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# Conservation introduction resulted in similar reproductive success of *Camellia changii* compared with augmentation

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**Abstract** *Camellia changii* is an endemic and rare species in China with an extremely narrow range. It is an insect-pollinated and self-incompatible species. We conducted two types of conservation translocation experiments, augmentation and conservation introduction at two sites, Ehuangzhang and Tianxin, respectively, using 4-year-old grafted *C. changii* reproductive-size plants. In addition to survival and growth rate, we compared reproductive traits including flowering phenology, pigment production in flowers, pollinator visiting frequency, and reproductive success (flower, fruit and seed productions) for 2 years at augmentation and introduction sites. The survival rate of transplanted plants was 100 % after 2 years at both sites. The individuals at the augmentation site grew significantly faster than those at the introduction site ($P < 0.05$). Transplanted plants at the augmentation site produced 26–28 flowers per plant per year, significantly less than did those at the introduction site (35–38 flowers) during the 2-year (2013–2014) observation period ($P < 0.05$). The content of anthocyanin, a main pigment in the petal of flowers, at the introduction site was less than that at the augmentation site. Compositions of the pollinating species of *C. changii* at both sites were similar, but the dominant-pollinating species and their visiting frequency were different. Butterflies dominated the augmentation site and bees the introduction site. Nevertheless, the annual fruit and seed productions of both translocations in 2013 and 2014 were very similar. Our findings suggest that *C. changii* subject to the out-of-range conservation introduction did not suffer a decline in reproductive success when compared with augmentation. These findings suggest that certain rare and endangered plants may have the ability to cope with challenges that are brought on by conservation introduction in the face of climate change, at least in the short term.

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## Introduction

In the face of anthropogenic climate change, some rare and endangered plants may become extinct because of inability to migrate or to adapt (Loss et al. 2011; Corlett and Westcott 2013). Conservation introduction, i.e., moving species to new locations that are out of historic range but anticipated to be suitable for the species in the near future given the ongoing climate change (IUCN/SSC 2013), has been seen as a potential solution to the problem, along with the conventional species restoration approaches (Maschinski and Haskins 2012; Ren et al. 2014b). Conservation introduction is also known as assisted colonization or managed relocation, and has been a subject of debate regarding its feasibility and potential impacts (Ricciardi and Simberloff 2009; Minteer and Collins 2010; Seddon 2010; Liu et al. 2012; Maschinski and Haskins 2012). Two conventional and non-controversial restoration techniques are *augmentation* (in which individuals are added to an existing population), *reintroduction* (in which captive-grown individuals or seeds are released into an area formerly inhabited by that species) (Maschinski and Haskins 2012; IUCN/SSC 2013). Among the important concerns related to species restoration, conventional or new approaches, is whether the restored populations can receive adequate pollination services and reproduce successfully (Godefroid et al. 2011; Liu et al. 2012; Maschinski and Haskins 2012).

When dealing with the preservation of threatened species, a thorough knowledge of their interaction with and their dependency on pollinators is necessary to the development of proper management strategies (Martinell et al. 2010). There have been many studies on the pollination biology of rare and endangered plants, but few on reintroduced populations' pollination biology (Martinell et al. 2010). Nevertheless, a few studies on pollination ecology of introduced species may shed some light on potential issues encountered by populations subject to conservation introduction. For example, Memmott and Waser (2002) found that a lower number of pollinator species visited flowers of alien or newly arrived plant species compared with native plants, despite the fact that these insects were extreme generalists. In addition, Olesen et al. (2002) reported that introduced plants and pollinators do not interact as much as expected by chance. Finally, Dixon (2009) suggested that the climate change could have negative impacts on pollination services due to the alteration in timing of greening, flowering, and senescence, and overall shortening of the growing season for the plants. Therefore, understanding and managing pollinator services in rare plant restoration are of paramount importance.

*Camellia changii* Ye (Family Theaceae) is an evergreen tall shrub or small tree in South China. It is endemic to an extremely narrow range, with 1,039 individuals left in the sole, wild population. The species was included in the conservation list of extremely small populations in China in 2012 (Ren et al. 2014a). As an excellent ornamental plant (Fig. 1), the wild population faces threats due to both human removal as well as natural influences such as global climate change (Ren et al. 2014a).

Several studies have previously been undertaken for *C. changii.* Luo (2008) described *C. changii* community characteristics and found that climatic factors are likely important in determining its distribution pattern. *Camellia changii* had lower genetic diversity than other *Camellia* species in China (Luo et al. 2007), which may indicate less adaptability of the species to environmental changes. The species is insect-pollinated, self-incompatible, and relies on outcrossing for fruit set. Butterflies and bees are the main effective pollinators of *C. changii*, but their visiting frequency at the extant population is low (Luo et al. 2011). The ovary has a high abortion rate (64 %) under natural conditions, possibly due to inadequate pollen quantity and/or quality (Luo et al. 2011). Successful propagation of *C. changii* has been undertaken via cutting and grafting and other techniques (seed germination and tissue culture) for its cultivation (Ren et al. 2014a). Conservation translocations have also been carried out recently. Yet, little is known about the reproductive ecology of translocated populations of *C. changii* (Ren et al. 2014a).

In this study, we examined survival, pollination ecology, and reproductive traits of *C. changii* following conservation introduction and augmentation. We hypothesize that different climate and habitats will


**Fig. 1** The flower (**a**), fruit (**b**), and an individual *Camellia changii* tree (**c**)

affect flower traits that influence pollinator attractions, composition, and visitation frequency as well as reproductive success following conservation introduction. We focused on the following questions: How do augmented and introduced *C. changii* plants differ in (1) the flowering traits including flowering phenology, flower number, and petal color? (2) floral visitor composition and visitor frequencies? and (3) fruit and seed productions?

## Materials and methods

### Study sites

The study was conducted simultaneously at the Ehuangzhang Nature Reserve, where the sole natural population of *C. changii* is located (hereafter referred to as Ehuangzhang, Fig. 2), and the Tianxin Nature Reserve, an 'out of range' site for *C. changii* that is 390 km north of Ehuangzhang (hereafter referred to as Tianxin), Guangdong Province, southern China, from February 2013 to December 2014.

Ehuangzhang Nature Reserve (21°0′–21°58′N, 111°21′–111°36′E) is located in Yangchun County, southwestern Guangdong Province, southern China (Fig. 2). It has a lower subtropical monsoon climate. The average annual temperature is 22.1 °C, and the average annual rainfall is approximately 3430 mm. The zonal vegetation type is the lower subtropical monsoon broadleaf forest. However, the existing plant communities are secondary forests in different successional stages of recovery following deforestation. The soil type is lateritic soil (Ren et al. 2014a).

Tianxin (24°59′–25°58′N, 111°98′–113°11′E) is 12,000 ha in area located in Lianzhou City, northwestern Guangdong (Fig. 2), and has a central subtropical monsoon climate. It is approximately 390 km from Ehuangzhang. The mean annual temperature is 19.5 °C, and the mean annual rainfall is 1571 mm. Tianxin is also located within a lateritic soil zone. The vegetation is dominated by evergreen broadleaf forests typical of the subtropics. Due to human disturbance, the current vegetation is secondary forest (Ren et al. 2012). Rainfall is predicted to decrease by approximately 35 % and air temperature

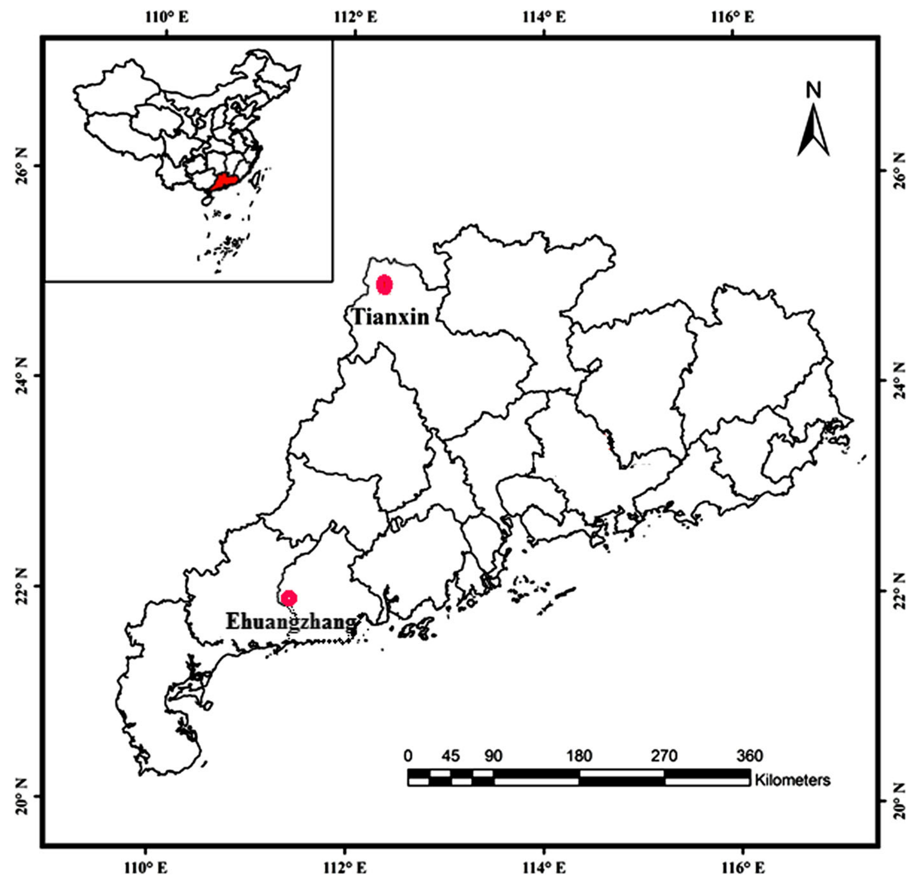


**Fig. 2** Locations of Ehuangzhang and Tianxin, Guangdong, South China

increases approximately 1.2 °C at Ehuangzhang, the augmentation site, in the next 20 years (Du 2007). This is the primary reason that we selected Tianxin Nature Reserve which has the climate similar to the predicted climate at Ehuangzhang as a conservation introduction site to conserve this species.

## Experimental design

We conducted an augmentation experiment at Ehuangzhang (elevation 230 m) and a conservation introduction experiment at Tianxin (elevation 280 m). We selected plots with similar vegetation and soil properties. The plots were located in the experimental area of the nature reserve and had no native flora of special concern.

At preparatory experiment, both the grafted and cutting plants survived and grew well, but grafted plants showed better survival and growth at both sites. Therefore, we selected grafted plants as materials. In March 2009, we grafted 300 *C. changii* scions (2–3 cm each) to 300 rootstocks of 1-year-old *C. gauchowensis*. The scions were obtained from the largest clump of wild plants at Ehuangzhang. Of the 300 grafted plants, more than 280 grew successfully. They began to bloom in 2012. In January 2013, we planted 45 of the grafted plants at Ehuangzhang and at Tianxin, respectively. All the planted plants (Height = 75 ± 3 cm) were healthy, similar in size, had a similar number of flowers in 2012, and were randomly assigned to the two sites.

At each of the two sites, a 1-ha experimental field was divided into three plots. Each plot was planted with 15 grafted plants in 3 m × 3 m grid systems in such a way that each plant was 3 m apart from its closest neighbors. Field investigation (Ren et al. 2014a) indicated that there were less than 15 *C. changii* individuals at each sub-population within the extant natural population. We, therefore, planted 15 individuals at each plot. There were 45 plants in total at both sites. We watered the plants several times after transplanting, and then left them to grow naturally. *C. changii* plants are sun-tolerant, therefore, we cleared all trees and shrubs from all plots before the plants were transplanted to eliminate competition from other plants. The plots were not fenced, fertilized, or mulched, and were left unmanaged after the initial clearing, planting, and watering.

### Assessment of survival and growth

We recorded the survival and height of each transplant every six months for two years from January 2013 to December 2014.

### Floral phenology observation

Floral phenology was investigated at all three plots at both sites (Spira et al. 1992; Kudo 1993; Wei et al. 2009). All *C. changii* individuals in each plot were observed to determine the flowering period, flower lifespan, and number of flowers produced during the 2-year period. We counted the number of flowers each plant produced on weekly basis over a 2-year period (2013 and 2014) because this species flowers all year round. The total number of flowers a plant produced over a whole year was obtained by summing up the weekly counts. The mean number of flowers per plant per year was calculated by averaging the total number of flowers a plant produced each year over all 45 individuals at each site.

### Pollinator observations and fruit and seed productions

*Camellia changii* was pollinated mainly during the day (Luo et al. 2011). Flower visitors were observed in all three plots at both sites during the peak flowering period for 12 days (3 days in May, July, September, and October, respectively) each year. All observations were conducted on sunny days and were performed at 6:30–7:30, 10:00–11:00, 14:00–15:00, and 17:00–18:00 of each observation day. In each observation, we selected 50 flowers at random to observe at each plot. We identified the species of insect visitors and recorded the number for each visiting species, the number of flowers each insect visited, and the behavior and visiting frequency of each insect on the flowers (Wei et al. 2009). At least one individual of each visiting species was captured for voucher and further identification. We quantified the visiting frequency as the number of visits per hour of each pollinator species on one flower (Spira et al. 1992; Kudo 1993; Martinell et al. 2010). The mean number of visits per hour was calculated over 50 flowers at each site. For each plant, we recorded the number of fruit and seeds in each fruit (Martinell et al. 2010) each year. The mean number of fruit and seed productions was calculated over all 45 individuals. The seed viability was tested by Yan's method (Yan 1992).

### Anthocyanin content of petals

The petals of *C. changii* are red to the human eyes. There are subtle differences in flower color that may lead to differences in floral attraction to pollinators. In order to determine the differences in flower color, we measured the anthocyanin content of each petal. For each plot, we collected all petals from five flowers of each individual to measure the anthocyanin content. Petals were cut into small pieces, then extracted with methanol and HCl (99:1, V/V) at 4 °C in the dark for 5 days. Absorption was measured at 530 and 650 nm with an ultraviolet–visible spectrophotometer (UV-3802, Unico), and then calculated the content of anthocyanin (Murray and Hackett 1991; Reddy et al. 1995).

### Statistical analysis

We calculated that the percent of plants survived at the final census for each plot. We used one-way ANOVA to compare the survival and growth of *C. changii* plants at the two sites ($P < 0.05$). Differences in number of flowers, content of anthocyanin, visiting frequency, fruit production, and seed production at each site were tested using two-way ANOVA. Assumptions of the above parametric analyses, i.e., normal distribution and equal residuals among groups, were satisfied. All statistical tests were performed using SPSS 13.0 for Windows (SPSS, Chicago, IL, USA).

## Results

### Plant survival and growth

The survival rate of transplanted plants was 100 % after 2 years at both sites. However, the height increases were different: The initial height of all plants was 75 ± 3 cm, but the individuals at Ehuangzhang (the augmentation site, 95 ± 3 cm in 2014) grew faster after planting than those planted at Tianxin (the introduction site, 89 ± 5 cm in 2014) (mean ± SD, $P < 0.05$).

### Floral phenology and anthocyanin content of petals

Mature *C. changii* individuals bloom nearly all year round, but they generally produce more flowers from mid-May until February of the following year, with only sporadic blossoms occurring in the remaining months. Therefore, flowers and fruits may be observed at the same time on any specific individual. The flowers started blooming earlier at Ehuangzhang than at Tianxin, with blossoms appearing 5 days earlier at Ehuangzhang in 2013 and seven days earlier in 2014. There were no significant differences between Ehuangzhang and Tianxin in other floral phenology characteristics.

The flower of *C. changii* is composed of 5–9 red petals in a single-layer arrangement. Each flower has more than 30 stamens with yellow filaments. Flowers open at early morning (approximately 6:30) and have a life span of 5–6 days. A flower bud develops into a blooming flower in approximately 14 days, and another 5–6 days are needed for a fertilized flower to show sign of a fruit. Almost 90 days are needed for fruits to fully mature.

Each individual at Ehuangzhang had a total of 26–28 flowers, in contrast to the 35–38 flowers found on each individual at Tianxin during the year ($P < 0.05$). In general, the number of flowers observed on each individual at Ehuangzhang during the bloom period was less than that at Tianxin (Table 1). Each individual plant at Ehuangzhang had 4–6 blooming flowers on any given day, whereas those at Tianxin had 6–8 blooming flowers ($P < 0.05$). However, the anthocyanin content in the petals of flowers at Tianxin was less than that in petals of flowers at Ehuangzhang in both 2013 and 2014 (Table 1; $P < 0.05$).

### Pollinators, visiting frequency, and reproductive success

In total, we identified 15 species of pollinators for the two sites during the observation periods. All visitor species were identified as pollinators on the basis of (i) *C. changii*'s pollen grains were found on their bodies and (ii) pollen grains were deposited to the stigma when they visited virgin flowers. There were 14 species at Ehuangzhang and 11 species at Tianxin; 10 species were common to both sites (Table 2). The major pollinators belonged to Lepidoptera (butterflies), Hymenoptera (bees), and Orthoptera (crickets/grasshoppers). We found that the Lepidoptera insects generally foraged for nectar, and Hymenoptera insects mainly gathered pollen. The greatest number of visitors as well as the highest frequency of visitation occurred in the morning at both sites.

**Table 1** The mean annual flower abundance per individual, anthocyanin content of petals per individual, number of fruits in each plot, and number of seeds in each plot at the two sites from 2013–2014

| | 2013 | | 2014 | |
|---|---|---|---|---|
| | Ehuangzhang | Tianxin | Ehuangzhang | Tianxin |
| No. flowers per plant | $28 \pm 5^a$ | $35 \pm 3^b$ | $26 \pm 6^a$ | $38 \pm 5^b$ |
| Anthocyanin content of petals (μmol/g) | $1.1 \pm 0.4^a$ | $0.8 \pm 0.3^a$ | $1.1 \pm 0.3^a$ | $0.9 \pm 0.2^a$ |
| No. fruits per plot | $18 \pm 5^a$ | $19 \pm 3^a$ | $20 \pm 3^a$ | $20 \pm 5^a$ |
| No. full seeds per plot | $54 \pm 5^a$ | $55 \pm 2^a$ | $57 \pm 3^a$ | $56 \pm 5^a$ |

Note that Ehuangzhang is the augmentation site, while Tianxin is the conservation introduction site. Values are mean ± SD. Values within the same row for a certain year (2013 or 2014) are not significantly different from one another if they share the same letter, are if they bear different letters ($P < 0.05$)

**Table 2** Floral visitors and mean visitor frequencies* of *C. changii* at the Ehuangzhang (augmentation site) and Tianxin (conservation introduction site)

| Insects order/species | Ehuangzhang | Tianxin |
|---|---|---|
| Lepidoptera | | |
| *Pelopidas sinensis* | 6.2 ± 1.8 | 2.0 ± 1.0 |
| *Papilio helenus* | 4.1 ± 2.0 | 1.0 ± 0.6 |
| *Papilio memnon* | 6.0 ± 1.2 | 1.0 ± 0.5 |
| *Vindula erota* | 3.1 ± 0.7 | 1.0 ± 0.9 |
| *Pelopidas mathias* | 8.1 ± 2.1 | 2.0 ± 1.2 |
| Hymenoptera | | |
| *Solenopsis invicta* | 2.0 ± 0.8 | |
| *Pheidole megacephala* | 1.0 ± 0.5 | |
| *Apis cerana* | 1.0 ± 0.6 | 6.1 ± 1.8 |
| *Apis mellifera* | | 8.2 ± 2.0 |
| *Ceratina* sp. | 0.6 ± 0.2 | 2.2 ± 0.9 |
| Orthoptera | | |
| *Tettigoniidae* sp 1 | 0.3 ± 0.1 | |
| *Tettigoniidae* sp 2 | 0.2 ± 0.1 | 0.5 ± 0.2 |
| *Tettigoniidae* sp 3 | 0.6 ± 0.2 | |
| *Gryllidae* sp | 2.0 ± 0.3 | 2.0 ± 1.2 |
| Coleoptera | | |
| *Chrysomelidae* sp | 0.2 ± 0.1 | 0.2 ± 0.1 |

Frequency means the number of times of occurrence on a flower per hour for a pollinator species. 50 flowers were assessed to derive this mean at each site. Values are mean ± SD

The visitors at Ehuangzhang were mainly Lepidoptera, Hymenoptera, and Orthoptera insects, with Lepidoptera visiting at the highest frequency. At Tianxin, visitors were Hymenoptera and Lepidoptera insects, and Hymenoptera insects visited at a higher frequency than did Lepidoptera (Table 2).

Without any human assistance, the reproductive success at both sites was similar. At Ehuangzhang (augmentation site), the number of fruits per plot was 18 ± 5 and 20 ± 3 in 2013 and 2014, respectively, and at Tianxin (introduction site) 19 ± 3 and 20 ± 5, respectively. Total seed production (number of full seeds per plot) at Ehuangzhang was 54 ± 5 and 57 ± 3 in 2013 and 2014, respectively; and at Tianxin 55 ± 2 and 56 ± 5, respectively (Table 1). Two-way ANOVA showed no significant difference in the production of fruits and seeds between years nor between sites. No significant year-by-site interaction was detected. All the seeds from both sites germinated during 2013–2014 under laboratory conditions.

## Discussion

The translocated populations of *C. changii* at both the augmentation and conservation introduction sites enjoyed high survival rates (100 %). Each site also received seemly adequate pollination services resulting in similar levels of fruit and seed productions during our study period. We discuss the major translocation success indicators and the differences between the two translocation populations below.

### Short-term survival and growth

Survival rate of plants subject to conservation introduction was as high as that of augmentation. It is possible that using grafted, reproductive-size plants played an important role in the high survival seen in our study. In general, using large plants as transplanting material led to higher survival and probability of the transplants reproducing (Akeroyd and Jackson 1995; Guerrant and Fiedler 2004). In addition, grafted plants can better resist biotic and abiotic stress and adapt to a wider range of habitat variations because the host rootstocks facilitate the growth and nutrition absorption of the whole plant (Harada 2010; Ren et al. 2014b). It is clear that having appropriate propagule material is one of the most important factors contributing to translocation success (Akeroyd and Jackson 1995; Guerrant and Fiedler 2004), especially in the short term, as measured in our study. In contrast, significant differences were seen in plant growth rate among the two sites. The individuals at Tianxin, the introduction site, grew slower than that at Ehuangzhang, most likely because Tianxin is at a latitude 4° further north and has a slightly colder climate than that of at Ehuangzhang. Slower plant growth rate may have implications on long-term population dynamics and needs to be studied further.

### Floral phenology, color, and pollinator visits

*Camellia changii* showed some differences in floral phenology between the two reintroduction sites. The flowers of plants at Tianxin (conservation introduction site) bloomed slightly later. The number of flowers at that site was significantly higher ($P < 0.05$), yet the amount of anthocyanin, which determines the degree of red coloration of the petals, was significantly lower among the individuals at Tianxin ($P < 0.05$). This result supports our hypothesis. These differences may be due to that the weather is colder and the light intensity is weaker at Tianxin (19.5 °C, 25°N) than that at Ehuangzhang (22.1 °C, 21°N). Climate is well known to influence phenology and vernalization of this species (Mckinney 1940; Khanduri et al. 2008). The plants' exposure to cold temperatures can facilitate the transition from leaf buds to flower buds during the flower development stage (Minorsky 2002). In addition, light intensity can affect the biosynthesis of anthocyanin, which affects the degree of red coloration of flower petals (Zhou and Singh 2002). The weaker light intensity at Tianxin may have led to the formation of a lighter colored petal.

Composition of pollinating species of *C. changii* at both sites was similar, but more common pollinating species differed, with butterflies dominating in Ehuangzhang, while bees dominated in Tianxin. Bees (series Apiformes), flies (order Diptera), and butterflies and moths (Lepidoptera) are the predominant pollinators for plants in ecosystems at Guangdong province. Both Ehuangzhang and Tianxin have the same general faunal complex found in lower subtropical areas, and most insects are expected to be similar at the area. The broad groups of pollinators (bees, butterflies, flies, and vertebrates) do not usually show markedly different responses to land-use change due to ecological memory (Winfree et al. 2011; Abeli et al. 2013; Sun et al. 2013; Aslan et al. 2014). In this particular instance, however, dominant-pollinating species of *C. changii* differed between Ehuangzhang (butterflies) and Tianxin (bees) was most likely due to human impacts. We found some commercial beehives near Tianxin Natural Reserve during the experiment periods, and the presence of man-made bee colonies nearby may be the main reason that we found more bee pollinators at Tianxin.

Another possible reason for difference in pollinator dominance was the petal color difference. During observations in the field, butterflies visited the flowers with bright red petals at Ehuangzhang with high frequencies, while frequency of visitation by butterflies on the lighter red petals at Tianxin was lower. It seems that flower color affected the butterfly visitation, though we cannot exclude the influence of odor given that this factor was not measured. However, butterflies, especially *Papilio* spp., have been reported to be highly dependent on visual information for their flower-foraging behavior (Kinoshita et al. 2015). Many studies, most using artificial flowers, have shown that butterflies have the ability to respond to color and learn new associations between color and nectar rewards, e.g., Phol et al. (2011). In some studies, a color change has been shown to act as a cue to discourage pollinators from visiting older flowers with little reproductive value for the after-arrival effects (Weiss 1991). Luo et al. (2011) once noted that the petal color of *C. changii* gradually becomes lighter with age due to a lower of fluorescence. The flowers on the first day blossom are brighter and have a higher possibility of rewarding the visitor with greater amounts of nectar. Thus, butterflies may have already learned to select bright red flowers with more nectar.

### Fruit and seed productions

Despite the statistically significant differences in phenology, flower production, and dominant pollinators, the reproductive success in terms of number of fruits and full seeds was similar at the two sites. This suggests that resource limitation was a factor limiting fruit and see production. Specifically, plants at the two sites were of similar size and they were able to perhaps only allocate a similar amount of overall energy into fruit production, which resulted in similar level of fruit and seed productions. It is not clear whether or not pollen limitation is also a factor, as suggested by Luo et al. (2011) in their study. Neither quantified pollen on stigmas.

### Is conservation introduction a viable tool for *C. changii*?

Our study showed encouraging signs that conservation introduction may be used as a viable tool to prevent the extinction of *C. changii.* The species, despite having lower genetic diversity than other Camellia species in China (Luo et al. 2007), was able to withstand the lower temperature at the introduction site, was well served by an abundance of pollinators and produced comparable number of fruit and seeds. Being a generalist in terms of its pollination demand certainly helps in this situation. Nevertheless, longer term study is needed to see whether the survival, growth, and reproductive success pattern observed here will persist, and whether these conservation translocations can result in a 2nd generation and beyond. Equally important is the monitoring and detection of possible negative impacts that this species may cause at the conservation introduction site, in the short and long term.

Global climate change will influence the reproductive biology of plants by affecting plant–animal mutualisms (Bond 1995). If we considered our conservation introduction as a trial run for a plant amenable to generalized pollinators, we are cautiously optimistic that this approach may work for *C. changii* and other similar species, especially with the use of grafted rootstock, which boosts the plant's hardiness in the new environment.

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