Flower size preferences of the honeybee (*Apis mellifera*) foraging on *Mimulus guttatus* (Scrophulariaceae)

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ABSTRACT

For pollinators to exert selection on floral traits, they must be able to detect and respond to differences in floral morphologies. I examined foraging preferences by the bee *Apis mellifera* (Apidae) visiting naturally occurring flowers of *Mimulus guttatus* (Scrophulariaceae). The results indicate that *A. mellifera* preferentially selects larger *M. guttatus* flowers in their foraging bouts. Since differences in flower size in *M. guttatus* have previously been shown to have a significant heritable component, I suggest that *A. mellifera* has the potential to be an important selective force in the floral morphology of *M. guttatus*.

Keywords: *Apis mellifera*, flower size, *Mimulus guttatus*, pollination biology, pollinator choice.

INTRODUCTION

The co-evolution of floral characters with pollinators has long been considered a primary cause of morphological divergence and speciation in angiosperms (Darwin, 1859). The genus *Mimulus* provides support for this notion, including species with large red flowers visited by hummingbirds; species with pink, yellow and blue flowers with bulky landing pads accessible to bees and flies; and numerous self-pollinating species that attract few or no visits to their relatively reduced flowers. Selection imposed by pollinators may be important in explaining floral diversity, but to experimentally demonstrate this phenomenon, one must show that (1) individual plants in a population vary in the flower traits they possess, (2) pollinators can be responsive to those differences, (3) those trait differences are heritable and (4) those traits are associated with fitness.

It is widely known that bees are able to discriminate between flowers with varying amounts of reward without actually visiting the flower (Heinrich, 1979; Marden, 1984; Wetherwax, 1986; Kato, 1988; Duffield *et al.*, 1993; Goulson *et al.*, 2001). Pollinators utilize a wide variety of morphological cues to distinguish between flowers, including size, colour, ultraviolet reflectance and floral symmetry (Waser, 1983; Kearns and Inouye, 1993; Conner and Rush, 1996; Møller and Sorci, 1998; Elle and Carney, 2003). Natural selection may act on those plant characters that pollinators utilize for discriminating between rewarding and

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unrewarding flowers if variation in those characters has a genetic component, and if those characters are associated with fitness.

Bee pollinators of *Mimulus guttatus* are capable of discriminating between flowers based on pollen quality and reward depletion (Robertson *et al.*, 1999). However, the cues that these pollinators utilize for finding rewarding flowers are poorly understood. Flower size may be an important indicator of reward to potential pollinators in *Mimulus*, since it has been shown to be positively correlated with pollen production (Ritland and Ritland, 1989; Robertson *et al.*, 1994; Fenster and Carr, 1997). In this study, I examine foraging preferences of the bee *Apis mellifera* visiting flowers of *M. guttatus*, and I describe a choice test for determining whether or not bees detect and discriminate between flowers of different sizes. I utilize corolla width as an indicator of flower size, as it is highly correlated with other flower size measures (Macnair and Cumbes, 1989; Carr and Fenster, 1994; Fenster and Ritland, 1994; Fenster and Carr, 1997). If honey bees are capable of distinguishing between flowers, then they may be an important selective force for floral morphology of *M. guttatus*.

MATERIALS AND METHODS

Study system

Mimulus guttatus is an herbaceous wild flower that grows along the moist banks of streams and seeps throughout western North America. Populations differ greatly with respect to the degree of outcrossing, and individual plants may produce either entirely selfed, entirely outcrossed or a mixture of both selfed and outcrossed seeds (Ritland and Ganders, 1987; Ritland, 1990; Dudash and Ritland, 1991; Dole and Ritland, 1993; Willis, 1993a). When selfed, progeny from those populations exhibit marked amounts of inbreeding depression (Ritland, 1990; Dole and Ritland, 1993; Willis, 1993a,b, 1996, 1999a,b; Latta and Ritland, 1994; Carr and Dudash, 1996). *Mimulus guttatus* is typically considered bee pollinated, and pollinators in California include *Apis mellifera, Bombus* spp. (Apidae) and *Dialictus* spp. (Halictidae) (Gardner and Macnair, 2000). Flowers produce copious amounts of pollen, which has been considered the primary reward for bee pollinators (Robertson *et al.*, 1999). However, the flowers of many California populations have a swollen floral nectary located at the base of the ovary that also produces a substantial nectar reward for pollinators (personal observation).

Previous studies have shown that *M. guttatus* has significant amounts of heritable variation for a variety of floral traits, including corolla width and length, style width and length, ovary size, ovary and pollen number, and seed yield (Robertson *et al.*, 1994; Fenster and Carr, 1997). Much of the variation in the corolla traits can be partitioned into a 'flower size' principal component (Robertson *et al.*, 1994). These traits also show a good deal of environmental plasticity, such that plants grown in favourable wet conditions produce larger and more numerous flowers, more pollen and increased seed set.

Study site and identification of pollinators

I conducted pollinator-visitation experiments from 08.00 to 12.00 h (Mountain Standard Time) on 20 April 2002 in a population of *M. guttatus* located in the California Sierra

foothills at the Don Pedro Recreation Area, Moccasin Point Campground, at Hwy 120/49 and Jacksonville Road. The study site elevation is approximately 300 m and the vegetation is predominantly oak savannah. The most common pollinators were honeybees (*Apis mellifera*), but I observed occasional visits from two species of bumblebee (*Bombus*), three species of sweat bees (Halictidae) and one species of fly (Bombyliidae). Only *A. mellifera* was abundant enough to record a significant number of visits for statistical analysis, and I observed as many as 30 honeybees foraging at any particular time. The bees were collecting both pollen and nectar, and were depositing pollen grains primarily from their thorax onto the touch-sensitive stigmas, so these bees can be considered actual pollinators rather than simply visitors.

Pollinator choice

In wet habitats, such as the one where this experiment was performed, thousands of *M. guttatus* plants grow extremely close to one another producing tens of thousands of flowers. In such circumstances, it is very difficult to monitor visitation rates of naturally occurring, individual plants or flowers because they receive relatively few visitors in a single day (personal observation). Instead of monitoring individual plants with known flower sizes, I located bees in mid-flight and measured, to the nearest 0.5 mm, the corolla width of the first flower that they probed for nectar or pollen. I then located the closest flower to the one visited by the bee (and on a separate plant), and measured and recorded the corolla width of that flower as well. 'Chosen' flowers and 'nearest neighbour' flowers were never further than 20 cm apart. This essentially constituted a choice test, where the bees 'chose' the flowers that they probed but not the 'nearest neighbour' flowers.

After recording the data from one bee's visit, I located a different bee and repeated the same measurements. Bees were not marked, so it is possible that flower choice could have been assayed more than once for some individual bees. Since there were as many as 30 bees flying in the patch at once, and since I observed many bees leave the site and new bees arrive throughout the observation period, it is likely that multiple measurements on individual bees were rare. This method, in addition to increasing the sample size of bee visits, has an added advantage in that it is fairly easy for a single field researcher to record these data. Data for corolla width for the 'visited' versus 'nearest neighbour' flowers were statistically compared for 144 observations using a paired *t*-test using the PC version of JMP (version 4.0.4).

RESULTS AND DISCUSSION

This study provides evidence that pollinators respond to differences in flower size of *Mimulus guttatus*, and preferentially select larger flowers when foraging. The corolla widths of visited flowers were significantly larger than those of nearest neighbours (paired *t*-test, $t = 2.09$, d.f. = 143, $P = 0.038$ [two-tailed *t*-test], $P = 0.019$ [one-tailed *t*-test]). The mean corolla width for the visited flowers was 2.27 ± 0.03 cm (mean ± 1 standard error), and the mean corolla width for the nearest neighbour flowers was 2.20 ± 0.03 cm.

Flower size preferences of bees have been well documented, and the fact that bees visit flowers with larger displays is not new (Lunau, 1991; Blarer *et al.*, 2002). However, since

M. guttatus is quickly being developed as a model system for answering a wide range of evolutionary questions, basic assumptions about the role of floral characters in this species need to be tested. This study is somewhat limited in that observations were made in a single day, and only on one particular species of pollinator. However, this was due to the fact that on most days, few pollinators are observed visiting nearby *M. guttatus* populations. (In an unrelated experiment, I was monitoring populations on a daily basis for the entire flowering season of 2001.) On 20 April 2002, I took advantage of a unique situation in which literally dozens of bees were foraging simultaneously at a natural population of *M. guttatus*, and I was able to record a large number of visits in a relatively short period of time. I do not suggest, however, that days on which large numbers of pollinators are present are more important in determining overall outcrossing rates of *M. guttatus*. Nor do I suggest that because *A. mellifera* is the most commonly observed pollinator that it is necessarily the most important. *Apis mellifera* is not native to California, and thus has been interacting with *M. guttatus* for a relatively short period of time. However, honeybees are now certainly an important component of the pollinator fauna of *M. guttatus* throughout most of its range. I have observed them pollinating *M. guttatus* populations ranging from northern Oregon to southern California. Since native bumblebees (*Bombus* spp.) have been shown to have similar preferences for larger flowers in a variety of other systems (Lunau, 1991; Blarer *et al.*, 2002), these relatively common visitors of *M. guttatus* may also have similar flower size preferences as observed in *A. mellifera*. Further research documenting flower choice in native bumblebee pollinators is required to verify this.

Since variation in flower size has been shown to have a significant genetic component in *M. guttatus* (Robertson *et al.*, 1994; Fenster and Carr, 1997), bee pollinators have the potential to exert directional selection towards larger flowers. Although individual *M. guttatus* flowers display the ability to autogamously self-pollinate (Dole, 1992), the seed set of non-visited flowers is substantially lower than that of open-pollinated flowers (L. Fishman and J. Willis, unpublished). *Mimulus guttatus* also exhibits extreme inbreeding depression in the field and greenhouse for many fitness components. Thus, flowers that are not visited by pollinators produce a reduced number of seeds, and those seeds exhibit marked amounts of inbreeding depression. If increased pollinator visitation results in a greater total seed set, a relatively greater proportion of outcrossed progeny and an increase in pollen dispersal, then large-flowered plants may exhibit increased fitness due to pollinator preference.

The results of this experiment suggest that honey bees may be an important selective force in the floral morphology of *M. guttatus*. Indeed, since significant heritable variation is associated with flower size, the potential for this trait to respond to selection exists. However, if other selective forces are acting such that smaller flower sizes show a selective advantage in certain environmental conditions, then other forms of selection may be acting to maintain the genetic variation associated with this trait. For instance, habitat variation in water availability may be one important factor in maintaining genetic variation in corolla size despite directional selection imposed by pollinators, since water loss can occur in plants with larger corollas (Galen *et al.*, 1999). Future work needs to be performed to positively ascertain whether or not increased pollinator visitation in natural populations results in an associated increase in fitness. If this is found to be the case, studies examining other environmental variables may be helpful in determining what selective forces are maintaining genetic variability for flower size.

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REFERENCES

Blarer, A., Keasar, T. and Shmida, A. 2002. Possible mechanisms for the formation of flower size preferences by foraging bumblebees. *Ethology*, **108**: 340–351.

Carr, D.E. and Dudash, M.R. 1996. Inbreeding depression in two species of *Mimulus* (Scrophulariaceae) with contrasting mating systems. *Am. J. Bot.*, **83**: 586–593.

Carr, D.E. and Fenster, C.B. 1994. Levels of genetic variation and covariation for *Mimulus* (Scrophulariaceae) floral traits. *Heredity*, **72**: 606–618.

Conner, J.K. and Rush, S. 1996. Effects of flower size and number on pollinator visitation to wild radish, *Raphanus raphanistrum. Oecologia*, **104**: 234–245.

Darwin, C. 1859. *The Origin of Species*. London: John Murray.

Dole, J.A. 1992. Reproductive assurance mechanisms in three taxa of the *Mimulus guttatus* complex (Scrophulariaceae). *Am. J. Bot.*, **79**: 650–659.

Dole, J.A. and Ritland, K. 1993. Inbreeding depression in two *Mimulus* taxa measured by multigenerational changes in the inbreeding coefficient. *Evolution*, **47**: 361–373.

Dudash, M.R. and Ritland, K. 1991. Multiple paternity and self-fertilization in relation to floral age in *Mimulus guttatus* (Scrophulariaceae). *Am. J. Bot.*, **78**: 1746–1753.

Duffield, G.E., Gibson, R.C., Gilhooly, P.M. *et al.* 1993. Choice of flowers by foraging honey-bees (*Apis mellifera*): possible morphological cues. *Ecol. Entomol.*, **18**: 191–197.

Elle, E. and Carney, R. 2003. Reproductive assurance varies with flower size in *Collinsia parviflora* (Scrophulariaceae) *Am. J. Bot.*, **90**: 888–896.

Fenster, C.B. and Carr, D.E. 1997. Genetics of sex allocation in *Mimulus* (Scrophulariaceae). *J. Evol. Biol.*, **10**: 641–661.

Fenster, C.B. and Ritland, K. 1994. Quantitative genetics of mating system divergence in the yellow monkeyflower species complex. *Heredity*, **73**: 422–435.

Galen, C., Sherry, R.A. and Carroll, A.B. 1999. Are flowers physiological sinks or faucets? Costs and correlates of water use by flowers of *Polymonium viscosum. Oecologia*, **118**: 461–470.

Gardner, M. and Macnair, M.R. 2000. Factors affecting the co-existence of the serpentine endemic *Mimulus nudatus* Cruuan and its presumed progenitor, *Mimulus guttatus* Fischer ex DC. *Biol. J. Linn. Soc.*, **69**: 443–459.

Goulson, D., Chapman, J.W. and Hughes, W.O.H. 2001. Discrimination of unrewarding flowers by bees: direct detection of rewards and use of repellent scent marks. *J. Insect Behav.*, **14**: 669–678.

Heinrich, B. 1979. Resource heterogeneity and patterns of movement in foraging bumblebees. *Oecologia*, **40**: 235–245.

Kato, M. 1988. Bumble bee visits to *Impatiens* spp.: pattern and efficiency. *Oecologia*, **76**: 364–370.

Kearns, C.A. and Inouye, D.W. 1993. *Techniques for Pollination Biologists*. Niwot, CO: University Press of Colorado.

Latta, R. and Ritland, K. 1994. The relationship between inbreeding depression and prior inbreeding among populations of four *Mimulus* taxa. *Evolution*, **48**: 806–817.

Lunau, K. 1991. Innate flower recognition in bumblebees (*Bumbus terrestris, B. lucorum*; Apidae): optical signals from the stamens as landing reaction releasers. *Ethology*, **88**: 203–214.

Macnair, M.R. and Cumbes, Q.J. 1989. The genetic architecture of interspecific variation in *Mimulus. Genetics*, **122**: 211–222.

Marden, J.H. 1984. Remote perception of floral nectar by bumblebee. *Oecologia*, **64**: 232–240.

Møller, A.P. and Sorci, G. 1998. Insect preference for symmetrical artificial flowers. *Oecologia*, **114**: 37–42.

Ritland, C. and Ritland, K. 1989. Variation of sex allocation among eight species of the *Mimulus guttatus* complex. *Am. J. Bot.*, **76**: 1731–1739.

Ritland, K. 1990. Inferences about inbreeding depression based upon changes of the inbreeding coefficient. *Evolution*, **44**: 1230–1241.

Ritland, K. and Ganders, F.R. 1987. Covariation of selfing rates with parental gene fixation indices within populations of *Mimulus guttatus. Evolution*, **41**: 760–771.

Robertson, A.W., Diaz, A. and Macnair, M.R. 1994. The quantitative genetics of floral characters in *Mimulus guttatus. Heredity*, **72**: 300–311.

Robertson, A.W., Mountjoy, C., Faulkner, B.E., Roberts, M.V. and Macnair, M.R. 1999. Bumble bee selection of *Mimulus guttatus* flowers: the effects of pollen quality and reward depletion. *Ecology*, **80**: 2594–2606.

Waser, N.M. 1983. The adaptive nature of floral traits: ideas and evidence. In *Pollination Biology* (L. Real, ed.), pp. 241–285. New York: Academic Press.

Wetherwax, P.B. 1986. Why do honeybees reject certain flowers? *Oecologia*, **69**: 567–570.

Willis, J.H. 1993a. Partial self-fertilization and inbreeding depression in two populations of *Mimulus guttatus. Heredity*, **71**: 145–154.

Willis, J.H. 1993b. Effects of different levels of inbreeding on fitness components in *Mimulus guttatus. Evolution*, **47**: 864–876.

Willis, J.H. 1996. Measures of phenotypic selection are biased by partial inbreeding. *Evolution*, **50**: 1501–1511.

Willis, J.H. 1999a. The contribution of male sterility mutations to inbreeding depression in *Mimulus guttatus. Heredity*, **83**: 337–346.

Willis, J.H. 1999b. The role of genes of large effect on inbreeding depression in *Mimulus guttatus. Evolution*, **53**: 1678–1691.