

# Induced mutations affecting pollinator choice in *Mimulus lewisii* (Phrymaceae)

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**Abstract** The flowering plants are one of the most phenotypically varied and wide-ranging groups of organisms on earth, and yet, we have limited understanding of the contribution of animal pollinators to the diversification of floral form. To explore the interaction between variation in floral form and pollinator behavior, we observed the foraging behavior of bumblebees (*Bombus impatiens*) when presented with both wild-type *Mimulus lewisii* plants and each of three chemically induced single-locus mutants with altered floral phenotypes, including loss of the three lower petals, loss of nectar guides, and a change in petal color patterning. We found that each of the mutants attracted successful pollinator visits at just 29–80% of the rate relative to wild-type flowers, suggesting that effective recruitment of bumblebee pollinators requires the landing platform provided by the lower petals, and visual cues provided by the nectar guides and petal color pattern. Since single-locus recessive mutations are capable of ablating the lower petals, nectar guides, and color pattern, such changes in floral form provide insight into the driving forces behind plant adaptation.

**Keywords** Color contrast · Floral form · *Mimulus* · Nectar guides · Petal number · Pollinator choice

## Introduction

The diversity of flower shapes, colors, sizes, and scents in the more than 250,000 extant angiosperm species is remarkable. Despite a large body of literature on the topic, the contribution of animal pollination to the evolution of this diversity has remained unresolved (Waser 1998). Although some authors have argued that the role of animal pollination in driving speciation is minimal (Ricklefs and Renner 1994; Armbruster and Muchhala 2009), most of the earth's flowering plants rely on animal pollinators for sexual reproduction and reproductive success (Kearns et al. 1998), and this often intimate relationship clearly influences fitness, reproductive isolation, and speciation (Darwin 1877). This has been demonstrated in a variety of studies involving examination of the fossil record (e.g., Crepet 1984), phylogenetic studies (e.g., Johnson et al. 1998), and pollinator observation experiments (e.g., Manning 1956; Castellanos et al. 2004). In their recent analysis of macroevolutionary patterns, Coyne and Orr (2004, p. 441) state:

Our major conclusion is that two sets of key factors—traits increasing sexual selection in animals, and traits promoting animal pollination in plants—appear to increase the rate of speciation.

Grant (1949) described two types of pollinator-dependent reproductive isolation: mechanical isolation, in which pollinators visit multiple species but do not cross-pollinate (each plant species' pollen is deposited on a different part of the pollinator's body), and ethological isolation, in which sister plant species are pollinated by different species (or guilds) of pollinators. While mechanical isolation is dependent upon the reproductive morphology of the plant, ethological isolation is based entirely on a pollinator's

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preference for a particular floral form and a plant's ability to generate flowers that will be appealing to that pollinator.

The preferences of certain types of pollinators can be reflected in “pollination syndromes”—suites of floral traits correlated with the pollinators that are attracted to them (e.g., red, tubular flowers are part of the hummingbird pollination syndrome). Although the evolutionary relevance of this concept has been questioned (Waser 1998; Aigner 2001), there is substantial support for the idea that during the evolution of pollinator specialization, flowers often do converge on phenotypes characteristic of pollinator guilds (Stebbins 1970; Johnson and Steiner 2000; Fenster et al. 2004). While producing specialized flowers that are attractive to a single reliable and effective pollinator must inevitably occur at the expense of pollinator variety, this is clearly a successful strategy as greater speciation rates have been detected in angiosperm lineages with more specialized pollination systems (Grant 1949, 1994; Crepet 1984; Johnson et al. 1998; Dodd et al. 1999; Hodges et al. 2003). Reproductive isolation mediated by pollinator choice has also been explicitly demonstrated both in computer models (Gegear and Burns 2007), and for a number of taxa in nature, including *Mimulus* (Bradshaw and Schemske 2003; Ramsey et al. 2003; Streisfeld and Kohn 2006), *Aquilegia* (Hodges et al. 2002; Fulton and Hodges 1999), and *Petunia* (Ando et al. 2001; Hoballah et al. 2007).

Additional research into pollinator behavior provides some indication of which traits are most important for attracting particular pollinators. For example, bumblebees prefer flowers reflecting light at wavelengths 400–425 nm with two-color patterning (Gumbert 2000; Heuschen et al. 2005), and having large, symmetrical flowers, or floral displays (Møller 1995; Ishii 2006), but are also capable of learning to reach a nectar reward in much more morphologically complex flowers (Laverly 1994). Hummingbirds have been shown not to have an innate color preference (Grant 1966), but they quickly learn by trial and error in which flowers provide a greater reward and are thus driven by experience and payoff, associating reward with specific floral characteristics such as red color, tubular corolla shapes, or greater flower size (Grant 1966; Sutherland and Vickery 1993; Fenster et al. 2006). Moths learn to associate specific odors with nectar rewards (Cunningham et al. 2004; Riffell et al. 2008), but also may possess an innate attraction to certain colors (Kelber 1997).

What remains relatively unknown is how individual mutations altering floral characteristics affect visitation by pollinators, how selection acts on mutant phenotypes to maintain a pollinator-preferred form, or how many floral phenotype changes are required for a shift in pollinator guild to take place. Most of the work investigating the role of specific floral traits in pollinator attraction has

involved phylogenetic analysis of closely related taxa (Johnson et al. 1998; Wilson et al. 2004; Whittall and Hodges 2007; Smith et al. 2008), artificially altering a flower's form through manual manipulation (Waser and Price 1985; Meléndez-Ackerman et al. 1997; Johnson and Steiner 1997; Meléndez-Ackerman and Campbell 1998; Castellanos et al. 2004), using simulated flowers (Manning 1956; Kelber 1997; Fenster et al. 2006; Gomez et al. 2008), or examining pollinator response to segregating floral traits in interspecific hybrids (Schemske and Bradshaw 1999; Whitney and Glover 2007; Kimball 2008; Martin et al. 2008).

Although manual manipulation or simulations allow for maximum control of a trait of interest, alteration of floral characteristics by induced mutations at single loci more appropriately mimics the random mutations that occur in nature and allows us to query pollinators with forms that are both physically and genetically feasible in natural populations. Previous work in *Antirrhinum* (Glover and Martin 1998; Comba et al. 2000; Dyer et al. 2007; Shang et al. 2011) and *Petunia* (Hoballah et al. 2007) has shown that this approach does yield valuable information on the influence of floral traits on pollinator behavior and the potential of those traits to contribute to speciation. In this study, we take a similar approach, using chemical mutagenesis to induce mutations that alter particular floral traits and examine the effect of those phenotypic changes on the foraging behavior of naïve pollinators.

#### *Mimulus* as a system of study

The genus *Mimulus* contains over 120 species displaying a wide range of floral morphology, mating system, ploidy level, and pollination syndrome unparalleled in current plant model systems. A rapid generation time (~3 months seed to seed), abundant seed set (~1,000 seeds per fruit), and available genomic resources (Wu et al. 2008) make *Mimulus* a useful system in which to study the genetics and evolution of pollinator-mediated selection. Multiple shifts to hummingbird pollination from a bee-pollinated ancestor have occurred throughout the genus, including in the sister taxa, *Mimulus lewisii* and *M. cardinalis* (Beardsley et al. 2003). Previous work has shown that, in sympatry, these two species are >99.9% reproductively isolated by virtue of attracting different pollinator guilds—*M. lewisii* is pollinated by bumblebees and *M. cardinalis* by hummingbirds (Ramsey et al. 2003). In a demonstration of the importance of single loci in shifting from one pollinator guild to another, hummingbirds can be recruited to *M. lewisii* by substituting the *M. cardinalis* allele at the *YELLOW UPPER* locus, which changes the color of the *M. lewisii* flower from pink to yellow (Bradshaw and Schemske 2003).

It thus seems plausible that chemically induced mutations at single loci in *M. lewisii* could result in substantial changes in pollinator attraction. In this study, we use three different recessive mutants to examine how various changes to flower phenotype affect choice in a bumblebee pollinator. The three mutations examined include an alteration in floral structure that reduces petal number from five to two (*chinless*), a removal of the nectar guide pigmentation and trichomes (*guideless*), and a reduction in petal color pattern and contrast (*light areas2*).

## Materials and methods

### Plant materials

*Mimulus lewisii* seeds were collected by Amy Angert along the South Fork of the Tuolumne River near Yosemite National Park in late spring of 2000. The seeds were transported to the Biology Greenhouse at the University of Washington, sown in Sunshine #4 (Sun Gro Horticulture, Bellevue WA), sub-irrigated with capillary mats, and fertilized once or twice a week as needed. Supplementary light was provided (except in summer) by sodium vapor lamps, with a 16-h light:8-h dark photoperiod. One of the resulting *M. lewisii* plants was self-pollinated and maintained by single seed descent for eight generations to produce the inbred line LF10.

### Mutagenesis

Each mutant line used in this study was generated with the DNA alkylating agent ethyl methanesulfonate (EMS; Sigma M0880; CAS Number: 62-50-0) as part of a larger mutant screen that took place between February 2004 and December 2007 (Owen and Bradshaw, unpublished). Approximately 4,000 LF10 seeds were soaked in one of three concentrations of EMS (25, 30, 35 mM) for 19 h and then rinsed extensively with water.  $M_1$  (first mutant generation) seeds were suspended in 0.1% agarose, sown immediately in Sunshine #4, and grown in the greenhouse as described elsewhere.  $M_1$  plants were self-pollinated to produce  $M_2$  seeds segregating for any mutations induced by EMS treatment. For each  $M_1$  plant line, 18 or 24  $M_2$  seeds were planted in 72-plug trays (60 cm<sup>3</sup> per plug), grown to flowering age, and inspected for mutant phenotypes.  $M_2$  plants were observed every 1–3 days June through July 2003 and February through April 2008. After flowering, each plant was examined for any floral abnormalities. Plants with mutant floral phenotypes were recorded, photographed, and transplanted. Each mutant plant was self-pollinated to perpetuate the

mutant phenotype in homozygous form and backcrossed to the wild-type LF10 line to produce heterozygotes. Heterozygotes were self-pollinated to produce an  $F_2$  population.

Bumblebee foraging behavior was observed for *chinless*, *guideless*, and *light areas2* mutants. To ensure homogeneity in the genetic background of mutant and wild-type plants, all plants used in each experiment are full sibs, originating from the  $F_2$  populations segregating for the recessive mutant phenotype of interest.

### *Mimulus* mutants

The *chinless* mutant (Fig 1b) completely lacks the three lower petals typical of *M. lewisii* (Fig. 1a). Although the mutant flowers sometimes have thin, ragged strips of petal tissue along the bottom of the corolla tube where the lower petals should be, the loss effectively removes the landing platform required by bumblebees to gain entrance into *M. lewisii* flowers and thus should hinder pollination. The *chinless* mutant closely resembles the natural species *M. douglasii*, which also lacks lower petals. The pollinator of *M. douglasii* is unknown.

Like many insect-pollinated species, wild-type *M. lewisii* flowers have two yellow stripes that extend along the bottom of the corolla throat, known as nectar guides (Fig. 1a). These guides consist of carotenoid-pigmented tissue studded with trichomes. The nectar guides are thought to direct bumblebees to the pollen and nectar at the back of the corolla throat and provide color contrast for attraction (Manning 1956; Penny 1983; Lunau et al. 1996; Gumbert 2000). In some species, nectar guides are only visible under ultraviolet light, making them visible to bumblebees but invisible to the human eye (Lunau et al. 1996). In wild-type *M. lewisii*, however, the flowers absorb ultraviolet light uniformly so all colors and pigments relevant to pollinator recognition are in the visible spectrum (Schemske and Bradshaw unpublished). In the *guideless* mutants, the yellow nectar guides and trichomes are completely absent (Fig. 1c). The loss of the guides reduces color contrast and should have a negative effect on pollinator attraction (Gumbert 2000).

Wild-type *Mimulus lewisii* flowers also possess distinct dark pink (anthocyanin; Wilbert et al. 2007) petal stripes with white (anthocyanin-less) borders around the yellow nectar guides and inside the corolla throat that provide additional color contrast for the nectar guides (arrows, Fig. 1a). The *light areas2* mutant lacks these characteristic white regions and dark petal stripes and has a generally muted, homogenized pink coloration (Fig. 1d). This mutant retains its nectar guides so the alteration to color contrast is quite subtle and might be expected to have minimal effect on pollinator choice.



**Fig. 1** Mutants of *M. lewisii*. **a.** Wild-type *M. lewisii*. Light areas are indicated with *arrows*. **b.** *Chinless* mutant. **c.** *Guideless* mutant. **d.** *Light areas2* mutant

#### Pollinator observations

In nature, *M. lewisii* is pollinated primarily by the yellow-faced bumblebee, *Bombus vosnesenskii* (Schemske and Bradshaw 1999; Ramsey et al. 2003). For this study, we used *Bombus impatiens* as the pollinator, because they are comparable in body size to *Bombus vosnesenskii* and were commercially available (Green Spot Ltd., Nottingham, New Hampshire).

For each *M. lewisii* mutant line,  $F_2$  seeds were sown on Sunshine soil (Sun Gro Horticulture, Bellevue, Washington), in 10-cm square pots (1 l). Upon flowering, 20 plants with the mutant phenotype and 20 plants with the wild-type phenotype were selected from each  $F_2$  population and transplanted into 25-cm round pots (7 l). These were then arranged in a checkerboard pattern on two benches in a small greenhouse and numbered. One bumblebee hive was placed at the end of the greenhouse aisle, equidistant from each bench.

To preclude the possibility of the bumblebees developing long-term learned preferences, foraging observations for each floral mutant phenotype were made for

2–4 h on a single day with naïve bumblebees, previously fed only a sugar solution provided within the hive. Bumblebee visits were recorded as attempted visits (approached flower but failed to enter the corolla tube sufficiently to affect pollination) or successful visits (made contact with the anthers and pistil). The orientation of the bumblebee within the flower (rightside-up or upside-down) was noted. To examine the effect of floral constancy in the foraging bumblebees, we also recorded transitions between and among flower phenotypes for each mutant form.

Before release of the bumblebees, we recorded the number of available flowers of each phenotype. Chi-square goodness-of-fit tests were performed under the null hypothesis that bumblebees show no preference for visiting the wild-type or mutant plants, i.e., that the number of bumblebee visits is proportional to the availability of each floral phenotype in the experimental array. The relative success of each mutant (compared with wild type) was estimated as the number of successful bumblebee visits observed versus the number expected.

## Results

For each of the three mutants phenotypes in this study (*chinless*, *guideless*, and *light areas2*), the  $F_2$  pattern of inheritance was consistent with a single-locus recessive (Table 1). This suggests that point mutations can have drastic effects on floral traits likely to affect pollinator attraction and pollination success, such as petal number, presence/absence of nectar guides, and anthocyanin pigment pattern. In our observations of bumblebee pollination behavior, wild-type flowers were universally preferred over mutant phenotypes (Table 2). The lack of lower petals in the *chinless* mutant reduced (compared with wild type) the proportion of successful visits by bumblebees by 71%. The *guideless* and *light areas2* mutations were less severe, but still decreased successful pollinator visits by ca. 20%.

Although *chinless* mutant flowers experienced 71% fewer successful bumblebee visits than wild-type flowers, it was not for lack of effort on the part of the bumblebees. While bumblebees could enter wild-type flowers without difficulty (Fig. 2a), those attempting to visit *chinless* flowers were only able to grip the edge of the corolla opening, leaving the rest of their bodies to dangle below (Fig. 2b). The occasional successful visitation was only achieved when the mutant flower had filaments of tissue where the lower petals normally would be, providing the bumblebee with “stepping stones” into the corolla tube. The absence of a landing pad (the lower petals) significantly reduced visitation success. Bumblebees were successful in entering *chinless* flowers in only 27% of attempted visits (31/115). In contrast, bumblebees visiting

wild-type flowers experienced a success rate of 90% (95/105) ( $P < 0.001$ ,  $\chi^2 = 62.3$ ,  $df = 1$ ).

Examination of bumblebee orientation during visits to *guideless* flowers revealed a significant difference in pollinator positioning within the corolla when compared with wild type. In 55% (43/78) of all visits to *guideless* flowers, bumblebees entered the flower upside-down (Fig. 2c), while only 10% (12/116) of visits to wild-type flowers were of similarly improper orientation ( $P < 0.01$ ,  $\chi^2 = 17.5$ ,  $df = 1$ ). The bumblebees appeared to use the nectar guides to orient themselves to enter the wild-type flower with their back to the stamens and stigma. This pattern of behavior was quite consistent, such that when bumblebees approached wild-type flowers that happened to be upside-down, they would walk around the flower opening in order to enter with their ventral surface against the nectar guides (Fig. 2d).

We used Bateman’s Index to analyze our flower-to-flower transitions data as a measure of floral constancy (Waser 1986; Gegear and Laverty 2005). Bateman’s index ranges from  $-1$  to  $+1$ , with a  $-1$  rating indicative of completely inconstant behavior, 0 is random foraging, and  $+1$  is completely constant. Our analysis did not show strong constancy for the *chinless* mutant (BI = 0.198), the *guideless* mutant (BI = 0.072), or the *light areas2* mutant (BI = 0.269). All displayed BI values that are much closer to random than constant, suggesting that the tendency of pollinators to visit a particular flower morph exclusively was not prevalent in these experiments. This is not surprising given that the mutant and wild-type flowers were similar in color. Wilson and Stine (1996) found similar

**Table 1** Segregation data for mutant lines

Mutant	Plants in $F_2$ segregating population mutant/wild type	$P$ one locus 1:3 ( $\chi^2$ , $df = 1$ )	$P$ two loci 1:15 ( $\chi^2$ , $df = 1$ )
<i>Chinless</i>	12:60	0.10 ( $\chi^2 = 2.67$ )	<0.001 ( $\chi^2 = 11.57$ )
<i>Guideless</i>	13:34	0.67 ( $\chi^2 = 0.18$ )	<0.001 ( $\chi^2 = 33.29$ )
<i>Light areas2</i>	8:21	0.75 ( $\chi^2 = 0.10$ )	<0.001 ( $\chi^2 = 20.40$ )

Proportions of mutant and wild-type plants for each line were tested for significance with one-locus and two-loci models of Mendelian inheritance using chi-square tests, ( $df = 1$ )

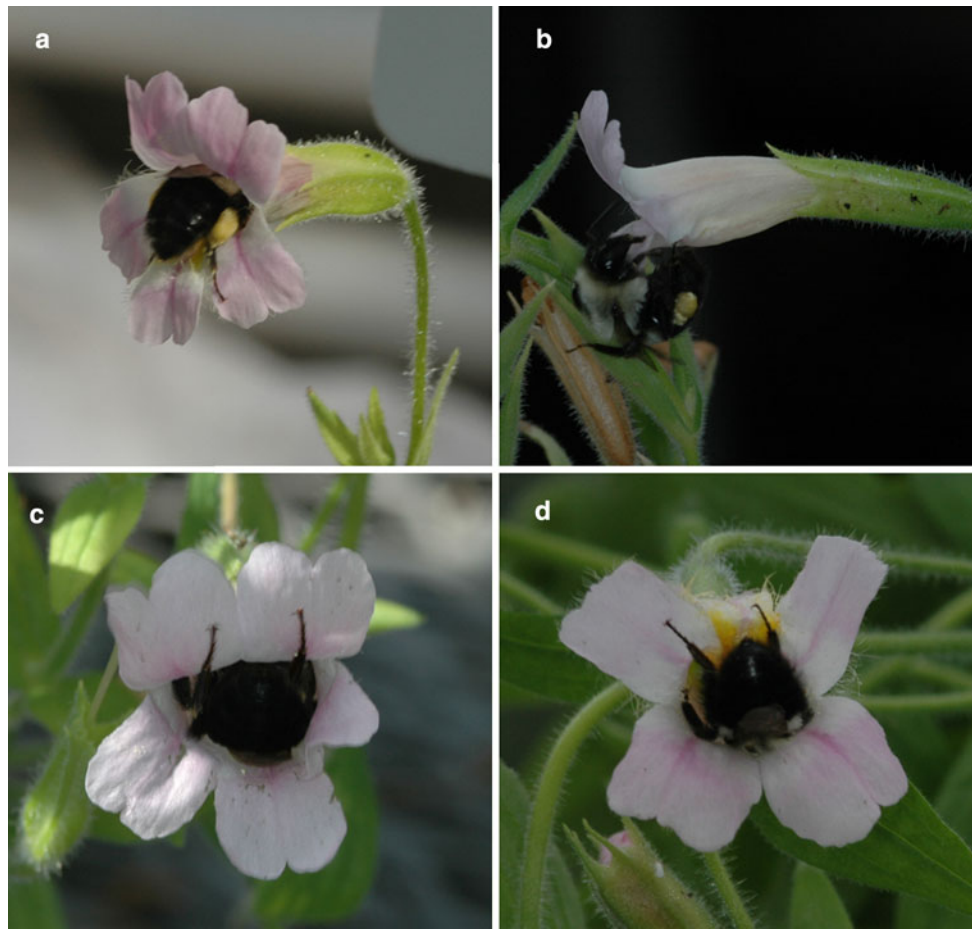
$P$  values less than 0.05 indicate mutant segregation is significantly different from the inheritance model being tested (either one locus or two loci)

**Table 2** Visitation of the bumblebee *Bombus impatiens* foraging on mixed plots of wild-type and mutant flowers of *M. lewisii*

Mutant	Flower count mutant/wild type	Successful bumblebee visits mutant/wild type	$P$ ( $\chi^2$ , $df = 1$ )	Relative success mutant/wild type (%)
<i>Chinless</i>	124:20	31:95	<0.001	29
<i>Guideless</i>	43:43	78:116	0.006	80
<i>Light areas2</i>	57:57	88:138	<0.001	78

Successful bumblebee visits only visits in which the bumblebee was able to enter the flower and make contact with the anthers and pistil

$P$  values of less than 0.05 indicate significant difference in successful visits between mutant and wild-type flowers



**Fig. 2** Visitation to mutant flowers by *Bombus impatiens*. **a.** Normal visitation to a wild-type *M. lewisii* flower. **b.** Unsuccessful visitation to a *chinless* mutant flower. **c.** Improper upside-down visitation to *guideless* mutant. **d.** Normal visitation to upside-down wild-type *M. lewisii* flower

results, showing that the bumblebee *Bombus vagans* exhibited less constancy when morphologically distinct flowers were similar in color.

## Discussion

How the behavior of animal pollinators affects floral diversity in the angiosperms is currently not well understood. This goal of this study was to determine the contribution of specific floral characteristics to pollinator choice by examining the effect of genetically induced changes to floral form on the visitation behavior of bumblebee pollinators. Every floral change to wild-type *M. lewisii* flowers examined here induced a negative response in the attraction of bumblebee pollinators. Indeed, even the subtlest alteration in flower color (*light areas2*) showed a strong effect, significantly reducing pollinator visitation. For the *chinless* mutant, the dramatic reduction in visitation and successful pollination was expected. Corolla shape has been previously shown to influence pollinator choice (Møller 1995;

Galen and Cuba 2001; Gomez et al. 2008), and for bumblebees, a landing platform is required to enter a flower and collect the nectar and pollen. The loss of the lower petals in the *chinless* mutant removes that platform, preventing the bumblebee from entering the flower and effectively excluding bumblebees as pollinators.

Pollinator recruitment can also be affected by floral changes less severe than the complete loss of lower petals. A significant reduction in visitation by bumblebees is also accomplished by altering a flower's color contrast. This is the case for both the *guideless* and the *light areas2* mutants. Complete removal of the yellow nectar guides, as in the *guideless* mutant, greatly reduces color contrast, and, not surprisingly, this also significantly reduces pollinator visitation. The color change in the *light areas2* mutant is much subtler than that in the *guideless* mutant, but unexpectedly, it also experienced a significant reduction in visitation. The *light areas2* phenotype not only removes the light areas around the corolla opening, but also homogenizes the color of the petals, washing out the darker pink petal stripes (Fig. 1c). It is also possible that the *light*

*areas2* mutant has a color phenotype in the ultraviolet spectrum, which would affect the ability of the flower to attract bumblebee pollinators. Further examination of the UV profile of the mutant flowers is required to determine the contribution of visual cues in the UV spectrum to the visitation behavior of the bumblebees. However, previous studies examining color contrast in the visible spectrum have shown that the more a flower contrasts with the background, the more it is able to attract bumblebee visitors (Lunau et al. 1996; Gumbert 2000). Pigmentation pattern has also been shown to have a significant effect on the attraction of bumblebee pollinators, which use pattern to distinguish between rewarding and non-rewarding flower morphs (Scora 1964; Petrikin and Wells 1995; Johnson and Dafni 1998; Shang et al. 2011). Our data further support the idea that bumblebees are attracted to pattern and contrast and indicate that even mutations of seemingly small phenotypic effect may have significant effects on pollinator choice.

Observations with the *guideless* mutant also revealed something about the nature of nectar guides. Nectar guides have long been recognized as a component of pollinator attraction but are often thought of as acting primarily as “signposts” that attract and direct pollinators to a nectar or pollen reward (Manning 1956; Scora 1964; Penny 1983; Pohl et al. 2008; Thomson and Wilson 2008; Suárez et al. 2009). Previous research into their specific role has mostly involved manual manipulation of real and artificial flowers, or training the pollinators being tested (Manning 1956; Penny 1983; Waser and Price 1985; Johnson and Dafni 1998; Pohl et al. 2008). Recent work in *Mimulus luteus* has also suggested that the size and shape of nectar guides can influence the type of pollinator attracted, which may promote floral divergence (Medel et al. 2003). Our data also support the importance of nectar guides in pollinator attraction. However, the significant increase in upside-down bumblebee visits we observed in *guideless* mutant flowers points to an orientation function of the nectar guides as well. Bumblebees appear to home in on the parallel marks of the nectar guides and follow them into the flower much like an airplane pilot following runway lights. For the flower, this ensures proper orientation of the bumblebee during visitation for maximum contact with the anthers and stigma. The morphological fit of a pollinator and flower significantly affects pollination effectiveness (Campbell et al. 1996), and while previous studies have suggested that nectar guides may orient pollinators for ease in nectar collection (Manning 1956; Dafni and Kevan 1996; Johnson and Dafni 1998; Lunau 2000), we propose that the orientation function of nectar guides is primarily beneficial to the plant, encouraging bumblebee pollinators to enter the flower in a manner that guarantees successful pollination.

## The adaptive value of pollinator exclusion

These data demonstrate that floral characteristics such as lower petals, nectar guides, and color patterning are important to the attraction and successful visitation of bumblebee pollinators and, if altered, result in a significant reduction in pollination. But the question remains: Can a mutation that reduces pollinator visitation be adaptive? It may be that during a pollinator shift, exclusion is just as important as attraction. This appears to be the case in *Penstemon* where “pro-bird” traits that encourage visitation by hummingbirds were correlated with “anti-bee” traits that discourage bumblebee pollination (e.g., exertion of reproductive organs, narrowed corolla tube) (Castellanos et al. 2004). Castellanos et al. suggest that different traits may evolve in concert, hypothesizing that a “pro-bird” trait may only be adaptive when coupled with an “anti-bee” trait. In this case, if a species adapted to bumblebee pollination developed a novel “pro-” mutation that increased attraction of some alternative pollinator, it would be pushed off its adaptive peak unless purifying selection brought it back to its former state, or an “anti-bee” change also took place, allowing it to be swept up a neighboring peak associated with the alternative pollinator. This idea has been suggested before (Stebbins 1970; Whibley et al. 2006), and with the tremendous number of insect and bird species globally, it is not unreasonable to think of the adaptive landscape of floral form as having many peaks, each representing a floral form that converges on a single pollinator or pollinator guild.

Attraction of a new pollinator would not only depend on floral characteristics but also the relative abundance of different pollinator types (Martin et al. 2008), and the efficacy of these pollinators, with the most effective pollinator favored (Stebbins 1970; Schemske and Horvitz 1984, 1989; Armbruster 1988; Campbell et al. 1996). Fluctuations in that landscape by changes in the composition of the pollinator populations could also have an impact on floral evolutionary trajectories. For instance, if hummingbirds or moths were more abundant than bumblebees over several flowering seasons, it could be advantageous for a plant to attract a new pollinator and then exclude bumblebees to more finely adapt to that new pollinator. An increase in a pollinator population would likely result in greater competition at its traditional feeding places and may also encourage that pollinator to seek out non-traditional food sources. Goulson et al. (1998) found that bumblebees systematically avoided flowers that had been previously visited by other bees, reducing competition between foragers. Modeling experiments suggest that resource competition can promote the evolution of long corolla tubes by encouraging greater visitation constancy (Rodríguez-Gironez and Santamaria 2007).

Waser (1998) claims that plants sit atop broad adaptive peaks with specialization occurring during limited periods of strong selection and that it may be useful to “explore whether adaptive peaks are broad, and whether stronger selection will occur if phenotypes fall off their sides.” The work presented here examines exactly that using chemical mutagenesis to push normal phenotypes off their adaptive peaks and then measure the effect on pollinator behavior. Recent population genetic models predict that plants that are rare among the local plant community (such as a new mutant plant) are more likely to evolve a specialized pollination strategy than if they are abundant (Sargent and Otto 2005), and our results indicate that pushing these phenotypes even slightly (e.g., *light areas2*) results in a significant reduction in visitation by bumblebee pollinators.

*Mimulus lewisii* is dependent upon pollination by bumblebees for its reproduction—99.9% of pollinator visits to *M. lewisii* are made by bumblebees (Bradshaw and Schemske 2003). Hence, a disruption to visitation by bumblebees, such as those observed as a result of the mutations reported here, would likely result in a reduction in fitness. In order to determine the evolutionary consequences of these mutations in nature, future experiments must include observations in the field, measuring visitation by all available native pollinators and fitness over several generations. In addition, it is possible that pleiotropic effects on other floral traits contributed to the pollinator behavior observed here. In particular, future experiments must control for the possible influence of odor and nectar reward, as floral odor, nectar availability, concentration, and volume have also been shown to influence bumblebee visitation behavior (Cnaani et al. 2006; Makino and Sakai 2007; Dobson et al. 2008).

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