

Article

The Floral Biology, Breeding System and Pollination Efficiency of *Schima superba* Gardn. et Champ. (Theaceae)

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Received: 21 July 2017; Accepted: 11 October 2017; Published: 24 October 2017

Abstract: *Schima superba* Gardn. et Champ. is a perennial, evergreen tree valued for its eco-protection and commercial values in China. In this study, we investigate the breeding system, reproductive ecology and pollination biology of *S. superba* in a seed orchard. The flowers are hermaphrodite and protogynous. The viability of the pollen is inactivated rapidly, and the stigma maintains a high receptivity within the flower lifespan. Flowers typically offer pollen and nectar to visitors. The flowers possess a typical insect pollination syndrome, and three visitors (*Apis cerana cerana* Fabricius, *Protaetia brevitarsis* Lewis, and *Popillia mutans* Newman) are observed on flowers during the study period. The visitation frequency per minute and capability of pollen removal and deposition of *A. cerana* are significantly higher than *P. brevitarsis* and *P. mutans*, although the pollinator efficiency is lower than those shown by the two beetles. Fruit set (28.27%) and seed set (6.57%) percentages resulting from open-pollination are significantly lower than those resulting from cross-pollination (fruit/seed set, 43.73%/11.66%), and the pollen limitation index (*L*) was 0.34, suggesting that seed production is pollen-limited in the seed orchard. The pollen/ovule ratio (P/O) and outcrossing index (OCI) values are 6686.67 and 4, respectively. The self-incompatibility index (ISI) was estimated to be 0.95. Results from hand-pollination, pollen tube growth experiments and the ISI value show that *S. superba* is late-acting self-incompatible. The synthetic results indicate that *A. cerana* is the most efficient pollinator of *S. superba*, and seed production is frequently limited by pollinators, fruit abortion, and pollen quality.

Keywords: breeding system; pollination; floral biology; *Schima superba* Gardn. et Champ.

1. Introduction

Breeding systems are important and often neglected aspects of reproductive biology; the main research content includes floral characteristics, pollination and mating systems [1]. The floral biology, pollination pattern, and behavior are closely related to reproductive fitness and are thus subjected to strong selective forces [2]. The reproductive success of plants is often limited by lack of pollination, mate availability and fecundity and mate limitation [3]. Flower arrangement and reward availability within plants are also important factors that influence mating success [4]. The timing of pollen presentation and stigma receptivity at both the individual and population levels may strongly correlate with mate availability, and species with long flowering periods may avoid pollination limitation and reproductive deficit as a consequence of an increased overlap with insect activity [5]. Individuals with hermaphrodite flowers may resort to self-pollination, which leads to maximum seed setting but minimum genetic variation in their offspring [6]. However, geitonogamy may also mitigate pollen limitation in self-compatible plants [7]. Therefore, floral syndrome and breeding system investigations are not only conducive to understanding the pollination

mechanism and mating patterns but also have scientific significance for further investigating the evolution and environmental compatibility of plants.

Pollination is a key stage of generative propagation, and the dispersal of plants is closely linked with pollinators. In addition, pollination has important application value for biodiversity. Approximately 65% of flowering plants are insect-pollinated [8]. Insect pollination promotes the rapid development of flowering plants. Pollination mediates male-female interactions and is dependent on abiotic and biotic factors [6]. The pollination success of pollinator-dependent species is highly influenced by the frequency of visits, the intrafloral foraging behavior of pollinators and the breeding system [9]. In self-incompatible plants, the visitation rate and foraging behavior of pollinators within plants influence the amount of self-pollen transferred and may eventually lead to pollen limitation [9]. A lack of faithful pollinators, a few pollinators or low pollination efficiency in insect-pollinated plants can result in low pollination intensity and reduced seed yield, and the resulting flower-visiting behavior would also affect the seed yield and quality [10]. Pollination efficiency is directly related to pollination amount and quality and is affected by flower-visiting behavior [11]. Therefore, the interaction among population structure, pollination behavior, and pollination efficiency is very complex and requires synthetic and meticulous research.

Theaceae is a family of subtropical and tropical trees in Asia with approximately 17–20 genera and 500 species [12]. Members of this family are predominantly pollinated by insects [6,13]. Previous studies on the breeding biology of Theaceae have mainly focused on *Ternstroemia laevigata* [13], *Ternstroemia dentate* [13], *Camellia japonica* L. [14], and *Schima wallichii* (DC.) Korth [6]. *Schima superba* Gardn. et Champ. is the most widely distributed species of *Schima* plants in China. This plant species is not only used as a biological firebreak and an ecological protection tool, but it is also considered a valuable timber tree [15]. However, we know little about the reproductive biology of *S. superba*. Studies examining pollination and breeding biology have great potential for revealing insect/plant interactions and their underlying importance in speciation and diversification, genetic fitness, reproductive efficiency and success in areas of applied forest science [16]. Thus, we analyzed the floral syndrome, breeding system and pollination efficiency of *S. superba*: (1) What are the characteristics of the breeding system of *S. superba*? (2) What are the barriers to fruit and seed set in the flowering and pollination processes? (3) What are the effective pollinators of *S. superba*, and what are their effects on fruit and seed set? The answers to these questions can contribute to artificial pollination, the development of crossbreeding and variety improvement, as well as promote seed production.

2. Materials and Methods

2.1. Study Species and Site

Schima superba Garden. et Champ. is a monoecious, large evergreen tree native to China [17]. The leaves are leathery or slightly leathery; the flowers are white and the ovary hairy [17]. Based on long-term observations, the fruit-bearing period of *S. superba* lasts 1.5 years and seed maturation occurs in approximately October of the following year. The present investigation was conducted in a seed orchard in Jinhua City, eastern China (29°08'16" N, 119°27'47" E). The average annual rainfall is 1438.9 mm, the elevation is 42 m, and the mean annual temperature is 17.7 °C. The surrounding vegetation consisted mainly of *Erigeron annuus* (L.) Pers., *Setaria viridis* (L.) Beauv., *Plantago depressa* Willd., *Cinnamomum camphora* (L.) Presl., *Liriodendron chinense* (Hemsl.) Sarg. and *Celtis sinensis* Pers., etc. The selected trees were of similar age and were cultivated through cuttings.

2.2. Floral Biology

The total number of inflorescences per individual was counted based on 30 randomly selected trees. We recorded the total number of flowers, flowering period, flower lifespan, and timing of anther dehiscence. We measured the length of the stigma and stamen (LS and LOS) and petal and corolla (LP and LC) and calculated the mean values of 60 replicates. All floral measurements were made to 0.01 mm using digital calipers. The number of pollen grains was estimated using a hemocytometer [18],

and ovule numbers were counted directly, based on 60 replicates. Twenty flowers were randomly selected and bagged before anthesis, and the nectar volumes were measured using 20- μ L Hirschmann “minicap” calibrated capillary tubes. Stigma receptivity and pollen viability were measured three times a day (at 09:00, 15:00, and 21:00) for a single live flower using a benzidine- H_2O_2 test [18] and an in vitro culture method, respectively. The flowers used to measure floral structure, pollen grains, nectar volumes, stigma receptivity, and pollen viability were collected separately.

2.3. Breeding System

To determine the breeding system, we carried out five pollination treatments on five trees with a large amount of flowers: (1) apomixis: 20 artificial emasculation flowers were bagged without pollination; (2) self-pollination using the same flower (SPSF): 20 flowers were hand-pollinated with the pollen from the same flower; (3) geitonogamy (GSP): 20 flowers were hand-pollinated with pollen from other flowers from the same individual; (4) crossing (CP): 20 flowers were hand-pollinated with pollen from another individual; (5) open-pollination (NP): 20 flowers were tagged and left for open pollination. Flowers subjected to hand-pollination were bagged with sulfuric acid paper bags and bagged again after hand-pollination. These experiments were conducted from 15 May to 30 May in 2013, 2014, and 2015. The fruit set of each treatment was counted two (July) and five months (October) later, and the seed set was counted 17 months later (October of the next year). For the natural fruit/seed set, we randomly selected 20 inflorescences to count and record the fruit/seed number per inflorescence. Analysis of variance (ANOVA) was performed to evaluate the difference of fruit/seed set for different treatments followed by Bonferroni post-hoc test. Fruit and seed set data were arcsine-transformed prior to analysis. The purpose of using the control and open pollination was to determine whether the pollen supplementation treatments affected fruit set [19]. We calculated an index of self-incompatibility (ISI) to determine the breeding system [20]. The index ranged from 0 to 1 and was the ratio between self and cross pollination, where 0.25 is considered the upper limit for self-incompatible species. Finally, the reproductive efficacy index (REI) was defined as the ratio between seed set from open-pollinated and cross-pollinated flowers; it also ranged from 0 to 1 and was used to estimate the relative efficacy of natural pollination [21]. The significance of ISI and REI was calculated to ensure that they were significantly different from zero based on 1000 bootstrap samples, using a 95% confidence interval, through bootstrap analysis using R version 3.4.1. (R Foundation for Statistical Computing, Vienna, Austria)

Pollen tube growth experiments were also carried out to compare the compatibility of self- and cross-pollination. We bagged inflorescences on the day before the flowers opened and performed self- and cross-pollination treatments at 2, 4, 8, 12, 16, 24, 36, and 48 h. We picked 20 flowers per treatment, removed the styles and immediately fixed them in Formalin-acetic acid-alcohol (FAA) solution (formaldehyde, acetic acid, 70% ethanol at 5/5/90, $v/v/v$), transferred them to ethanol (70%) and stored them at 4 °C. Pollen tubes were measured according to the aniline blue method [18]. The growth rate (v) of the pollen tubes was calculated as the length of the pollen tube (Lpt), divided by the total style length (Ls) ($v = Lpt/Ls$).

2.4. Visitors

To determine the pollinators and their visitation rates, counting of the visiting insect species was performed for all 20 randomly selected trees. The presence of floral visitors was recorded during sunny days from 15 May to 21 June in 2015 and 2016. The duration and frequency of visits and the behavior of the visitors were recorded. The selected trees were observed in two observation blocks, based on one-hour intervals, that is, between 08:00–09:00, 10:00–11:00, 13:00–14:00, and 16:00–17:00. The frequency of visitors was assessed in terms of flowers/minute.

To estimate the difference between pollinators, counts of removed and deposited pollen grains were made subsequent to an initial visit by pollinators. Prior to visitation, 90 flowers from 20 trees were bagged with sulfuric acid paper bags to exclude visitors. The removed and deposited pollen grains

were counted after the flowers were first visited by the visitors according to the method described by Han et al. [22]. These samples were then transported to the laboratory to count the remaining pollen grains and those deposited on the stigmata. Sixty buds from ten trees were used to estimate the pollen production per flower. The anthers per flower were placed in a 5 mL centrifuge tube, and the quantity of pollen was determined according to the method described by Dafni (1992), based on 5 replicates per flower. Pollinator efficiency was calculated using the following formula:

$$\text{pollinator efficiency} = \log_{MR}^{MD} \quad (1)$$

where MD is the mean number of pollen grains deposited on the stigmata, and MR is the mean number of removed pollen grains [23]. The capability of pollen removal and deposition was calculated using the following formula:

$$\text{the capability of pollen removal} = \text{the mean visiting frequency} \times \frac{R}{\text{the pollen production per flower}} \quad (2)$$

where R is the number of removed pollen grains; and

$$\text{the capability of pollen deposition} = \text{the mean visiting frequency} \times D \quad (3)$$

where D is the number of pollen grains deposited on the stigmata. Analysis of variance (ANOVA) was performed to evaluate the difference in visitation frequency, pollinator efficiency, and the capability of pollen removal and deposition between pollinators followed by the Bonferroni post-hoc test.

3. Results

3.1. Floral Biology

The flowering of *Schima superba* Garden. et Champ. occurs mainly from the middle of May to the middle of July. Flowers opened after sunrise at approximately 08:00 a.m., reached maximum aperture between 10:00–11:00, and withered in the afternoon on the fourth day (Figure 1a,b). The flowers were arranged on a single racemose inflorescence containing an average of seven flowers (Table 1). The average number of inflorescences per tree was 86.68. Flowers opened randomly in the inflorescence, and typically more than two flowers bloomed at the same time. The flowers are conspicuous, with a white, dialypetalous and actinomorphic corolla (mean length = 31.57 ± 2.69 mm), and have five connate white petals (mean length = 16.79 ± 2.18 mm). An average of approximately 80.2 stamens (mean length = 7.91 ± 2.42 mm, ranged from 6.9 to 12.2) are inserted around the style head, and the stigma is, on average, 8.30 ± 0.85 mm long (Figure 1a, Table 1). The flower is herkogamous with a mean value (mean length of stamens/mean length of stigma) of 0.95, and 96.7% of the examined flowers had longer stigmata than stamens. There also was a spatial separation between the stigmata and stamens in all flowers. The majority of the flowers presented viscous nectar with an average volume of 252.27 ± 16.67 μ L. We observed nectar at the bottom of the filament, which smelled sweet after the flowers bloomed. The number of pollen grains and ovules produced per flower was $100,300 \pm 47,056$ and 15.00, respectively. Hence, the P/O ratio was 6686.67.

Table 1. Floral characteristics of *Schima superba* Garden. et Champ (S.E.).

Items	NIP	NFI	LC (mm)	LP (mm)	LS (mm)	LOS (mm)	Pollen Grains	Ovules	Pollen-Ovule Ratio
Mean (SE)	86.68 (21.42)	7 (2)	31.57 (2.69)	16.79 (2.18)	8.30 (0.85)	7.91 (2.42)	100,300 (47,056)	15.00	6686.67
Range	40.50–147.55	4–11	27.86–35.12	11.73–20.35	6.80–9.60	4.01–12.25	92,104–165,105	-	-
N	30	30	60	60	60	60	30	30	30

Note: N, sample size; NIP, number of inflorescence/plant; NFI, number of flowers/inflorescence; LC, length of corolla; LP, length of petal; LS, length of stigma; LOS, length of stamen.

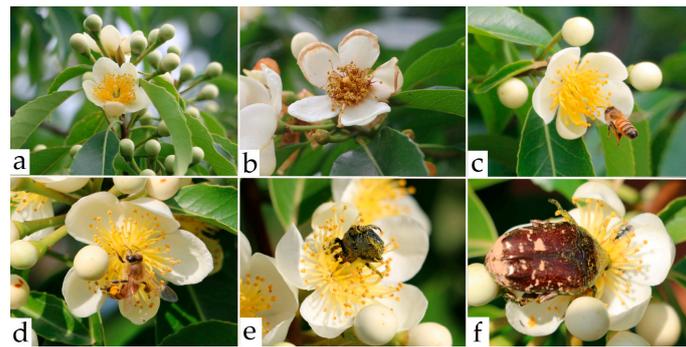


Figure 1. The flowers and pollinators of *Schima superba* Garden. et Champ.

3.2. Pollen Viability and Stigma Receptivity

The viability of pollen showed a sharp decreasing trend: the viability was relatively stable initially; thereafter, the viability of the pollen decreased rapidly to 5.45% at 09:00 on Day 4 (Figure 2). Based on the daily average viability, the percentage of viable pollen (in decreasing order) was observed on Day 1 > Day 2 > Day 3 > Day 4. The stigma is papillose, wet after pollination and situated at a similar height as the anthers. The stigma receptivity started before anther dehiscence, which points to a slightly retained protogynous type of dichogamy in *S. superba*. The stigma remained highly receptive for the entirety of the flowers' lifetime (Figure 2).

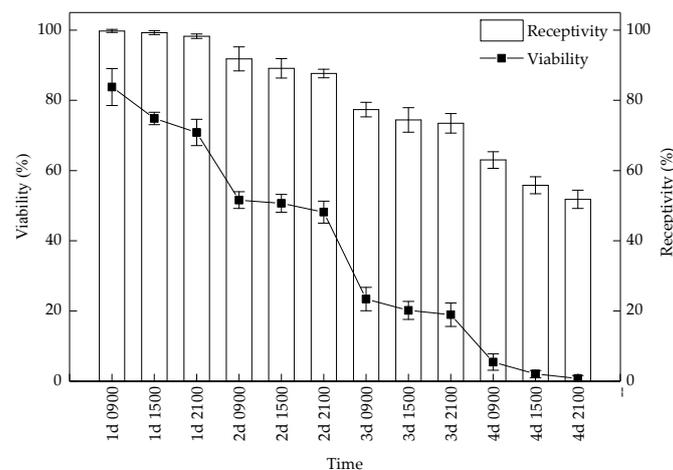


Figure 2. Pollen viability and stigma receptivity in *Schima superba* (S.E. percentage per day).

3.3. Breeding System

No fruits were obtained from the bagged flowers, indicating the absence of agamospermy for this species. The fruit and seed set of cross-pollination (CP) were significantly higher than geitonogamy self-pollination (GSP) (fruit set: $F_{1,43} = 273.724$, $P < 0.001$, seed set: $F_{1,27} = 407.019$, $P < 0.001$ for seed set) and self-pollination with the same flower (SFSP) (fruit set: $F_{1,43} = 265.224$, $P < 0.001$, seed set: $F_{1,27} = 407.011$, $P < 0.001$) (Figure 3). The reproductive efficacy index (REI) of *S. superba* was low (0.56 ± 0.03 , which ranged from 0.50 to 0.63 at the 95% confidence interval), and the fruit and seed set from open-pollination (OP) was significantly lower than in the CP treatment (fruit set: $F_{1,43} = 23.145$, $P < 0.001$, seed set: $F_{1,27} = 40.633$, $P < 0.001$). The CP treatment was more successful than the SP and OP treatments: maximum fruit (41.99%) and seed (11.66%) setting was observed in the CP treatment, followed by the NP treatment (27.70% and 6.57%, respectively). The GSP treatment yielded the least fruit (1.91%) and seed (0.20%), which were equivalent to the results obtained with the SFSP treatment (fruit set, 2.05%; seed set, 0.40%), and there was no significant difference between GSP and SFSP

(fruit set, $F_{1,43} = 0.084$, $P = 0.773$; seed set, $F_{1,27} = 0.042$, $P = 0.184$). The fruit abortion was very high; the fruit abortion rates of the SFSP, GSP, CP and NP treatments were 88.8%, 88.9%, 32.3%, and 33.1%, respectively (Figure 3).

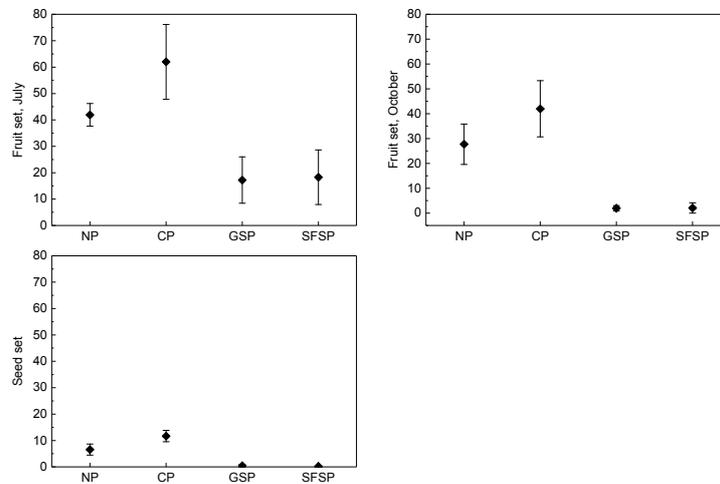


Figure 3. The effect of pollination treatments on the fruit and seed set of *Schima superba* (S.E.). Note: NP, open-pollinations; CP, cross-pollination; GSP, geitonogamous self-pollination; SFSP, self-pollination with the same flower.

The pollen tube growth experiments showed that pollen growth after the crossing treatment was more rapid than in the self-pollination treatment, as assessed during the first 2 h (9.82% vs. 2.48%). These two treatments reached their greatest difference at 12 h (81.23% vs. 49.45%), and then the bottom of the styles was reached after 36 h (Figures 4 and 5). At 48 h, the pollen tube of the crossing treatments reached the ovary, but the pollen tube of the self-pollinated flowers did not (Figure 4). The self-incompatibility index (ISI) was estimated to be 0.95 ± 0.01 (0.93–0.97 at 95% confidence interval), suggesting that *S. superba* behaved mostly as a late-acting self-incompatible (LSI) species.

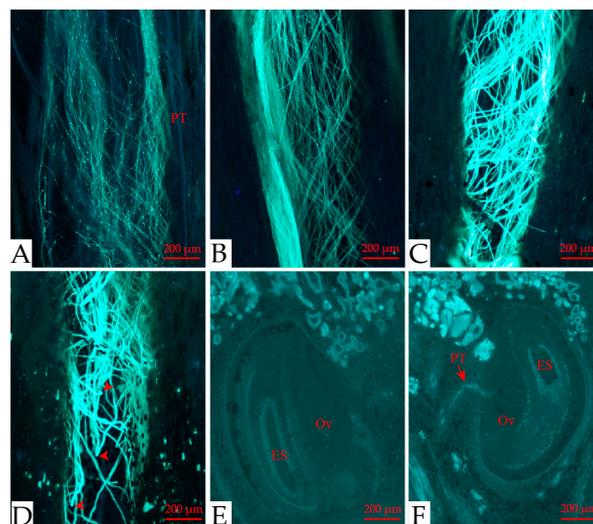


Figure 4. Pollen tube growth of *Schima superba* for self- and cross-pollination treatments in style and ovary. Note: (A) pollen tubes resulting from cross-pollination grew to the medial style at 12 h; (B) pollen tubes resulting from self-pollination grew to the medial style at 12 h; (C) pollen tubes from cross-pollination grew to the base of the style at 36 h; (D) pollen tubes resulting from self-pollination grew to the base of the style at 36 h with part retardation (shown by red arrows); (E) cross-pollinated pollen tubes entered the ovary; (F) none of the self-pollinated pollen tubes entered the ovary. SEM resolution of 200 μm .

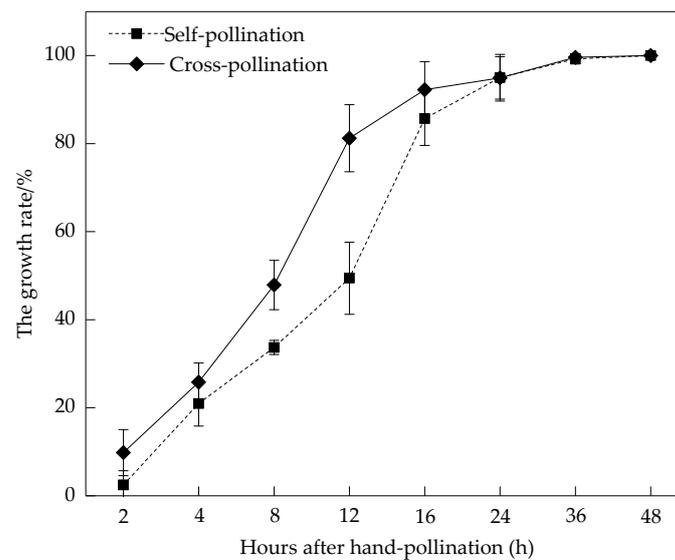


Figure 5. The pattern of pollen tube growth in the styles of *Schima superba* under two pollination treatments (S.E.).

3.4. Pollinator Visitation

A large number of *Apis cerana cerana* Fabricius, *Protaetia brevitarsis* Lewis and *Popillia mutans* Newman were observed within the whole flowering phase of *S. superba*. Therefore, we determined that the three insects were the main pollinators of *S. superba* through observation. In the seed orchard, *Pieris rapae*, *Nephargynnis anadyomene*, *Epicopeia mencia* and *Musca domestica* were occasionally observed with flower-visiting behavior; however, they were not the main pollinators because of very small quantity in the seed orchard. *A. cerana* landed on any part of flowers and drew the nectar from the bottom of the stamen using their mouthparts (Figure 1c,d). *P. brevitarsis* and *P. mutans* had similar visiting behaviors. The beetles were rewarded with pollen found in the staminode and inner petals. The pollen adhered to the mouthparts, feet, and sternum of the beetles as they fed, though some pollen also adhered to the back of the animal (Figure 1e,f). We also observed that the beetles would often try to dive their mouthparts into the nectary (at the bottom of the stamens) to suck nectar. Their legs, chest, and abdomen could touch the stigma when they walked on the flowers; thus, we believe that the beetles' pollination strategy is abdominal pollination. There was a major difference in the abundance, identity, and diversity of pollinators between plants. The average pollinator visit (abundance, identity, and diversity) per plant was 1 (0–4) for *A. cerana*, 1.1 (0–8) for *P. brevitarsis*, and 10.2 (5–18) for *P. mutans*.

The floral measurement results shed some light on the flower-pollinator interactions (Figures 6 and 7). The large corolla made it possible for the putative pollinators to land on and enter the flower. The highest visiting frequency occurred between 10:00–11:00 (Figure 6). There were no significant differences in visiting frequency between *P. brevitarsis* and *P. mutans* ($F_{1,129} = 9.283$, $P = 0.053$). Nevertheless, the visiting frequency of *A. cerana* was significantly higher than *P. brevitarsis* ($F_{1,127} = 53.282$, $P < 0.001$) and *P. mutans* ($F_{1,127} = 43.191$, $P < 0.001$) (Figure 7). This means that *A. cerana* had absolute superiority in visiting frequency compared with the two beetles, as evidenced by their ability to visit more flowers than beetles in a given amount of time. The pollinator efficiency of *P. mutans* was slightly higher than the two other pollinators (Figure 7). There was no significant difference between *P. brevitarsis* and *P. mutans* with respect to the capability of pollen removal ($F_{1,64} = 67.403$, $P = 0.672$) and deposition ($F_{1,64} = 31.632$, $P = 1.000$). Nevertheless, the capability of pollen removal and deposition of *A. cerana* was significantly higher than *P. brevitarsis* (capability of pollen removal: $F_{1,54} = 116.122$, $P < 0.001$, capability of pollen deposition: $F_{1,54} = 18.063$, $P < 0.001$) and *P. mutans* (capability of pollen removal: $F_{1,59} = 78.512$, $P < 0.001$, capability of pollen deposition: $F_{1,59} = 10.693$, $P < 0.001$) (Figure 7).

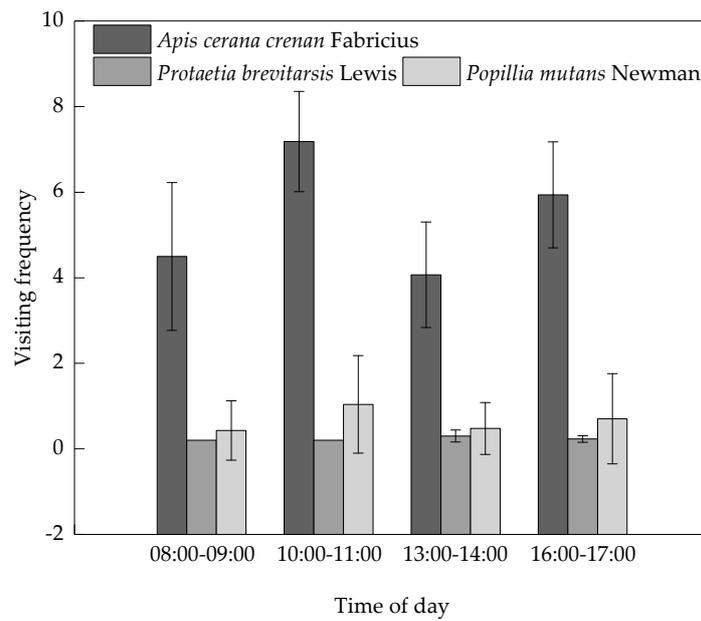


Figure 6. Pollinator visitation frequency for *Schima superba* (S.E.).

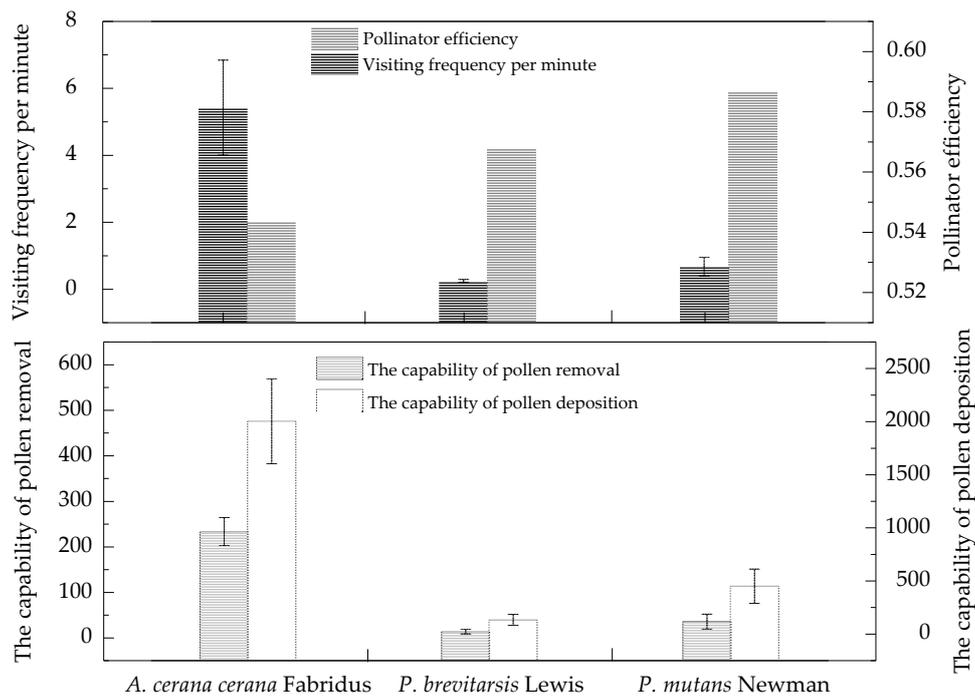


Figure 7. The general visiting frequency, pollinator efficiency, capability of pollen removal and deposition for three pollinators after the first visitation of a *Schima superba* flower (S.E.).

4. Discussion

Phenological and reproductive biology studies are useful for planning conservation strategies as well as formulating measures for cultivating such species at a large scale [24]. The more flowers and the larger flower diameter the plants have, the more pollinators are attracted, which leads to superior fruit set and pollen output rate [25–27]. The flower diameter of *S. superba* Gardn. et Champ. exceeded 27.86 mm. This species belongs to species with large flowers according to the technical standard of Abe [28], and this may attract pollinators to visit flowers and increase pollination opportunities.

The natural flower life ranged from 4 to 5 days, which categorizes the plant as a longer flowering type [28]; this enhances the opportunity of floral visits and ensures the stability of pollen flow. Flowers of *S. superba* have abundant and well-exposed rewards that are potentially easily exploited by a wide array of flower visitors: nectar can be reached by both short and long mouthparts. Several traits contribute to attracting beetles and protecting the ovary from being eaten by them: flowers that are white, larger and gathered into an inflorescence with an inferior ovary [29]. These traits are potentially a result of mutualism between beetles and plants [30]. Protogyny in *S. superba* may be another adaptation to pollination by beetles [29]. Anthesis in *S. superba* is diurnal, beginning at approximately 08:00–09:00 am after sunlight irradiation. In sexual reproduction, the floral syndrome and pollination behavior are reciprocal adaptations, whereas a high amount of viable pollen and many receptive stigmata are prerequisites for effective pollination. The duration of pollen viability and receptivity directly affects the effectiveness of pollination and the success rate of fertilization at different flowering stages [31]. In the current study, pollen viability decreased from the first day of flower opening to the time at which the pollen fell off the flower, which may be associated with the loss of water upon sunlight exposure [16]. Therefore, we predicted that rapid inactivation of pollen was one of the primary reasons for the low fruit set of *S. superba*. In contrast, higher receptivity within a flower's natural life (that is, with high female reproductive fitness) could enhance the fruit set of *S. superba* under adverse environmental conditions as well as improve the number of pollinators and pollination efficiency. There was a major difference in the abundance, identity, and diversity of pollinators between plants, which could be the reason for lower fruit set in some plants. Therefore, we can improve the seed production of *S. superba* seed orchards through artificial cultivation of bees.

According to the high calculated index (0.95) of self-incompatibility (ISI) and the high observed pollen/ovule ratio (P/O) (6686.67), we believe that *S. superba* is mostly self-incompatible with obligatory outbreeding [21,32]. This, together with the results of the pollen tube growth experiments, suggests that incompatibility may be late-acting self-incompatibility (LSI) [33], and the incompatibility may occur in the ovary. In the present study, pollen tubes reached the base of the style 36 h after self-pollination and cross-pollination, and the growth rate of the crossed tubes was slightly higher than that of the self-tubes, similar to what has been described for other plants of the same family, such as *Camellia oleifera* Abel. and *Camellia sinensis* (L.) O. Ktze. [34]. Furthermore, the pollen tubes did not penetrate the ovule after self-pollination (Figure 4E). This may be the result of the generation of resistant materials in the ovule or micropyle (e.g., callose) that prevented the self-pollinated pollen tubes from penetrating the ovule [34]. Fruit and seed set is the standard by which mating success is estimated. The extremely low seed set of geitonogamous and autogamous self-pollination (GSP and SFSP) indicated that the breeding system of *S. superba* is obligate xenogamy, similar to the results from the ISI and P/O. This breeding system can prevent a decline in seed genetic quality due to self-pollination in a seed orchard. The herkogamy of *S. superba* could also reduce self-pollination and prevent inbreeding depression [35–37]. The fruit and seed set resulting from cross-pollination (CP) is higher than that resulting from open-pollination (OP), suggesting that the seed orchard was probably pollinator-limited [38]. It also showed that artificial pollination can significantly improve the fruit and seed set of *S. superba*. The pollen limitation of *S. superba* may be due to both the quantity and quality of the pollen deposited on the stigmata, as we detected a rapid devitalization of pollen (Figure 2). The low reproductive efficacy index (0.66) of *S. superba* may be related to both its late-acting self-incompatibility system and to the beetles' infrequent visits, which, in turn, may have resulted in pollen limitation [39]. This explanation is also confirmed by the observed significantly higher fruiting success of cross-pollination compared with open-pollination. We also detected a higher decreasing rate in the cross- and open-pollination treatments, suggesting that resource limitation may be an important factor because of the high amount of humidity and nutrients demanded during a growth and development period as long as 1.5 years. In the future, to reduce the percentage of fruit abortion, improved management and adequate fertilization methods should be implemented to ensure the necessary humidity and nutrients required for adequate fruit growth.

Mapping floral characters and pollinators onto phylogenies shows that the relationship between flowers and their pollinators is prone to coadaptation [40]. The flowers of *S. superba* show many characteristics of insect pollination, such as a strong smell, an abundance of nectar and a stigma well separated from the nectary. We found that *S. superba* is pollinated by three insects (*Apis cerana cerana* Fabricius, *Protaetia brevitarsis* Lewis and *Popillia mutans* Newman), unlike other Theaceae plants such as *Schima wallichii* (DC.) Korth [6], *Ternstroemia laevigata* and *Ternstroemia dentate* [13]. Based on the pollen viability over time (Figure 2), it is plausible that the three pollinators are the primary and key pollinators of *S. superba* [16]. *P. mutans* is more abundant than *P. brevitarsis* and *A. cerana*; however, the visitation frequency of the two beetles is significantly lower than that of *A. cerana*, which may limit fruit/seed production. Therefore, *A. cerana* is an important and efficient pollinator of *S. superba*. The pollen removal of *A. cerana* in this study was significantly lower than that reported for single visits by *Apis* to other plants [41]. This observation could be explained by the large flower size and numerous pollen grains of *S. superba*. Thomson found that larger *Bombus* queens made more contact with *Erythronium grandiflorum* Pursh (Liliaceae) stigmata [42], and Snow also reported that larger bees deposited more pollen on *Cassia* flowers than smaller bees [43]. Although removal and deposition may increase with body size, in the present study, the smaller *P. mutans* removed and deposited more pollen than *P. brevitarsis* (which has a larger body size than *P. mutans*), suggesting that small beetles could potentially remove and deposit more or as much pollen as larger species. Suzuki hypothesized that flowers had a higher pollinator visiting frequency, and the flowers could increase the amount of fruit set [44]. The amount of removed and deposited pollen grains by *P. mutans* is numerically higher than *P. brevitarsis* and *A. cerana* but not significantly so. Moreover, the capability of pollen removal of *A. cerana* was significantly higher than the two beetles, i.e., 21.58 and 6.83 times higher than *P. brevitarsis* and *P. mutans*, respectively. The capability of pollen deposition of *A. cerana* was also significantly higher than the two beetles, i.e., 17.88 and 4.67 times higher than *P. brevitarsis* and *P. mutans*, respectively. In general, a higher visitation frequency translates to a higher fruit set [41]. Thus, we believe *A. cerana* was the most efficient pollinator of *S. superba*.

5. Conclusions

In summary, we investigated the floral biology, breeding system and pollination ecology of *Schima superba* Gardn. et Champ. The breeding system of *S. superba* is characterized as late-acting self-incompatible. The viability of pollen decreased rapidly, but the stigmata maintained high receptivity during the flowers' life spans. Three visitors were defined as pollinators: *A. cerana cerana* Fabricius appeared to be the most effective pollinator. Finally, we confirm that the seed production of *S. superba* is frequently limited by pollinators, fruit abortion, and pollen quality. A comprehensive analysis revealed that seed production could be improved through the artificial cultivation of bees in an *S. superba* seed orchard.

Acknowledgments: Research was supported by the National Science & Technology Pillar Program during the 12th Five-year Period (2012BAD01B04), and Zhejiang Science and Technology Major Program on Agricultural New Variety Breeding, China (2016C02056-3).

Author Contributions: Hanbo Yang and Rui Zhang conceived and designed the experiments; Hanbo Yang performed the experiments; Hanbo Yang and Ping Song analyzed the data; Zhichun Zhou contributed reagents/materials/analysis tools; and Hanbo Yang drafted the manuscript.

Conflicts of Interest: The authors declare that there are no financial or personal relationships with other people or organizations that could inappropriately influence this work, and that there are no professional or other personal interests of any nature or in any product, service and/or company, which could influence the positions presented herein or affect the review of the manuscript.

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