# AN ULTRAVIOLET FLORAL POLYMORPHISM ASSOCIATED WITH LIFE HISTORY DRIVES POLLINATOR DISCRIMINATION IN *MIMULUS GUTTATUS*[1]

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- *Premise of the study:* Ultraviolet (UV) floral patterns are common in angiosperms and mediate pollinator attraction, efficiency, and constancy. UV patterns may vary within species, yet are cryptic to human observers. Thus, few studies have explicitly described the distribution or ecological significance of intraspecific variation in UV floral patterning. Here, we describe the geographic distribution and pattern of inheritance of a UV polymorphism in the model plant species *Mimulus guttatus* (Phrymaceae). We then test whether naturally occurring UV phenotypes influence pollinator interactions within *M. guttatus*.
- *Methods:* We document UV patterns in 18 annual and 19 perennial populations and test whether UV pattern is associated with life history. To examine the pattern of inheritance, we conducted crosses within and between UV phenotypes. Finally, we tested whether bee pollinators discriminate among naturally occurring UV phenotypes in two settings: wild bee communities and captive *Bombus impatiens*.
- *Key results:* Within *M. guttatus*, perennial populations exhibit a small bulls-eye pattern, whereas a bilaterally symmetric runway pattern occurs mainly in annual populations. Inheritance of UV patterning is consistent with a single-locus Mendelian model in which the runway phenotype is dominant. Bee pollinators discriminate against unfamiliar UV patterns in both natural and controlled settings.
- *Conclusions:* We describe a widespread UV polymorphism associated with life history divergence within *Mimulus guttatus*. UV pattern influences pollinator visitation and should be considered when estimating reproductive barriers between life history ecotypes. This work develops a new system to investigate the ecology and evolution of UV floral patterning in a species with extensive genomic resources.

**Key words:** *Bombus impatiens*; bumblebee; ethological isolation; floral constancy; life history; nectar guide; Phrymaceae; pollination ecology; pollinator isolation; ultraviolet.

Floral traits shape diverse ecological and evolutionary processes within angiosperms, from interactions with pollinators and antagonists to the evolution of mating systems and reproductive isolation (Schiestl and Johnson, 2013). Pollinator-mediated selection is frequently considered the prevalent force shaping flower color, pattern, shape, and reward (Galen, 1996; Fenster et al., 2004). However, floral traits also influence interactions with antagonists such as florivores or seed predators (Irwin et al., 2003; Strauss and Whittall, 2006; de Jager and Ellis, 2014), as well as physiological performance under drought or heat stress (Schemske and Bierzychudek, 2001; Warren and

Mackenzie, 2001; Coberly and Rausher, 2003). Such complex selective landscapes are thought to drive the repeated evolution of floral polymorphisms within species (Ellis and Anderson, 2012), although nonselective processes such as gene flow and genetic drift also influence floral trait distributions (e.g., Husband and Barrett, 1992). Understanding the geographic distribution and ecological correlates of floral trait variation is a critical first step toward determining the underlying mechanisms driving floral evolution (Hansen et al., 2000; Nattero and Cocucci, 2007; Anderson and Johnson, 2008).

Most well-studied floral polymorphisms reflect the biases of human perception, involving readily observable morphological or color differences. However, more cryptic floral signals also affect pollinator behavior, including scent profiles (Saleh et al., 2006; Parachnowitsch et al., 2012), floral humidity (von Arx et al., 2012), and floral electrical fields (Clarke et al., 2013). Ultraviolet light is one such signal that is visible to insects and many vertebrates but cryptic to most mammals (Goldsmith, 1980; Burkhardt and Maier, 1989; Peitsch et al., 1992; Tovée, 1995). Many flowers reveal patterns in the ultraviolet (UV) spectrum (Kugler, 1963; Guldberg and Atsatt, 1975; Utech and Kawano, 1975; Chittka et al., 1994) that function in the attraction, constancy, and handling efficiency of insect pollinators (Kühn, 1924; Jones, 1978; Rae and Vamosi, 2013; Horth et al., 2014;

Koski and Ashman, 2014). In addition, UV-absorbing pigments can serve other important ecological functions, including protection from florivores (Gronquist et al., 2001) and UV irradiance (Koski and Ashman, 2015). Thus, as in less cryptic floral traits, UV floral patterns are likely subject to a range of biotic and abiotic selective forces that may vary throughout a species range (Anderson and Johnson, 2008; Gómez et al., 2009), and intraspecific variation in UV patterning is presumably common. However the distribution, inheritance, or ecology of naturally occurring UV floral variation has only been described within a few plant species (Cruden, 1972; Yoshioka et al., 2005; Koski and Ashman, 2013; Horth et al., 2014).

Ultraviolet floral variation within species could have important consequences for pollination ecology. Floral selectivity, in which individual pollinators temporarily specialize on a floral phenotype during foraging (Gegear and Laverty, 2005), can cause pollinator discrimination against rare floral phenotypes (Waser and Price, 1981) or generate assortative mating among commonly available morphs (Jones and Reithel, 2001; Kennedy et al., 2006; Aldridge and Campbell, 2007; Hersch and Roy, 2007; Marques et al., 2007; Hopkins and Rausher, 2012). However, assortative pollination is often leaky and is most likely to impact adaptive divergence if floral traits have a simple genetic basis and covary with other major forms of divergence (Kay and Sargent, 2009). Recently, Koski and Ashman (2015) suggested that the size of UV nectar guides may increase across latitudinal clines to shield gametes from UV irradiance; in this case, UV pattern would likely co-vary with other climate-related traits. Despite the potential for UV floral patterns to vary within species and mediate floral selectivity in insect pollinators (Horth et al., 2014; Koski and Ashman, 2014), few studies have tested the consequences of naturally occurring UV floral variation on pollinator foraging behavior. Thus, our understanding of the evolutionary consequences of this common floral signal remains limited.

Here, we describe a cryptic UV floral polymorphism in *Mimulus guttatus* DC (common monkeyflower, Phrymaceae). Previous work in this species has identified two floral UV patterns: a bilaterally symmetric nectar guide in which the entire lower petal absorbs UV ("runway" pattern; Rae and Vamosi, 2013), and a smaller nectar guide in which UV absorption is limited to the upper portion of the lower petal near the corolla throat ("bulls-eye" pattern; Bodbyl Roels, 2012). Life history is a major axis of ecological divergence within *M. guttatus* and among closely related species; annual and perennial ecotypes differ in many traits linked to a chromosomal inversion region (*DIV1*) and have been suggested as incipient species based on habitat and flowering time isolation (Lowry et al., 2008; Lowry and Willis, 2010; Friedman, 2014). Given this ecological divergence, we tested whether UV phenotype is correlated with life history within *M. guttatus* and in two closely related annual species, *M. laciniatus* Gray and *M. nasutus* Greene, which share the annual inversion arrangement (Lowry and Willis, 2010; Oneal et al., 2014). To clarify the genetic basis of this polymorphism, we performed crosses within and between UV phenotypes. Finally, we tested whether this UV polymorphism drives floral selectivity in two settings: natural populations of *M. guttatus* with wild bees, and outbred lines of *M. guttatus* with captive *Bombus impatiens*. UV floral variation could impact adaptive divergence in *M. guttatus* if UV pattern is correlated with life history strategy, has a simple genetic basis, and influences pollinator foraging behavior. Understanding the distribution and functional significance of this UV polymorphism

is a critical first step toward investigating the ecology and evolution of ultraviolet patterning within this model species for ecological genomics and speciation (Lowry et al., 2008; Wu et al., 2008; Lowry and Willis, 2010).

## MATERIALS AND METHODS

*Study system*—*Mimulus guttatus* is a widely distributed and highly polymorphic herbaceous annual or short-lived perennial that grows in moist habitats throughout western North America and is thought to be the progenitor of several closely related annual species in central California (Macnair et al., 1989; Brandvain et al., 2014; Oneal et al., 2014). Taxa in this species complex are at least partially interfertile and produce bright yellow zygomorphic flowers (Vickery, 1964). *Mimulus guttatus* has a mixed mating system (Ritland and Ritland, 1989) and is primarily pollinated by large-bodied bees, including bumblebees (*Bombus* sp.), honey bees (*Apis mellifera*), and large-bodied solitary bees (Martin, 2004; Bodbyl Roels and Kelly, 2011; Rae and Vamosi, 2013). Other insects, including small solitary bees, lepidopterans, and beetles, visit *M. guttatus* flowers but rarely contact the stigma (M. DeMarche, personal observations). Rae and Vamosi (2013) documented the runway UV pattern in *M. guttatus* populations in British Columbia and demonstrated that this UV-absorbing nectar guide is important for the attraction and orientation of wild bee pollinators. Bodbyl Roels (2012) found the smaller bulls-eye UV pattern in two populations of *M. guttatus* in California and Oregon (Table 1). UV absorption in *M. guttatus* is mediated by flavonol pigments (B. Blackman, University of Virginia, personal communication), similar to other species in this genus (e.g., Bloom and Vickery, 1973).

Given the extensive morphological and life history variation within this complex, combined with incomplete crossing barriers, taxonomic treatments of this group have variously recognized between 4 and 20 species (reviewed by Nesom, 2012). The most recent taxonomic treatment splits *Mimulus guttatus* into multiple morphological species (Nesom, 2012). However, recent evidence challenges this view by suggesting that genomic variation in this complex is partitioned geographically rather than according to ecological, life history, or morphological characters (Brandvain et al., 2014; Oneal et al., 2014; Puzey and Vallejo-Marín, 2014). For this reason, and to be consistent with other work in this complex, we treat these life history strategies as ecotypes within the broader circumscription of *M. guttatus* sensu lato (Grant, 1924).

*Distribution of the UV polymorphism*—To clarify the geographic distribution of UV patterning, we examined a total of 37 populations of *M. guttatus* throughout central California, the center of diversity for this species complex (Table 1) (Vickery, 1964). Sixteen populations were phenotyped from field-collected seed grown in the greenhouse at UC Santa Cruz, and an additional 21 populations were examined in the field. We grew at least 10 individuals, from separate maternal families, from each of 16 populations from March 2012 to February 2013. Seeds were sown in 3.5-in$^2$ square pots filled with Pro Mix HP potting soil (Premier Tech, Québec, Canada), and adult plants were repotted into 6-in round pots as required. Plants were grown to flowering under a 14 h day/10 h night schedule and received weekly treatment with Peters General Purpose 20-20-20 fertilizer (JR Peters Inc., Allentown, Pennsylvania, USA). We measured the UV phenotype of all flowering individuals (see below). To increase our geographic sampling coverage, we visited an additional 21 populations throughout central California during peak flowering (May 2013 for lower elevations, July and August 2013 for higher elevations). At each population, we measured the UV phenotype on 10–37 haphazardly selected individuals at least 1 m apart. We supplemented populations in this study with information on the UV phenotype from three additional populations of *M. guttatus* in California and Oregon from Bodbyl Roels (2012). Among populations, we tested whether UV phenotype is associated with life history (see below) using Pearson's $\chi^2$ contingency test in R v. 3.1.2 (R Development Core Team, Vienna, Austria). We excluded populations with fewer than 10 phenotyped individuals from this analysis (sample sizes were limited by availability of flowering plants in some populations; see Table 1).

*Characterization of the UV phenotype*—In all 37 populations, we assessed the magnitude and pattern of ultraviolet reflectance in each flower by comparing the reflectance spectra of the upper and lower portions of the bottom petal and a side petal. Reflectance spectra were obtained using a Jaz spectrometer

with a UV/VIS xenon light source and XSR fiber optic probe (Ocean Optics, Dunedin, Florida, USA). We confirmed the accuracy of this method by visualizing a subset of flowers using a Panasonic GH-2 digital camera modified for UV photography with a U-filter (Baader Planetarium, Mammendorf, Germany). The presence or absence of UV reflectance in the bottom portion of the lower petal is a discrete polymorphism and does not vary among flowers within individuals or among greenhouse, growth chamber, or field environments (M. DeMarche, unpublished data).

*Characterization of life history ecotypes*—We characterized each population as either annual or perennial based on plant duration in the field. Annual populations live for a single year and senesce after fruiting, whereas perennial populations live for multiple years, survive fruiting, and produce stolons or rhizomes. These life history strategies are associated with habitat differences in water availability; annual populations occur in areas with temporary water sources (e.g., seeps or meadows), and perennial populations grow near a constant water supply (e.g., coastal seeps or montane streams). Previously, common garden experiments have confirmed that life history characterizations based on duration in the field reflect consistent, genetically based differences in morphological and phenological traits (van Kleunen, 2007). Perennial populations in coastal habitats and montane habitats in the Sierra Nevada have been further recognized as distinct ecotypes from other inland perennial populations (*M. grandis* and *M. corallinus*, respectively, sensu Nesom, 2012); we note populations that belong to these ecotypes in Table 1.

***Determination of the pattern of inheritance***—We observed both UV phenotypes segregating within each of three mixed populations (see results), suggesting this UV polymorphism exhibits Mendelian inheritance. Given this observation, we tested for dominance in UV patterning by performing crosses within and between UV phenotypes. In November 2012, we performed cross pollinations between populations using individuals from the greenhouse study with known UV phenotype. We performed three crosses between UV phenotypes: Long Barn (runway) × Soda Creek (bulls-eye), Scott Creek (bulls-eye) × Upper Truckee (runway), and Eagle Meadows (bulls-eye) × Red Hills (runway) (Table 1). We also performed one cross within each UV phenotype: Eagle Meadows (bulls-eye) × Silver Fork (bulls-eye) and Upper Truckee (runway) × Red Hills (runway). In December 2012, we grew 16 progeny from each cross in two growth chambers under a 22°C 14 h day/18°C 10 h night schedule. Upon flowering, we measured the UV phenotype of 1–3 fresh flowers per individual. We also visualized UV patterns for a subset of individuals using UV photography (see above). Under the assumption that parental populations are homozygous, we expect all progeny within a cross to exhibit the same UV phenotype. However, for one cross, progeny exhibited both UV patterns (see results); in this case, we tested whether progeny exhibited the expected 1:1 ratio (under the hypothesis that the runway parent was heterozygous) with Pearson's $\chi^2$ test.

***Assessing floral selectivity***—We tested whether bee pollinators exhibit floral selectivity in response to UV phenotype in three complementary experiments. Since most populations of *M. guttatus* have a single dominant UV pattern (see Results), we tested whether pollinators would discriminate against the novel UV pattern by conducting choice trials using wild bee communities and captive *Bombus impatiens* colonies. We also took advantage of a population segregating for both UV phenotypes (see Results) to observe wild bee foraging bouts when both phenotypes are present.

*Choice trials*—To test whether bees discriminate against novel UV phenotypes, we presented bees that were actively foraging on *M. guttatus* with a choice of both UV phenotype using the interview stick method (Thomson, 1981). We used freshly cut flowers of *M. guttatus*, representing each UV phenotype, placed in microcentrifuge tubes filled with moist floral foam and affixed to the end of a 1-m pole. We presented actively foraging pollinators with this choice test by extending the pole slightly above the inflorescence currently being visited so that experimental flowers were equidistant and clearly visible to the pollinator (Thomson, 1981). When a bee entered the corolla throat of an experimental flower, we considered this a "choice" and recorded the proportion of choices for each phenotype. Bees contact the stigma as they enter the corolla throat to forage, so this visitation behavior is closely related to pollination. In both experiments, we tested whether pollinator choice depended on the familiar UV phenotype using generalized linear mixed models (GLMMs) with logit link functions in the lme4 package in the program R v. 3.1.2 (R Development Core Team, Vienna, Austria). We tested

the significance of fixed effects by comparing full and reduced models with likelihood ratio tests (LRTs).

*Wild bee communities*—From May through August 2013, we assessed pollinator discrimination in six natural populations of *M. guttatus* with a single UV phenotype (Table 1, Fig. 4). To minimize the potential for population differences in other floral traits to drive pollinator choice, we used two sets of populations to provide flowers for trials: Red Hills (runway) and Kyburz (bulls-eye), and Traverse Creek (runway) and Girard Creek (bulls-eye) (Table 1). Individuals from these four populations were grown in the UC Santa Cruz greenhouse from field-collected seed and transported to field populations during trials. For each trial, we classified bee pollinator identity as either *Apis*, *Bombus*, or solitary. In each population, all choice trails were conducted between 10:00 and 16:00 hours within a single day. The number of choice trials within each population varied depending on population size and pollinator abundance (mean = 38.5, range = 10-66). We fit GLMMs with local UV phenotype, pollinator identity, and experimental flower source as fixed effects and population as a random effect.

*Captive bee colonies*—To control for any floral traits that may differ between runway and bulls-eye populations, we took advantage of a mixed population (Tule River, Table 1) to generate outbred lines that differ in UV phenotype. In June 2013, we grew 10 individuals from each of 10 field-collected maternal families in the UCSC greenhouse and identified the UV phenotype of each flowering individual using spectrometry. Two maternal families yielded entirely runway progeny whereas eight families produced progeny of both phenotypes. Based on evidence that the runway phenotype is dominant (see Results), we cross-pollinated individuals from the two runway-only maternal families to create four outbred runway lines. We also cross-pollinated bulls-eye individuals from separate maternal families to create four outbred bulls-eye lines. This outbred design avoids any potential effects of inbreeding depression on floral traits such as size, pollen reward, or scent (Ivey and Carr, 2005). In November 2013, we grew 10 individuals per outbred line and identified the UV phenotype of each using spectrometry. Individuals from runway lines with the bulls-eye phenotype were excluded from choice trials.

We conducted choice trials using separate *Bombus impatiens* colonies (Koppert Biological Systems, Howell, Michigan, USA, Research Mini hives) reared in two sealed rooms equipped with HID metal halide grow lights (Sylvania Metalarc 400 W M59T) to provide a natural light spectrum. In each room, bees were trained on a particular UV phenotype for 3 d before choice trials. During training, bees foraged freely on 6–10 individuals of *M. guttatus*, representing 3–4 outbred lines, of a single UV phenotype. Individuals of *M. guttatus* were replaced as pollen was depleted. We uniquely marked each bee using oil-based paint markers and recorded the number of flowers visited by each bee. Bees were considered "trained" on a particular phenotype after visiting 40 flowers. Although *B. impatiens* is not a wild pollinator of *M. guttatus*, it is similar in size to closely related western bumblebee species and has been shown to be an effective pollinator of *M. guttatus* in several greenhouse studies (Arathi and Kelly, 2004; Bodbyl Roels and Kelly, 2011; Wise et al., 2011).

After training, we tested floral selectivity by presenting interview stick choice trials to bees as they foraged on *M. guttatus*. We continued to provision each room with 6–10 individuals of *M. guttatus* from 3–4 outbred lines of the trained UV phenotype. We allowed individual bees to visit at least 5–6 flowers at the start of each foraging bout before presenting them with a choice trial. Individual bees were not interviewed more than four times in a given day or more than six times total. Choice trials consisted of freshly cut *M. guttatus* flowers from outbred lines representing each UV phenotype. For each choice trial, the outbred line and position (right or left) of each phenotype was fully randomized. We also measured flower size as the corolla width of all flowers used in choice trials and calculated the size difference between choice flowers as runway corolla width minus bulls-eye corolla width. We fit GLMMs with training UV phenotype and flower size difference as fixed effects and *Bombus* individual and outbred line as random effects.

*Natural foraging bouts in a mixed population*—We tested whether bee pollinators exhibit nonrandom foraging in a population segregating for both UV phenotypes (Tule River, Table 1). We observed consecutive foraging bouts for 10 individual pollinators (eight *Bombus californicus* and two *Apis mellifera*) over 2 d (26 April 2013 and 16 May 2013). During each bout, we tracked the

visitation order and then identified the UV phenotype of consecutively visited plants of *M. guttatus*. We only included foraging bouts that encompassed multiple individuals of *M. guttatus*, resulting in a total of 60 between-plant transitions. We tested whether the overall visitation rate (i.e., preference) varied among individual pollinators following Jones (1997); we found no evidence for heterogeneous preferences ($\chi^2 = 11.45$, df = 9, $P = 0.25$), so we combined all bouts (Jones, 1997). We classified transitions between individuals of *M. guttatus* as either within the same UV phenotype or between different UV phenotypes, and used Pearson's $\chi^2$ to test whether the observed within-phenotype transition rate was significantly greater than the null expectation (indicating assortative visitation). We calculated the null expectation for within-phenotype transitions as $p^2 + (1-p)^2$ where $p$ is the frequency of the runway phenotype, assuming that both UV phenotypes are randomly distributed in space. This population occupies a relatively small clearing (~50 m$^2$) in a seasonal stream, and although we did not explicitly map the distribution of UV phenotypes, we did not observe spatial clumping with respect to UV pattern during phenotyping. Given the high dispersal ability of *Mimulus* seeds in water (Waser et al., 1982), fine-scale clustering of related individuals is unlikely. Further, wild bees often bypassed neighboring plants during foraging such that bouts were not restricted to local groups of plants. Thus, the population proportion is a reasonable approximation of the rate at which pollinators would encounter each phenotype when foraging.

Data are available from the Dryad Digital Repository (http://doi.org/10.5061/dryad.3q6n0).

## RESULTS

***Characterization of the UV phenotype***—In all populations, we found that the top and side petals of *M. guttatus* flowers exhibited the typical bee–UV green reflectance spectra (Fig. 1F), whereas UV-absorbing patches on the bottom petal were bee-green (Fig. 1E) (bee colors described by Chittka et al., 1994). UV photography confirmed that the presence or absence of UV-absorbing pigments in the lower portion of the bottom petal was a discrete polymorphism (Fig. 1). Bulls-eye and runway phenotypes were readily distinguishable by the presence or absence of characteristic peaks of ultraviolet reflectance at approximately 300 nm and 350 nm in the lower portion of the bottom petal (Fig. 1E, F). These peaks coincided with the 340 nm peak sensitivity for ultraviolet vision in bees (Peitsch et al., 1992).

In general, we found that the bulls-eye UV nectar guide was prevalent in populations of *M. guttatus* distributed along the coast of California and at mid to high elevations in the Sierra Nevada, whereas the runway nectar guide was common at lower elevations in the Central Valley, the North Coast range, and the foothills of the Sierra Nevada (Fig. 2, Table 1). This geographic distribution is largely consistent with the distribution of perennial and annual ecotypes, respectively, and we found strong evidence for an association between UV floral phenotype and life history strategy among populations in this species ($\chi^2 = 19.11$, df = 1, $P < 0.01$, $N = 29$). The bulls-eye UV pattern occurred in coastal, inland, and montane perennial populations (Table 1). Correspondingly, annual populations of *M. guttatus* and closely related annual species *M. nasutus* and *M. laciniatus* exhibited the runway phenotype (Table 1).

We found little evidence of variation in UV phenotype within populations: only 3 of 37 populations contained both phenotypes. However, the sample sizes for some populations were small (see Table 1). Within mixed populations, the proportion of runway individuals was 12.5% in Upper Truckee ($N = 24$), 73% in Yosemite Lakes ($N = 15$), and 57% in Tule River ($N = 37$). These mixed populations occured at midelevations in the Sierra Nevada and within close spatial proximity to populations of both UV phenotypes (Table 1, Fig. 2). Within mixed populations, flower size did not differ between UV floral phenotypes (*t* test: Upper Truckee: $t = 1.53$, df = 5.71, $P = 0.18$, $N = 24$; Yosemite Lakes: $t = 0.1306$, df = 8.87, $P = 0.90$, $N = 15$; Tule River: $t = -1.12$, df = 24.75, $P = 0.28$, $N = 37$).

***Inheritance of the UV phenotype***—Progeny from between-population crosses exhibited discrete variation in the presence or absence of UV-absorbing pigments in the lower portion of the bottom petal. Although some individuals exhibited low levels of UV reflectance (approx. 5–10% reflectance), UV photography confirmed that these intermediate reflectance spectra indicated variation in the concentration of UV-absorbing pigments in the lower petal rather than variation in the spatial pattern of pigment deposition (Fig. 3D). Both intermediate and runway reflectance spectra were observed among flowers of the same individuals, whereas intermediate spectra were never observed in individuals with bulls-eye flowers. For these reasons, we classified any flowers with intermediate reflectance spectra as the runway phenotype to reflect discrete variation in the spatial pattern of pigment deposition. It was clear that there was continuous variation in the concentration of UV-absorbing pigments within and among hybrid individuals with the runway phenotype (Fig. 3). However, a hierarchical cluster analysis (based on UV-range reflectance measurements) confirmed our classifications for a subset of hybrid individuals ($N = 40$), correctly grouping bulls-eye or runway/intermediate phenotypes 98% of the time.

Crosses within UV phenotype resulted in 100% progeny with the parental phenotype. The Upper Truckee (runway) × Red Hills (runway) cross produced only runway progeny ($N = 10$, Fig. 3A) whereas the Eagle Meadow (bulls-eye) × Silver Fork (bulls-eye) cross only produced bulls-eye progeny ($N = 10$, Fig. 3B). Two of the three crosses between UV phenotypes yielded entirely runway progeny, although one of these crosses produced only three flowering individuals (Eagle Meadows (bulls-eye) × Red Hills (runway): 100% runway, $N = 13$, Fig. 3C; Long Barn (runway) × Soda Creek (bulls-eye): 100% runway, $N = 3$, Fig. 3D). This supported the hypothesis that the presence of UV-absorbing pigments in the lower petal was a simple Mendelian trait, in which the runway phenotype was dominant.

The third cross (Scott Creek [bulls-eye] × Upper Truckee [runway]) produced equal proportions runway and bulls-eye progeny (55% runway, $N = 11$, Fig. 3E). The runway parent in this cross came from the mixed Upper Truckee population, and the observed ratio of runway and bulls-eye offspring was consistent with a model of simple Mendelian inheritance in which the runway parent was heterozygous ($\chi^2 = 0$, df = 1, $P = 1$).

***Floral selectivity***—*Choice trials—Wild bee communities—*Wild bee flower preference strongly depended on the local UV phenotype in natural populations of *M. guttatus* ($\chi^2 = 9.58$, df = 1, $P < 0.01$). There was no effect of pollinator identity or population pair used in trials (pollinator: $\chi^2 = 3.31$, df = 2, $P = 0.19$; population pair: $\chi^2 = 0.45$, df = 1, $P = 0.50$). Wild bees foraging on runway populations of *M. guttatus* exhibited a 59% average preference for the runway phenotype (95% CI: 51–67%), whereas bees foraging on bulls-eye populations exhibited a 66% average preference for the bulls-eye phenotype (95% CI: 53–77%) (Fig. 4).
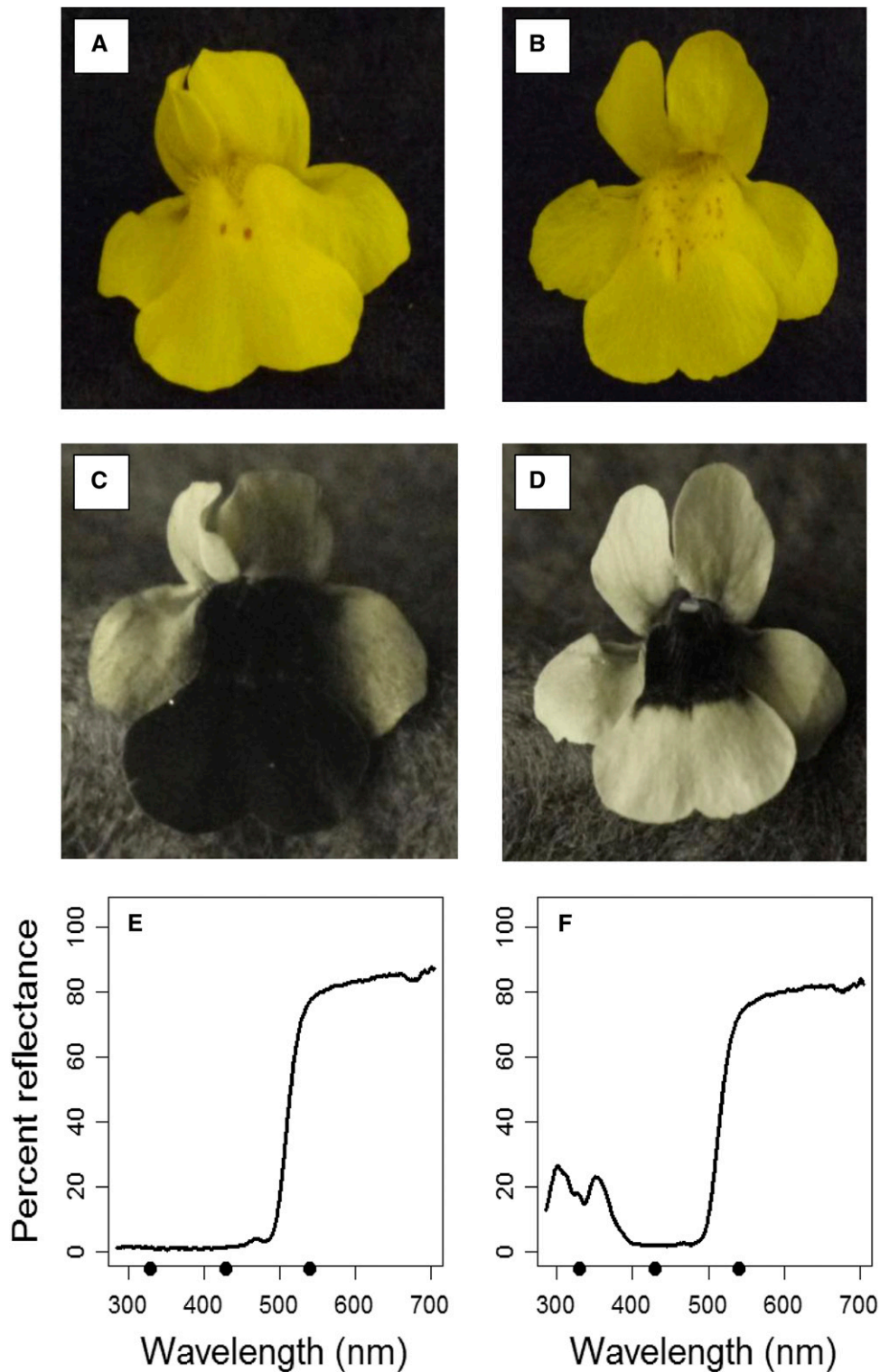
Fig. 1. *Mimulus guttatus* exhibits a discrete ultraviolet floral polymorphism. Images of runway (left) and bulls-eye (right) flowers in the visible (A, B) and ultraviolet (C, D) spectrum. Characteristic reflectance spectra for UV-absorbing (bee-green, E) and UV-reflecting (bee UV-green, F) portions of the flower. Dots on *x*-axis represent wavelengths of peak sensitivity in bee vision.

*Captive bee colonies*—We marked, trained, and interviewed 47 individual *Bombus impatiens* for a total of 140 choice trials. Captive bee flower preference depended on the UV phenotype used for training ($\chi^2 = 17.35$, df = 1, $P < 0.01$). *Bombus impatiens* trained on the runway phenotype exhibited a 68% preference for runway flowers (95% CI: 54–78%), whereas *B. impatiens* trained on the bulls-eye phenotype exhibited a 69% preference for bulls-eye flowers (95% CI: 51–83%) (Fig. 5).
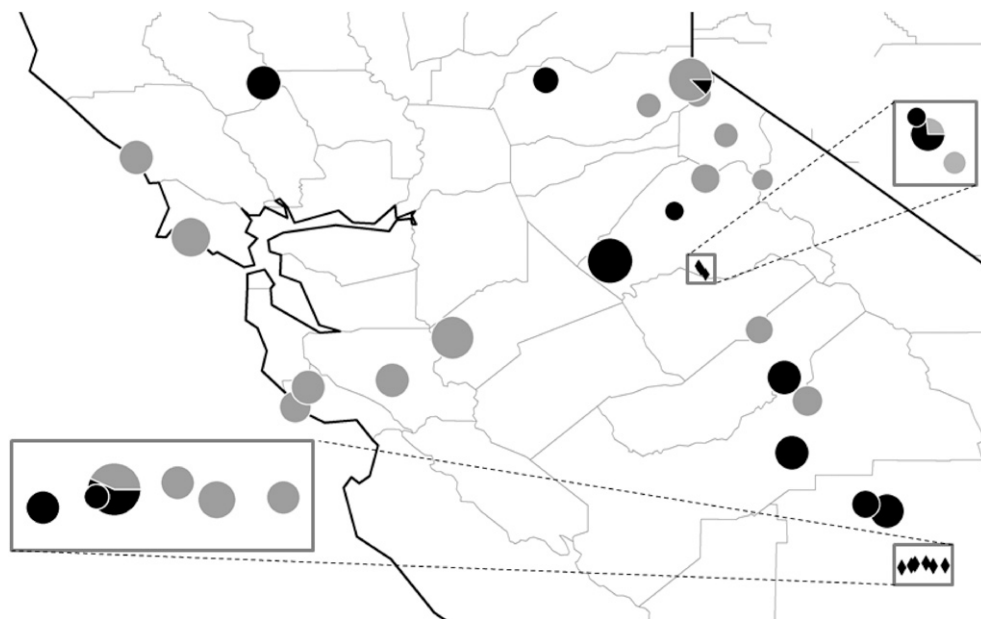
Fig. 2. Locations and UV phenotype of 31 populations of *Mimulus guttatus* in central California. Pie charts show the frequency of runway (black) and bulls-eye (gray) individuals in each population, where the size of the chart is proportional to sampling effort ($N = 5$–37). Some populations in Table 1 not shown here for clarity.

There was no effect of relative flower size on pollinator discrimination (Flower size: $\chi^2 = 0.6543$, df = 1, $P = 0.42$).

*Natural foraging bouts in a mixed population*—We observed 42 transitions between plants with the same UV phenotype and 18 transitions between plants with different UV phenotypes. We estimated that 70% of between-plant transitions occurred within UV phenotype during foraging bouts (95% CI: 57–81%, $N = 60$). This was significantly different from the point estimate of the null expectation (51%) ($\chi^2 = 7.94$, df = 1, $P < 0.01$), suggesting assortative visitation with respect to UV pattern in a mixed population. However, this was not significantly different from the 61% null expectation under the upper confidence limit for the proportion of the runway phenotype in this population ($\chi^2 = 1.85$, df =1, $P = 0.17$).

## DISCUSSION

Ultraviolet nectar guides are common within angiosperms and serve important functions in the attraction and efficiency of insect pollinators (Utech and Kawano, 1975; Chittka et al., 1994). Experimental manipulations of UV patterns in wild and artificial flowers have demonstrated that UV signals can influence pollinator preference and constancy (Jones and Buchmann, 1974; Rae and Vamosi, 2013; Horth et al., 2014; Koski and Ashman, 2014). Yet empirical evidence based on naturally occurring UV floral variation within species remains scarce. Jones (1978) first suggested that UV floral patterns cause pollinator isolation between closely related species of *Cercidium* in sympatry. More recently, there has been some observational evidence that UV nectar guide variation drives assortative mating in *Brassica rapa* (Yoshioka et al., 2005). In this study, both wild and captive bees discriminate against novel, naturally occurring UV patterns. Learned pollinator discrimination can generate stabilizing selection within populations, preventing the invasion of novel floral phenotypes (Levin, 1972; Waser and Price, 1981; Owen and Bradshaw,

2011). In most populations of *M. guttatus*, we only find evidence of a single UV phenotype, as expected if pollinators discriminate against rare UV patterns. However, our sampling design prioritized geographic coverage rather than extensive sampling within populations, and the alternate UV phenotype could be present at low frequencies in additional populations. The fate of mixed populations will depend on the strength and pattern of floral selectivity when both UV phenotypes are abundant. We find preliminary evidence that naturally foraging bees exhibit fewer transitions between UV patterns than expected based on the population proportion. Moreover, the occurrence of mixed populations at elevational boundaries between annual and perennial ecotypes suggests that perhaps they are maintained by dispersal from surrounding populations.

The evolutionary consequences of floral polymorphisms depend on the genetic basis of the underlying floral traits (Schiestl and Johnson, 2013). Pollinator isolation can evolve rapidly when floral traits are determined by few genes of large effect, especially if they exhibit dominance (Bradshaw et al., 1995; Schemske and Bradshaw, 1999; Hopkins and Rausher, 2012). Conversely, intermediate hybrid floral phenotypes can act as pollination "bridges" to facilitate introgression (Wesselingh and Arnold, 2000; Ippolito et al., 2004; Hersch and Roy, 2007). The presence or absence of UV pigmentation in the lower petal of *M. guttatus* appears to have a simple genetic basis in which the runway phenotype is dominant, although examination of F2 progeny is necessary to confirm this hypothesis. A simple genetic basis is supported by the lack of intermediate UV phenotypes in either mixed populations or outbred lines. The dominance of the runway phenotype suggests that floral selectivity may be asymmetrical, in which pollen transfer is more likely to occur between hybrids and individuals with the runway phenotype.

The potential for UV pattern to contribute to pollinator isolation in *M. guttatus* will depend on the strength of floral selectivity in sympatry and the pattern of pollen carryover. Assortative mating due to floral selectivity is rarely strong enough to drive speciation singly (Chittka et al., 1999), and our finding of incomplete

TABLE 1. List of *Mimulus guttatus* populations, ordered from northwest to southeast.

| Taxon | Population | UV phenotype | N | Elevation (m a.s.l.) | Latitude | Longitude | Life-history ecotype |
|---|---|---|---|---|---|---|---|
| *M. guttatus* | Oswald State Park [a, b] | Bulls-eye | 10 | 30 | 45.76 | −123.96 | Coastal perennial |
| | Iron Mountain [a, b] | Runway [c] | 50 | 650 | 44.52 | −122.19 | Annual |
| | Upper Truckee | Mixed | 24 | 1911 | 38.877 | −120.004 | Perennial |
| | Traverse Creek | Runway | 9 | 681 | 38.873 | −120.818 | Annual |
| | McLaughlin | Runway | 15 | 625 | 38.858 | −122.407 | Annual |
| | Grass Lake | Bulls-eye | 9 | 2308 | 38.795 | −119.961 | Montane perennial |
| | Kyburz [b] | Bulls-eye | 11 | 1371 | 38.768 | −120.290 | Perennial |
| | Girard Creek | Bulls-eye | 12 | 1515 | 38.731 | −120.240 | Perennial |
| | Silver Fork [b] | Bulls-eye | 3 | 1959 | 38.664 | −120.219 | Montane perennial |
| | Forest Meadow [b] | Bulls-eye | 6 | 2224 | 38.634 | −120.245 | Montane perennial |
| | Kinney Creek | Bulls-eye | 8 | 2508 | 38.564 | −119.804 | Montane perennial |
| | Goat Rock | Bulls-eye | 15 | 25 | 38.438 | −123.122 | Coastal perennial |
| | Eagle Meadows | Bulls-eye | 14 | 2046 | 38.320 | −119.920 | Montane perennial |
| | Soda Creek | Bulls-eye | 6 | 2632 | 38.312 | −119.599 | Montane perennial |
| | Bald Mountain | Runway | 5 | 1693 | 38.137 | −120.094 | Annual |
| | Long Barn [b] | Runway | 3 | 1525 | 38.100 | −120.123 | Perennial |
| | Pt. Reyes [a] | Bulls-eye | 20 | 10 | 37.990 | −122.816 | Coastal perennial |
| | Peoria Basin [b] | Runway | 10 | 293 | 37.933 | −120.520 | Annual |
| | Red Hills | Runway | 26 | 313 | 37.857 | −120.457 | Annual |
| | Big Oak Flat | Runway | 6 | 1225 | 37.829 | −119.958 | Annual |
| | Yosemite Lakes | Mixed | 15 | 1128 | 37.811 | −119.946 | Perennial |
| | Big Creek | Bulls-eye | 7 | 1257 | 37.782 | −119.919 | Perennial |
| | Fish Camp | Bulls-eye | 10 | 1638 | 37.469 | −119.616 | Perennial |
| | Frank Raines | Bulls-eye | 23 | 300 | 37.425 | −121.343 | Annual |
| | Minarets | Runway | 15 | 824 | 37.202 | −119.475 | Annual |
| | Kirby Canyon | Bulls-eye | 15 | 135 | 37.187 | −121.682 | Annual |
| | Ciatana Creek [b] | Runway | 15 | 533 | 37.172 | −119.515 | Annual |
| | Lucille's Court | Bulls-eye | 15 | 258 | 37.147 | −122.155 | Annual |
| | Pine Ridge | Bulls-eye | 12 | 1585 | 37.070 | −119.345 | Montane perennial |
| | Scott Creek | Bulls-eye | 13 | 4 | 37.036 | −122.228 | Coastal perennial |
| | Trimmer Springs | Runway | 15 | 179 | 36.780 | −119.433 | Annual |
| | Dry Creek | Runway | 10 | 244 | 36.489 | −119.017 | Annual |
| | Three Rivers | Runway | 15 | 263 | 36.451 | −118.897 | Annual |
| | Sequoia Monument | Bulls-eye | 15 | 1052 | 36.160 | −118.677 | Perennial |
| | Coffee Canyon [b] | Runway | 14 | 655 | 36.154 | −118.739 | Annual |
| | Tule River | Mixed | 37 | 602 | 36.153 | −118.741 | Annual |
| | Long Canyon | Runway | 8 | 510 | 36.146 | −118.759 | Annual |
| | Shady Way | Bulls-eye | 15 | 1524 | 36.145 | −118.569 | Perennial |
| | Wilson Creek | Bulls-eye | 19 | 1318 | 36.143 | −118.637 | Perennial |
| | Springville | Runway | 15 | 320 | 36.135 | −118.814 | Annual |
| *M. nasutus* | Bald Mountain | Runway | 6 | 1693 | 38.137 | −120.094 | Annual |
| | Minarets | Runway | 5 | 824 | 37.202 | −119.475 | Annual |
| | Wishon | Runway | 6 | 754 | 36.162 | −118.710 | Annual |
| *M. laciniatus* | Bald Mountain | Runway | 10 | 1693 | 38.137 | −120.094 | Annual |

[a] Data on UV phenotypes are from Bodbyl Roels (2012); elevation and location were approximated based on description therein.

[b] Not included in Fig. 2 to improve map clarity.

[c] Some individuals from highly inbred lines exhibited aberrant UV phenotypes (see Bodbyl-Roels [2012] for details).

Populations with fewer than 10 individuals were excluded from statistical analyses.

pollinator discrimination supports this. Yet floral selectivity can act in concert with other barriers to contribute to reproductive isolation (Marques et al., 2007; Yang et al., 2007; Kay and Sargent, 2009; Hopkins and Rausher, 2012), particularly early on in the speciation process when individual barriers are incomplete. We find that UV phenotype is strongly correlated with life history divergence within *M. guttatus*. Any pollinator isolation between UV phenotypes would be correlated with other reproductive barriers, including habitat and flowering time isolation, operating between life history ecotypes. Assortative pollination with respect to UV pattern could impact adaptive divergence in this system, though further observations of pollinator foraging behavior and pollen transfer rates are necessary to test the strength of pollinator isolation relative to other barriers.

The strong association between UV nectar guide pattern and life history divergence in the *M. guttatus* species complex suggests further hypotheses for UV floral evolution in this system. Annual populations of *M. guttatus* and closely related annual species, including *M. laciniatus* and *M. nasutus*, share a chromosomal inversion polymorphism relative to perennial *M. guttatus* (*DIV1*) (Lowry and Willis, 2010; Oneal et al., 2014). Given the lack of genomic divergence between annual and perennial ecotypes outside the inversion (Oneal et al., 2014), the tight correlation between UV pattern and life history in this study suggests a link to the *DIV1* inversion region. Habitat or life history differences between annuals and perennials could also shape UV floral trait evolution. Floral pigments perform a range of ecological functions, including responses to drought or heat stress (Strauss and Whittall, 2006; Whittall and Carlson, 2009) and defense from florivores (Gronquist et al., 2001). Annual taxa in the *M. guttatus* species complex have repeatedly adapted to physiologically stressful habitats, including geysers,
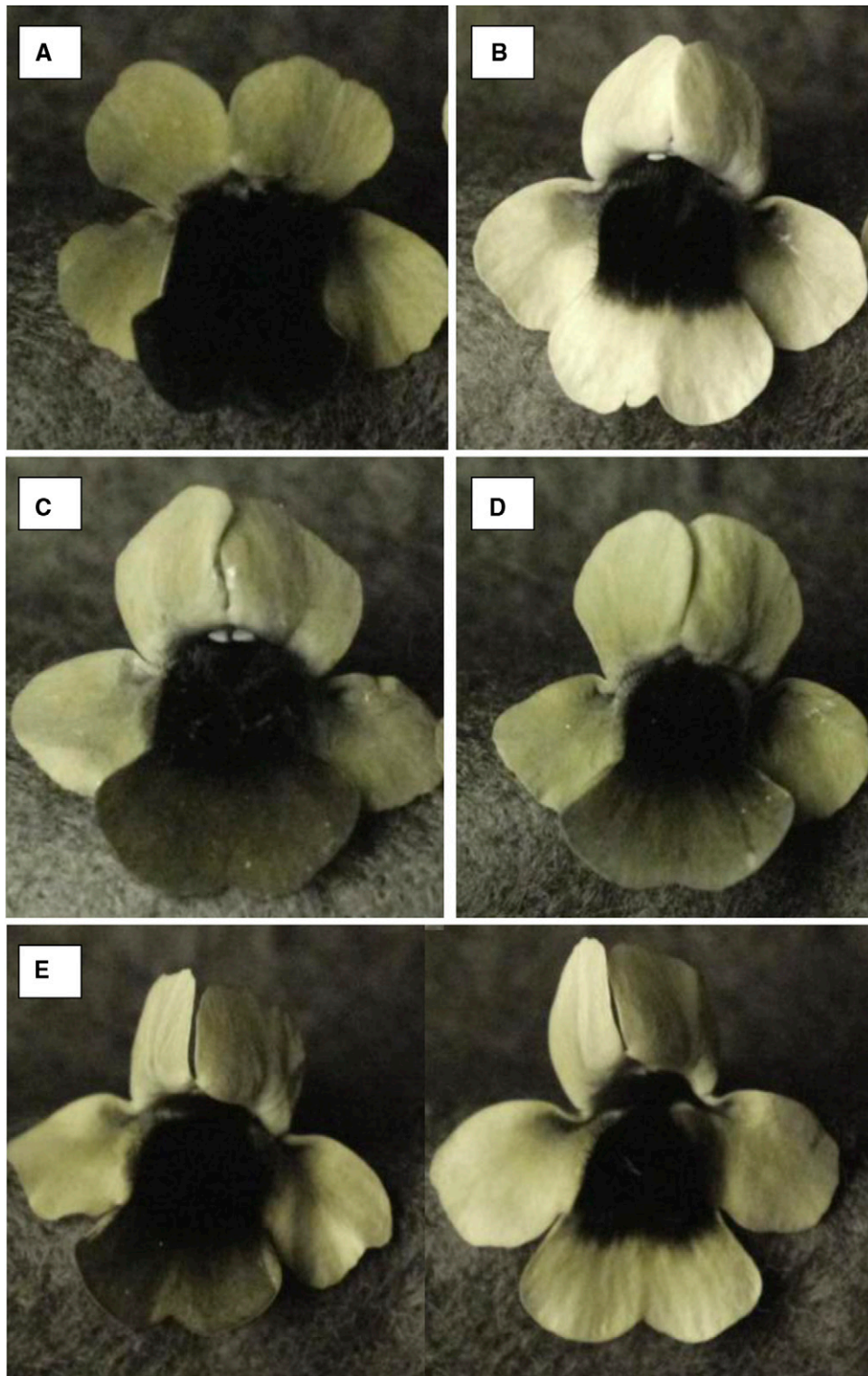
Fig. 3. Characteristic UV floral patterns in progeny from between-population crosses. (A) Upper Truckee (runway) × Red Hills (runway), (B) Eagle Meadows (bulls-eye) × Silver Fork (bulls-eye), (C) Eagle Meadows (bulls-eye) × Red Hills (runway), (D) Long Barn (runway) × Soda Creek (bulls-eye), (E) Scott Creek (bulls-eye) × Upper Truckee (runway).

copper mine tailings, and serpentine soils (Wu et al., 2008); increased production of UV-absorbing pigments in annuals could be related to physiological adaptations to stress. Moreover, perennial populations of *M. guttatus* contain higher leaf concentrations of defensive compounds than annual populations, suggesting that perennial and annual ecotypes may differ in defense vs. avoidance strategies (Holeski et al., 2013). *Mimulus guttatus* interacts with a suite of florivorous insects (Meindl
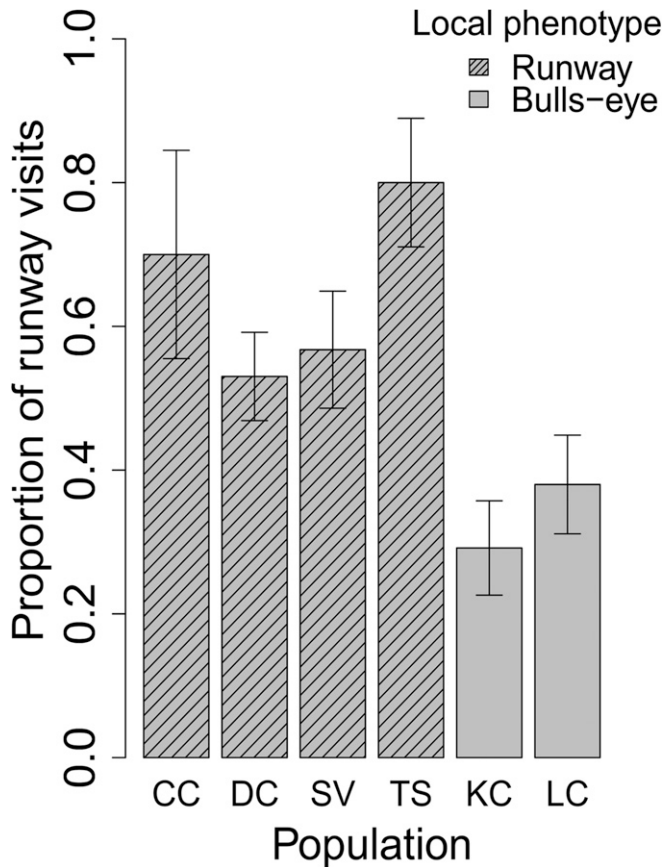
Fig. 4. Wild bees demonstrate preference for the local UV phenotype in field choice trials. Data are the proportion of visits to the runway phenotype (±SE) in six populations of *M. guttatus*. Population information is provided in Table 1. *Abbreviations:* Coffee Canyon (CC), Dry Creek (DC), Springville (SV), Trimmer Springs (TS), Kirby Canyon (KC), and Lucille's Court (LC).



Fig. 5. Captive *Bombus impatiens* discriminate against the novel ultraviolet phenotype. Data are the mean proportion of visits (±SE) to the runway flower in choice trials for bees trained on runway and bulls-eye phenotypes. Dashed line represents the null hypothesis of 50% visitation.

et al., 2013), and UV floral signals could play a role in mediating these interactions. Future research should test whether increased floral UV pigmentation confers any physiological or defensive advantages that may indicate a role for nonpollinator selection in shaping the distribution of this trait. Interestingly, our observations conflict with the recent suggestion that UV irradiance drives the evolution of larger UV patterns, as found in *Argentina anserina* (Koski and Ashman, 2015). Rather, we find a smaller bull's-eye pattern in high elevation populations of *M. guttatus*, where UV irradiance is greatest, underscoring the need for a broader understanding of UV floral variation within additional plant species to gain general insights.

In summary, the pattern of UV absorption in the nectar guides of *Mimulus guttatus* is a discrete, geographically widespread polymorphism that is associated with life history divergence in central California. Bee pollinators discriminate against novel UV patterns and exhibit some floral constancy in a mixed population, suggesting that UV floral variation within species can shape pollinator foraging behavior. The association between UV pattern and life history divergence provides a potential link for pollinator discrimination to influence adaptive divergence, though estimates of pollen carryover are necessary to test this prediction. Finally, crosses support a model of simple Mendelian inheritance for this trait, in which the runway phenotype is dominant. Taken together, this work highlights the potential for
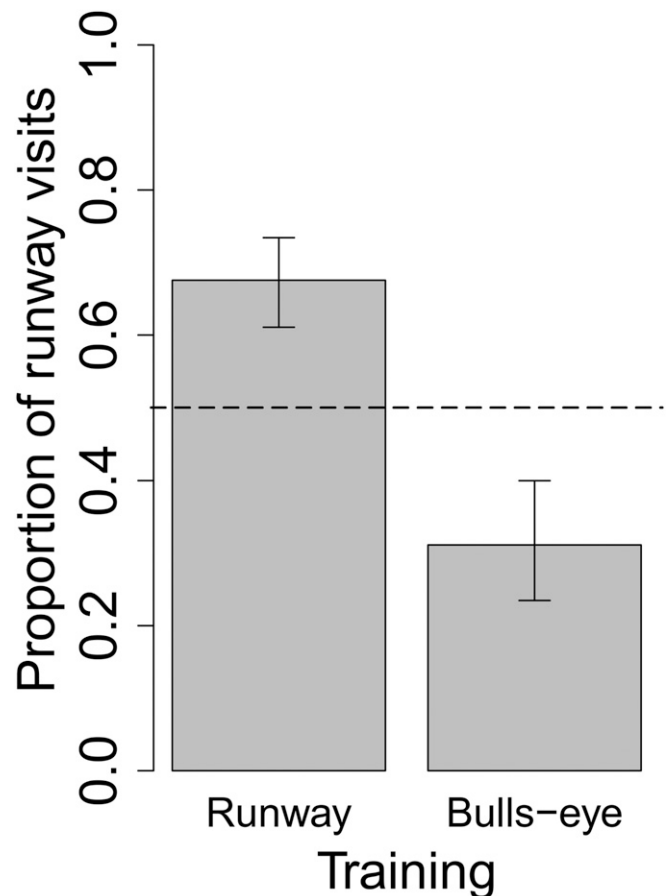
UV floral patterns to vary within species and influence local plant–pollinator interactions throughout a species' range.

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