Post-print of a Study on the Reproductive Biological Characteristics of Wild Buckwheat (Fagopyrum gracilipes)

Authors: Bing Zhou, Yan Xiaohong, Su Qitao, Zhang Zhengguang, Wang Ning, Lihua Chen

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Abstract

Through a combined method of field observation and artificial control experiments, the reproductive biological characteristics of *Fagopyrum gracilipes* (genus *Fagopyrum* Mill.) were investigated from aspects of flowering dynamics, floral basic characteristics, breeding system, pollination biology, and seed traits, and the contribution of each trait to its reproduction was discussed. The results are as follows: In Weining, Guizhou, the flowering and fruiting period of *F. gracilipes* is typically from June to October each year, with the flowering period of a single inflorescence and a single flower being 13–21 days and 1–3 days, respectively. The flowers are relatively small, with a diameter of 3.99 ± 0.12 mm, style and anther heights of 1.30 and 1.65 mm, respectively. Flower diameter is significantly positively correlated with tepal length and tepal width, and style height is extremely significantly positively correlated with anther height. The pollen-ovule ratio of *F. gracilipes* is 371 ± 16.40, the outcrossing index is 2, and bagging experiments demonstrate that it is self-compatible and cross-compatible, indicating that its breeding system is facultative selfing, partially cross-compatible. *Fagopyrum gracilipes* has relatively few flower-visiting insects, primarily consisting of 9 species from 7 families of Hymenoptera, Diptera, and Coleoptera, with Syrphidae insects being the main pollinators. The fruits of *F. gracilipes* exist in two types: winged and wingless, which is conducive to adapting to different dispersal modes. The seeds are relatively small, with a 1000-grain weight of 1.05 ± 0.04 g, and the germination rate is relatively low, with a cumulative germination rate of 19.60 ± 2.14% at 30 days after sowing; however, germination is synchronous, mainly concentrated within the first 5 days. In summary, the flexible breeding system of *F. gracilipes* provides a guarantee for producing large quantities of seeds, and the diverse fruit dispersal modes and synchronous seed germination characteristics create a foundation for it to occupy broader habitats and become a dominant species in the community.

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Full Text

Preamble

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Authors: ZHOU Bing, YAN Xiaohong, SU Qitao, ZHANG Zhengguang, WANG Ning, CHEN Lihua
Affiliation: School of Life Sciences, Jinggangshan University, Ji' an 343009, Jiangxi, China

Abstract

This study investigated the reproductive biology of *Fagopyrum gracilipes*, a wild buckwheat species, through a combination of field observations and controlled experiments. We examined flowering dynamics, floral morphology, breeding system, pollination biology, and seed traits, and evaluated the contribution of these characteristics to reproductive success. In Weining, Guizhou, *F. gracilipes* typically flowers and fruits from June to October annually, with individual inflorescences lasting 13–21 days and single flowers persisting for 1–3 days. The flowers are relatively small, with a diameter of 3.99 ± 0.12 mm; style and anther heights measure 1.30 mm and 1.65 mm, respectively. Floral diameter shows significant positive correlations with both tepal length and width, while style height exhibits a highly significant positive correlation with anther height.

The pollen-ovule ratio is 371 ± 16.40, and the outcrossing index is 2. Bagging experiments demonstrate that *F. gracilipes* is both self-compatible and cross-compatible, indicating a facultative selfing system with partial cross-compatibility. Flower visitors are relatively scarce, comprising nine species from seven families across Hymenoptera, Diptera, and Coleoptera, with Syrphidae flies serving as the primary pollinators. The species produces two fruit morphs —winged and wingless—which facilitates adaptation to different dispersal modes. Seeds are small, with a thousand-seed weight of 1.05 ± 0.04 g, and exhibit low but synchronized germination, with a cumulative germination rate of 19.60 ± 2.14% after 30 days, concentrated primarily within the first five days. In summary, the flexible breeding system ensures substantial seed production, while diverse fruit dispersal mechanisms and synchronized germination provide the foundation for colonizing extensive habitats and achieving dominance in plant communities.

Keywords: *Fagopyrum gracilipes*; breeding system; pollination biology; seed; reproduction

Introduction

Reproductive success is fundamental to plant population persistence and expansion, representing the outcome of long-term adaptive evolution to environmental conditions. Plant reproductive biology encompasses all traits and processes related to reproduction, including flowering phenology, breeding systems, pollination biology, and reproductive allocation (Zhang & Jiang, 2001; Barrett, 2002; Huang, 2006; Ren et al., 2012). Although reproductive processes vary among species, plants generally enhance female fitness through increased seed quantity and quality, and male fitness through improved pollen acquisition and transfer efficiency (Ren et al., 2012). Current research in reproductive biology primarily focuses on the reproductive characteristics of endangered plants (Pellegrino et al., 2006; Cursach & Rita, 2012; Mo et al., 2016), the relationship between reproductive traits and invasiveness in alien species (Zhou et al., 2013; Castro et al., 2013; Yan et al., 2016), and the ecological functions of specialized floral traits (Wen et al., 2015; Suetsugu, 2015; Wang et al., 2016). In contrast, relatively few studies have examined the reproductive biology of economically important plants, particularly their wild relatives (Zhang et al., 2013). Elucidating the reproductive characteristics of these wild relatives provides crucial theoretical foundations for breeding improved cultivars and identifying valuable traits.

China is the center of origin for buckwheat, with nearly all *Fagopyrum* Mill. species distributed in southwestern regions. Buckwheat has attracted considerable attention and cultivation due to its high nutritional value and medicinal properties (Ruan & Chen, 2008; Tang et al., 2010). The genus comprises two groups: large-achene and small-achene species, which exhibit low interspecific hybridization compatibility between groups but relatively higher compatibility within groups (Chen, 1999; Chen et al., 2004). Extensive research has addressed resource utilization (Wu et al., 2010; Wang et al., 2011), morphology (Chen, 2004; Ren & Chen, 2008; Tang et al., 2010), genetics (Zhou et al., 2012; Kishore et al., 2013; Li et al., 2013), and development applications (Park et al., 2012; Tsai et al., 2012) in *Fagopyrum*, yet reproductive biology studies remain limited (Zhang et al., 2013; Chen et al., 2015; Wu et al., 2018).

Fagopyrum gracilipes is an annual herb in the small-achene group, primarily distributed in high-altitude, cool regions of southwestern China, where it inhabits grasslands, moist valleys, field margins, and grows intermixed with crops such as maize (Chen, 2012). Field surveys reveal that *F. gracilipes* readily forms dominant populations within its distribution range, particularly in crop fields. As the species lacks vegetative propagation, its population dominance likely relates closely to sexual reproductive characteristics. However, which specific reproductive traits contribute significantly to this success remains unexplored. This study investigates the reproductive biology of *F. gracilipes* by examining floral morphology, breeding system, flower visitors, and seed characteristics to provide theoretical insights into its reproductive mechanisms and population dominance, while offering reference data for buckwheat breeding programs.

Materials and Methods

1.1 Study Site

The study site was located at Caohai, Weining Yi, Hui, and Miao Autonomous County, Guizhou Province, covering approximately 200 m². The region features a subtropical monsoon climate with an average annual rainfall of ~750 mm, mean annual temperature of 11.2 °C, and 1,812 hours of annual sunshine. Geographically situated between 104°12 -104°18 E and 26°49 -26°53 N, the area exhibits high biodiversity and species richness, providing ideal habitat for *F. gracilipes.*

1.2 Experimental Methods

1.2.1 Flowering Dynamics and Floral Morphology Unopened flower buds were marked and observed daily until anthesis. On the day of opening, flowers were monitored continuously to record changes in perianth segments, stamens, and pistils. Individual inflorescences were randomly marked and observed until the last flower withered to calculate flowering duration. Twenty freshly opened flowers were randomly selected to measure peduncle length, floral diameter, tepal length and width, and anther and style heights using vernier calipers.

1.2.2 Breeding System Determination Pollen-Ovule Ratio: Twenty unopened, non-dehisced flowers were randomly selected. All anthers were collected in 1.5 mL centrifuge tubes, and 0.5 mL of 1.0 mol/L HCl was added to dissolve anther walls. The suspension was vortexed, and 10 L aliquots were transferred to slides for microscopic pollen counting (five replicates per flower) to determine mean pollen quantity per flower. Ovaries were cross-sectioned with a blade to count ovules under a microscope. The pollen-ovule ratio (P/O) was calculated and evaluated according to Cruden (1977).

Outcrossing Index (OCI): Based on floral diameter and stamen-pistil characteristics, the breeding system was assessed following Dafni (1992). Scoring criteria were: (1) flower or inflorescence diameter <1 mm = 0, 1-2 mm = 1, 2-6 mm = 2, >6 mm = 3; (2) simultaneous anther dehiscence and stigma receptivity or earlier stigma receptivity = 0, anther dehiscence preceding stigma receptivity = 1; (3) stigma and anther at same height = 0, height difference present = 1. The sum of these scores yields the OCI value, which classifies breeding systems as: OCI=0 (cleistogamy), 1 (obligate selfing), 2 (facultative selfing), 3 (self-compatible, pollinator sometimes required), or 4 (partial self-compatibility, outcrossing, pollinator required).

Bagging Experiments: During the flowering period, consistent developmental stages were selected on individual plants for the following treatments: (1) control (no manipulation); (2) bagged without emasculation; (3) emasculated before anthesis, unbagged; (4) emasculated before anthesis with artificial xenogamous pollination; (5) emasculated before anthesis, unpollinated, and bagged. Each plant received 2–4 replicates per treatment. Fruit set was recorded periodically after treatment.

1.2.3 Flower Visitor Observation Flower visitors were observed during peak flowering on sunny days from 8:30 to 18:00 for a minimum of three days. Visitor behavior was documented and photographed, and specimens were collected with insect nets for laboratory identification.

1.2.4 Floral Module Characterization At peak flowering, 30 plants were randomly excavated to count leaf number, inflorescence number, and branch orders (primary, secondary, tertiary), and to measure plant height and root length.

1.2.5 Seed Characteristics and Germination Fifty plump seeds were measured for height, width, vertical length, and transverse length. Five sets of 100 plump seeds were weighed to calculate thousand-seed weight. Additional plump seeds were soaked for 5 hours and placed on moist filter paper in petri dishes (50 seeds per dish, four replicates) for germination testing at 70% humidity and 25±1 °C. Germination was recorded daily for 30 days to calculate germination rate.

1.2.6 Statistical Analysis All data were processed using Excel and SPSS 19.0. Pearson' s two-tailed test was used to analyze correlations among floral morphological traits.

Results

2.1 Flowering Phenology and Floral Morphology

2.1.1 Flowering Dynamics In Weining, Guizhou, *F. gracilipes* flowers and fruits from June to October annually, with peak flowering in July–August. Inflorescences are racemes containing 10–40 flowers, with 2–3 flowers per bract. Flowers are small, with five deeply lobed perianth segments ranging from pale red to white, eight stamens (three inner, five outer) shorter than the perianths, and three styles with three carpels and one locule. Individual inflorescences flower for 13–21 days, while single flowers last 1–3 days. During anthesis, one flower in the lower bract opens first, followed sequentially by upper bract flowers, while other lower bract flowers open simultaneously. Perianth segments progress from enclosing to slightly open to fully expanded, changing color from pale red to white to brown. Filaments and styles elongate during expansion, with anthers and stigmas at nearly equal heights; inner whorl anthers dehisce first, followed by outer whorl anthers after two hours. Stamens wither before perianth segments. Flowers lack fragrance but produce small amounts of secretions during opening.

2.1.2 Floral Morphological Characteristics Table 1 presents basic floral traits of *F. gracilipes*, which show considerable variation. Peduncle length, style height, and anther height exhibit relatively high variation, with mean values of 1.63 mm, 1.30 mm, and 1.65 mm, respectively, and coefficients of variation >19%. Floral diameter and tepal length show less variation, with coefficients of variation <15% and mean values of 3.99 mm and 2.40 mm, respectively.

Correlation analysis reveals significant relationships among floral traits. Floral diameter correlates significantly with tepal length (r=0.462) and highly significantly with tepal width (r=0.555). Peduncle length correlates significantly with style height (r=0.528) and highly significantly with anther height (r=0.637). Tepal length and width show significant correlation (r=0.447), while style and anther heights are highly significantly correlated (r=0.693) (Table 2).

2.2 Breeding System

2.2.1 Pollen-Ovule Ratio Individual flowers of *F. gracilipes* produce 270–580 pollen grains, with a mean of 371 ± 16.40 grains per flower. Each pistil contains three carpels with one locule and one ovule, yielding a pollen-ovule ratio (P/O) of 371 ± 16.40. According to Cruden (1977), this indicates a facultative selfing breeding system.

2.2.2 Outcrossing Index Table 3 summarizes OCI measurements. With a floral diameter of 3.99 mm, *F. gracilipes* scores 2. Stigmas are receptive before anther dehiscence (protogyny), scoring 0. Stigmas and anthers are at nearly equal heights, scoring 0. The resulting OCI of 2 classifies the breeding system as facultative selfing according to Dafni (1992).

2.2.3 Bagging Experiments Table 4 presents bagging and artificial pollination results. Emasculation with bagging and no pollination yielded 0% fruit set, confirming the absence of apomixis. Natural pollination produced 28.69% fruit set, while bagging without emasculation yielded 26.67%, indicating high self-compatibility. Emasculation without bagging and emasculation with artificial xenogamous pollination produced 13.89% and 23.64% fruit set, respectively, demonstrating cross-compatibility. Thus, *F. gracilipes* exhibits facultative selfing with a predominant selfing component.

2.3 Flower Visitors

Flower visitors began appearing on *F. gracilipes* around 8:30 on sunny mornings, though overall visitor abundance was low. Observations identified nearly 10 species from Diptera, Hymenoptera, and Coleoptera (Table 5 and Figure 1 [Figure 1: see original paper]). Primary visitors included the syrphid flies *Episyrphus balteatus* and *Sphaerophoria* sp. 1, the sarcophagid fly *Boettcherisca* sp. 1, and the chrysomelid beetle *Aulacophora* sp. 1.

2.4 Floral Module Growth Characteristics

Table 6 shows growth characteristics of *F. gracilipes* modules during flowering. Considerable individual variation exists. Leaf number (25–248) and plant height (30.09–73.25 cm) are relatively stable, with mean values of 92.87 leaves and 53.92 cm, and coefficients of variation of 2.58% and 15.28%, respectively. Plants produce up to three branching orders; primary branch number is relatively stable, while tertiary branching is highly variable, with coefficients of variation of 35.02% and 462.38%, respectively. Inflorescence number ranges from 6 to 79, averaging 32.73 with a coefficient of variation of 54.56%.

2.5 Seed Characteristics and Germination

Fagopyrum gracilipes seeds are small, with height, vertical length, short length, and transverse width of 1.80 mm, 1.05 mm, 0.88 mm, and 1.49 mm, respectively, and a thousand-seed weight of 1.05 g (Table 7). As shown in Figure 2 [Figure 2: see original paper], germination rate is low under 70% humidity and 25±1 °C, with cumulative germination of only 19.60% after 30 days. However, germination is highly synchronized, concentrated primarily between days 2 and 5.

Discussion

Plant reproductive success results from the integrated function of breeding systems and pollination biology (Harmon-Threatt et al., 2009). Ramírez and Nassar (2017) categorize angiosperm breeding systems into apomixis, autonomous selfing, facilitated selfing, and self-incompatibility, representing the combined influence of genetic information and environmental factors. Breeding systems are not static and may exhibit geographic variation in response to environmental changes. For example, Ren et al. (2004) demonstrated that although clonal propagation is the primary regeneration mode in water hyacinth (*Eichhornia crassipes*), southwestern China and Hainan populations exhibit diverse breeding systems, while other geographic populations show compromised outcrossing systems and self-compatibility due to the absence of certain floral morphs. Similar geographic variation occurs among populations of alpine woodsorrel (*Oxalis alpina*) (Weller et al., 2016).

Breeding systems are typically assessed through pollen-ovule ratio, outcrossing index, and bagging experiments. Our results show that *F. gracilipes* has a P/O ratio of 371, an OCI of 2, and is both self- and cross-compatible, indicating facultative selfing with predominant selfing. Breeding system type is closely associated with floral traits. For instance, dioecious species must be outcrossing, while hermaphroditic species exhibit diverse breeding systems (Castro et al., 2004; Costa et al., 2013). Some buckwheat species possess heterostyly, conferring strict self-incompatibility (Yasui et al., 1998; Zhang et al., 2013), whereas homostylous species, particularly small-achene buckwheats, tend toward facultative selfing (Yasui et al., 1998). The genus has undergone at least three transitions from heterostyly to homostyly: twice in the *Cymosum* group and once in the *Urophyllum* group (*F. urophyllum*–*F. gracilipes*) (Yasui et al., 1998; Wu et al., 2018). These transitions shift reproductive strategy from strict outcrossing to self-compatibility. Although selfing carries certain disadvantages, self-compatibility ensures zygote formation and abundant seed production, providing crucial insurance for population survival and dispersal (Baker, 1955). Our data show that *F. gracilipes* produces an average of 37 inflorescences per plant, with 10–40 flowers per inflorescence, ensuring high seed output and facilitating its establishment as a community dominant.

Pollination biology is another critical aspect of reproductive success. Visitor abundance, particularly pollinator abundance and efficiency, strongly influences reproductive output. Castro et al. (2013) found that although *Polygala vayredae* attracted 24 visitor species, only four effective pollinators deposited pollen on stigmas during single visits, contributing to low reproductive success. Zhang et al. (2013) reported 38 visitor species for *F. dibotrys*, yet its fruit set was only ~20% due to intramorph incompatibility and limited effective pollination. Selfing-predominant plants typically attract fewer visitors. Our study confirms low visitor diversity for *F. gracilipes*, with approximately 10 species, primarily Hymenoptera and Diptera. Similar patterns occur in facultative selfing species such as *Phytolacca americana* (Zhou et al., 2013).

Floral structure, color, scent, and secretion type and quantity significantly influence pollinator attraction (Cerana, 2004; Klahre et al., 2011). Most flower visitors seek pollen and nectar rewards (Heinrich & Raven, 1972), and plants evolve specific floral traits to attract or accommodate particular pollinators (Gong & Huang, 2007). For example, *Impatiens* species with long floral tubes and spurs are primarily pollinated by long-tongued bumblebees and hawkmoths (Mao et al., 2011). Flower size and symmetry affect attractiveness; larger flowers within a type attract more insects (Worley et al., 2000), and bilaterally symmetrical flowers transmit visual signals more effectively than radially symmetrical ones (Fenster et al., 2009; Ushimaru et al., 2009). *Fagopyrum gracilipes* has small, radially symmetrical flowers (3.99 mm diameter) on relatively short plants, offering limited food rewards (mean 371 pollen grains per flower), which restricts pollinator attraction. However, as a self-compatible species, pollinator presence has minimal impact on fruit set, likely explaining its low visitor diversity.

Seed and fruit traits directly affect population persistence and dispersal. Wind-dispersed Asteraceae such as *Conyza sumatrensis* produce pappus-bearing achenes (Hao et al., 2009), while animal-dispersed species like *Bidens frondosa* develop hooked fruits (Yan et al., 2016), and drought-adapted species such as *Plantago virginica* produce mucilaginous seeds (Luo et al., 2015). *Fagopyrum gracilipes* produces both winged and wingless fruit morphs (Chen, 2012), with winged types facilitating water dispersal and wingless types enabling local colonization. Seed germination characteristics further influence population dynamics. *Fagopyrum gracilipes* shows low germination rates (<20% after 30 days) but high synchrony within the first five days. With large seed numbers, this strategy generates numerous seedlings that can colonize extensive habitats. Similar patterns occur in *P. americana*, with 18.50% cumulative germination after 20 days (Zhou et al., 2013). Additionally, the small thousand-seed weight (1.05 g) classifies *F. gracilipes* as a small-seeded species. Although small seeds contain limited nutrient reserves that may negatively affect seedling vigor (Guo et al., 2006), they enable greater population densities and broader habitat occupation (Liu et al., 2003) and are better adapted to wind and water dispersal.

In conclusion, *F. gracilipes* possesses a prolonged flowering period, a flexible breeding system combining facultative selfing with partial cross-compatibility, low but synchronized germination, high seed output, and effective fruit dispersal mechanisms. These reproductive traits collectively facilitate habitat colonization and community dominance. Understanding these characteristics provides theoretical foundations for elucidating its reproductive mechanisms and offers valuable references for cultivated buckwheat breeding. While this study provides comprehensive insights, further research is needed on flowering phenology, pollen and stigma morphology, and potential differences in germination characteristics between winged and wingless fruit morphs, as well as the underlying mechanisms generating these dimorphic fruits.

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Note: Figure translations are in progress. See original paper for figures.