Influence of Stigma Colors on Reproductive Success of *Epimedium pubescens*

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Abstract

The flower colors are often considered as the important signals rewards to the pollinators, which can greatly effect on the plant reproduction. Compared with the petal or corolla color polymorphism, vegetal organs have fewer changes in color and reported. The stigma color polymorphism and its reproductive ecology of *Epimedium pubescens* populations was studied, and the reproductive ecology of the stigma color polymorphism in *E. pubescens* populations are compared. The results show that the *E. pubescens* with the white stigma are in the majority among the populations, and there are no differences between plants with two stigma shapes and flower shapes. In environmental factors, the humidity of the red stigma was obviously higher than that of the white stigma. Pollinators were found to have no preference with respect to for *E. pubescens* with two stigma colors. The pollen of the red stigma was higher than that of the white stigma in pollen deposition and removal, which means that the pollination efficiency of *E. pubescens* with the red stigma was higher than that of the white stigma. The comparison of the hand-pollinate experiment with the natural seed setting showed that the female fitness of the *E. pubescens* with the red stigma was on an average higher than that of the white stigma. In conclusion: the differentiation of stigma color affects the success of *E. pubescens* reproducton due to higher pollination efficiency. © 2018 Friends Science Publishers

Keywords: *Epimedium pubescens*; Stigma colors; Reproductive success; Pollination efficiency

Introduction

Flower color polymorphism means color differences between different individuals and groups in the same species. This phenomenon is used to explain the evolutionary mechanism among plants (Levin and Brack, 1995; Clegg and Durbin, 2000; Tang and Huang, 2012). For example, the *Ipomoea purpurea* has purple, pink and white corollas (Clegg and Durbin, 2000; Durbin et al., 2003). The *Butomus umbellatus* has pink and white female groups (Huang and Tang, 2008). Diverse ecologic factors may add selective pressure to flower color and maintain the color polymorphism. It is found in many species that the environmental heterogeneity and the stress tolerance are related to the maintenance of the blue and white color polymorphism (Warren and Mackenzie, 2001). On the other hand, color polymorphism may be a disruptive selection result of pollinators (Melendez-Ackerman et al., 1997; Eckhart et al., 2006; Streisfeld and Kohn, 2007) or variation of resistance to natural enemies (Frey, 2004).

Research on the color polymorphism mainly focuses on the intraspecific variation of petal colors. The variation of sexual organ colors truly exists (Jorgensen et al., 2006). A few studies involve the pollen color polymorphism – *Lythrum* (Darwin, 1877), *Erythronium* (Thomson, 1886), *Linum* (Wolfe, 2001), *Campanula* (Lau and Galloway, 2004), and *Nigella* (Jorgensen et al., 2006). The color polymorphism of the female sex organ is only found in two studies - *Crocus scepusiensis* (Rafinski, 1979) and *Butomus umbellatus* (Tang and Huang, 2010).

*Epimedium pubescens* belongs to the Berberidaceae, and is an important medicinal herbaceous plant with a panicle and has 4 petals on each flower. The petal transforms into a horn-shaped nectar spur. In the past 10 years, we have found the pollen color is polymorphic in some groups (Wang et al., 2017, 2018) and the stigma color mutates during our field study on the reproductive ecology of *E. pubescens*, which has never been reported before. This paper takes *E. pubescens* as the research object, explores the influence of stigma color variation on the pollination biology and reproductive success of *E. pubescens*, and provides a scientific theoretical basis for the large-scale artificial cultivation, introduction and domestication of *Epimedium* plants.

Materials and Methods

Study Site

The study site is set in Jincheng Mountain National Forest
Park in Jialing River Basin, Nanchong City (35°45.94' N, 106°27.83' E), with a total area of about 56 km². The annual average temperature is 17.6°C. The average temperature in the coolest month (January) is 5.4°C, and in the hottest month (July) 21.7°C. With a wide temperature difference all year round, plants have a very long growing period. The annual rainfall is about 1,063 mm. The site has a typical monsoon humid climate.

Study Materials

E. pubescens is a perennial herb native to China, and is mainly distributed in Sichuan and Shaanxi. The average altitude of distribution is 500~1,500 m. Generally it grows in a shady and humid broadleaved deciduous forest on a slope. It has about 60 bisexual flowers with the thyrse. Its flower is about 10~20 cm in size, with a diameter of about 1 cm. It has 2 sepals, the inner sepal is a white lanceolate or narrow lanceolate with a length of about 4 mm. The stamen is about 4 mm in length; the anther is about 2 mm long; the stigma is about 2 mm long; the flowering phase is in March and April; the fruiting season is in May.

Measurement of Enironmental Factors

The FIELDS Cout3413 illuminometer was used to measure the illumination intensity, and the KIMO HD100 hygrothermograph was used to measure the temperature and humidity.

Measurement of Plant and Flower Shape Parameters

Plants with heterostyly in a population were recorded; 20 to 30 of the plants were randomly selected to measure their morphological characteristics with a tape; at the same time, 20 to 30 blooming flowers were selected to measure their morphological characteristics with a vernier caliper.

Observation of Flower Visitors

This experiment was conducted during the full-blossom period of E. pubescens. Three blooming plants within 2 m × 2 m quadrat were selected respectively from every heterostyly for marking. The hour was taken as the unit of time. Flower visitors' visit times, actions and their duration of stay on each flower from 8:30 a.m. to 5:30 p.m. were recorded and observed for the calculation of flower-visiting frequency.

Detection of Pollination Efficiency

To compare the pollen output and the pollen depositing on the stigma of the heterostyly, 25 flower buds on the heterostyly were respectively and randomly selected during the flowering phase, and put into small EP tubes with 50% of ethyl alcohol for the natural control. Then threads in different colors were selected to mark the heterostyly (about 50 flowers for each stigma), and covered with mesh bags. Flowers visited immediately were cut off. The stamen and pistil were respectively cut off and put into small EP tubes with 50% ethyl alcohol, and then their stigma types were marked. Falloff numbers of pollen on the stigma and residual pollen number in the stamen were recorded under an optical microscope (LEICA DM500) in the laboratory.

Artificial Pollination Experiment

Artificial pollination experiment: Ten inflorescences of heterostyly were respectively selected. When flowers on the inflorescence just bloomed (30 to 40 flowers are treated in each type), their stamens were removed, and the stigma with threads in different colors was marked. The first treatment took the red stigma as the female parent, and the pollination pollen sources respectively came from different plants with the red stigma and plants with the white stigma. The second treatment took the white stigma as the female parent, and the pollination pollen sources respectively came from different plants with the white stigma and plants with the red stigma. At last, they were marked with tags and covered with mesh bags. Their fruits were collected when expanded, and the ripening rate and the seed setting rate were calculated.

Natural control group: Randomly selected 15 plants with heterostyly in the population. Their blossom and bud numbers were recorded and stigma types were marked without other treatments. The ripening rate and the seed setting rate were calculated after the fruits ripened.

Data Analysis

Data statistics and analysis were conducted using the single factor and T test (independent sample test) of statistical software SPSS17.0.

Results

Measurement of Environmental Factors

Growing environments of stigmas in two different colors are given in Table 1. Single factor analysis showed that there was no significant difference in temperature and relative light intensity. However, the relative humidity of the habitats was significantly different, and the red stigma plants were significantly higher than white stigma plants.

Measurement of Plant Morphology and Flower Morphology

In population survey, according to different stigma colors, it was divided into 2 phenotypes: red stigma type (Fig. 1A) and white stigma type (Fig. 1B). The ratio of red to white stigmas in the population was about 3:1. Plants and flower morphology of red stigma and white stigma were selected for measurement, and the results of T test indicate that there was no significant difference in each observation items (Table 2).
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Table 1: General situation of growing environmental factors of plants of E. pubescens with heterotypic stigma

<table>
<thead>
<tr>
<th>Stigma Type</th>
<th>Temperature (°C)</th>
<th>Relative Humidity (%)</th>
<th>Light Intensity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Stigma</td>
<td>14.66±0.49a</td>
<td>62.21±0.92a</td>
<td>55.73±5.34a</td>
</tr>
<tr>
<td>White Stigma</td>
<td>18.66±0.24a</td>
<td>50.58±2.14b</td>
<td>69.33±3.55a</td>
</tr>
<tr>
<td>F</td>
<td>3.53</td>
<td>2.959</td>
<td>48.429</td>
</tr>
<tr>
<td>Sig</td>
<td>0.05</td>
<td>0.005</td>
<td>0.075</td>
</tr>
</tbody>
</table>

Note: The data in the table are expressed in the mean value ± standard error (n=3). The different lowercase letters (a, b, c, d) indicate significant difference at the level of P=0.05

Effective Pollinators and their Flower Visiting Frequency

E. pubescens with two different stigma colors shared an effective pollinator - Andrena (Fig. 2), whose flower visiting behaviors were mainly nectar sucking and pollen collecting. Data showed that records of flower-visiting insects of E. pubescens with white and red heterotypic stigmas, the number of E. pubescens visitors in the cloudy day was relatively small than the sunny weather. Single factor analysis showed that there was no significant difference in insect flower-visiting frequency between flowers with red or white stigma (Fig. 3).

Pollination Efficiency

The t- test statistics revealed that there was a significant difference in pollination efficiency of heterotypic stigmas' effective pollinators. Both the pollen removal rate and the number of stigmas falling of the red stigma were significantly higher than those of the white stigma (Table 3).

Artificial Pollination Experiment

The artificial pollination experiment shows the pollination of the same type in seed bearing rate was higher than that of the heterotypic and the seed setting rate of red stigma female parent was higher than that of the white stigma. However, there was no significant difference in seed setting rate of the four pollination treatments (Table 4).

Comparison of Spontaneous Seed Bearing

There was no significant difference in the spontaneous seed bearing rate between the red stigma and the white stigma. However, the seed setting rate of the red stigma was much higher than that of the white stigma (Table 5).

Discussion

In general, stigma heterotopia refers to herkogamy. People find that herkogamy with various types is quite common in the plant kingdom and its type is various (Zhang, 2004). However, it is rather rare to find a stigma heterotype varying in color in kindred plants. For example, the red stigma and the white stigma appearing in the E. pubescens natural population had no significant difference in single flower shape and plant shape (Fang and Huang, 2008).

The variation of stigma color results probably from the differences of geographical conditions, such as Crocus scepusiensis (Iridaceae) (Rafinski, 1979).
The natural environment might result from mixed pollen in the population. Thus, the seed bearing rate in artificial pollination was significantly higher than that in the natural environment, which might result from mixed pollen in the population. Findings in artificial pollination that the seed bearing rate of female parent of *E. pubescens* with the red stigma was higher than that with the white stigma is in good harmony with the investigation results of spontaneous seed bearing. This indicated that *E. pubescens* with the red stigma increases female fitness by raising its seed bearing and yield (Tang and Huang, 2010). Therefore, another reason for the low seed bearing rate in *E. pubescens* population might be that there were more plants with the white stigma than that with the red stigma.

**Table 4:** Xenogamy and cross pollination treatments of *E. pubescens* with heterotypic stigma

<table>
<thead>
<tr>
<th>Female parent</th>
<th>Male parent</th>
<th>Number of processed flowers (No.)</th>
<th>Number of seed bearing (No.)</th>
<th>Seed bearing rate %</th>
<th>Seed setting rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red stigma</td>
<td>Red stigma</td>
<td>31</td>
<td>22</td>
<td>70.96</td>
<td>81.55±3.89a</td>
</tr>
<tr>
<td></td>
<td>White stigma</td>
<td>30</td>
<td>20</td>
<td>66.66</td>
<td>78.69±3.96a</td>
</tr>
<tr>
<td>White stigma</td>
<td>Red stigma</td>
<td>27</td>
<td>16</td>
<td>59.25</td>
<td>77.11±4.59a</td>
</tr>
<tr>
<td></td>
<td>White stigma</td>
<td>30</td>
<td>19</td>
<td>63.33</td>
<td>80.61±5.44a</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.18</td>
</tr>
<tr>
<td>Sig.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.90</td>
</tr>
</tbody>
</table>

**Table 5:** Comparison of fruit setting and seed setting between red stigma and white stigma

<table>
<thead>
<tr>
<th>Item</th>
<th>Red stigma</th>
<th>White stigma</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit setting rate</td>
<td>35.96±4.31a</td>
<td>26.32±4.19a</td>
<td>2.57</td>
<td>0.12</td>
</tr>
<tr>
<td>Seed setting rate</td>
<td>83.81±1.44a</td>
<td>75.93±2.10b</td>
<td>10.20</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**Fig. 3:** Comparison of effective pollinator’s flower-visiting frequency of with heterotypic stigma in *E. pubescens*.

However, plants of *E. pubescens* with red and white stigmas are distributed in the same population among which the number of plants with white stigma was higher than that with the red. Meanwhile, plants with red stigma prefer to grow in a relatively humid environment. Their ratio in the experimental population was about 3:1. Their pollinator was *Andrena*, and the pollination was done mainly through nectar sucking and pollen collecting. No difference in the flower-visiting frequency of the two stigmas indicated that the pollinator had no preference for their stigma colors and the color variation was not caused by its mediation. The pollen removal rate and the number of fallen pollen after each flower visit of the white stigma was both lower than those of the red stigma. This revealed shows that the red stigma’s pollination efficiency is relatively higher in the population.

According to the research of color polymorphism in terms of *E. pubescens*, mixed pollen of the two colors would decrease their seed bearing rate (Wang et al., 2017, 2018). The fact that the seed bearing rate in artificial pollination experiment was significantly higher than that in the natural environment might result from mixed pollen in *E. pubescens* population under natural circumstances. The pollinator has no preference for *E. pubescens* of the two stigma colors. Plants with the red stigma are of higher pollination efficiency than those with the white. According to the comparison between artificial pollination and spontaneous seed bearing, female fitness of *E. pubescens* with the red stigma is higher than that with the white, while the variation of stigma color affects the reproductive success of *E. pubescens*.

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**References**


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