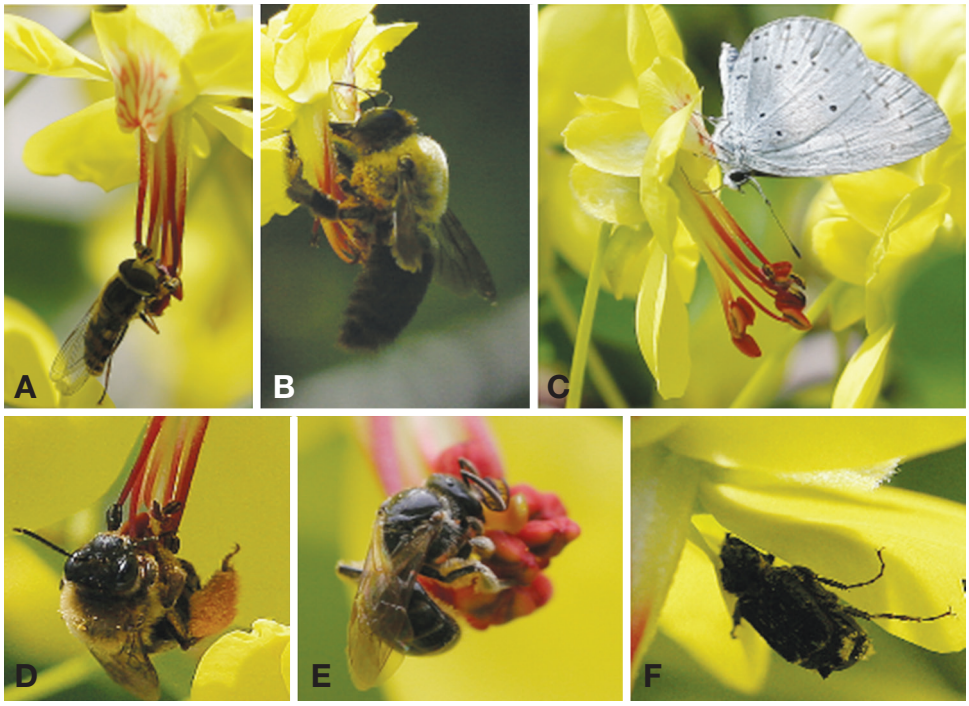


Fig. 4. Insects visiting *Caesalpinia decapetala*. A. *Metasyrphus corollae*. B. *Xylocopa appendiculata circumvolans*. C. *Celastrina argiolus*. D. *Bombus ardens ardens*. E. *Megachile japonica*. F. *Anastrangalia sequensi*.



Fig. 5. Pollen grain attachment ventral view of Apidae. A. *Megachile japonica*. B. *Bombus ardens ardens*. C. *Xylocopa appendiculata circumvolans*.

grasped style and stamens with legs and they inserted proboscis into standard petal deeply, seeking nectar (Fig. 4B). While probing nectar, its ventral thorax was pushed against the anther and stigma, thereby receiving pollen grains or, if it was already laden with pollen grains, transferring pollen grains to the stigmatic surface. This behavior could be regarded as appropriate for pollination. Among all flower visitors, particularly large-bodied carpenter bee behaved on flowers in this manner that caused contact with anthers and stigmas. The most frequent visiting bee was the *X. appendiculata circumvolans* (N = 25), followed by *B. ardens ardens* (N = 4) and *M. japonica* (N = 3) (Table 1).

Pollen grain attachment

Pollen grain was found in mouth part of flies, but not butterflies, because butterflies did not contact reproductive organs of *C. decapetatla* while sucking nectar. Most of the pollen grain attached on the bodies of bees remained on the ventral side, not dorsal. Pollen grain was not only covered on the ventral side of *M. japonica* and *B. ardens ardens* but also the mass of pollen grain was attached to the pollen basket on the tibia of hind legs (Fig. 5A, B). In the case of *X. appendiculata circumvolans*, a large amount of pollen grain was particularly attached to the ventral thorax (Fig. 5C). As a result of electronic

microscope scanning, all pollen grains on the bodies of bees were classified as a pollen grain of *C. decapetala*.

Discussion

The most frequent visitor of Korean *C. decapetala* was the *X. appendiculata circumvolans*, followed by *Bombus ardens ardens* and *Megachile japonica*. But they exhibited different behaviors while visiting. Their visiting purposes are different with each species; *B. ardens ardens* and *M. japonica* visit flowers to gather pollen grain whereas *X. appendiculata circumvolans* visits flowers to gather nectar (Table 1). *B. ardens ardens* and *M. japonica* visit particularly male flowers which produce pollen grain. They did not approach nectary gland. Their proboscis might be so short that they could not reach nectary gland even though they actually gather nectar as food in other plants. These visiting preferences of *B. ardens ardens* and *M. japonica* give no chance to deliver pollen grain to the stigma. Consequently, these behaviors are not effective to pollination of *C. decapetala* even though their bodies are covered with pollen grain.

Sometimes bees take pollen grain from some flowers and nectar from others : for example, *Anthophora plumipes* visits peonies in gardens for pollen grain only. Kugler (1940) reported that species of *Lasioglossum* readily collect pollen grain from flowers whose nectar they cannot reach. Harper and Wood (1957) suggested, that some long-tongued bees never visit certain flowers with rather easily accessible nectar. There are no records of visit of *Megachile* to ragwort (*Senecio jacobaea*). The visit preference of bees is generally associated with sugar type of nectar; sucrose-rich nectars tend to be associated with deep (or concealed) nectaries and with long-tongued bees. Hexose-rich nectars are often associated with freely-exposed nectaries and visits by short-tongued bees (Proctor *et al*., 1996).

Large-bodied bee, *X. appendiculata circumvolans* visits both male flowers and female flowers of *C. decapetala* to get nectar. The flowers are protandrous and the inflorescence is a columnar shape which the lowest flowers open first, but there is an overlap of flowering between adjoining flowers. This means that the carpenter bee first visits the oldest flowers which are in the female stage, and any pollen grain deposited in them is likely to come from another plant. As the bee moves upwards it acquires pollen grain from the youngest flower in the male stage; after visiting the highest available flower the insect must fly off to another inflorescence (which usually means going to another plant) in order to avoid revisiting flowers that have just been depleted. Bee-pollinated plants with columnar type of inflorescence show similar behaviors. Particularly bumble bees, when confronted with an inflorescence of columnar form, such as that of foxglove, visit first the lowest available flowers and then work upwards. The protandry of flowers in such an inflorescence thus constitutes a highly efficient outbreeding mechanism. Benham (1969) has suggested that this could be an arrangement to ensure that insects move in the upward direction and occurrence of such arrangements may have led to upward movement becoming instinctive. A further feature of this plant which might reinforce the tendency to start at the bottom is the fact that nectar secretion is greatest during the female stage.

Carpenter bee is known for an effective pollinator to various plants. Naoto (2013) reported specialized pollination by carpenter bees in *Calanthe striata* (Orchidaceae) in Japan. *C. striata* has nectarless flowers and is pollinator dependent. The flowers were pollinated exclusively by carpenter bee, *X. appendiculata circumvolans*, although the bees occasionally wasted pollen grain by delivering it to the stigmatic surface pollen grains that retained their anther caps. *C. striata* blooms in spring when post-overwintering carpenter bees have not yet started foraging for brood production. It can therefore exploit an abundance of opportunistic/naive foragers. This timing may also increase the possibility of pollinator visits, because no rewarding co-flowering plants are available in the orchid habitats. In the deceptive pollination system of *C. striata*, flowering time is supposed to be a crucial factor. Dodson and Frymire (1961) reported that large, open-flowered orchids of eastern hemisphere, such as *Cymbidium* Sw. *Grammatophyllum* Blume and *Vanda* W. Jones ex R. Br., were pollinated for the most part by *Xylocopa*. Dressler (1981) pointed out that carpenter bees are the usual pollinators of a number of orchids, especially large gullet flowers, such as *Arundina* Blume, *Cymbidium*, *Eulophia* R. Br. ex Lindl., *Schomburgkia* Lindle, and *Vanda*.

In Korea, carpenter bee is effective to pollination of *C. decapetala*. The visiting behaviors are as follows. Soon after carpenter bees perceive guide mark, they forage rightward and grasp style and stamens tightly with legs which makes stigma and anther push against the thorax of themselves. With this behavior, the pollen grains of male flower transfer to ventral thorax of carpenter bee. As a carpenter bee moves to another flower which is in the female stage, the deposited pollen grains on the thorax are delivered to the stigma. Because of this, carpenter bee is considered a legitimate pollinator of *C. decapetala*. The length of *C. decapetala* from stigma to nectary gland is 16 mm. In order that visitor can touch reproductive organs and reach a nectary gland simultaneously, the body length of visitor should be longer than 16 mm. The body length of carpenter bee is approximately 22 mm. Carpenter bee is an ideal large-bodied visitor that is longer than 16 mm. The body size of visitor plays

a crucial role for pollination to *C. decapetala*.

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