Petal Color, Flower Temperature, and Behavior in an Alpine Annual Herb, Gentiana leucomelaena (Gentianaceae)

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Petal Color, Flower Temperature, and Behavior in an Alpine Annual Herb, 
Gentiana leucomelaena (Gentianaceae)

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Abstract

Gentiana leucomelaena (Gentianaceae), an alpine herbaceous species of the Qinghai-Tibetan Plateau, has two colors of flowers (blue and white) that bloom in early spring. In order to determine the effect of petal color on flower interior temperature and behavior in the gentian, we investigated the differences in timing of flower opening and closure and the interior temperature of blooming flowers between the two colors, while recording the ambient temperature, light intensity, and relative humidity over the flowering season from March to May of 2009. When the flowers were open, the anther temperature was higher in the white flowers than in the blue flowers in various weather patterns; in particular it was about 2°C higher on sunny days. Relative to the ambient temperature, the anther temperature was 1.27°C higher in the white flowers, but was 0.41°C lower in the blue flowers. Compared to blue flowers, white flowers opened later but closed earlier in the day at a higher ambient temperature. The two-factor two-level experiment (10°C vs. 20°C and 10,000 lux vs. darkness) indicates that temperature is the factor eventually determining the timing of flower opening and opening rates, but light may accelerate flower opening at the same temperature. The dye experiment, in which blue flowers were painted with red and purple food coloring, showed that the purple flowers had higher anther temperature, opened later but closed earlier, relative to the red ones. These results suggest that flower interior temperature is affected by both flower color and behavior in the species. In addition, we surveyed the percentages of the two flower colors in the field during the flowering season and also experimentally placed individuals with flower buds into growth chambers with contrasting day/night temperatures (12°C/2°C, 15°C/2°C, and 20°C/2°C), so as to examine the temperature effect on flower color frequency. A greater proportion of white flowers emerged in the early stage of the flowering season and in the low-temperature chambers, but blue ones dominated the late season and in the high-temperature chambers. This suggests that the color differentiation in the species is associated with temperature. The different strategies of adaptation to temperature might have allowed for flower color polymorphism.

Introduction

Flower color polymorphism is widely believed to be driven by animal pollinators (Lloyd, 1969; Chittka and Menzel, 1992; Heiling et al., 2003; Chittka and Raine, 2006). Flower appearance, as primarily determined by flower color and size, is often positively associated with visitation rates of pollinators and hence cross-fertilization rates and seed production of plants (Waser and Price, 1981; Melendez-Ackerman and Campbell, 1998; Harder and Barrett, 2006). However, recent evidence indicates that environmental physical factors may also act as one of the important selective agents for flower color diversity (Grace and Logan, 2000; Warren and Mackenzie, 2001; Steyn et al., 2002).

Petal colors can significantly influence flower interior temperature due to the pigment difference in absorption and reflection of the light spectrum (Jewell et al., 1994; Seymour, 2001; Ivancic et al., 2008; Seymour et al., 2009). This effect can be of significance for the adaptation of plants living in stressful habitats. For example, in drought- and heat-stressed environments, individuals with pigmented flowers usually survive better than anthocyanin-free (or less) morphs (Grace and Logan, 2000; Warren and Mackenzie, 2001; Steyn et al., 2002), while white-flowered individuals are often susceptible to the stress and hence suffer a large loss in fitness benefit (Coberly and Rausher, 2003). In contrast, in arctic or cold alpine regions, flower color may have evolved to help keep interior temperatures warm and stable against fluctuating ambient temperatures (Seymour and Schultz-Motel, 1998; Ivancic et al., 2008; Li and Huang, 2009), which is critical to the development of pollen, ovules, and fertilization (McKee and Richards, 1998a; Li and Huang, 2009). For example, the white petals of *Crocus chrysanthus*, an arctic herb species, redirect light spectra into flower interiors, trapping heat energy to warm the gynoecia (McKee and Richards, 1998b). At the same ambient temperature, white flowers are usually warmer than yellow flowers in interior structures but not in corolla (McKee and Richards, 1998b; Molgaard, 1989), because light-colored petals
absorb short wavelengths more efficiently than dark ones (Büdel, 1959; Tikhomirov et al., 1960). Studies examining the relationships among petal colors, flower interior temperature, and pollinator visitation rates mostly employ a comparative methodology, i.e., interspecifically or among varieties of a single species (McKee and Richards, 1998b; Kjellberg et al., 1982; Knutson, 1981). However, there might be a confounding factor involved in these studies. For instance, as the three varieties of *Crocus* differ in both petal color and flower structure (having feathering or not; McKee and Richards, 1998b), it is hard to distinguish whether the temperature difference between flowers is due to the color effect.

Besides petal coloration, the species living in alpine or arctic cold regions may employ other mechanisms to help keep flower interiors warm; e.g., flower movements (Bynum and Smith, 2001). Flowers in some species can track the sun 24 hours a day (Kevan, 1975) while others can open/close every day (He et al., 2005) so as to lessen the fluctuating magnitude of flower interior temperature. Such flower behaviors are often thought to be plant responses to the changes in light intensity (Karvé et al., 1961; Saito and Yamaki, 1967; Kawai and Takimoto, 1980, 1981; Ichimura and Suto, 1998; Cashmore et al., 1999; van Doorn and van Meeteren, 2003), ambient temperature (Cockshull et al., 1981; Tanaka et al., 1987; Kinet, 1993; Ichimura and Suto, 1998; McKee and Richards, 1998b), and humidity (Davy de Virville and Obaton, 1922a, 1922b; Halket, 1931; van Doorn and van Meeteren, 2003). Because flower interior temperature is affected by both petal color and flower movements, it is expected that there should be a connection among the physical factors (flower color and movement). However, there is no report focusing on that relationship, to the best of our knowledge.

*Gentiana leucomelaena* is a widespread herbaceous species with altitudes ranging from 1900 m to 5000 m on the Tibetan Plateau. This species is often referred as the blue-white gentian because it has two flower colors (blue and white) within populations and within single individuals. The flower has apparently at least one open/closure cycle every day. In this study, we investigated the differences in the opening and closure and the interior temperature of blooming flowers between the two flower colors (while recording the ambient temperature, light intensity, and relative humidity) in order to determine the relationships among ambient temperature, flower interior temperature, flower color, and behavior. This study occurred over the flowering period of *Gentiana leucomelaena* from March to May of 2009. As noted, we predicted that the white flowers would have a higher interior temperature compared to the blue flowers due to more heat being trapped by the light color. We also predicted that the white flowers would open later but close earlier than the blue ones provided that they required higher temperature for flower development. In addition, we investigated the percentages of the two flower colors during the flowering season, and we employed growth chambers to experimentally test whether temperature affected flower color frequency. Because the species flowered in early spring when the temperature was very low, we hypothesized that white flowers were more likely to emerge in early flowering season and in low-temperature conditions.

**Methods**

This study was conducted in the permanent plots of the Hongyuan Alpine Meadow Ecosystem Research Station of Chinese Academy of Sciences (31°50'–33°22'N and 101°51'–103°23'E), located in the eastern Qinghai-Tibetan Plateau. The altitude is 3494 m a.s.l. The mean annual precipitation is 749.1 mm, 80% of which occurs in the growing season from May to October. The mean annual temperature is 1.4 °C, with the maximum and minimum monthly means being 10.9 °C and −10.3 °C in July and January, respectively (Gao et al., 2008).

The meadow community is dominated by *Agrostis trinii*, *Agrostis hugoniana*, *Potentilla anserina*, and *Anemone trullifolia* var. *linearis*. Total vegetation coverage is more than 95% and plant height is about 30 cm. The soil is of meadow soil type, which is characterized by high organic content (215–280 g kg⁻¹) and low availability of N (673.75 mg kg⁻¹) and P (6.32 mg kg⁻¹) (Song et al., 2007).

**SPECIES**

*Gentiana leucomelaena* is an annual herbaceous species of the Gentiana sect. Chondrophyllae. It grows along the streams, in meadows, and the scrubs of alpine regions in China, with altitude ranging from 1900 to 5000 m above sea level.

The species is 5–10 cm in height; flowers are 1.2 cm on average in corona diameter and 1.0 cm in corona length, with a campanulate-shaped corona consisting of ten connate petals. There are two colors of corolla, blue and white, both having dark blue spots in the corolla throat. Usually, there are more than 10% of individuals bearing both blue and white flowers, but the ratio of the individuals with blue flowers to those with white flowers vary with time during the flowering season. Both self-fertilization and cross-fertilization can lead to seed production. Primary pollinators are flies, ants, and honeybees. The honeybees are observed to visit blue flowers more frequently than white flowers, but the other pollinator species groups have no observable preference. Seed size is very small (0.061 g per 1000 seeds).

**TEMPERATURE EFFECT ON FLOWER COLOR FREQUENCY AND FLOWER BEHAVIOR**

We established five 5m × 5m subplots in March 2009 in order to examine the variation in flower color frequency during the flowering season. For each subplot, we recorded flower numbers for both colors every 10 days from 26 March to 15 May 2009. There were seven censuses in total over the 50 day period.

We transferred 270 seedlings and associated indigenous soil from the field into the lab on 23 March 2009 before the flower buds opened. Each seedling was put into a cylindrical container (5 cm in diameter and 6 cm in depth). The containers were assigned to nine dishes, each dish with 30 seedlings. The dishes were then placed into three growth chambers, each chamber having three dishes. The temperature was set as 20 °C/2 °C, 15 °C/2 °C, and 12 °C/2 °C, respectively, for the three chambers, but light intensity, relative humidity, and photoperiod were the same for each (10,000 lux in light intensity, 60% in relative humidity, and 12 hrs light/dark cycle). Plants were regularly watered. After the plants began to flower, we recorded the flower number for each color and tagged each flowering individual on the stem every day. We observed the flower color and behavior outside of the chambers.

In addition, a two-factor two-level full factorial experiment was conducted to determine the relative importance of temperature and light on flower behavior. Four dishes, each of which contained 60 flowers (30 individuals of each color), were put into four different chambers with contrasting temperatures and light intensities (10 °C + 10,000 lux, 20 °C + 10,000 lux, 10 °C with...
RESULTS

RELATIONSHIP BETWEEN FLOWER COLOR, INTERIOR TEMPERATURE, AND BEHAVIOR

Thirty individuals of each color were tagged for repeated measurements beginning in March. Under differential weather conditions such as on sunny, overcast, and windy days, anther and petal temperature was measured hourly using an infrared thermometer (AR300, temperature range -32 °C to 300 °C, made in Hong Kong Shema, China) while the light intensity, ambient temperature, and relative humidity were simultaneously recorded using a light meter (1-5818-02, made in Kyoritsu, Japan) and temperature/humidity meter (AR827, made in Shanghai Fuzhong Co., China), respectively. The temperatures were also measured at the time when the flowers were opening and closing.

To test whether petal color affects flower interior temperature and behavior we conducted a petal-dyeing experiment. In late March 2009, both sides of the petals of blue flowers were dyed with white, purple, and red food coloring on sunny days, as was done by Begum et al. (2004). The food coloring used is not toxic to plants. We recorded the flower temperature and timing of opening and closure for these dyed flowers, and the ambient temperature, light intensity, and humidity were measured.

DATA ANALYSIS

The data on physical environmental factors, flower phenology, and behavior were tested for normality before analysis. The percentages of flower opening rate, flower closure rate, and flower color frequency were arcsine-transformed for normality before statistical analysis. The relationships between flower interior temperature and environmental factors (e.g. temperature, light, and humidity), and between flower temperature and behaviors were determined using Pearson Correlation Analysis. One-way ANOVA was used to test the statistical significance of the differences in the ambient, flower anther, and petal temperatures at which the plants flowered; the timing of flower opening and closure; and the time spent in flower opening. All statistical analyses were performed with SPSS Version 16.0 (SPSS Inc., Chicago, Illinois, U.S.A.).

**Results**

TEMPERATURE EFFECT ON FLOWER COLOR FREQUENCY AND FLOWER BEHAVIOR

Flower color frequency changed greatly during the flowering season. From 26 March to 15 April, the proportion of white-flowered plants was greater than that of the blue-flowered plants. Afterwards, the blue-flowered frequency increased rapidly until the end of the flowering season (Fig. 1A). Regression analyses showed that the ambient temperature was closely correlated with the flower frequency, i.e., white flowers were more likely to be associated with low temperature than blue flowers ($p < 0.001$). The flower color frequency was also significantly correlated with light intensity and photoperiod (both $p < 0.05$) but not relative humidity.

The chamber temperature influenced flower color frequency. At the low temperature ($12^\circ C/2^\circ C$), the frequency of white flowered individuals was significantly higher than that of the blue flowers. In contrast, at the $20^\circ C/2^\circ C$, the percentage of white flowers decreased to a much lower level, relative to that of the blue flowers. The flower color frequency did not differ significantly at the middle temperatures ($15^\circ C/2^\circ C$) (Fig. 1B).

Flower behavior was also affected by temperature. At low temperatures, there was no flower opening for either color even after 2 hrs, whether the chamber was lighted or in darkness. Only at high temperatures did the flowers open. Nevertheless, light accelerated the flower opening (Fig. 2), particularly for blue
flowers. Blue flowers in the lighted chamber were fully open at 20 °C after 21.5 min, while the white flowers were all open after 33.8 min. In the dark chamber (20 °C, 0K lux), blue and white flowers took about 59.2 min and 85.9 min for full opening of all the flowers, respectively.

**RELATIONSHIP BETWEEN FLOWER COLOR, INTERIOR TEMPERATURE, AND BEHAVIOR**

The ambient temperature at which flowers opened and closed was influenced by the flower color. The timing and temperature of opening and closure were significantly different between the white and blue flowers under different weather conditions (all $p < 0.001$). On sunny days, blue flowers usually began to open at 8:35 a.m. (Beijing time) when the ambient temperature was around 10.11 °C, while white flowers did not begin to open until the temperature increased to 12.05 °C at about 9:13 a.m. (Figs. 3A, 4A). Usually, the blue flowers began to close when the temperature dropped below 12.05 °C, whereas the white flowers often began to close at 14.27 °C (Fig. 3B). The temperatures differed significantly for both flower opening and closure between the two colors (both $p < 0.001$; Fig. 3B). Similar situations occurred on overcast and windy days (Figs. 3, 4B, 4C). The temperatures at which flowers began to close was higher in white flowers than in blue ones, and hence the white flowers closed earlier than the blue ones under the three different weather conditions. In addition, regressive analyses showed that flower opening and closure times of both colors were significantly influenced by temperature but not light and relative humidity (Table 1).

The mean anther temperature (while flowers were open) was significantly higher in the white flowers compared to the blue flowers on sunny days ($p < 0.001$), but the difference was not significant on the overcast days (Fig. 5A). In sunshine, the anther temperature was on average (0.41 °C) lower, while the petal temperature was 0.42 °C higher than the ambient temperature in the blue flowers. In the white flowers, the mean petal and anther temperatures were 0.43 °C and 1.27 °C, respectively, higher than the ambient temperature. On overcast days, however; mean petal and anther temperature were lower than the mean ambient temperature in both the white and blue flowers. In sunshine, the mean anther temperature was 0.67 °C lower in the blue flowers, but was 0.84 °C higher than the petal temperature in the white flowers. On overcast days, however, the mean petal temperature was higher than the anther temperature in both the white and blue flowers (0.12 °C and 0.14 °C, respectively). The petal temperatures were significantly higher on sunny than overcast days in both the white and blue flowers (Fig. 5B). In addition, the ambient temperature was positively correlated with the petal and anther temperature in both white and blue flowers (Table 2). Light intensity was significantly and positively correlated with flower temperature in various weather conditions, except on sunny days for the blue flowers (Table 2).

The timing of flower opening and closure was significantly affected by the dying treatments. Red flowers opened earlier than purple and white flowers, but the order for flower closure was reversed. The ambient temperature, at which the flowers opened, was 9.65 °C for the red dyed flowers, and 12.01 °C for the purple dyed ones. The mean daily anther temperature was significantly higher in the purple flowers than in the red ones (Fig. 6).

**Discussion**

_Gentiana leucomelaena_ flowers in early spring, when the temperature is low and fluctuates greatly between day and night.
Low temperature should be one of the most important factors influencing the eventual development of seeds and the species persistence. The anther temperature is often higher and steadier than ambient temperature for both colors in the gentian. Nevertheless, the anther temperature differed significantly between white and blue flowers in various weather conditions. This temperature difference is associated with the differences in petal color and whether flowers were opened or closed. This suggests that there are perhaps two different strategies employed by the two colors for flowering and seeding.

The effect of petal color on flower interior temperature has been reported in several previous studies. For example, temperature is raised from 0.7 to 2.0°C in white flowers, whereas blue and lilac-colored flowers were increased from 3.4 to 4.2°C on sunny days (Tikhomirov et al., 1960). McKee and Richards (1998b) have also found that white and purple flowers have a greater temperature increase than yellow flowers in *Crocus* species. However, there are also contrasting results in previous studies. For example, in *Papaver radicatum* in North Greenland, yellow-flowered individuals obtained a temperature 1.4 to 1.7°C higher than white-flowered individuals in sunshine (Mølgaard, 1989). Dark colored arctic flowers can reach higher temperatures than light colored flowers (Büdel, 1959; Tikhomirov et al., 1960). It is therefore suggested that plants invest in the expensive petal pigmentation to increase intrafloral temperatures and in turn benefit from enhanced reproductive efficiency (Mølgaard, 1989). This discrepancy in the color effect on flower interior temperature may be due to difference in flower structure and environmental conditions. On one hand, the temperature difference sometimes resulted from the effect of flower structure but not color. Our study materials have the same flower structure and shapes except the petal colors. The finding that anthocyanin-less ones increased...

**FIGURE 4.** The diurnal variations in flower opening percentages and flower closure percentages in three typical weather conditions including sunny days (A), windy days (B), and overcast days (C). The sample size (N) is 150 for each color in (A), (B), and (C).
The physical mechanism underlying the color effect lies in that the flower interior micro-greenhouse had the capability to absorb shortwave sunlight and reflect the longer wavelengths, which retained the energy and raised the internal flower temperatures (Kevan, 1989). McKee and Richards (1998b) have even indicated that white and purple flowers are warmer than yellow ones because the yellow flowers transmit no light of less than 500 nm, while white- and purple-flowered individuals absorbed the short wavelengths insolation, especially below 400 nm. However, we did not examine the light wavelengths during the flowering season for this study.

For the species that flower in early spring, flower opening and closure is usually supposed to be related to the ambient temperature (Andrews, 1929). In the blue-white gentian, we have shown that flowers opened only when the ambient temperatures reach a minimum level. Because temperature changes are accompanied with variations in light intensity and humidity, other factors may not be excluded from potentially affecting flower behavior. This has happened for the flowers of *Portulaca*. The flowers did not fully open in darkness even if the air temperature was increased from 20 °C to 30–35 °C; full opening was observed only under light and higher temperature conditions (Ichimura and Suto, 1998). This suggests that light conditions are a precondition for flower opening in that species. Nevertheless, in the current study, although flower opening and closing behaviors were significantly correlated with temperature and light intensity, the predictor for flower opening is temperature, not light intensity. We observed that the flowers opened as the ambient temperature reached 20 °C even in darkness, and that when the ambient temperature is 10 °C, all flowers are closed even in high light conditions. These results indicate that the flower opening and closure is largely controlled by temperature. Similar results were obtained by van Doorn and van Meeteren (2003) and Ichimura and Suto (1998), who found that a small temperature rise in the

The regression coefficients of temperature, light, and humidity against petal and anther temperatures on sunny, windy, and overcast days.*,**, *p < 0.05; **, *p < 0.01; ns, not significant.

<table>
<thead>
<tr>
<th></th>
<th>Petal temperature (°C)</th>
<th>Anther temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blue</td>
<td>White</td>
</tr>
<tr>
<td>Sunny</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>0.512**</td>
<td>0.548**</td>
</tr>
<tr>
<td>Humidity</td>
<td>−0.036 ns</td>
<td>−0.033 ns</td>
</tr>
<tr>
<td>Light</td>
<td>0.079 ns</td>
<td>0.149*</td>
</tr>
<tr>
<td>Windy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>0.673**</td>
<td>0.691**</td>
</tr>
<tr>
<td>Humidity</td>
<td>−0.503*</td>
<td>−0.591*</td>
</tr>
<tr>
<td>Light</td>
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<td>0.701*</td>
</tr>
<tr>
<td>Overcast</td>
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<tr>
<td>Temperature</td>
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<td>0.713**</td>
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<td>Humidity</td>
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<td>−0.527**</td>
</tr>
<tr>
<td>Light</td>
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<td>0.680**</td>
</tr>
</tbody>
</table>

The sample size (N) is 150 for each color.

The regression coefficients of temperature, light, and humidity against flower opening and closure time. **, *p < 0.01; ns, not significant.

TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Flower opening duration (hrs)</th>
<th>Flower closure duration (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blue flowers</td>
<td>White flowers</td>
</tr>
<tr>
<td>Temperature</td>
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<td>0.730**</td>
</tr>
<tr>
<td>Humidity</td>
<td>−0.062 ns</td>
<td>−0.136 ns</td>
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<tr>
<td>Light</td>
<td>0.341 ns</td>
<td>0.305 ns</td>
</tr>
</tbody>
</table>

The sample size (N) is 150 for each color.

FIGURE 5. Mean petal temperatures (A) of blue and white flowers (B) and anther temperatures during flower opening on sunny and overcast days. Values are given as mean ± 1SE. Significant differences between the blue and white flowers in the same survey are indicated by different capital letters, and significant differences among different surveys for the same flower color are indicated by different lower-case letters (both are at *p < 0.05 level). The sample size (N) is 150 for each color in (A) and (B).

FIGURE 6. Mean ambient, petal, and anther temperatures of the dyed flowers while flowers are open on sunny days. Values are given as mean ± 1SE. Significant temperature differences among the colors are indicated by different lower-case letters (*p < 0.05 level). The sample size (N) is 150 for each color.
morning was adequate for full flower opening of *Ficaria*, *Galanthus*, *Tulipa*, *Crocus*, and *Portulaca* hybrid cv. ANR1.

We not only demonstrated that the petal color is associated with the difference between ambient and flower interior temperatures, but also show that the petal color polymorphism influenced the flower opening and closing behavior. The blue flowers opened earlier and closed later than the white ones under the various weather conditions. In the dyed petal experiment, red-dyed flowers opened earlier than the purple or white individuals, while the sequence of the flower closure is reversed. Such color effects are possibly because dark and deep colored petals absorb longer wavelengths more efficiently than light colored ones, which can elevate corolla temperature (Tikhomirov et al., 1960). In addition, petal colors affected the rate of opening and closing behaviors. The blue flowers opened more rapidly than the white flowers with the same increase of temperature, but *vice versa* for flower closure at the same temperature decline. Thus, it can be concluded that petal color affected flower behavior via temperature control.

In addition, we have shown that there were a larger proportion of white flowers than blue ones in the early flower season when the ambient temperature was very low, and that the white flowers open and close at higher ambient and interior temperature than the blue ones. The experiment, in which flower buds were treated with contrasting temperatures, also showed that white flower frequency was higher at lower temperatures. These results apparently suggest that flower color variation is temperature related in this species.

Both colors should facilitate anther development in the flowering seasons by opening and closing at the proper time that is critical to pollen growth (McKee and Richards, 1998a). Molgaard (1989) has observed that the white flowers of *Papaver radicatum* occur in the North Greenland coast where sunshine is limited, while the yellow flowered plants are more common inland and at higher elevations (Molgaard, 1989). The difference in the anther temperature suggests that there perhaps is a differentiation in the temperature requirement for pollen development between the two color flowers. However, although we demonstrated that ambient and flower temperature, petal color, and behavior are closely correlated with each other in the species, the evolutionary mechanisms underlying the coexistence of the two colors cannot be fully understood from the data collected in this study.

In conclusion, the anther temperature is higher in white flowers than blue ones at the same ambient temperature, and white flowers open later but close earlier than blue ones. This suggests that the difference in flower temperature between the two colors is due to both the effects of petal color and associated flower open/closure behavior in the species. Temperature influences flower color frequency in the species, indicating that the flower color polymorphism can be driven by adaptation to the environmental factor, temperature.

**Acknowledgments**

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