Discovery of Gynoecium Color Polymorphism in an Aquatic Plant

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Abstract

Flower color polymorphism exhibited by natural populations provides an opportunity for understanding the evolutionary mechanisms contributing to the diversity of floral morphology. However, little is known about the color polymorphism of female organs in flowering plants. Here we report gynoecium color polymorphism in *Butomus umbellatus* (Butomaceae), an emergent, aquatic monocot. Populations from Mishan, northeastern China comprised two morphs; gynoecia are either pink, as observed in other areas, or white. We measured floral traits and female fecundity in the two gynoecium color morphs in the field. There was no significant difference in plant height, pedicel length, and flower size including petal, sepal and gynoecium between the two morphs, but plants with pink gynoecia had wider inflorescence stalks, larger inner whorl anthers and produced more pollen and ovules than those with white gynoecia. Correspondingly, we found that seed production was significantly higher in the pink than in the white morph. This new finding suggested selection against white gynoecia in part because of low fecundity, consistent with the rarity of the white gynoecium morph in this species.

Key words: *Butomus umbellatus*; female fecundity; floral traits; flower color polymorphism; gynoecium morphs; pollen and ovule production.


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Flower color is among the simplest of natural phenotypes but presents a richly complex process of adaptation (Clegg and Durbin 2000). Flower color polymorphism, where two or more different flower color phenotypes occur in the same population or species, has been used to examine evolutionary mechanisms in plants (Levin and Brack 1995; Clegg and Durbin 2000). Various ecological factors may exert selection on flower color and thereby maintain color polymorphisms. Environmental heterogeneity and stress tolerance may explain pink/blue and white flower color polymorphisms in numerous species (Warren and Mackenzie 2001). On the other hand, flower color polymorphism could be maintained by balancing selections from variations in pollinator components (Meléndez-Ackerman et al. 1997; Eckhart et al. 2006; Streisfeld and Kohn 2007) or from defense against natural enemies (Frey 2004).

Studies on flower color polymorphisms have paid much attention to intraspecific variation in petal color, yet color polymorphisms of sexual organs do exist in flowering plants (Jorgensen et al. 2006). Pollen color dimorphisms have been reported in several species including *Lythrum* (Darwin 1877), *Erythronium* (Thomson 1986), *Linum* (Wolfe 2001), *Campanula* (Lau and Galloway 2004), and *Nigella* (reviewed in Jorgensen et al. 2006). The only study examining female organs involved stigma color dimorphisms in *Crocus scepusiensis*, in which morph frequencies of orange and white stigmas were quantified, as well as petal color polymorphism, at Gorce Mountains in southern Poland (Rafinski 1979). To date, we know little about pistil or gynoecium color polymorphism, or about the evolution and maintenance of this form of color polymorphisms.

In the present study, we report for the first time gynoecium color polymorphism in flowering rush, *Butomus umbellatus* (Butomaceae). To begin exploring the maintenance of gynoecium color polymorphism, we investigated morph frequency and compared floral traits and fecundity between two color morphs in the field populations.

Results

The first population we found in Hexiangyuan wetland had 1 144 and 1 133 individuals with pink and white gynoecia, respectively, in a plot of $30 \times 45 \text{ m}^2$. All flowers within an individual have either pink or white gynoecia throughout the flowering period (Figure 1). Therefore, the variation in gynoecium color was not due to developmental plasticity or color changes during growth.
Figure 1. Gynoecium color polymorphism in Butomus umbellatus.

One phenotype has pink gynoecia (left) and one has white gynoecia (right).

The ratio of the two morphs was not significantly different from 1:1 as demonstrated by G-test ($G = 0.053$, $P > 0.05$). The two other populations that we found next had distinctive morph ratios. One had 2 710 individuals only with white gynoecia in a plot of $58 \times 45 \text{m}^2$, the other had 880 and 70 individuals with pink and white gynoecia, respectively, in a plot of $52 \times 15 \text{m}^2$.

Numbers of umbels per plant, numbers of flowers per umbel and lengths of inflorescence stalks were not significantly different between the two morphs (Table 1). But the diameter of inflorescence stalks in the pink morph was wider than the white morph. There were no significant differences in pedicel length, petal and sepal size (length $\times$ width), stamen height of both inner and outer whorls, anther length of outer whorls, and gynoecium width between the two morphs. One umbel usually produced 23 flowers and late-blossoming flowers had longer pedicels than early flowers ($t = 19.3$ in the pink morph and $t = 17.6$ in the white morph, respectively, both $P < 0.0001$), although pedicel length of late flowers was marginally different between the two morphs. However, the pink morph had larger anthers in the inner whorl than the white morph (Table 2). The pink morph produced significantly more pollen and ovules than the white morph. The following measurement of female fertility indicated that the pink morph produced more seeds with higher seed set than the white morph (Table 1).

### Discussion

Our investigation found that a white gynoecium phenotype appeared in Butomus umbellatus in northeastern China. White-gynoecium variants were rare in this aquatic plant according to our field survey, and previous investigations have not observed the color polymorphism (Song 1998; Eckert et al. 2000; Lui et al. 2005; Z. P. Song at Fudan University, China, pers. comm., 2005; C. G. Eckert at Queen's University, Canada, pers. comm., 2006). However, this white morph was pictured at Ocontee Lake in Wisconsin, USA (K. Yatskievych at Missouri Botanical Garden, pers. comm., 2006)). Flower color polymorphism often displays spatial and temporal variation in morph frequency in various populations (Schemske and Bierzychudek 2001). Clonal reproduction through rhizomes may facilitate variable morph frequency among the three populations in B. umbellatus.

<table>
<thead>
<tr>
<th>Gynoecium color</th>
<th>Umbels per plant</th>
<th>Flowers per umbel</th>
<th>Pollen number per flower</th>
<th>Ovules per flower</th>
<th>Seeds per capsule</th>
<th>Seed set (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pink</td>
<td>2.2 ± 0.1</td>
<td>23.5 ± 0.5</td>
<td>1 677 030 ± 69 450</td>
<td>565.8 ± 18.8</td>
<td>100.1 ± 6.9</td>
<td>17.6 ± 1.0</td>
</tr>
<tr>
<td>White</td>
<td>2.3 ± 0.1</td>
<td>23.2 ± 0.2</td>
<td>1 022 460 ± 175 060</td>
<td>502.1 ± 17.6</td>
<td>41.5 ± 3.0</td>
<td>8.2 ± 0.5</td>
</tr>
<tr>
<td>$F$ value</td>
<td>$F_{1135} = 1.4$</td>
<td>$F_{1135} = 0.44$</td>
<td>$F_{135} = 67.4$</td>
<td>$F_{160} = 6.2$</td>
<td>$F_{160} = 66.2$</td>
<td>$F_{160} = 79.9$</td>
</tr>
<tr>
<td>$P$</td>
<td>0.24</td>
<td>0.51</td>
<td>&lt;0.0001</td>
<td>0.0159</td>
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</tr>
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$F$ and $P$ values are from one-way ANOVAs.
Table 2. Differences in floral traits between pink-gynoecium and white-gynoecium morphs (mean ± SE) tested by paired \( t \)-test.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Pink</th>
<th>White</th>
<th>T-value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflorescence stalk height (cm)</td>
<td>108.8 ± 2.2</td>
<td>101.0 ± 4.8</td>
<td>1.716</td>
<td>0.10</td>
</tr>
<tr>
<td>Pedicel diameter (mm)</td>
<td>5.15 ± 0.14</td>
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<td>0.831</td>
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<td>Pedicel length (mm)</td>
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<td>0.73</td>
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<td>Outer whorl stamen length (mm)</td>
<td>8.14 ± 0.24</td>
<td>8.09 ± 0.10</td>
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<td>Outer whorl anther length (mm)</td>
<td>8.75 ± 0.25</td>
<td>8.59 ± 0.15</td>
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<td>Inner whorl stamen length (mm)</td>
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<td>0.86</td>
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<td>Petal size (mm)</td>
<td>4.17 ± 0.08</td>
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Heights of inflorescence stalks, numbers of inflorescences and numbers of flowers per plant, all these features that may reflect plant size, were not different between the two morphs. Yet, the pink morph with wider inflorescence stalks produced more pollen and ovules per flower and consequently more seeds than the white morph. Floral visitors to *B. umbellatus* included bees, flies and syrphid flies (Tang and Huang, unpubl. data, 2007), consistent with previous observations in other areas that *B. umbellatus* is pollinated by generalists (Song 1998; Bhardwaj and Eckert 2001; Lui et al. 2005). Seed production mainly depended on insect visitation and autonomous pollination yielded few seeds in this species (Song 1998; Eckert et al. 2000). Seed sets of open-pollinated flowers in three populations in northeastern China were 4.7%, 11.0% and 14.3% and could be up to 21.3%, 25.9% and 28.5%, respectively, after supplemental pollination (Song 1998), suggesting pollen limitation in this emergent aquatic plant. The difference in seed production between two gynoecium morphs could be attributed to two factors. First, the white morph produced fewer ovules than the pink morph. Second, the seed set of the open-pollinated plants was higher in the pink morph. It is likely that the pink morph provides more rewards (higher pollen production) to pollinators and consequently yields higher pollinator visitations in this aquatic plant.

Pollinators are usually attracted to the flowers in the first place by floral color or scent although most flowers provide pollen or nectar as a food reward (Proctor et al. 1996). Pollinators may use these signals as cues of quality or quantity of reward (Weiss 1991; Meléndez-Ackerman et al. 1997; Armbruster et al. 2005 and references therein). Therefore, variation in flower colors may affect pollinator visitation rates and, in turn, plant reproductive success (Waser and Price 1981; Stanton et al. 1989; Rausher and Fry 1993; Gigord et al. 2001; Jones and Reithel 2001). For example, white flower variants occur in natural populations of many brightly colored species. Experimental studies in *Delphinium nelsonii* indicated that a reduction of seed set in white-flowered plants was caused by pollinator discrimination (Waser and Price 1981). However, several studies have not observed pollinator discrimination against certain petal colors (Levin and Brack 1995; Jersákova et al. 2006). For example, selection against white-flowered plants in *Phlox drummondii* evidently resulted from a reduction in survivorship to flowering and in flower production rather than pollinator discrimination (Levin and Brack 1995). However, flower color polymorphism could be maintained by balancing selections from spatial or temporal variation in pollinator assemblage (Brown and Clegg 1984; Meléndez-Ackerman et al. 1997; Subramaniam and Rausher 2000; Eckhart et al. 2006; Streisfeld and Kohn 2007) or from defense against natural enemies (Simms and Bucher 1996; Frey 2004) or from pleiotropic effects or indirect selection on plant performance in contrasting physical environments (Schemske and Bierzychudek 2001; Warren and Mackenzie 2001; Coberley and Rausher 2003).
Color variation in sexual organs may directly relate to plant reproductive success. Clearly, further survey in the field is needed to document the whole picture of morph frequency and the roles of ecological forces in the evolution and maintenance of gynoecium color polymorphisms in this species. The rarity of color polymorphism in female organs in flowering plants suggests that gynoecia experience stronger selection than other floral organs such as petals. To our knowledge, this observation on B. umbellatus represents the first report of gynoecium color polymorphism in plants. Although the mechanism underlying gynoecium color polymorphism in B. umbellatus remains unclear, our study suggested that the white morph produced fewer seeds than the usual pink morph. This variation might be generated by mutations at loci regulating pigment synthesis, as is color variation in petals in other species (Levin and Brack 1995). Thus, B. umbellatus provides a unique opportunity for us to understand the evolutionary processes governing gynoecium color.

Materials and Methods

Study species and populations

Butomus umbellatus L. is an emergent, aquatic monocot in the monotypic family Butomaceae. It is native to Europe and north temperate Asia and was introduced to eastern North America about 100 years ago (White et al. 1993), usually growing in shallow water around the margins of lakes and slow-moving rivers. Plants can reproduce both sexually through seed and clonally via rhizome fragmentation and vegetative bulbils, which are borne on rhizomes and occasionally on inflorescences (Eckert et al. 2000). Inflorescences are cyrstate umbels borne on thin, cylindrical, upright stalks and consist of 15–50 light pink flowers (Wilder 1974). Flowers consist of three pink sepals, three slightly larger pink petals, nine stamens (in an outer whorl of six and an inner whorl of three) and six conduplicate carpels, each of which contains about 200 ovules (Singh and Sattler 1974; Kaul 1976; Lieu 1979). Nectar is secreted from nectaries located at the base of the carpels (Bhardwaj and Eckert 2001).

In a survey of aquatic plants in northeast China during summer 2005, we noted that gynoecia were white in some individuals of B. umbellatus at Hexiayuan wetland, Mishan City, Heilongjiang Province, China (45°30′32″ N 131°51′18″ E). This white gynoecium morph (Figure 1), different from the usual pink genoecium, has not been described before. Following exploration for the color polymorphism in other areas, two populations were found near Mishan City but not observed in our survey in the other four counties in Heilongjiang Province. To document the frequency of white morphs, we counted all individuals in a plot in the three populations. Hexiayuan wetland was around 20 000 m², dominated by Nelumbo nucifera. Plants of B. umbellatus were growing in shallow water near the bank. A simple goodness-of-fit test (G-test) was used to test whether the observed morph ratio was different from 50:50 (Sokal and Rohlf 1995).

Differences in floral traits and female fertility

We counted umbels and flowers per umbel in 78 and 59 individuals with pink and white gynoecia, respectively, in Hexiayuan wetland. We randomly selected 20 umbels of each morph and measured height of the inflorescences (plant height) and floral characters. To quantitatively compare the two gynoecium morphs, we measured 10 floral traits of each flower to 0.1 mm using a caliper micrometer, including pedicel length, petal length and width, sepal length and width, stamen length and anther length in inner and outer whorls, and gynoecium length. Because pedicel length of early blooming flowers is generally different from the late blooming flowers, we measured pedicel length at the two flowering stages. We collected newly dehisced anthers from one flower each of these 40 individuals and brought them back in separated centrifugal tubes, following the method of Huang (2003) to measure pollen production. The pollen from all anthers in a flower was suspended in 50 drops of water, and pollen production per flower based on scoring pollen grains contained in five drops under a light microscope was estimated subsequently.

To compare female fertility between two morphs, we estimated seed production in the Hexiayuan population. In the late flowering stage of sampled plants, we counted ovules and seeds in one flower in 29 and 33 individuals with pink and white gynoecia respectively. Seeds are plump and cylindrical, with longitudinal ridges on the seed coat (Eckert et al. 2000), permitting us to distinguish seeds from undeveloped ovules in capsules.

One-way ANOVAs were used to compare umbel and flower production, seed and ovule production, and seed set (the ratio of seeds to ovules per fruit) between two morphs. Floral traits were compared by a paired T-test to test the null hypothesis of zero mean difference between pairs.

Acknowledgements

The authors wish to thank Jiao-Kun Li, Zuo-Dong Li and Lu-Lu Tang for their help in the field; Zhi-Ping Song, Christopher G. Eckert, and Kay Yatskievych for instructive suggestions for flower color in Butomus umbellatus, Sarah Corbet for correcting the English and reviewing an earlier draft of the manuscript and the Executive Editor of JIPB for inviting us to write this manuscript.

References


