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BREEDING SYSTEM AND POLLINATION BIOLOGY OF *PAEONIA DELAVAYI* (PEAONIACEAE), AN ENDANGERED PLANT IN THE SOUTHWEST OF CHINA

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Abstract

Breeding system and pollination biology of *Paeonia delavayi* (Peaoniaceae) from Shangri-La, Yunnan Province, southwest of China were studied. Flowering phenologies and flower visitors were observed or collected from 2008 to 2011. The pollen viability, stigma receptivity and pollination efficiency of different visitors were detected and tested. The florescence lasted for 6-9d in a single flower from mid-May to late June. A high percentage of flower damage promoted early anther dehiscence. Flowers started disseminating pollen at 1-2 d after flowering, and lasted for 5-6 d. Pollen viability could be preserved for more than 10 d at normal temperature. High seed rate from the stigma was observed at 1 d before flowering to 3d after flowering, and the dissemination hysteresis was defined as protogyny. The P/O ratios were 6,124 to 9,713:1, suggesting that the larger quantity of pollen to increased the seed setting rate. Three species of bees, eight species of beetles, seven species of syrphid flies, four species of ants, and three species of butterflies were observed on the flowers. *P. delavayi* rewarded to the visitors by releasing fragrance, providing pollen and nectar. On the bodies of the visitors under stereomicroscope and scanning electron microscope (SEM), much pollen from the plants of similar flowering period inner community were found which indicated that these incompatible visitors were not species-specific pollinators. The bagging experiments showed that *P. delavayi* was self-incompatible and no apomixes. Anemophily only played a minor role in the fertilization. A few seeds with poor plumpness can be produced by geitonogamy. Seed setting rate of artificial xenogamy was higher than natural pollination. Artificial control of the visitors' species showed bees being the most important pollinators. Beetles and ants participated in pollination to some extent and were unstable. Syrphid flies and butterflies were very unreliable with low pollination efficiency. Reproductive success depended largely on cross-pollination assisted by pollinator activities, especially the bees.

Key words: Ants; Beetles; Bees; Endangered plant; Flowering time; Breeding system; *Paeonia delavayi*; Pollination Biology; P/O ratio; Stigma receptivity.

Introduction

Paeonia delavayi, as a plant of Paeoniaceae, *Paeonia* and Sect. Moutan (Hong & Pan, 1999), is mainly distributed from central to northwest Yunnan, and some in southwest Sichuan and southeast Tibet. As an endemic species in southwest of China (Wang, 1997), *P. delavayi* is distributed in the southernmost part under the wild tree peony group. The China Plant Red Data Book listed *P. delavayi* as an endangered species (Feng, 1992), and as a Grade II state protected plant of the Regulations of the People's Republic of China on Wild Plants Protection in 1996. Since 2008, we have conducted a large number of investigations in distribution areas of *P. delavayi*. A total of 12 populations were found in Kunming, Dali, Lijiang, Shangri-La, Deqin, and Weixi, Yunnan, as well as in Nyingchi, Tibet (Li *et al*., 2009). Based on the investigations, *P. delavayi* grows individually or in clusters on forest edges, under secondary forests, or among bushes of mountains at elevations between 2400 and 3600 m. Human intervention has led to frequent changes in the population structure of *P. delavayi*, and its effective seeding productivity decreased, with asexual reproduction characteristics (Li *et al*., 2012). In recent years, the morphology, palynology, molecular biology, and seed germination characteristics of *P. delavayi* have been investigated in a number of studies (Wang, 2007; Li *et al*., 2011). However, no detailed report on its breeding system in the wild, including flowering habits, pollination ecology and biological characteristics, etc.

At present, researches on *Paeonia* pollinations are limited extremely. Grant (1964) reported in his observational studies that the herb *P. californica*, mainly distributed in the United States, had self-pollination characteristics. Through radioiodine labeling, Schlising (1971) indicated that the beetles carried and disseminate pollens among flowers of different plants. Turpin & Schlising (1976) conducted in-depth observational studies and found that a total of four families, 17 species of bees, and one species of beetles participated in pollination. Based on the research of the visiting insects on *P. lactiflora* in the Gaogesitaihanwula Natural Preserve in Inner Mongolia, Hong & Liu (2006) demonstrated that with the absence of apomixes and cleistogamy and with weak selfing, *P. lactiflora* insects played a major role in cross pollination. With regard to woody plants of *Paeonia*, the pollination experiment were conducted by Luo *et al*., (1998) in connection with *P. suffruticosa* subsp. *spontanea*, distributed in southern Shanxi Province, China, in was show that the species had neither apomixes nor automatic self-pollination. In addition, the species with unisexual flowers producing few seeds, has weak selfing and low seeding rate. Moreover, a total of only five species of bees and four species of scarabs were involved in its pollination. The studies on *Paeonia* pollination were extremely limited. Focusing on *P. delavayi*, this study was performed as a preliminary research on the breeding and biological characteristics of its pollination, including its flowering time, floral characters, pollen viability, stigma receptivity period and pollen-ovule ratios (P/O). This study aims to investigate the pollination mechanism of *P. delavayi*, its breeding system and its relationship with the reproductive biological characteristics, to provide a theoretical basis for the effective protection and rational utilization of germplasm resources of wild *P. delavayi*.

Materials and Methods

Study site: The study was carried out near the ski resort, approximately 25 km west of Shangri-La County (27° 57' N, 99° 35' E) in Northwest of Yunnan Province, China. The area is the most large, concentrated and abundant distribution region of *P. delavayi*, and has a temperate highland semi-humid monsoon climate, with an altitude about 3,318 m, annual average temperature of 5.9°C, monthly coldest average temperature of -0.4°C, monthly hottest average temperature of 13.3°C, frost-free period of 123.8 d, annual precipitation of 648.6 mm, annual evaporation of 1,616.8 mm, annual sunshine time of 2,155.9 h, annual average relative humidity of 69%, and accumulated temperature above 10°C of 1,539.2°C (the above meteorological data was obtained from Diqing Meteorological Bureau); the soil type is composed mainly of brown soil and limestone.

Vegetation composition: The forest zone in the region is a conifer-dominated community, and *P. delavayi* is distributed on forest edges and canyon shrub zones, with tree-layer canopy density of 0.1 to 0.2, and occasionally scattered in the understory with higher canopy density. Moreover, the area has a shrub layer coverage of 35% to 55% and herb layer coverage of 10% to 25%. The arbor, an accompanying plant, composed mainly of *Abies fabri*, *Pinus densata* and *Populus yunnanensis*; shrubs composed mainly of *Salix rehderiana*, *Rhododendron lapponicum*, *R. pulchrum*, *Quercus senescens*, *Rosa forrestiana* and *Berberis alpine*; herbs and lianas composed mainly of *Clematis florida*, *Pteridium revolutum*, *Plantago asatica*, *Sinopodophullum hexandrum*, *Iris ruthenicar*, *Gentiana veitchiorum*, etc. In this region, plants with similar flowering time as that of *P. delavayi* include *Euphorbia esula*, *Herba taraxaci*, *Rhododendron lapponicum, Berberis alpine*, *Gentiana veitchiorum, Podophyllum hexandrum*, *Duchesnea indica*, *Clematis florida, S. rehderiana*, and *I. ruthenicar*.

Flowering time and floral characters observation: From 2008 to 2011, we observed flowering phenologies both in the florescence and single flowering period, by describing and analyzing the corolla features and stamen development modes.

P/O ratios calculation: We obtained 20 budding flowers of *P. delavayi*, peeled the petals, and collected 4 anthers from the inside and 6 from the outside, respectively. The total number of anthers and ovules of the same flower were recorded (Abid, 2010; Niesenhatm, 1992). The anther were placed in a 14 ml centrifuge tube, and allowed to dry. Subsequently, distilled water was added, mixed well, and diluted to a certain concentration. A sample of the diluted solution was microscope and the number of pollen was recorded. The process was repeated 5 to 10 times to determine the pollen/ovule (P/O) ratio.

Pollen viability and stigma receptivity test: *P. delavayi* possesses high pollen viability. With *P. delavayi* as A hybridization test was performed in from the Central Plains, and found that *P. delavayi* pollen could still germinate after storage at 4°C for 1yr and after pollination seeded successfully, maintaining a high germination rate at room temperature for 10 d (Lv *et al.,* 2010). For the stigma receptivity, fresh pollens were obtained from the same population, and the pollen experiment was conducted with the flower at 2 d before and 5d after the opening of petals. For flowers before opening of petals, isolation of after emasculation was performed directly, had the flowers for the pollination experiment after flowering to experience the isolation after emasculation when the stigma was not bare and had no pollen dissemination, and with the surrounding flowers of the same flowering degree as reference (Khan & Perveen, 2011). The pollination time was recorded at -2, -1, 1, 2, 3, 4, and 5 (from 2 d before to 5 d after flowering) in the morning and afternoon, and the seeding rate was recorded when harvesting the seeds.

Pollinators: During the flowering of *P. delavayi*, we observed various types of insect activities regularly from 2009 to 2011 in Shangri-La of Yunnan Province. The insects were captured for identification and detection of the pollen load by scanning electron microscopy (SEM). The activity insects were observed from 8:00 am to 8:00 pm. A cluster about 30 plants was selected to record the flower-visiting frequency, movement mode, and stay time of different insects in the flowers.

Breeding system experiments: The following bagging experiments were performed to determine the characteristics of the breeding system of *P. delavayi*. The flowers used were selected from regions with similar plant age, flowering period, population density and environment to ensure the accuracy of the experimental data. The flowers were inspected frequently, the bags were exposed for aeration and prevention of mildew infestation and protect against the influence of insect activities, wind and rain. When the seeds matured in mid and late September of each year, follicles were collected in accordance to different bagging, and the ripening rate, ripening capacity and seed plumpness were recorded.

Isolation without emasculation: The flower bud with not yet opened corolla and unexposed stigma was directly covered with sulfuric acid paper for the determination of its selfing characteristics.

Isolation after emasculation: The flower bud which was going to open was stripped of all stamens, and then covered with sulfuric acid paper to detect the presence of apomixes.

Anemophilous pollination: To detect wind pollination ability, a mesh bag was used before flower opening to prevent insect pollination. In addition, 2 cm × 5 cm glass slides were placed at 20 cm apart at different distances around the fully-bloomed flowers, and were collected after 24 h and pollen were counted.

Artificial geitonogamy: During full blooming stage, pollen were collected to pollinate unopened flower bud from the same plant. Pollen were obtained for natural drying before disseminated, to pollinate the other flowers, and isolation emasculation was carried out for pollinated before flowering.

Artificial xenogamy: The method was the same as that of artificial cross pollination on the same plants.

Natural pollination: Flowers with close flowering periods in other experiments were marked and their pollination status was detected under natural conditions.

Control pollinators test: Some flowers from plant clusters for controlled pollination experiment were selected to understand the pollination effects of various insects (Luo *et al*., 1998). Within the specified flowers, bees, beetles, ants and flies were individually allowed to enter and to compare the seeding rate and number of seeds by different species of insect.

Results

Flowering time and floral phenology: Florescence in Shangri-La ski resort was from mid-May to late June. The flowering time varied slightly every year, from May 10 to June 12 in 2009, May 18 to June 13 in 2010, and May 13 to June 18 in 2011. The flowering period, which may be related to drought and extreme weather, was obviously delayed and short in 2010. *P. delavayi* has a single flowering period of 6 to 9 d, which is significantly longer than normal cultivars. The flowering period was closely related to temperature, light and other environmental factors. Under desirable environmental conditions, plants of more than three years old can bloom normally, and sprouting plants can bloom and seed in the following year. The stigma tops and the round anthers appear first followed by the outer anther when the petal was opened. A blooming flower is in a semi-open state, i.e., when not fully expanded and in a cup shape, the inner anthers start disseminating, and the sunny anthers start a little earlier. With the opening of the petals, anthers gradually disseminate from inside to outside, last up to 5 to 6 d, and sometimes until after the petals became dry or withered, but pollen still remain or not cleared.

Flowers of pest damaged: For flowers suffering by herbivores, as shown in Fig. 1, the petals were removed before opening and dissemination could be observed in the edge of the damaged anther, in which small creeping larvae were present (Figs. 1-1, 1-2). After the petals are naturally opened, the stigma is penetrated by the larvae (Figs. 1-3, 1-4), and then the excreta overflows. After the peeling of the petals, larger larvae can be seen once the stigma opened is (Figs. 1-5, 1-6). From our survey, the rate of damaged seeds of *P. delavayi* was up to 80%, in which the insects may come from injection breeding of Syrphid flies. Further observations and researches are still required.

Pollen-ovule ratio (P/O): Based on anther sampling of *P. delavayi*, the pollen and ovule (P/O) ratios obtained was 6,124 to 9,713:1, and a great number of pollen provided possibility for higher success rate of pollination.

Pollen viability: For the pollen viability of *P. delavayi*, we referred to the findings of Lv *et al.,* (2010). *P. delavayi* pollens still have viability after one year of storage at 4°C, with higher viability for pollen stored for 10 d at normal temperature. Using the *P. delavayi* pollen stored for one year, we crossed-hybridized it with other cultivars of *P. suffruticosa*, and obtained seeds.

Fig. 1. Stigma damaged at different stages of flowering.
1, 2. Anther damaged before floral opening. 3, 4. Stigma injected at flowering stage. 5, 6. Stigma occupied after flowering.

Stigma receptivity: In connection with stigma receptivity of *P. delavayi*, we collected pollen to directly pollinate flowers at 2 d before and 5 d after flowering. The time recorded was -2, -1, 0, 1, 2, 3, 4, and 5 d, and the number of pollinated flowers in different periods were 15 to 25. As shown in Fig. 2, the pollination success rate from 1 d before to 2 d after flowering was higher than 70%; while declined rapidly from 3 d after flowering until the stigma lost its ability to accept pollens and become dark and hard.

The pollen of the same flowering plants: We collected pollen from flowers of different species with similar blooming period in a cohabitation group, and performed scanning electron microscopy (SEM) (Fig. 3), with its external shape to study the influence with *P. delavayi* pollination and show visitors pollination law and pollination characteristics. The pollen were from *Euphorbia esula, Herba taraxaci, Rhododendron pulchrum, Berberis spp., Gentiana veitchiorum, Sinopodophullum hexandrum* (pink and white), *Duchesnea indica, Clematis florida, Salix rehderiana, Iris ruthenicar* and *P. delavayi.* They were used for a comparative analysis of pollen carried by the insect specimens of the visitors.

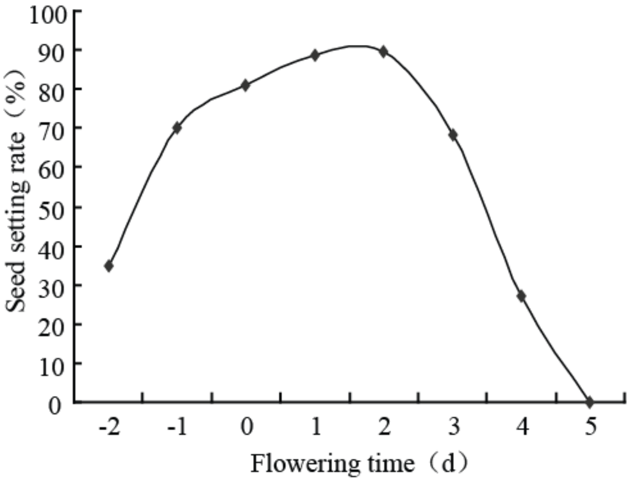

Fig. 2. Stigma receptivity period of *Paeonia delavayi*, Artificial pollinated from 2 d before floral open to 5 d after flowering.

Total insects visitors: Through careful observation and recording during *P. delavayi* flowering, we identified the visiting insects according to the photographs and collected specimens. The results showed that a total of 6 orders and 24 species of insects were functioning in *P. delavayi* flowers, including 3 species of bees, 8 species of beetles, 7 species of syrphid flies, 4 species of ants, and 3 species of butterflies (Table 1).

Pollen with the visitors surface: On the surface of *Apis cerana, Macropis (Sinomacropis) immaculate* and sawfly bodies, several pollen were discovered, all over the beak, head, abdomen, wings, many on the legs, and a large amount in the middle portion of the legs. Through observation of pollen on each part of the bee, under stereological microscope and SEM, we found that different types of bees carried different pollen. For *A. cerana*, except for pollen from *P. delavayi*, we did not find other pollen from plants with similar flowering period (Fig. 4-4). After the pollen scattered on the legs of *M. (Sinomacropis) immaculata*, pollen from three other species of cohabitation populations (Figs. 4-6, 4-7, 4-8), although few in numbers, were discovered, including *Euphorbia esula, Sinopodophullum hexandrum*, and *I. ruthenicar*. In addition to *P. delavayi* pollen on the sawfly body, some special pollen (Fig. 4-12), which were not found in any plants with similar flowering period, account for a large proportion. The pollen might have been carried from Asteraceae species; however, we did not know which kind of plant it was.

Behavior of the visiting insects: The overall flower-visiting period was concentrated between 10:00 to 16:00h, (Fig. 5 shows the results for a sunny day, and bees are rarely seen moving in flowers in a rainy day), and visiting insects in other time are mostly ants. With stereomicroscope and scanning electron microscope, we observed and analyzed the collected insect species. Fig. 6 shows the pollen load carried by the insects.

Bees: Bee activities were mainly concentrated between 10:00 to 15:00 h, at the time when the petals first opened and pollen dissemination had not begun. Bees rarely stayed within the petals; however, they stayed for a long time after the appearance of dissemination, passing through the stigma when crawling on the whole anther, leaving pollen on the stigma. The visiting frequency of bees increased gradually, with the mean value of 22, 78, 103 or 130 on each day during the 4-day observation of 6 flowers. Moreover, flowers with good lighting conditions were visited more frequently. Flower-visiting bees still had nocturnal activities, and the stigma became dark and hard After 4 to 5 d of flowering. At the end of the pollination process, the bees only collected the remaining pollen and nectars within the flowers.

The activity characteristics of the three bees species were not the same. *A. cerana* started to visit at 10:00, with a peak time between 12:00 and 15:00, in different degrees of flowering including the flowers of first blooming and basically complete dissemination and flowers where even petals were withered. *A. cerana* generally crawled outside the anther in the morning (Fig. 7-1), often plunging into the bottom of the anther to suck nectar (Fig. 7-2), or remaining on the petals at the bottom dipped in nectar, with several pollen attached to their legs, abdomens, mouthparts, and bodies. *M. (Sinomacropis) immaculata* appeared around 11:00, during flower visitation before 12:00, they crawled on pollens, and climbed 1 or 2 times on the stigma in each visiting, with the pollen attached to their legs and abdomen (Figs. 7-6, 7-7). The average visitation time was about 30 s. In addition, flower nectar increased after sunshine and further increased at noon. The visiting time was slightly longer, sucking more nectar, with the average flight distance of less than 100 cm. Sawfly appeared almost simultaneously with *M. (Sinomacropis) immaculata*; however, it moved and stayed on the pollen surface for a longer time, repeatedly passed through a stigma top, had smaller flight distance, visited blooming flowers in the same plant cluster one by one, and then visited another plant cluster.

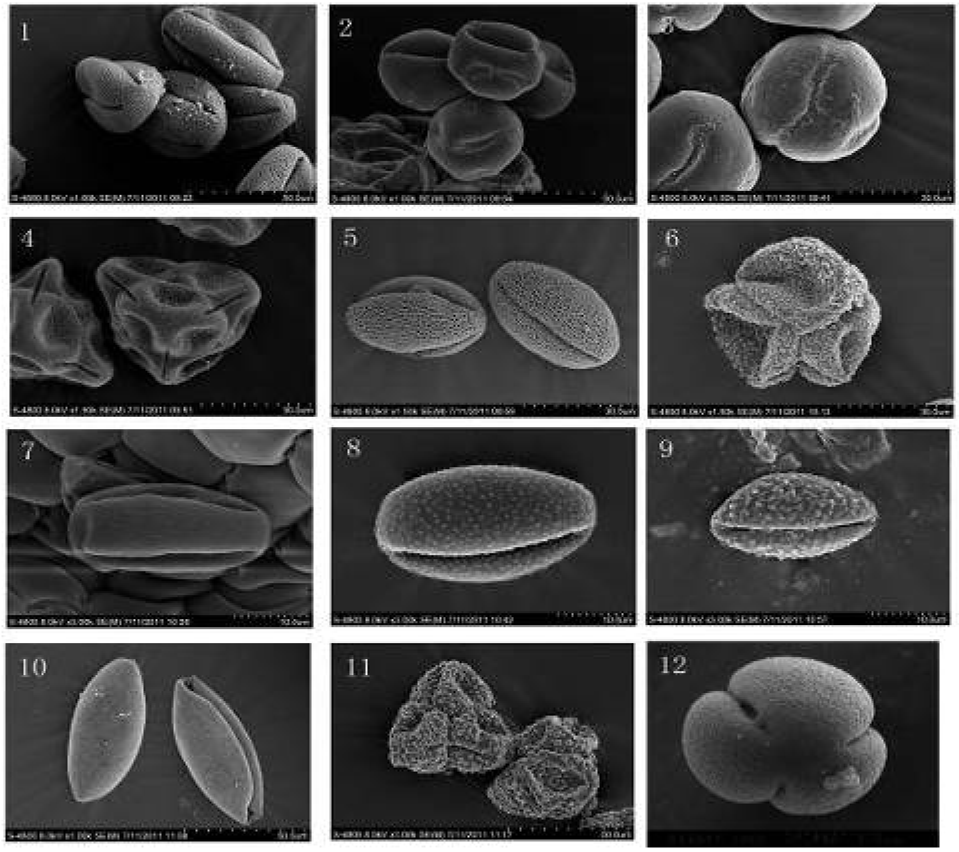

Fig. 3. Pollen grains of plants with similar flowering periods. 1: *Euphorbia esula* 2: *Herba Taraxaci* 3: *Rhododendron pulchrum* 4: *Berberis spp.* 5: *Gentiana veitchiorum* 6: *Sinopodophullum hexandrum* (pink) 7: *Duchesnea indica* 8: *Clematis florida* 9: *Salix rehderiana* 10: *Iris ruthenicar* 11: *S. hexandrum* (White) 12: *Paeonia delavayi*.

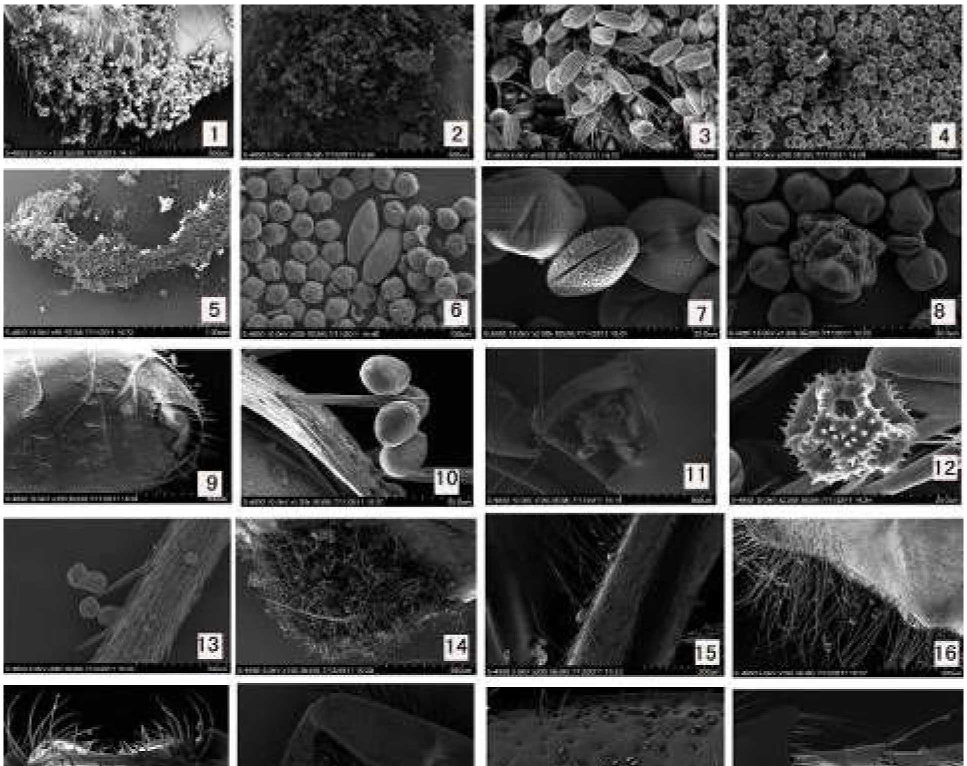

Fig. 4. Pollen grains on the surface of visiting insets
1, 2, 3, 12: *Sawfly* sp. 4: *A. (Sigmatapis) cerana cerana* 5, 6, 7, 8: *M. (Sinomacropis) immaculata* 9, 10, 11: *Formica temani* 13: *Camponotus japonicus* 14, 15, 16: *Volucella trifasciata* 17, 18, 19, 20: *Mimela chinensis*

Table 1. Insects visiting the flowers.

Insect of visiting

Hemiptera
Pentatomidae
1. *Plautia fimbriata* (Fabricius)
2. *Nezara viridula* (Linnaeus)
Miridae
1. *Heteroligus trivittulatus* (Reuter)
2. *Miridae*
Dermaptera
Forficulidae
1. *Forficula davidi* Burr
Coleoptera
Cantharidae
1. *Lycocerus pubicollis* (Heyden)
Rutelidae
1. *Mimela chinensis* Kirby
Nitidulidae
1. Nitidulidae
Lepidoptera
Adelidae
1. *Nemophora amurensis* Alphéraky
Pieridae
1. *Pieris canidia* (Sparrman)
Hesperiidae
1. *Lobocla proxima* (Leech)

Hymenoptera
Formicidae
Formicinae
1. *Camponotus japonicus* Mary
2. *Formica sinae* Emery
3. *Formica fusca* Linnaeus
4. *Formica temani* Bondroit
Apoidea
Apidae
1. *A. (Sigmatapis) cerana cerana* Fabricius
Melittidae
1. *M. (Sinomacropis) immaculata*
Tenthredinidae
1. Sawfly
Diptera
Syrphidae
1. *Melanostoma scalare* (Fabricius)
2. *Episyrphus balteatus* (De Geer)
3. *Volucella trifasciata* (Linnaeus)
4. *Eristalis cerealis* Fabricius
5. *Scaeva selenitica* (Meigen)
6. *Syrphus serarius* Wiedemann
7. Syrphidae
Tabanidae
1. *Hybomitra ruoergaiensis* Xu et Song

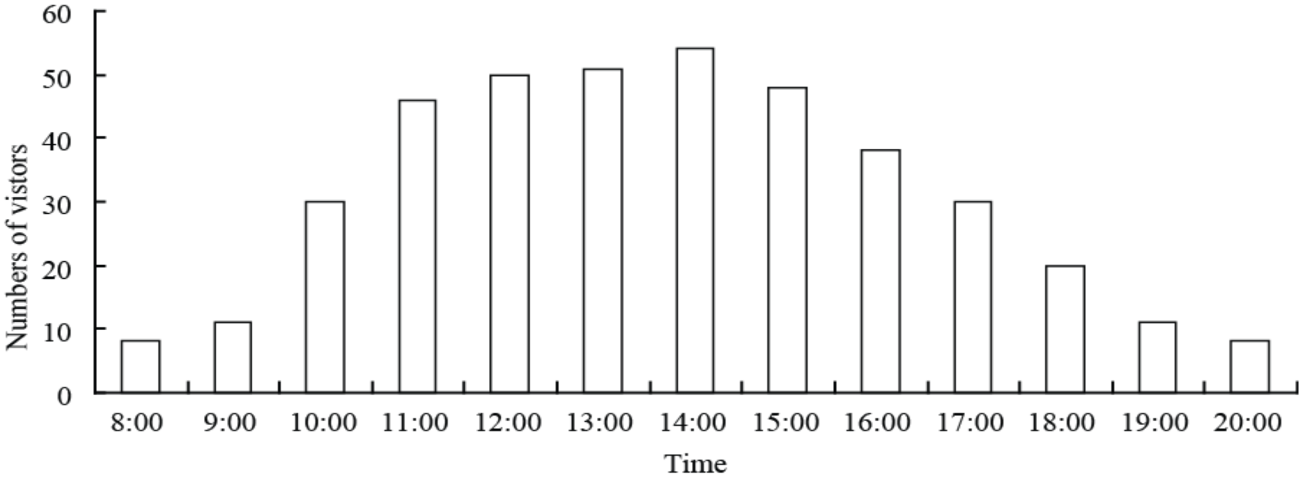

Fig. 5. Visitation rates of flower total visitors, from 8:00 to 20:00 in a day.

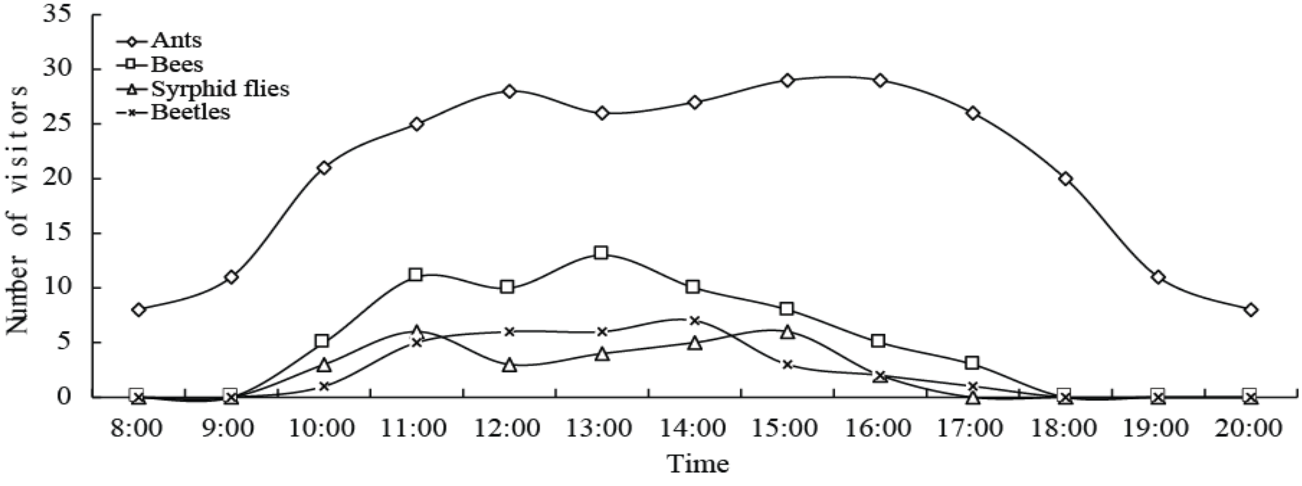

Fig. 6. Visitation numbers of different kinds of insects.

Fig. 7. Visiting insets (1)
1, 2 *A. (Sigmatapis) cerana cerana*; 3, 4 *Syrphidae*; 5 *Melanostoma scalare*; 6, 7 *M. (Sinomacropis) immaculata*; 8, 12 Miridae; 9 *Hybomitra ruoergaiensis*; 10 *Lobocla proxima*; 11 *Nemophora amurensis*.

Syrphid flies: Syrphid flies visited more varieties of flowers. However, most of them stayed only for a few seconds, rarely moved in flowers, passed on the stigma and rarely captured pollen.

Seven species of Syrphidae were found on *P. delavayi* flowers (Figs. 7-3, 7-4, 7-5, 7-10, 8-22), including *Melanostoma scalare*, *Episyrphus balteatus*, *Volucella trifasciata*, *Eristalis cerealis*, *Scaeva selenitica*, *Syrphus serarius*, and *syrphidae* sp. As one of the observed insect groups with most pollination, they moved irregularly, stayed for 1 s to 30 s, rarely crawled in flowers, stopped directly in the petals or pollen, and rarely appeared on the stigma. They had 2 peaks of visiting frequency occurring around 11:00 and 15:00 (Fig. 5). Through stereomicroscope and SEM, we found that there were some few *P. delavayi* pollen on the mouthparts, abdomens, and legs of *V. trifasciata* and *S. serarius* (Figs. 4-14, 4-15, 4-16); however, no pollen were detected on other species.

Fig. 8 Visiting insets (2)
13 *Syrphus serarius*; 14 *Formica sinae*; 15, 20 *Heteroligus trivittulatus*; 16 *Camponotus japonicus*; 17 *Plautia fimbriata*; 18 *Sawfly*; 19 *Forficula davidi*; 21 *Formica temani*; 22 *Episyrphus balteatus*; 23 *Lycocerus pubicollis*; 24 *Araneid.*

Ants: The activity patterns of ants in flowers were significantly different from the other insects. Ants have the largest number, with an average of at least 6 in each plant cluster. Ants stayed the longest, and some of them even stayed in the petals throughout the day (nocturnal activities not observed). Ants remained within the filaments in the morning, and most moved to the anthers when surface temperature increased. They often crawled past the stigma, and entered into the filament or came off after temperature droped in the afternoon.

Ants were the most main flower-visiting insects of *P. delavayi* (Figs. 8-14, 8-16, 8-21), including *Camponotus japonicus*, *Formica sinae*, *Formica fusca*, and *Formica temani*. Pollen were observed on the bodies of *F. sinae*, *F. fusca*, and *F. temani*, *P. delavayi*, and pollen were also found in some specimens of *C. japonicus*. Most of the pollen, although few and scattered, were attached to the mouthparts and the cilia (Figs. 4-9, 4-10, 4-11, 4-13). Moreover, ants had extremely high visiting frequency (Fig. 6), appearing all day long, which peaked at noon. *C. japonicus* crawled on the whole flower; however, the pollen load was less.

Beetles*:* The activity of beetles including *Plautia fimbriata*, *Nezara viridula*, *Heteroligus trivittulatus*, Miridae, *Forficula davidi*, *Lycocerus pubicollis*, *Mimela chinensis*, and *Miridae* patterns of beetles were similar to that of bees of becfles (Figs. 7-8, 7-9, 7-12; Figs. 8-13, 8-15, 8-17, 8-19, 8-20, 8-23). However they made fewer visit and stayed longer in flowers. The actions of beetles were more slowly and only less pollen could be attached on their bodies. Moreover, a number of pollens were often left when the beetles passed through the stigma.

The beetles, except for *P. fimbriata*, *F. davidi*, and *L. pubicollis*, *P. delavayi* pollen were discovered (Figs. 4-17, 4-18, 4-19, 4-20), and the pollen they carried were less than those in bees but more than those in ants. Their visiting frequency was relatively less (Fig. 6); however, they stayed much longer, moved more slowly, and migrated less.

Other groups were mainly *Nemophora amurensis* of *Lepidoptera*, *Pieris canidia*, *Lobocla proxima*, and two species of spiders (Fig. 7-11; Fig. 8-24). Most of such visitors stayed on the petals, and did not visit the flowers or chewed the pollen. No *P. delavayi* pollen was found in these specimens, and there is no significant correlation between their staying time and visiting frequency; therefore, no statistical analyses were made.

Breeding system: The results of different bagging experiments of *P. delavayi* are presented in Table 2. Twenty four flowers from isolation after emasculation didn't produce effective seeds, indicating the absence of apomixes. Only one of the 18 flowers from isolation without bagging produced 1 seed, which demonstrated the probability of weak selfing. Among the 16 flowers in anemophilous pollination, 3 produced seeds, indicating that *P. delavayi* could be wind pollinated. However, the efficiency was not high, with only 18.8% seed setting rate. Through detection of pollen flow (Fig. 9), the maximum horizontal flight distance of *P. delavayi* pollen was about 2 m, compared with large amount of pollen, the pollen collected from the 2 cm × 5 cm glass slides was extremely small, and wind pollination was almost negligible in a calm wind. Among the 20 flowers in artificial geitonogamy, 4 had seeds, translating to 20.0% seeding rate. In the three handling modes, the seed production of fructification was not high, and only 1 to 2 seeds were observed in some follicles. In addition, almost all barren flowers formed follicles, which had neatly arranged infertile ovules. More seeds were obtained in the artificial xenogamy and natural crossing exprements, and the seeding rate of artificial xenogamy (87%) was higher than that under natural conditions, indicating that insect pollination in the natural state cannot fully meet the requirements for stigma to receive pollen.

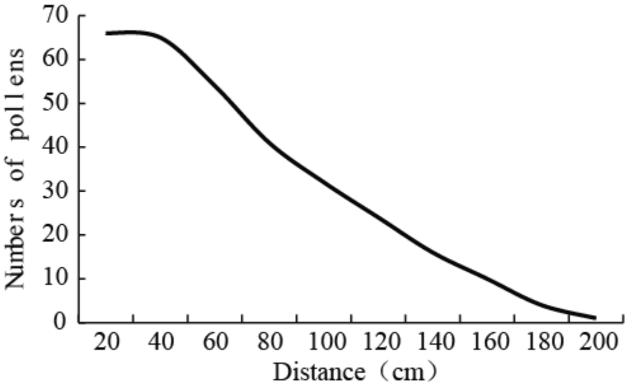

Fig. 9. Pollen flow of *Paeonia delavayi*, the pollen collected from the 2 cm × 5 cm glass slides.

Control pollinators: For the pollination effects of bees, beetles, ants, and syrphid flies in the same area, 4 plant clusters with close flowering periods were selected. Observation was performed from the start of stigma appearance and continued for 4 d. The observed flowers were bagged at night to exclude the influence of insects' nocturnal activities. The statistical results are shown in Table 3.

Table 2. Seeding rates of different treatments.

Treatments	No. of flowers	No. of fruits	Seeding rate (%)
Isolation without emasculation	18	1	5.5
Isolation after emasculation	24	0	0
Anemophilous pollination	16	3	18.8
Artificial geitonogamy	20	4	20.0
Artificial xenogamy	23	20	87.0
Natural pollination	27	21	77.8

Table 3. Seeds of different visitors.

Visitors	No. of flowers	No. of fruits	Seeding rate (%)	No. of seeds per flower
Bees	6	6	100	11.7
Beetles	4	2	50.0	8.5
Ants	7	3	42.8	5.3
Syrphid flies	7	1	14.3	4.0

Bee pollination efficiency was significant. With slight difference in the number of seeds, all of the 6 observed flowers had seeds, with an average of 11.7 (including the seeds with damaged endosperm). For beetles, among the 4 observed flowers, 2 had seeds with an average of 8.5. Among the 7 observed ants visiting flowers, 3 had seeds with an average of 5.3, which were significantly lower than those in the bee pollination. Only 1 Syrphid flies visiting flower had 4 seeds in total. Because of the limitations of the experiment, seeds derived from the pollination of syrphid flies cannot be guaranteed. Therefore, their pollination efficiency was extremely unreliable despite the number of syrphid flies.

Discussion

Breeding system: *P. delavayi* is featured by its asexual reproduction, and the maintenance and expansion of the population is mainly based on the formation of new plants through sprouting and with little quantity of seedlings. However, in terms of the distribution area, seed multiplication always had an irreplaceable function with nutritious breeding (Liu, 1998), and sexual reproduction allocation may be varied (Bierzychudek, 1981; Zhong, 1995; Primack & Silander, 1975). The adaptation of seeds to environmental changes has some selective variation, whereas pollination is a key link in formation of seeds.

The bagging test results of the six treatments used to study the breeding system showed the absence of apomixes, and may have wind pollination ability and weak selfing. In addition, artificial crossing seeding rate was higher than the seeding rate under natural environmental conditions. The experiments showed that natural pollination conditions could not meet the pollination requirements of *P. delavayi* to some extent because of the limited number of visiting insects or interference by plants with similar flowering period. Luo *et al.,* (1998) and Schlising (1976) also obtained similar results in other *Paeonia* species. The formation of seeds depended largely on pollinator activities. Heterogeneous genes carried through cross-pollination can further enhance the variability and adaptability of the offspring, and thus, drive the evolution of the species. For *P. californica*, Grant (1975) believed that self-pollination was dominating, and it was due to self-pollination that made heterozygosis which was not eliminated through natural selection. On the contrary, Schilising (1976) thought that it was a cross-pollinated plant. Based on the activity regularity of bees, Luo *et al*., (1998) reported that cross-pollination was possible in a natural state, and that *P. jishanensis* could be a cross-pollinated plant. After the petals open, *P. delavayi* dissemination gradually start from inside to outside; one day before opening and 3 d after opening of petals, pollination setting rate was high, until the stigma darkened and hardened, and dissemination continued with protogynous characteristics. In the breeding system experiment, the seeding rate from artificial xenogamy was slightly higher than that in the natural state. In combination with the results of the bagging experiment, we believe that the *P. delavayi* is a cross-pollinated plant.

Pollination ecology: The pollination data provide the basis for population genetics and phylogeny (Zhou, 1998; Proctor & Yeo, 1979), and pollination is a cross-disciplinary study of biological characteristics related to pollination events and their law (Zhou, 2003). Flower color, shape, smell, etc., are generally believed to be the factors attracting insects for pollination, and pollen and nectar are important compensations provided to insects by the flowers (Liu *et al*., 1998; Knudsen & Mori, 1996; Real & Rathcke, 1991; Real, 1983). *P. delavayi* attracted flower visitors by providing attractive rewards, and in addition, influenced the insect's visiting time by controlling the reward time, thereby achieving a successful pollination. The rewards provided by *P. delavayi* to pollinators were pollen and substantial nectar. The insects that were effective most for seed production were *A. cerana, M. (Sinomacropis) immaculate*, and sawfly. In addition, several factors, such as illumination, strong wind, temperature, humidity, rainy weather, plants with similar flowering period, artificial beekeeping, and even year, can affect the number of flower-visitors, flower-visiting behavior, and visiting frequency, thereby influence pollination and fructification (McCall & Primack, 1992; Niesenbaum, 1992; Picken, 1984; Real, 1983). Meteorological factors also affect the floras of insects within their habitats, and thus, affect the number and efficiency of flower visitors. According to previous researches, plants with similar flowering period may serve as competing pollinators (Motten, 1986; Campbell, 1985; Campbell & Moften, 1985). The results indicated that the competitions were enhanced between the pollinators by large quantity plants of similar flowering period in the same habitats and it lead to visiting the flowers incompletely. On the body of *M. (Sinomacropis) immaculate*, we found of pollens of 3 species with the same flowering period as *P. delavayi*. This may have affected the visiting frequency for *P. delavayi*. However, when there were several insects in the habitat, such competition may not exist. Another possibility also exists, i.e. when there are more plants with the same flowering period in the same region, plants will attract a greater variety and large number of visiting insects. *N. (Acunomia)* *thoracica*, as we observed, carried a large amount of *P. delavayi* pollens on its surface, despite not coming for special visit to *P. delavayi*, a case similar in studies of other plants (Conner & Neumeier, 1995), but Crows (1995) not in reference thought that beetles were pollinators; however, they were found to be only eaters of flowers. Some researchers reported that the pollen of pepper have poor adhesion to beetles. Bernhardt *et al*., (1987) thought that all the beetles were not necessarily pollinators. Our experiment showed that the pollination of bees was the most reliable. Therefore, increasing the number of insects in the habitat can increase pollination and ensure reproductive success.

In terms of the specificity of insect flower-visiting, pollen of more than two species were found on bees and beetles; and bees carried pollen with the largest number and type. Moreover, bees were observed visiting other plants with similar flowering period, whereas beetles were not. Pollens of other plants were found on some beetles viz. *M. chinensis*, *Miridae* sp., and *L. pubicollis*. Ants and Syrphid flies carried only few pollen (Figs. 4-9, 4-11), and no pollen of other plants was discovered under SEM.

According to Schlising (1976) and Luo *et al*., (1998), the annual seeding rate of *P. suffruticosa* was sufficient to maintain and expand the existing size of the populations, however, for *P. delavayi*, we found a dynamic change in its number of populations. The size of populations was declining, and it mainly relied on its seeds and sprout branches. However, if the seed multiplication is limited, even without human interference, *P. delavayi* would have difficulty in ensuring an advantage in niche competition. During the three years of research, we found that, with the increase of populations in the habitat such as *S. rehderiana* and *P. revolutum*, as well as the expansion of cultivated land and continued human excavation, *P. delavayi*, with sprouting as major reproduction mode, suffered from increased recession, declined growth potential, smaller flowers, and constant decrease in flowering number and seeding rate. Therefore, seed propagation is a guarantee, and successful pollination is a necessary prerequisite to bring into play the adaptive advantages of sexual reproduction.

Conservation biology: *P. delavayi* is a cross-pollinated plant, and bees are the major pollinating insects. The main factors affecting pollination include the type and number of visiting insects, weather conditions, and type and number of plants with similar flowering period. Because of the limited number of bees or weather conditions, insects cannot visit all the flowers within the populations, resulting to a lower pollination and seeding rate under natural conditions than that of artificial crossing. The increasing canopy density of other plant populations in the *P. delavayi* habitat affected its flowering. In addition, rain influenced *P. delavayi* pollination greatly, and as the temperature dropped, insects seldom moved into the petals. Pollen were also scattered by the rain, insufficient sunshine affected the secretion of nectar, and several flowers failed to achieve successful pollination in the best period of pollination. This condition resulted in the decline of seeding rate under natural conditions. The artificial bee keeping in the flowering period of *P. delavayi* can enhance pollination which may result high seed setting. In addition, reduction of the canopy density of other plant populations and using artificial pollination in special weather (rainy days) conditions could create adaptability of future generations to environmental changes, promote the formation of seedling plants in habitats, and protect the populations stably of *P. delavayi*.

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