Postprint: Flowering Biology and Breeding System of Panax notoginseng

Authors: Li Kecheng, Wang Fugui, Wang Zaiwang, Zhang Xiaoyu, Youguli, Yang Shengchao, Liang Yanli

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Abstract

Panax notoginseng, a precious traditional Chinese medicinal material in China, faces problems such as low reproductive rates and extinction of wild resources. To investigate the flowering biology and breeding system characteristics of P. notoginseng and elucidate the reasons for its low seed set rate under natural conditions, this study examined its flowering biological parameters, pollination system, and artificial pollination. The results showed that: (1) The individual flower lifespan of P. notoginseng was 4–5 d, the inflorescence flowering period was 20–25 d, and the population flowering period was approximately 60 d. (2) There existed herkogamy and dichogamy, representing a typical protandrous type. (3) The pollen grains were medium-sized, possessing three colpi. (4) Pollen viability peaked on the first day of flowering, while stigma receptivity peaked on the 12th day of flowering. (5) The outcrossing index (OCI) was 4, and the breeding system was outcrossing-type, partially self-compatible, and required pollinators. The pollen/ovule ratio was (450.0–1,037.5), indicating an obligate outcrossing breeding system. (6) Pollination experiments revealed that the breeding system type was a mixed mating system with both selfing and outcrossing present simultaneously, and both insect and wind pollination could facilitate its pollination. (7) The flower-visiting insects included the Chinese honeybee (Apis cerana), the black-banded hoverfly Episyrphus balteatus, and the spined soldier bug Riptortus pedestris, with the Chinese honeybee being the primary pollinator. In conclusion, the breeding system of P. notoginseng is facultative outcrossing-type, partially self-compatible, requiring both wind and insect pollination. Pollen limitation and severe diseases during the flowering and fruiting stages are key factors affecting its seed set rate. These results provide a theoretical basis for the breeding and propagation of improved varieties of P. notoginseng.

Full Text

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Authors: LI Kecheng[1,2,3], WANG Fugui[1,2,3], WANG Zaiwang[1,2,3], ZHANG Xiaoyu[1,2,3], YOU Guli , YANG Shengchao[1,2], LIANG Yanli[1,2,3*]

Affiliations: 1. National-Local Joint Engineering Research Center on Germplasms Innovation & Utilization of Chinese Medicinal Materials in Southwest China, Yunnan Agricultural University, Kunming 650201, China 2. Yunnan Provincial Key Laboratory of Medicinal Plant Biology, Yunnan Agricultural University, Kunming 650201, China 3. College of Agriculture and Biotechnology, Yunnan Agricultural University, Kunming 650201, China 4. Xundian County Bureau of Agriculture and Rural Development, Kunming 655200, China

Abstract: Panax notoginseng is a precious traditional Chinese medicinal material that faces challenges of low reproduction rates and extinction of wild resources. To explore the characteristics of its flowering biology and breeding system and clarify the reasons for low fruiting rates under natural conditions, this study investigated its flowering biological parameters, pollination system, and artificial pollination. The results showed: (1) The single flower duration was 4–5 days, inflorescence flowering period was 20–25 days, and population flowering period was approximately 60 days. (2) Herkogamy and dichogamy were observed, representing a typical protandrous type. (3) Pollen grains were medium-sized with three germination furrows. (4) Pollen viability peaked on the first day of flowering, while stigma receptivity peaked on the 12th day. (5) The outcrossing index (OCI) was 4, indicating an outcrossing breeding system with partial self-compatibility that requires pollinators; the pollen/ovule ratio ranged from 450.0 to 1,037.5, suggesting obligate xenogamy. (6) Pollination experiments revealed a mixed mating system with both selfing and outcrossing, where both insect and wind vectors could facilitate pollination. (7) Flower-visiting insects included Apis cerana, Episyrphus balteatus, and Riptortus pedestris, with A. cerana being the primary pollinator. In conclusion, the breeding system of P. notoginseng is facultative outcrossing, partially self-compatible, and requires both wind and insect pollination. Pollen limitation and severe diseases during flowering and fruiting stages are key factors affecting fruiting rates. These findings provide a theoretical basis for improved variety breeding and propagation of P. notoginseng.

Keywords: Panax notoginseng, flowering biology, breeding system, flower-visiting insects, pollination biology

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First Author: LI Kecheng (1999–), Master' s student, research focus on medicinal plant resources, (E-mail) 372527883@qq.com.

Corresponding Author: LIANG Yanli, Ph.D., Professor, Doctoral Supervisor, research focus on medicinal botany, (E-mail) liangyanlimt@sina.com.

Flowering is the most conspicuous and critical life phenomenon in plant sexual reproduction (Widen, 1991). Flowering biology investigates phenology, floral morphology, and gamete development characteristics (Sun et al., 2014; Barrionuevo et al., 2021). Breeding systems refer to the frequencies of intra- and inter-specific selfing and outcrossing and their effects on plants. The characteristics of flowering biology and breeding systems are crucial for plant evolution (Murren et al., 2014; Schoen et al., 2019). Pollination biology examines floral structure components, pollination vectors, and palynology (Wang et al., 2009). Studies by Long et al. (2021) on Sinomanglietia glauca and Yang et al. (2023) on the endangered Primula filchnerae have elucidated key reasons for low natural fruiting rates and revealed endangered mechanisms, providing theoretical foundations for species conservation. Therefore, understanding flowering phenology and floral morphology, identifying breeding system types, and clarifying pollination modes and vectors are essential for pollination success and have significant implications for hybrid breeding and conservation strategies (Ye et al., 2023).

The genus *Panax* originated in the Himalayan region, comprising 8 species and 5 varieties. All species have medicinally valuable rhizomes, with the most widely used being *P. notoginseng*, *P. ginseng*, *P. quinquefolium*, *P. stipuleanatus*, and *P. japonicus* var. *major* (Peng et al., 2024; Zhang et al., 2024). Previous studies on flowering biology and breeding systems in *Panax* revealed significant differences in flowering phenology among species. *P. japonicus* var. *major*, *P. ginseng*, and *P. quinquefolium* flower from mid-to-late May through mid-to-late June, while *P. stipuleanatus* and *P. notoginseng* flower from mid-March to mid-April and mid-August to mid-September, respectively. Inflorescence flower numbers also vary considerably, with *P. ginseng* and *P. quinquefolium* having 30–50 flowers per inflorescence, whereas *P. notoginseng* has 50–300 flowers (Xia, 1964; He and Deng, 1985; Chen and Li, 1986; Zhao, 2015). Furthermore, studies by Zhuravlev et al. (2008) and Zhao (2015) demonstrated that *P. ginseng* and *P. japonicus* var. *major* possess both self- and cross-pollination capabilities, forming stable and flexible reproductive strategies to adapt to extreme environmental conditions. Research using allozyme markers on *P. quinquefolium* populations revealed selfing within populations and inbreeding depression (Mooney and McGraw, 2007). In summary, significant interspecific differences in flowering biology exist within *Panax*, and findings from *P. ginseng* and *P. japonicus* var. *major* may not be applicable to other species in the genus.

Panax notoginseng, belonging to the Araliaceae family, is a perennial herb and precious traditional Chinese medicinal material primarily distributed in south-

western China at altitudes of 1,500–1,800 m and latitude 25°N (Li et al., 2022). Wenshan, Yunnan, is the authentic production region. *P. notoginseng* possesses bidirectional regulatory functions of hemostasis and blood circulation, earning it the reputation of "Divine Grass of Southern China" and "Invaluable Herb" (Zhang et al., 2020). It demonstrates significant effects in lowering blood lipids and pressure, anti-aging, and anti-fatigue (Cai and Peng, 2021), holding substantial economic value. From January to September 2023 alone, the comprehensive total output value of the biopharmaceutical industry centered on *P. notoginseng* in Wenshan Prefecture reached 28.528 billion yuan.

Long-term exploitative harvesting has led to the extinction of wild resources, making artificial cultivation the sole source of *P. notoginseng* medicinal materials (Xia et al., 2012). Improved variety breeding and promotion are crucial for enhancing yield and quality. Recent research has focused on ginsenoside and notoginsenoside synthesis pathways and pharmacological mechanisms, with limited studies on flowering biology and breeding systems. Sun et al. (2003) found that *P. notoginseng* flowering peaked between 7:00–10:00, with pollen dispersal peaking at 10:00–14:00. Temperature and humidity significantly affect flowering, with optimal conditions at 20–27°C and 80–90% humidity. Pollen viability peaked 2 hours after flower opening, with stigma receptivity appearing on day 6 (Sun et al., 2009). Pollen viability persisted longer at low temperatures, completely losing germination capacity only after 20 days of storage (Wang et al., 2007). Pollen dispersal distance should be 4.5 m and is closely related to weather and pollinators (Yang et al., 2012).

Panax notoginseng reproduces via seeds, but its fruiting rate is low at approximately 50% (Wang et al., 2013). This low reproductive rate limits improved variety breeding and propagation. Currently, systematic studies on flowering biology and breeding systems are lacking, and basic sexual reproductive characteristics remain unclear. Therefore, this study investigated flowering biology, breeding system characteristics, and pollination biology to address three key questions: (1) elucidate sexual reproductive characteristics of *P. notoginseng*; (2) identify its breeding system type and reproductive assurance strategy; and (3) reveal reasons for low natural fruiting rates.

1.1 Experimental Site and Materials

The experiment was conducted from late July to late September in 2022 and 2023 at Xiaoxinzhai Village, Shupi Township, Qiubei County, Wenshan Prefecture, Yunnan Province, at an altitude of 1,606 m. The climate is subtropical monsoonal, with mild winters and cool summers, an average annual temperature of 19°C, abundant rainfall (779 mm annual average), and organic-rich humus soil with uniform fertility and water management. Plants were identified as 3-year-old *Panax notoginseng* by Professor YANG Shengchao from Yunnan Agricultural University.

During July–September 2022 and 2023, 50 healthy, non-flowering *P. notoginseng*

plants at the budding stage were randomly selected and tagged in the sample area. From the opening of the first flower on the first plant, daily observations were recorded for each plant' s flower number, floral morphological changes, and pollen dispersal time, with photographic documentation. Phenological stages were calculated following the methods of YANG Qiuxiong et al. (2021). Floral organ lengths and morphological characteristics at different opening stages were measured using a 0–200 mm vernier caliper (DL91200).

1.3 Scanning Electron Microscopy of Pollen Grains

During peak flowering, *P. notoginseng* pollen was collected and fixed with Carnoy' s solution. Fixed samples were rinsed with 0.1 $mol \cdot L^{1}$ phosphate buffer (PB, pH 7.4), then fixed with 1% osmium tetroxide at room temperature in darkness for 1–2 hours, followed by another PB rinse. Samples were dehydrated through an ethanol series from 30% to 100% in 10% increments, 15 minutes each, then treated with isoamyl acetate for 15 minutes. Samples were mounted on conductive carbon double-sided tape and sputter-coated with gold for 30 seconds. Thirty pollen grains were observed and photographed using an FEI QUANTA200 scanning electron microscope to examine pollen morphology, size, polar axis length, equatorial axis length, and surface ornamentation.

1.4 Determination of Pollen Viability and Stigma Receptivity

To determine the optimal method for assessing pollen viability, five different staining reagents were compared: 1% I -KI, 1% MTT, 1% TTC, acetocarmine solution, and peroxidase staining. Pollen viability was measured from 2 days before flowering to 3 days after flowering, as well as diurnal changes on the day of anthesis.

I -KI staining method: Thirty single flowers were sampled. Anthers were placed on slides, pollen exine was ruptured with a dissecting needle, one drop of water was added to disperse pollen, followed by one drop of I -KI solution. After covering with a coverslip, five fields of view were observed under an optical microscope to count blue-stained pollen grains.

MTT staining method: Thirty single flowers were sampled. Anthers were placed on slides, pollen exine was ruptured, and two drops of 1% MTT stain were added, mixed with forceps, covered, and observed after a moment. Viable pollen stained blue.

TTC staining method: Thirty single flowers were sampled. Anthers were placed on slides, pollen exine was ruptured, two drops of 0.5% TTC solution were added, mixed, covered, and incubated at 25–28°C for 15–20 minutes. Viable pollen grains stained red.

Acetocarmine staining method: Thirty single flowers were sampled. Anthers were placed on slides, pollen exine was ruptured, two drops of acetocarmine solution were added, mixed, covered, and observed. Viable pollen stained red.

Peroxidase method: Reagent I was prepared by mixing 0.5% benzidine, 0.5% -naphthol, and 0.25% sodium carbonate (10 mL each). Thirty single flowers were sampled. Anthers were placed on slides, pollen exine was ruptured, one drop each of Reagent I and 0.3% hydrogen peroxide were added, mixed, covered, incubated at 30°C for 10 minutes, then observed under microscope. Red-stained pollen was counted.

Stigma receptivity was determined using the benzidine-hydrogen peroxide method. From 3 days before flowering to 24 days after flowering, 30 single flowers were sampled daily. Stigmas were removed with forceps, placed in concave slides, and two drops of benzidine-hydrogen peroxide solution (1% benzidine: 3% hydrogen peroxide: water = 4:11:22, V/V) were added. After 30 seconds, bubble formation around the stigma indicated activity, with more bubbles indicating stronger receptivity (DUAN Shaofeng et al., 2021).

1.5 Estimation of Pollen/Ovule Ratio (P/O) and Outcrossing Index (OCI)

Following the methods of YU Chenghua et al. (2021), the P/O value was estimated by randomly selecting single flowers in the sample area. In groups of five flowers, all anthers were extracted with forceps, softened with 1–2 drops of 1.0 mol·L ^{1} HCl, transferred to 1.5 mL centrifuge tubes, diluted with distilled water, and mixed thoroughly. Pollen number was counted using a hemocytometer in triplicate. Ovule number was observed by longitudinally dissecting florets. The P/O value was calculated by dividing total pollen per flower by ovule number. Breeding system type was evaluated according to Cruden (1976), and OCI was determined following Dafni (1994) standards.

1.6 Controlled Pollination Experiments

Following YANG Run et al. (2022), 30 healthy, non-flowering *P. notoginseng* plants were randomly selected per treatment. Flowers were thinned (removing upper and lower non-viable flowers) to maintain approximately 100 flowers per inflorescence, then subjected to: (1) natural pollination as control; (2) bagging to test autonomous selfing; (3) stamen removal to test insect dependency; (4) emasculation and bagging to test apomixis; (5) emasculation and netting to test wind pollination; (6) geitonogamy (within-plant cross-pollination); and (7) xenogamy (between-plant cross-pollination). Fruit set rates were calculated one month after pollination.

1.7 Flower Visitor Observations

During peak flowering, three 9 m^2 plots were randomly established at the experimental base. On clear days, flower-visiting insects were continuously observed from 7:00–19:00 for three days, recording species, abundance, and visitation behavior. Insects were collected in ethanol and identified by Professor GUO Yulong from Yunnan Agricultural University (YANG Run et al., 2023).

1.8 Data Analysis

Data were processed using IBM SPSS Statistics 19.0. Figures were prepared using Adobe Photoshop 2018 and GraphPad Prism.

2.1 Single Flower Development Dynamics

As shown in [FIGURE:1], the single flower duration of *P. notoginseng* lasted 4–5 days, divided into four stages: bud stage, initial flowering, full bloom, and withering. (1) **Bud stage** ([FIGURE:1] A, B): Petals tightly enclosed the anthers, filaments were curled and unextended, and pistils and stamens were undeveloped. (2) **Initial flowering** ([FIGURE:1] C, D): Lasting approximately 0.5–1 day, filaments extended, petals opened to 45°, anthers appeared pale yellow with shallow grooves. (3) **Full bloom** ([FIGURE:1] E, F): Lasting 1–2 days, petals fully opened, filaments further elongated, five anthers dehisced sequentially, releasing white or pale yellow pollen. Anthers were significantly higher than the stigma, showing clear spatial separation, while the stigma began to elongate. (4) **Withering** ([FIGURE:1] G–I): Lasting 2–3 days, petals and stamens loosened and abscised sequentially, the stigma elongated rapidly, split into a "Y" shape, and secreted mucus, marking the end of the single flower period. The **ovary expansion stage** ([FIGURE:1] J–L) followed, with the stigma further splitting into a horn shape, ovary enlarging, color changing from light to dark green with dull luster, and ovules developing continuously.

2.2 Inflorescence Development Dynamics

As shown in [FIGURE:2], *P. notoginseng* has a solitary terminal umbel inflorescence. Inflorescences entered the bud stage in late June and peak flowering in mid-August. [FIGURE:3] shows that individual inflorescences contained 50–300 flowers, with an inflorescence flowering period of 20–25 days. Daily flower opening data indicated that days 9–14 represented the peak flowering period, with maximum flower number on day 14, after which flowering gradually decreased. At the inflorescence level, flowering initiated at the apex and base, progressing toward the center, with earlier-opening flowers dispersing pollen and senescing first.

2.3 Floral Morphological Characteristics

Single flowers measured 0.311–0.535 cm in diameter (mean: 0.453 ± 0.048 cm) and 0.497–0.802 cm in height (mean: 0.653 ± 0.067 cm), consisting of petals, stamens, and pistils. Flowers typically had five petals (rarely four or six) and five stamens (approximately 15% had six). Petals emitted a strong fragrance with no obvious nectar secretion. The ovary was inferior, bilocular, heart-shaped, typically containing two ovules (rarely three). As shown in [FIGURE:4], pistil and stamen lengths were similar before flower opening. On the day of anthesis, stamens elongated rapidly, creating spatial separation from the pistil ([FIGURE:4]

B). Subsequently, stamen length changed little, with complete abscission by day 3 post-anthesis. Pistil elongation was slow post-anthesis, peaking on day 6 and completely abscising by day 20 ([FIGURE:4] A).

2.4 Pollen Grain Morphology

As shown in [FIGURE:5], *P. notoginseng* pollen was single-grain, elliptical in equatorial view and nearly triangular in polar view, with a polar axis length of 21.17 ± 0.12 m, equatorial axis length of 24.43 ± 0.25 m, and a P/E ratio of 0.87, classifying it as oblate-spheroidal. According to palynological size classification, it belongs to medium-sized pollen grains with three germination furrows distributed equidistantly along the polar axis, not converging at the poles. Under G. Erdtman' s NPC classification system, it is N3P4C5 type pollen with irregular reticulate surface ornamentation.

2.5 Pollen Viability Determination and Dynamics

The optimal method for determining *P. notoginseng* pollen viability was established. As shown in

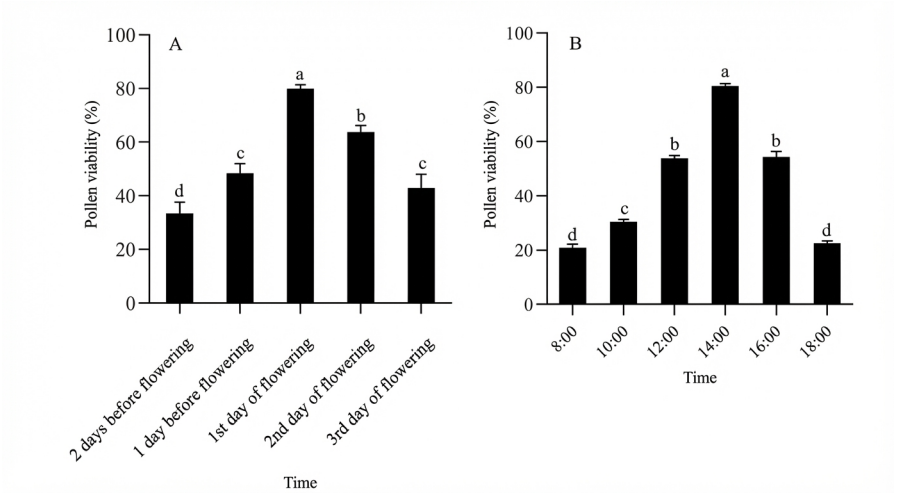

Figure 1: Figure 6

, acetocarmine and peroxidase methods stained both viable and non-viable pollen bright red with minimal color differentiation, making accurate discrimination difficult. The I -KI method stained only a few viable pollen grains blue, showing large discrepancies with actual viability. The TTC method showed clear color differences but involved complex procedures unsuitable for rapid assessment. The MTT method stained highly viable pollen blue-purple, weakly viable pollen light blue, and non-viable pollen remained unstained, offering simple

operation, short staining time, and clear results. Therefore, MTT was selected as the optimal method.

Using the MTT method, pollen viability changes were measured from 2 days before flowering to 3 days after flowering. As shown in [FIGURE:7], viability increased gradually from 2 days pre-anthesis, peaking on day 1 (79.92%), then declining. Diurnal variation was significant, with lowest viability at 8:00 (20.2%), increasing with temperature to peak at 14:00 (79.8%), then decreasing from 14:00–18:00.

2.6 Stigma Receptivity Dynamics

As shown in

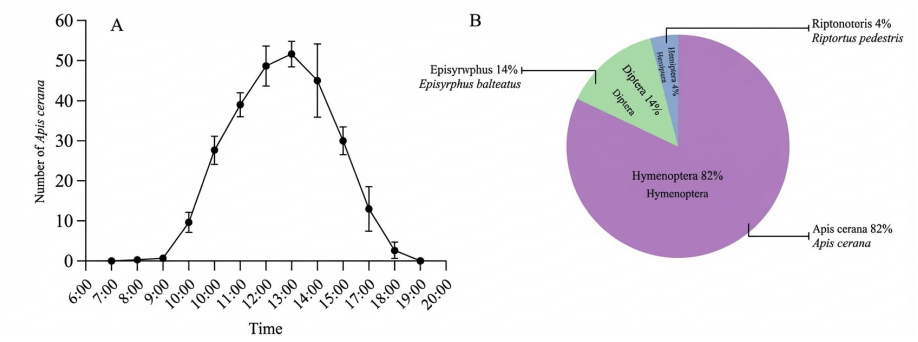

Figure 2: Figure 8

, stigma receptivity and morphology changed markedly from 3 days pre-anthesis to 20 days post-anthesis. From 3 days pre-anthesis to days 4–5, stigmas transitioned from pointed to slightly split, with receptivity changing from absent to weak. From days 6–11, stigmas changed from "Y-shaped" to horn-shaped with gradually increasing receptivity. Receptivity peaked during days 12–19, then declined, with stigmas turning red and losing receptivity after day 20.

As shown in , stigmas had no or weak receptivity from 3 days pre-anthesis to day 3. From days 4–9, most stigmas had low receptivity while some showed stronger receptivity. From day 9 onward, all stigmas became receptive, with the highest proportion showing strong receptivity on day 12 (83.3%). After day 15, some stigmas began losing receptivity. Therefore, days 9–15 represent the optimal period for hybridization.

2.7 Estimation of OCI and P/O Values

As shown in , according to Dafni (1994) standards, *P. notoginseng* flower diameter ranged 3.26 ± 0.87 mm to 4.73 ± 1.21 mm (scored as 2). Spatial separation between stamens and pistils ranged 1.20 ± 0.45 mm to 1.88 ± 0.26 mm (scored

as 1). Stamens matured and dispersed pollen on the day of anthesis when pollen viability peaked, but stigmas were not receptive, indicating protandry (scored as 1). The OCI value was 4, classifying *P. notoginseng* as outcrossing, partially self-compatible, and requiring pollinators.

As shown in , individual flowers contained 900 ± 225 to 3,500 ± 482.2 pollen grains with 2 ovules, yielding P/O ratios of 450.0 ± 112.5 to 1,037.5 ± 266.0. According to Cruden' s standards, the breeding system is facultative outcrossing.

2.8 Artificial Pollination Experiments

Controlled pollination experiments using bagging and emasculation treatments confirmed the mating system type. Under natural pollination, fruit set was 51.90%, confirming low fruiting rates and breeding difficulties. Emasculation with paper bagging yielded only 0.12% fruit set (below 1%), indicating negligible apomixis. Bagging without emasculation (autonomous selfing) produced 18.24% fruit set, demonstrating partial self-compatibility and autonomous selfing capability, though selfing rates were low. Emasculation with netting (wind pollination only) yielded 31.29% fruit set, confirming wind pollination capacity. Emasculation without bagging (wind + insect pollination) significantly increased fruit set to 66.27%, indicating insects play a crucial role. Xenogamy (between-plant pollination) achieved the highest fruit set at 69.95%, demonstrating strong outcrossing ability.

2.9 Flower Visitor Species and Behavior

As shown in [FIGURE:9] A–D, the main flower visitors were *Apis cerana* (Hymenoptera), *Episyrphus balteatus* (Diptera), and *Riptortus pedestris* (Hemiptera). As shown in

, *A. cerana* was the dominant pollinator, accounting for 82% of total visitors, followed by *E. balteatus* (14%) and *R. pedestris* (4%). Detailed observations of *A. cerana* showed numbers increasing from 10:00, peaking at 14:00, then declining from 15:00–19:00, with no activity after 19:00.

Apis cerana typically flew or crawled from inflorescence apex to base, using its mandibles and forelegs to scrape pollen from anthers. Pollen-laden appendages and head/thorax continuously contacted stigmas and anthers, accomplishing pollination ([FIGURE:9] A, B). *Episyrphus balteatus* hovered briefly before landing, feeding on pollen from dehisced anthers with its proboscis, with pollen on its thorax contacting stigmas during feeding ([FIGURE:9] C). *Riptortus pedestris*, a pest species, moved among plants by flight with long residence times, feeding on bud sap and crawling on inflorescences, thereby facilitating pollination ([FIGURE:9] D).

As shown in [FIGURE:11], different pollinators exhibited distinct visitation behaviors. Residence time on inflorescences ranked: *R. pedestris* > *A. cerana* > *E. balteatus*, with *R. pedestris* averaging 54.67 ± 7.50 seconds and *E. balteatus*

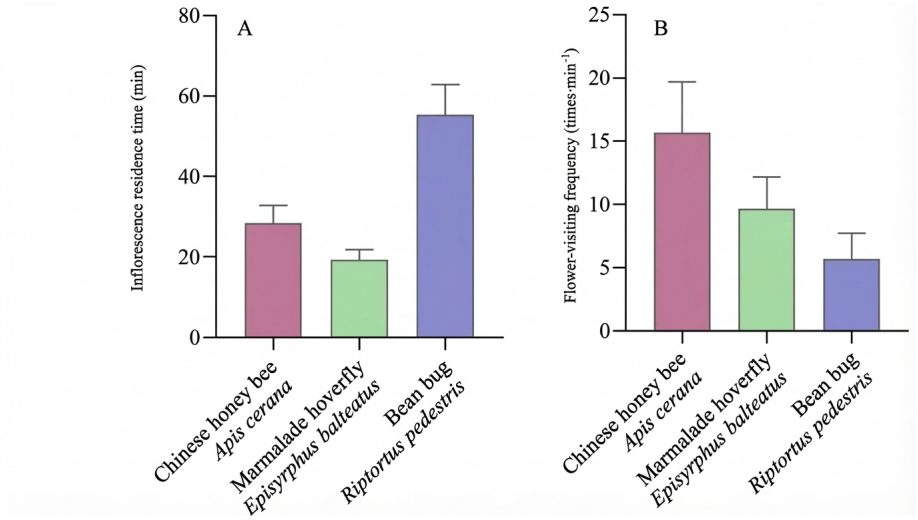

Figure 3: Figure 10

19.33 ± 2.52 seconds. Visit frequency ranked: *A. cerana* > *E. balteatus* > *R. pedestris*, with *A. cerana* at 15.6 ± 4.94 times · min ^{1} and *R. pedestris* at 5.66 ± 2.08 times · min ^{1}.

3.1 Flowering Phenology and Floral Morphology

Flowering phenology is critical for reproductive success, influencing pollinator abundance, species composition, and visitation behavior, which greatly affect fruit and seed set (CHAI Gexia et al., 2017). *Panax notoginseng* flowers from mid-August to mid-September, with single flower duration of 3–4 days, inflorescence flowering of ~20 days, and population flowering of ~60 days. Days 9–14 represent the peak flowering period, consistent with XIA Pengguo et al. (2012). The species exhibits continuous flowering at the population level with an extended flowering period. Compared with congeneric *P. ginseng* and *P. quinquefolium*, *P. notoginseng* shows similar inflorescence morphology and color but denser bud arrangement, particularly in the central region. With 50–300 flowers per inflorescence versus 30–50 in *P. ginseng* and *P. quinquefolium*, *P. notoginseng* invests more resources in sexual reproduction, likely related to its unique habitat adaptation (XIA Wenchang, 1964; CHEN Yu and LI Ketuan, 1986). The umbel inflorescence effectively aggregates single flowers, reducing pollinator search time. Large individual pollen quantities and asynchronous flowering among individuals attract more pollinators, increasing outcrossing opportunities and fruiting rates (Harder and Barrett, 1995; YE Jiatong et al., 2023), similar to findings in the congeneric *Eleutherococcus senticosus* (LIU Linde et al., 2002).

3.2 Pollen Morphology Identification

Recent scanning electron microscopy advances have enabled detailed observation and classification of pollen morphology, germination pores, and surface ornamentation. Pollen morphology shows strong genetic conservatism and can reflect phylogenetic relationships, serving as an important basis for species identification (CHEN Shengyu et al., 2024). This study found *P. notoginseng* pollen is nearly triangular in polar view and subspherical in equatorial view with three germination furrows, similar to REN Yueying et al.' s (2005) findings for *P. ginseng* and *P. quinquefolium*, confirming close affinity. However, submicroscopic studies on other *Panax* species like *P. stipuleanatus*, *P. vietnamensis*, *P. japonicus*, and *P. zingiberensis* remain limited, warranting further investigation to complete palynological studies of the genus.

3.3 Breeding System Type and Reproductive Assurance Strategy

This study revealed significant spatial separation (herkogamy) and temporal separation (dichogamy) between pistils and stamens. Pollen dispersal began on day 1 of anthesis when viability peaked, but pistils remained inside flowers as needle-like structures with no receptivity. Pistils elongated slowly, splitting into horn shapes, and only developed strong receptivity by day 9, confirming typical protandry consistent with SUN Yuqin et al. (2009). For hybridization, pollen should be collected on day 1 and used on days 9–14 as maternal parents to improve success rates. Similar dichogamy and herkogamy occur in *P. stipuleanatus*, *P. ginseng*, and *P. quinquefolium*, promoting outcrossing and reducing sexual interference and selfing (Cardoso et al., 2018). OCI estimation and controlled pollination experiments indicate a facultative outcrossing system with partial self-compatibility and strong outcrossing ability, similar to ZHURAVLEV et al. (2008) and ZHAO Xinli' s (2015) findings for *P. ginseng* and *P. japonicus* var. *major*. This suggests *P. notoginseng* enhances offspring fitness through outcrossing while maintaining some selfing capacity for reproductive assurance when pollinators are scarce.

3.4 Key Pollination Vectors

Pollination is a critical step in sexual reproduction, requiring specific vectors such as animals, wind, or water (WANG Luanfeng et al., 2024). Most Araliaceae species possess typical pollinator attraction traits including numerous flowers, fragrance, and abundant rewards. This study found *P. notoginseng* uses pollen as the primary reward without nectar secretion, unlike *E. senticosus* and *E. sessiliflorus* which secrete nectar, indicating divergent pollination strategies adapted to different habitats (LIU Linde et al., 2002). YANG Li et al. (2012) suggested pollination is closely related to weather and insects. Our controlled pollination experiments confirmed the requirement for both wind and insect vectors. Emasculation with netting (wind only) yielded 31.29% fruit set,

while emasculation without bagging (wind + insects) achieved 66.28%, demonstrating insects significantly improve fruit set. Assuming equal probability for wind and insect pollination, insects contributed 34.99% to fruit set, indicating similar contributions from both vectors. The pollinator community includes *A. cerana*, *E. balteatus*, and *R. pedestris*, indicating a generalized pollination system that accepts multiple visitors and promotes outcrossing (LI Qi et al., 2024). *Apis cerana* dominates (82% of visitors) and is the key pollinator. Peak activity occurred at noon on clear days, significantly higher than morning and evening, similar to *E. senticosus* pollinator behavior (LIU Linde et al., 2002), likely because higher temperatures accelerated anther dehiscence and increased pollen availability, attracting more bees (SUN Yuqin et al., 2003). Some Wenshan growers spray low-concentration sucrose and honey solutions to attract pollinators. Encouraging *A. cerana* cultivation could improve fruit set and increase farmer income.

3.5 Reasons for Low Natural Fruiting Rates

CHEN Taomei et al. (2024) demonstrated that low natural fruiting rates are closely related to resource limitation and pollen limitation. Although *P. notoginseng* produces many flowers, apical and basal flowers bend and abscise after pollen dispersal without setting fruit. This selective abortion mechanism is an important cause of low fruiting rates and the "many flowers, few fruits" phenomenon, similar to observations in *Vicia unijuga* (SHEN Ziwei and NAN Zhibiao, 2015). WANG Yingchun et al. (2001) suggested selective abortion improves offspring fitness, representing an evolutionary reproductive strategy for resource-poor environments. BURD (1998) proposed selective abortion of flowers and fruits ensures high-quality offspring by eliminating low-quality ones, enhancing habitat adaptation. Therefore, selective abortion in *P. notoginseng* inflorescences may facilitate selection of superior offspring, ensure rational resource allocation, and represent an adaptive reproductive strategy.

Pollen limitation is a major factor restricting pollination (LIANG Qiongyue et al., 2023). Flower visitor frequency was greatly affected by environmental conditions, decreasing during rainy and cloudy weather. SUN Yuqin et al. (2003) showed pollen dispersal was weather-dependent, with significantly reduced dispersal on rainy days. The flowering period coincides with Wenshan' s rainy season, likely causing inadequate pollen deposition during the optimal pollination window and hindering fertilization, consistent with findings on *Eleutherococcus brachypus* (WANG Zhongli et al., 1998). The umbel inflorescence shows asynchronous flowering among florets, with abundant self-pollen deposition on stigmas making cross-pollen deposition difficult, creating significant pollen competition, similar to observations in *Fatsia japonica* (DING Xuejiao, 2014). Additionally, uncleared pollen in inflorescences can develop gray mold after precipitation, severely affecting fruit set (JIANG Ni et al., 2017).

In summary, as a precious Chinese medicinal material facing wild resource extinction, low reproduction rates, and breeding difficulties, this study elucidates

the flowering biology and breeding system characteristics of *P. notoginseng* and reveals reasons for low fruiting rates, providing important implications for improved variety breeding and propagation.

References

BARRIONUEVO CN, BENITEZ-VIEYRA S, SAZATOMIL F, 2021. Floral biology of Salvia stachydifolia, a species visited by bees and birds: connecting sexual phases, nectar dynamics and breeding system to visitors' behavior[J]. Plant Ecol, 14(4): 580-590.

BURD M, 1998. "Excess" flower production and selective fruit abortion: a model of potential benefits[J]. Ecology, 79: 2123-2132.

CAI L, PENG P, 2021. Research progress on the pharmacological effects of Panax notoginseng[J]. Shandong Chem Ind Shan-tung Hua Kung, 50(3): 70-71. [蔡琳，彭鹏，2021. 三七药理作用的研究进展 [J]. 山东化工，50(3)：70-71.]

CARDOSO JCF, VIANA ML, MATIAS R, et al., 2018. Towards a unified terminology for angiosperm reproductive systems[J]. Acta Bot Basil, 32(3): 329-348.

CHAI GX, CAI MY, JIN XL, et al., 2017. The pollination biology of Michelia crassipes[J]. Guihaia, 37(10): 1322-1329. [柴戈霞，蔡梦颖，金晓玲，等，2017. 紫花含笑传粉生物学初探 [J]. 广西植物，37(10)：1322-1329.]

CHEN TM, XU X, ZHOU XY, et al., 2024. Research progress and protection strategy suggestions of Disanthus cercidifolius subsp. longipes endangered system[J/OL]. Mol Plant Breed: 1-15. [陈涛梅，徐侠，周鑫洋，等，2024. 长柄双花木濒危机制研究进展及保护策略建议 [J/OL]. 分子植物育种：1-15.]

CHEN Y, LI KT, 1986. Preliminary observations on the flowering habit of Panax quinquefolium[J]. Subtrop Plant Sci(2): 22-24. [陈裕，李克团，1986. 西洋参开花习性的初步观察 [J]. 亚热带植物通讯 (2)：22-24.]

CHEN SY, TANG XY, XIAO Y, et al., 2024. Pollen morphology and phylogenetic relationship of 14 Oxalis species[J]. Guihaia, 44(4): 682-698. [陈生煜，唐小云，肖云，等，2024. 14 种酢浆草属植物花粉形态及其亲缘关系分析 [J]. 广西植物，44(4)：682-698.]

CRUDEN RW, HERMAN-PARKER SM, 1977. Temporal dioecism: an alternative to dioecism?[J]. Evolution, 31(4): 863.

DAFNIA, 1994. Pollination ecology: a practical approach[J]. Brittonia, 46(2): 1-5.

DING XJ, 2014. Research on pollination biology and embryology in Fatsia japonica (Thunb.) Decne. et Planch[D]. Zhoushan: Zhejiang Ocean University: 38-42. [丁雪娇，2014. 八角金盘传粉生物学及胚胎学研究 [D]. 舟山：浙江海洋学院：38-42.]

DUAN SF, ZHU CY, YU CH, et al., 2021. Study on the biological characteristics of pollination of Gentiana rigescens Franch[J]. J Yuannan Agric Univ, 36(4): 660-665. [段绍凤，朱春艳，余成华，等，2021. 滇龙胆传粉生物学特征研究 [J]. 云南农业大学学报（自然科学），36(4)：660-665.]

HARDER LD, BAR RETT SCH, 1995. Mating cost of large floral display in hermaphrodite plant[J]. Nature, 372(6514): 512-515.

HE ZX, DENG XQ, 1985. Studies on flowing and fruiting of Panax notoginseng[J]. Guihaia(1): 65-70. [何振兴，邓锡青，1985. 三七开花结果习性的研究 [J]. 广西植物 (1)：65-70.]

JIANG N, CHEN QP, FENG SX, et al., 2017. Occurrence of Panax notoginseng grey mould and selection of funficides in Guangxi[J]. Chin J Trop Crops, 38(9): 1712-1719. [蒋妮，陈乾平，冯世鑫，等，2017. 广西三七灰霉病流行规律及药剂筛选 [J]. 热带作物学报，38(9)：1712-1719.]

LIANG QY, LIU CQ, LU QF, et al., 2023. The pollination biology of Begonia Guangxiensis, a narrowly distributed species[J]. Bull Bot Res, 43(3): 432-438. [梁琼月，刘长秋，陆奇丰，等，2023. 狭域物种广西秋海棠传粉生物学研究 [J]. 植物研究，43(3)：432-438.]

LI MQ, LIANG QW, YAN J, et al., 2022. Research progress on genetic improvement of Panax notoginseng[J]. Chin Tradit Herb Drugs, 53(10): 3241-3250. [李满桥，梁绮文，闫静，等，2022. 三七遗传改良的研究进展 [J]. 中草药，53(10)：3241-3250.]

LI Q, MA HZ, MAMUT J, 2024. Floral syndrome and breeding system of the spring flowering plant Iris scariosa (Iridaceae)[J]. Guihaia, 44(4): 756-765. [李琪，马菡泽，吉乃提汗·马木提，2024. 春季开花植物膜苞鸢尾的花部综合征与繁育系统研究 [J]. 广西植物，44(4)：756-765.]

LIU LD, ZHU N, SHEN JH, et al., 2002. Comparative studies on floral dynamics and breeding system between Eleutherococcus senticosus and E·sessiliflorus[J]. Acta Ecol Sin(7): 1041-1048. [刘林德，祝宁，申家恒，等，2002. 刺五加、短梗五加的开花动态及繁育系统的比较研究 [J]. 生态学报 (7)：1041-1048.]

LIU LD, LI W, ZHU N, et al., 2002. The relations among the nectar secretive rhythms, nectar compositions and diversities of floral visitors for both Eleutherococcus senticosus and E·sessiliflorus[J]. Acta Ecol Sin(6): 847-853. [刘林德，李玮，祝宁，等，2002. 刺五加、短梗五加的花蜜分泌节律、花蜜成分及访花者多样性的比较研究 [J]. 生态学报 (6)：847-853.]

LONG GG, CHEN FJ, CHEN YD, et al., 2021. Flowering and fruiting characteristics of Sinomanglietia glauca and its endangered reason analysis[J]. J NE For Univ, 49(10): 47-51. [龙桂根，陈发菊，陈应德，等，2021. 华木莲开花结实特性及其濒危的影响因素 [J]. 东北林业大学学报，49(10)：47-51.]

MOONEY EH, MCGRAW J, 2007. Effects of self-pollination and outcrossing with cultivated plants in small natural populations of Panax quinquefolius (Araliaceae)[J]. Am J Bot, 94(10): 1677-1687.

MURREN CJ, PURVIS KG, GLASGOW D, et al., 2014. Investigating lag phase and invasion potential of Vtex rotundifolia: a coastal dune exotic[J]. J Coastal Res, 30(4): 815-824.

PENG YD, MA N, YE YH, et al., 2024. Analysis of the chloroplast genome characteristics of three medicinal plants of Panax in the Qinba mountains[J/OL]. Guihaia: 1-14. [彭毅丹，马楠，叶奕含，等，2024. 秦巴山区 3 种人参属药用植物叶绿体基因组特征分析 [J/OL]. 广西植物：1-14.]

REN YY, MA HT, GAO W, et al., 2005. Ultrastructural study on pollen of Yellow, Red of Panax ginseng C.A. Meyer and and Panax quinguefolium L. by scanning electron microscopy[J]. Ginseng Research(4): 9-11. [任跃英，马红婷，高巍，2005. 黄果、红果人参、西洋参花粉扫描电镜超微结构研究 [J]. 人参研究 (4)：9-11.]

SCHOEN DI, JOHNSON MT, WRIGHT SI, 2019. The genetics of plant reproductive ecology, evolution, and genetics of plant reproductive systems[J]. New Phytol, 224(3): 999-1004.

SHEN ZW, NAN ZB, 2015. Selective abortion and fruit-set pattern of Vicia unijuga in Gannan Tibetan region[J]. Acta Pratac, 24(4): 132-139. [沈紫微，南志标，2015. 甘南地区歪头菜的选择性败育及结实格局 [J]. 草业学报，24(4)：132-139.]

SUN H, NIU Y, CHEN YS, et al., 2014. Survival and reproduction of plant species in the Qinghai–Tibet Plateau[J]. Acta Phytotaxon Sin, 52(3): 378-396.

SUN YQ, ZHOU SL, CHEN ZJ, et al., 2009. A study on the vitality and stigma viability of Panax notoginseng[J]. China J Chin Mater Med, 34(7): 913-915. [孙玉琴，周世良，陈中坚，等，2009. 三七花粉活力和柱头可授性研究 [J]. 中国中药杂志，34(7)：913-915.]

SUN YQ, CHEN ZJ, WANG CL, et al., 2003. Observation of blooming habits of Panax notoginseng[J]. Chin Med Mater(4): 235-236. [孙玉琴，陈中坚，王朝梁，等，2003. 三七开花习性观察 [J]. 中药材 (4)：235-236.]

WANG DK, SUN GF, GUO ZM, et al., 2007. Studies on viability determination of Panax notoginseng Pollen[J]. J. Anhui Agric Sci (28): 8811-8812. [王定康，孙桂芳，郭志明，等，2007. 三七花粉生活力测定研究 [J]. 安徽农业科学 (28)：8811-8812.]

WANG L, JIN B, LU Y, et al., 2009. Research progress in pollination biology of Ginkgo biloba L[J]. Acta Bot Boreal-Occident Sin, 29(4): 842-850. [王莉，金飚，陆彦，等，2009. 银杏传粉生物学的研究进展 [J]. 西北植物学报，29(4)：842-850.]

WANG LF, ZHANG TT, XIANG WQ, et al., 2024. Pollination biology of the rare and endangered submerged plant, Ottelia cordata[J]. Guihaia, 44(1): 157-166. [王銮凤，张同同，向文倩，等，2024. 珍稀濒危沉水植物水菜花传粉生物学研究 [J]. 广西植物，44(1)：157-166.]

WANG YC, HOU YW, ZHANG YJ, et al., 2001. Reproductive strategies of Tetraena mongolica maxim[J]. Acta Phytoecol Sin,(6): 699-703. [王迎春，侯艳伟，张颖娟，等，2001. 四合木种群生殖对策的研究 [J]. 植物生态学报 (6)：699-703.]

WANG YF, LI G, TANG L, et al., 2013. Preliminary research on introduction and cultivation adaptability of Panax notoginseng(Burk.)F.H.Chen in Xishuangbanna[J]. Chin Agric Sci Bull,(25): 136-141. [王艳芳，李戈，唐玲，等，2013. 三七在西双版纳引种适应性试验初报 [J]. 中国农学通报 (25)：136-141.]

Wang ZL, LIU LD, TIAN JW, et al., 1998. The development of gynoecium after anthesis and fertilization in Eleutherococcus brachypus(araliaceae)[J]. Chin Bull Bot (4): 22-28. [王仲礼，刘林德，田国伟，等，1998. 短柄五加开花后雌蕊的发育状态与受精作用的研究 [J]. 植物学报 (4)：22-28.]

WIDEN B, 1991. Environmental and genetic influences on phenology and plant size in a perennial herb, Senecio integrifolius[J]. Can J Bot, 69(1): 209-217.

XIA PG, CUI XM, WEI MT, et al., 2012. Progress of biological characteristic of Panax notoginseng[J]. J Chin Med Mater, 35(5): 831-835. [夏鹏国，崔秀明，韦美膛，等，2012. 三七的生物学特性研究进展 [J]. 中药材，35(5)：831-835.]

XIA WC, 1964. Study on the flowering habit and hybridisation technology of Panax ginseng[J]. Chin Pharm J(7): 310-312. [夏文昌，1964. 人参开花习性及杂交技术的研究 [J]. 中国药学杂志 (7)：310-312.]

YANG L, CUI XM, Sun YQ, et al., 2012. Preliminary study on pollen flow in Panax Notoginseng[J]. Res Pract Chin Med, 26(6): 11-14. [杨莉，崔秀明，孙玉琴，等，2012. 三七花粉流的初步研究 [J]. 现代中药研究与实践，26(6)：11-14.]

YANG L, ZHOU TH, WANG Y, et al., 2023. Flowering and pollination biology of rare and endangered plant Rrimula filchnerae Knuth[J]. Acta Bot Boreali-Occident Sin, 43(7): 1218-1226. [杨柳，周天华，王勇，等，2023. 珍稀濒危植物陕西羽叶报春的开花及传粉生物学研究 [J]. 西北植物学报，43(7)：1218-1226.]

YANG QX, PENG R, SUN NX, et al., 2021. Study on flowering phenology and mating system of Fritillaria taipaiensi[J]. Chin Med Mater, 44(4): 787-792. [杨秋雄，彭锐，孙年喜，等，2021. 太白贝母开花物候和交配系统研究 [J]. 中药材，44(4)：787-792.]

YANG R, LIANG YL, WANG S, et al., 2022. Study on flowering phenology and mating system of Dipsacus asperoides[J]. J. Chin Med Mater, 45(9): 2048-2053. [杨润，梁艳丽，王硕，等，2022. 川续断开花物候和交配系统研究 [J]. 中药材，45(9)：2048-2053.]

YANG R, HU QQ, ZHANG XD, et al., 2023. Study on the characteristics of pollination biology of Dipsacus asperoides[J]. J Yuannan Agric Univ (Nat Sci), 38(4): 644-651. [杨润，胡倩倩，张晓东，等，2023. 川续断传粉生物学特征研究 [J]. 云南农业大学学报（自然科学），38(4)：644-651.]

YE JT, MAO SZ, HU XH, et al., 2023. Flowering biology and breeding system of Ardisia humilis[J]. Guihaia, 43(2): 379-389. [叶家桐，毛世忠，胡兴华，等，2023. 矮紫金牛的开花生物学与繁育系统研究 [J]. 广西植物，43(2)：379-389.]

YU CH, ZHU CH, DUAN SF, et al., 2021. Flowering phenology and mating system of Gentiana rigescenss[J]. Chin J Trop Crops, 42(6): 1639-1645. [余成

华，朱春艳，段绍凤，等，2021. 滇龙胆开花物候与交配系统研究 [J]. 热带作物学报，42(6)：1639-1645.]

ZHANG H, ZHANG C, WANG F, et a1., 2024. Evolutionary mechanism of diversity formation of triterpenoid saponin metabolites of the Panax Species[J/OL]. Mol Plant Breed: 1-12. [张行，张辰，王富，等，2024. 人参属三萜皂苷代谢产物多样性形成的进化机制 [J/OL]. 分子植物育种：1-12].

ZHANG D, FENG LY, GAO LZ, 2020. Progress in the research and utilization of Panax notoginseng biological resources[J]. Biotic Res, 42(1): 61-66. [张丹，冯丽颖，高立志，2020. 三七生物资源研究与利用进展 [J]. 生物资源，42(1)：61-66.]

ZHAO XL, 2015. Study on reproductive biology of Panax japonicus var. major[J]. Mod Chin Med, 17(12): 1298-1301. [赵新礼，2015. 珠子参生殖生物学特性的研究 [J]. 中国现代中药，17(12)：1298-1301.]

ZHURAVLEV YN, KOREN OG, REUNOVA GD, et a1., 2008. Panax ginseng natural populations: their past, current state and perspectives[J]. Acta Pharmacol Sin, 29(9): 1127-1136.