Exploring Wild Plant Conservation from the Perspective of Reproductive Biology: A Case Study of Rhododendron Excellens

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Abstract: Wild plant protection has always been one of the key issues at home and abroad, which is of great significance to biodiversity protection and wild plant resource protection. The wild distribution of Rhododendron excellens is of high ornamental value, but in recent years due to tourism development and man-made destruction, the native plant population is shrinking, and the survival and reproduction of wild Rhododendron excellens is threatened. From the perspective of reproductive biology, this paper discusses the endangered mechanism of Rhododendron excellens, reveals the obstacles in the process of reproduction on the reproductive level, and puts forward some strategies for the successful conservation and recovery of wild Rhododendron excellens.

1 INTRODUCTION

Wild plant resource is an important ecological resource reserve and national property in our country, and it is an indispensable and important resource in the process of national development and construction. The protection of wild plant resource is not only related to the healthy development of human beings, more impact on the sustainable development of the global ecosystem. In recent years, with the continuous development of China's economic construction, there have been numerous environmental problems. Driven by economic interests, unrestrained exploitation and destruction have caused a large number of wild plant species to be destroyed to varying degrees, even led to the risk of extinction of many species, how to effectively protect wild plant resources is a major problem to be solved in the future.

Plant reproductive biology is the study of the laws of plant reproductive behavior, which includes three basic activities of plant growth, maintenance and reproduction. The life cycle of wild angiosperms mainly includes the stages of seed, seedling, adult plant, and seed, and the whole process of flowering, pollination, fertilization and seed-setting completes the propagation of plants and the continuation of species (Ren 2012). The natural pollination and seed propagation of some wild plants are threatened by human activities and other external factors, which hinder the normal propagation of plants, the decline of its own reproductive capacity is also a threat to its survival.

Based on the theory of reproductive biology, Field Investigation and experimental design, the present situation of Rhododendron excellens resources and pollination under natural conditions were investigated, to examine whether pollination failure is a potential risk factor for reproduction, to study the reproductive potential and the endangered mechanism of Rhododendron excellens under full pollination, on the premise of deep understanding of its biological characteristics and living environment, the protection and utilization of Rhododendron excellens are proposed.

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2 MATERIALS AND METHODS

2.1 Study Species and Sites

Rhododendron excellens (Rhododendron excellens Hemsl. et Wils), evergreen shrub, 1.5-3 m high, leaves evergreen, leathery, oblong-elliptic, corolla broadly funnelform, flowers large, white aromatic. Rhododendron excellens are found in mixed evergreen and deciduous woodlands or thickets at elevations of 1100-2400 m. The Rhododendron excellens are mainly distributed in Yunnan Province, and the distribution is small and concentrated, most of which are distributed in Wenshan Zhuang and Miao Autonomous Prefecture, southeast of Yunnan, and also in Yuxi, Red River and Lincang (Tian 2011). The experiment was conducted in the wild population of Ganbazi, Xiachang Township, Malipo County, Yunnan Province. Population coordinates of Ganbazi (N: 23 ° 07'27.47", E: 104 ° 49'21.63"), altitude 1850-1890m.

Figure 1: A photographs of Rhododendron excellens.

2.2 Research Methodology

2.2.1 Observation Record of Pollination Media and Determination of Visiting Frequency

Observation records of pollinator species and flower visiting frequency were conducted in the wild population of Ganbazi, Xiakinchang Township, Malipo County, Wenshan Zhuang and Miao Autonomous Prefecture, in May 2019, May 2020 and 2021 May 2020. The experiment was conducted in the blooming period of Rhododendron excellens for three years. During the field observation in the wild population of Ganbazi, a cluster of healthy growing plants was randomly selected, and 20 flowers were selected to be in full bloom, each cluster was observed for one hour, from 9:00 to 12:00 in the morning, from 2:00 to 6:00 in the afternoon, and from 7:30 to 10:00 in the evening. Each cluster was observed for about 10 hours a day and recorded at half an hour, change of observation site one hour after completion. In each time period, the number and species of successive visiting flowers were recorded in detail. Squatting on the ground at night to view the sky as a background from the bottom up, with a red flashlight to observe the weak red light.

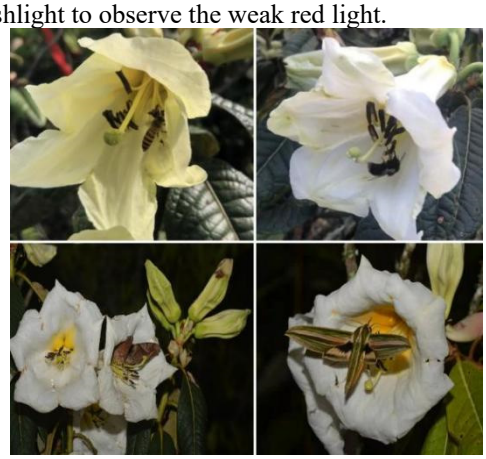

Figure 2: Pollinators of Rhododendron excellens.

2.2.2 Self-and Cross-pollination Experiments and Control Experiments

In May 2020, experiments were conducted in the wild population of Ganbazi in Xiakinchang Township to study the affinity, outcrossing potential and natural pollination of Rhododendron excellens, there were artificial self-pollination experiment, artificial cross-pollination experiment and control experiment. More than ten healthy plants were selected for each experiment, and a cluster of healthy inflorescences was selected for each treatment. Artificial self-pollination experiment and artificial cross-pollination experiment were carried out in the bud stage before the flower opened, and then the bag was taken off when the flower opened for self-pollination and cross-pollination, the flowers were then bagged until they wilted; the control experiment was listed at the bud stage, and the rest were left untreated.

In early November 2020, the three treatments were bagged at the experimental site, as some of the fruits were not yet ripe and early bagging prevented seed loss when the fruits cracked. At the beginning of December 2020, the fruits were basically ripe. The fruits were collected from three experimental treatments at the experimental site, and then brought back to the room for drying and waiting for the fruits to open naturally, fruit setting rate and seed number per fruit were counted in laboratory, in which fruit setting rate = fruit number/flower number × 100%. Seed number per fruit was weighed by 100-grain weight (Yang2020).

2.3 Statistical Analysis

In order to compare the frequency of flower-visiting, fruit-setting and seed-setting of different pollination treatments, Microsoft Excel 2020 was used to arrange, statistic and plot the data, and SPSS23.0 software was used to analyze the data, the difference of each group was compared by independent sample t test, the significance level was $P < 0.05$.

3 RESULTS AND ANALYSIS

3.1 Species of Pollinators

Over the course of three years, from 2020 to 2021, we counted about 90 hours of observations, observed different pollinators during the day and night, and recorded the frequency of flower visits in detail. The major flower-visiting insects in Rhododendron excellens are Hymenoptera, among which bumblebee is the main flower-visiting insect, bee is the secondary flower-visiting insect, butterfly and Drosophila are negligible due to the low frequency of flower-visiting. Four species of long-billed hawk moth, Cechetra Scotti, Notonagemia analis, Acosmeryx Naga and Daphnis Thous hypocrameri, have been observed visiting flowers at night in Rhododendron excellens. Flower visiting insects from the Rhododendron excellens visit more frequently when the weather is fine, all insects stop their activity when it is rainy, and all observations are counted on non rainy days.

3.2 Frequency of Flower Visits by Pollinators

Flower visiting frequency refers to the number of flower visiting insects in a unit time. When observing in the field, a certain number of flowers were selected and the number and species of flower visiting insects were recorded, thus the number of insects that visit a single flower per unit of time (Li 2014) is obtained. The number of insects and the frequency of visiting flowers varied greatly in different observation years, but there was no significant difference between 2019 and 2020, and the number of insects visiting flowers by day and night increased significantly in 2021 compared with the previous two years, the number and frequency of bumblebees were significantly higher in the daytime than in the previous two years.

Since the 2019 observations are scattered, this is a detailed analysis of the 2020 and 2021 data. In 2020, the frequency of flower visiting of bumblebee and honeybee is relatively low, the frequency of flower visiting of Bumblebee is 0.144 times/flower/hour, the frequency of flower visiting of honeybee is 0.128 times/flower/hour, the frequency of flower visiting of bumblebee is slightly higher than that of Honeybee, the frequency of butterflies and day moths is too low to be considered. During the observation period, the number and species of flower-visiting insects are relatively low and there is no obvious peak activity. In 2020,0.079 flower visits per hour were observed at night, and there was a more active peak from 7:30 to 8:30 at night. The frequency of flower visits at night was not high.

Bumblebees and honeybees were still the main flower-visiting insects in 2021. The frequency of flower-visiting of bumblebees was 1.167 and 0.198 respectively. The frequency of flower-visiting of bumblebees was significantly higher than that of honeybees, the frequency of flower-visiting of other insects is so low that it can be neglected. Bumblebees have a peak of flower-visiting at 5:00 pm, while bees have a peak of activity at 3:00 pm, as at 4:00 pm, the bloom-visiting activities of bumblebees and honeybees decreased dramatically at 12:00 p.m. -- 2:00 p.m., possibly due to higher temperatures and less insect activity. The frequency of flower visits was 0.3692021 at night, and there was an active peak between 7:30 and 8:30 at night.

3.2.1 Comparison of Flower Visiting Frequency of Pollination Media in Different Years

Data from 2020 and the 2021 show that the frequency of flower visits by pollinators varies significantly from year to year. In 2020, the flower visiting frequency of bumblebees was 0.144 times per flower per hour, which was significantly lower than that of 2021 bumblebees (1.167 times per flower per hour) ($p < 0.05$). There was no significant difference between bumblebees and 2021 bumblebees in 2020, 0.128 times per half hour and 0.198 times per hour, respectively. Compared with 2020, the flower visiting frequency of 2021 Bombus showed an explosive increase, while the flower visiting frequency of honeybee increased slightly. In 2020, the frequency of flower visiting was 0.079 times per flower hour at night, which was significantly lower than that of 0.369 times per flower hour at night ($p < 0.05$), and the frequency of flower visiting was also significantly increased for the 2021.

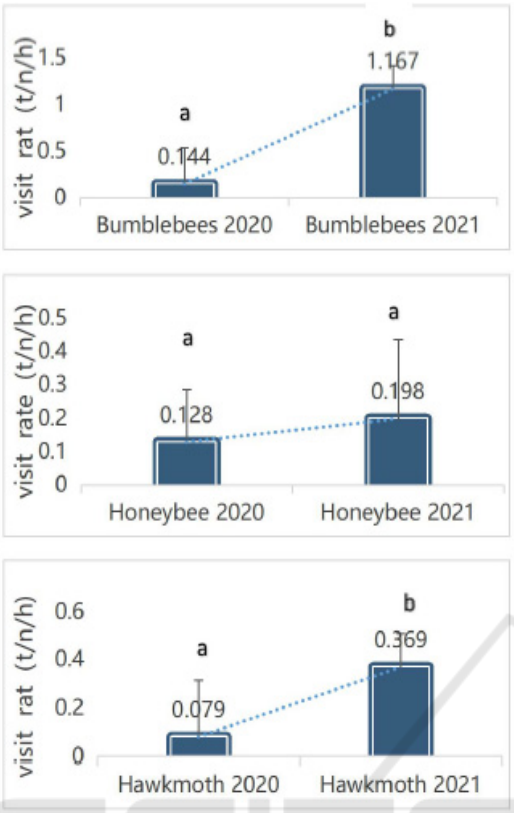

Figure 3: Frequency of insects visiting flowers in different years.

3.3 Results of Pollination Experiment and Control Experiment

The percentage of fruit set and the average number of seeds per fruit were obtained by treating the fruits and seeds of artificial self-pollination experiment, cross-pollination experiment and control experiment in laboratory, the fruit setting rate of the artificial cross-pollination experiment was 86%, and that of the control experiment was 82%. There was no significant difference among the three treatments ($p < 0.05$). The results showed that Rhododendron excellens had a certain degree of self-compatibility, and self-pollination also produced higher fruit-setting rate, the fruit-setting rate of artificial cross-pollination was slightly higher than that of the control.

The average number of seeds per fruit was 734 ± 50 in the artificial self-pollination experiment, among which the number of seeds of some fruits was 0, the minimum number of seeds was 120, and the maximum number of seeds was 4436 The average number of seeds per fruit was 5472 ± 452 in artificial cross-pollination experiment. There was only one fruit with 0 seeds, the next was 256 seeds, and the most was 15103 seeds In the control experiment, the average number of seeds per fruit was 3510 ± 312, part of the seeds was 0, the minimum number of seeds was 158, and the maximum number of seeds was 10378. There was significant difference ($p < 0.05$) in the number of seeds of each fruit in the three groups, and there was no significant synchronism between the number of seeds of each fruit, there was a great significance between the most and least number of seeds, some seeds did not develop, and the number of seeds produced was 0.

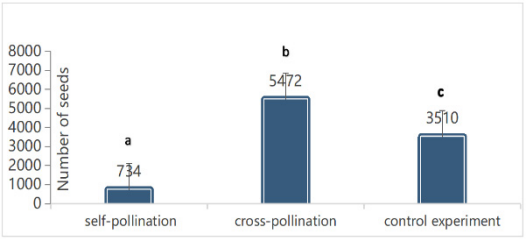

Figure 4. Average number of seeds produced per fruit under different treatments.

3.4 Analysis of Pollination Agents and Flower Visiting Frequency

Based on the results of field experiments, we can confirm that there are three theoretical effective pollinators of Rhododendron excellens, mainly bumblebees and honeybees during the day and Hawk Moths at night. Due to the integrated flower characteristics of the Rhododendron excellens, further quantitative tests of pollination effectiveness are needed to measure the contribution of different flower visitors to Rhododendron excellens fitness (Huang 2007). According to the frequency of flower visits by pollinators in different years, the frequency of insect visits by day and night in 2021 was significantly higher than that in 2020, especially the frequency of flower visits by day pollinators increased significantly, the frequency of flower visiting in night pollination medium also increased significantly. The increase in the frequency of visits to pollinators has ensured that Rhododendron excellens pollination is assured and has reduced pollination failures due to pollinator restrictions.

3.5 Analysis of Experimental Results

There was no significant difference in fruit setting rate between artificial self-pollination, cross-pollination and control, and there was partial self-compatibility in Rhododendron excellens, although there was no significant difference in the percentage

of fruit set, there was a significant difference in the average number of seeds produced by different types of fruit, the number of seeds produced by artificial self-flowering experiment was significantly less than that by cross-pollination experiment and control experiment, which indicated that the propagation of Rhododendron excellens in natural state needed pollination medium to be carried out, although a low frequency of flower visits by pollinators was observed in 2020, it may be that the influence of human factors on pollinators is less than the threshold of the minimal pollinators that maintain plant sexual reproduction, therefore, in the control experiment, the fruit setting rate and seed number of plants showe d no obvious limitation of pollination, and the cross-pollination experiment showed the most fruit setting rate and seed number, it also shows the reproductive potential of the Rhododendron excellens in the presence of sufficient pollinators, and the results of the present study suggest that there is no pollinator restriction in the Rhododendron excellens, however, the impact of human factors on the ecological environment of the wild population of the megaphone is still a major problem that needs to be solved urgently, and efforts should be made to regularize the construction of the MALIPO national nature reserve and limit all activities that cause human interference and destruction, maintaining the original habitat conditions of wild animals and plants is the responsibility and goal that must be fulfilled to protect the animal and plant resources in the future.

4 CONCLUSIONS

Our experiments show that Rhododendron excellens can not self pollinate and therefore must rely on pollinators for reproduction. Moreover, this species has a low degree of self-compatibility, similar to many other rhododendron plants (Weber 2004), so it must visit the flowers of animals for the alienation of pollination for full reproduction. Although sexual reproduction difficulties are common in some other endangered plants (Johnson 2004; Walsh 2019), we found that the natural fruit setting and seed setting rates of Rhododendron excellens are not low, indicating that it attracts pollinators and achieves a considerable degree of reproductive success, so we can rule out reproductive difficulties as a possible risk factor for the species. The results showed that environmental factors had no significant effect on the reproductive success of Rhododendron excellens, but the negative effects of limiting human factors did increase the frequency of bumblebees' visits to flowers during the day, Rhododendron excellens increases the chances of successful reproduction, so the importance of in situ conservation of wild plants needs to be addressed.

The Rhododendron excellens has a remarkable combination of pollinating characteristics, such as white corolla, aromatic smell and thin and rich nectar (Martins 2013). Its large funnel-shaped corolla is characteristic of a subtype of Hyphantria, which has been reported in the genera Lilium and Cactaceae (Liu 2019). It was found that the moth was indeed able to be visited, and the flower visiting behavior indicated that the moth was probably an effective pollinator (fig. 2). On the other hand, Rhododendron excellens flowers last for several days and are visited by a large number of bees during the day, so their pollination patterns may not be specialized. We don't know which of the daytime and nighttime visitors is effective or whether both are effective, but further experiments are needed to confirm this.

5 DISCUSSION

Experimental Underground Jinchangxiang Ganbazi wild population belongs to the MALIPO National Nature Reserve, which has extremely rich animal and plant resources and excellent ecological environment, and provides important conditions for the conservation and reproduction of species, however, due to the recent establishment of the reserve, various rules and regulations, regulations on management and protection are still in the stage of perfection. In previous years, human activities in the reserve were more frequent, there are many recreational facilities in the reserve, such as agricultural land and farm recreation facilities. Local residents have a serious grazing phenomenon. Tourists also barbecue and entertain in the reserve. random picking of plants and trampling of plants cause serious pollution, the structure and stability of animal and plant habitat were destroyed to a certain extent, which affected the abundance of pollinators and destroyed the stable relationship of mutualism between plants and pollinators. The paucity of pollination media may lead to difficulties in sexual reproduction in plants after 2020 due to the impact of a new global coronavirus, measures such as strict access control and registration of commuters have greatly reduced the number of tourists and idlers in Malipo's national nature reserves, and places of entertainment such as farmhouses are banned, the original habitat in the reserve has been restored and protected to the greatest extent under the combined effect of various factors,

and the original damaged plant and animal communities have also been restored to the greatest extent under the condition of sharp reduction of human disturbance, this may be an important reason for the large increase in the number and frequency of 2021 pollinators, further explaining the importance of protecting the habitat of wild plants, as well as the negative effects of man-made factors on wild animals and plants.

From the perspective of reproductive biology, this paper analyzed the visiting frequency of pollination media in different years and the seed setting rate and seed setting quantity under different experiments in the wild Rhododendron excellens, the natural reproductive potential and pollination of the wild horn were fully discussed, and the negative effects of human activities on pollination media were revealed through the experimental results, and some suggestions for solving the related problems were put forward, the purpose of this study is to provide a scientific basis for the propagation and growth of wild Rhododendron excellens. The deficiency of this study is that there is no quantitative test for the pollination effectiveness of different flower visitors and no experiment for seed germination, etc., follow-up related experiments have yet to be conducted. Pollination is one of the most important processes in the conservation of wild plants, and is an essential step in plant reproduction and species continuation. However, most of the research on wild plants focuses on the investigation and analysis of the original habitats of plants, in the wild plant pollination research is relatively few, this paper expects to wild plant protection in the pollination of specific research to make reference value.

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