# Flower color influences insect visitation in alpine New Zealand

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*Abstract.* Despite a long-standing belief that insect pollinators can select for certain flower colors, there are few experimental demonstrations that free-flying insects choose between natural flowers based on color. We investigated responses of insect visitors to experimental manipulations of flower color in the New Zealand alpine. Native syrphid flies (*Allograpta* and *Platycheirus*) and solitary bees (*Hylaeus* and *Leioproctus*) showed distinct preferences for visiting certain flower species. These responses were determined, in part, by flower color, as insects also responded to experimental manipulations of visible petal color in 7 out of 11 tests with different combinations of flower species and insect type. When preferences were detected, syrphid flies chose yellow over white petals regardless of flower species, whereas *Hylaeus* chose white over yellow *Ourisia glandulosa*. In some cases, the strength and direction of color preference depended on the context of other floral traits, in which case the response usually favored the familiar, normal combination of traits. Syrphid flies also visited in response to floral morphological traits but did not show preference based on UV reflectance. The unusually high preponderance of white flowers in the New Zealand alpine is not explained by complete generalization of flower color choice. Instead, the insect visitors show preferences based on color, including colors other than white, along with other floral traits. Furthermore, they can respond in complex ways to combinations of floral cues, suggesting that traits may act in nonadditive ways in determining pollinator visitation.

*Key words: alpine plants; bee;* Brachyglottis; Brachyscome; *flower color; New Zealand;* Ourisia; *pollinator preference; syrphid fly; UV.*

## INTRODUCTION

Flower colors have been attributed to natural selection by animal pollinators at least since the time of Darwin. In his 1862 book on orchids, Darwin (1862) remarked that "the white color of the conspicuous flower, and the strong sweet odor emitted at night, all show that this plant depends for its fertilization on the larger nocturnal Lepidoptera." In line with this argument, the traditional notion of pollinator syndromes has included color as one floral feature that is associated with particular kinds of pollinators (Faegri and van der Pijl 1966, Baker and Hurd 1968). An association of flower color and pollinator type does not necessarily have to result from an innate preference of a particular animal related to its visual perception. It could instead reflect a learned association between color and other features of the flower such as quantity of rewards (Menzel 1979). An alternative hypothesis to pollinator-mediated selection is that flower color could be selected due to pleiotropic effects on other traits. Anthocyanins are the most common floral pigments, and their expression in flowers is often correlated with pigmentation in vegetative tissue. This link can result in indirect selection on flower color because of vegetative effects on heat tolerance, drought tolerance, and production of defensive compounds against herbivores (reviewed by Strauss and Whittall 2006).

One of the challenges in testing whether pollinators select for flower color is disentangling the effect of flower color vs. effects of other correlated plant traits on pollinators (Mitchell-Olds and Shaw 1987). Three experimental approaches have been used: construction of model flowers out of other materials (e.g., Johnson and Dafni 1998), genetic manipulation of flower color (e.g., Bradshaw and Schemske 2003), and phenotypic manipulation (e.g., Waser and Price 1983). Each of these approaches has its own advantages and disadvantages (Raguso 2006), with the last method providing a relatively simple way to examine the effect of color, while keeping other traits of real flowers constant in a realistic presentation (Campbell 2009). Although Clements and Long (1923) pioneered this approach decades ago; only a few studies have since followed suit. The more recent studies include phenotypic manipulations of the human-visible color in *Delphinium* and *Ipomopsis* (reviewed by Rausher 2008), and of the UV-signal in *Eulophia* (Peter and Johnson 2008). Most of these experimental studies have focused on plants pollinated by a small subset of pollinator types: hummingbirds, bumble bees, halictid bees, and hawkmoths. Less is known about responses to flower color by other types of

solitary bees and by flies (Kay 1976), even though they are important pollinators on a worldwide basis (Kearns 1992, Lazaro et al. 2008). Whereas some aspects of color perception by flies have been elucidated (Arnold et al. 2009) and responses to model flowers noted (Johnson and Dafni 1998), behavioral responses to color under field conditions have not been demonstrated with phenotypic or genetic manipulations of flowers.

In this study we examined responses of flies and solitary bees to phenotypic manipulations of flower color and other floral traits in the New Zealand alpine zone. This habitat provides an extreme test of the role of pollinators in selecting for flower color, as it has one of the highest percentage of white flowers anywhere in the world, with 70% of species white or near-white (Wardle 1978). Many of the species with white flowers have brightly colored congeners elsewhere, including, in some cases, elsewhere in New Zealand or Australia (Lloyd 1985). This preponderance of white petals has been widely attributed to the absence of social bees and to a presumed lack of color preferences by the native pollinators (Wardle 1978, Godley 1979), which are mainly flies and solitary bees (Primack 1983, Donovan 2007). The prevailing view is that the native insects are extremely generalized in their flower choice, far more so than social bees (Lloyd 1985, Newstrom and Robertson 2005), even though the latter can also be generalized, with few restrictions to particular colors (Gumbert et al. 1999). The most extreme expression of this idea is the suggestion that the insect visitors to New Zealand alpine flowers lack color selectivity entirely (Wardle 1978). A modified version of this hypothesis allows for insects to prefer white and/or yellow, as yellow is the second most common color, but not red, blue, or purple, as these colors are largely missing (Heine 1938). These hypotheses are entirely untested as no detailed studies of pollinator preferences have been carried out in this habitat. The few studies of pollination in the New Zealand alpine include one community-wide study of insect visitors caught on flowers (Primack 1983) and studies of dependence on outcrossing vs. selfing (Garnock-Jones 1976, Schlessman 1986). Here we examined preferential visitation by insects to flowers of a certain phenotype, hereafter referred to with the shorthand "preference." Such "preference" could be the result of perceptual capability, detectability against the background, innate preference, or learned preference. We tested the hypothesis that insect visitors in this habitat lack preference based on flower color, compared the strength of any preference based on color to preference based on other floral traits, and examined whether flower color and other traits, especially petal size, interact in their effects on visitation. Such nonadditive, synergistic effects on pollinator visitation may be common (Raguso 2006), but are rarely examined (Herrera 2001, Raguso and Willis 2005).

We asked the following specific questions: (1) Do insect visitors in the New Zealand alpine show preferences between flower species? (2) Do the insects use petal color, UV reflectance, and/or other floral traits as cues in making these choices? (3) Do different floral cues interact in their effects on visitation?

## Methods

### Study system

Our study site was at the Rastus Burn Recreation Reserve within the Remarkables Range in Otago, New Zealand. Experiments were conducted between 1650 m and 1750 m elevation along the trail from the Remarkables Ski Area (45°03′11″ S, 168°48′46″ E) to Lake Alta. This area is mainly an alpine herb field with tussock grasses and a diversity of small alpine herbs and cushion plants (Mark and Bliss 1970, Patrick et al. 1992).

We focused on the following plant species: *Ourisia glandulosa* Hook. f. (Plantaginaceae), *Ourisia caespitosa* Hook. f. (Plantaginaceae), *Ranunculus gracilipes* Hook. f. (Ranunculaceae), *Brachyscome sinclairii* Hook. f. (Asteraceae), and *Brachyglottis bellidioides* (Hook f.) B. Nord. (Asteraceae) (see Plate 1; color plate in Appendix A: Fig. A1). These species were chosen because they were abundant in the area and self-incompatible (Bischoff 2008). As such, they likely all depend on pollinators to transfer pollen.

To assess insect preferences, we constructed experimental arrays of natural or manipulated flowers of increasingly subtle trait differences (Table 1). The following species pairs represented a natural gradient in subtlety of trait differences: (A) white-flowered *O. glandulosa* and yellow-flowered *R. gracilipes,* which differ not only in flower color but also markedly in flower morphology; (B) two members of the Asteraceae with similar size inflorescences, but one with white-ray florets (*B. sinclairii*) and one with yellow-ray florets (*B. bellidioides*); (C) two species of *Ourisia* that were more similar, except that *O. caespitosa* has a larger yellowish spot in the throat of the otherwise white flower and shorter upper petals (Appendix B). Reflectance spectra for pairs A and B show that in both cases the two species have flower colors distinguishable by bees in hexagon color space (Appendix C).

### General experimental methods

All experimental arrays were set up near (usually 5–10 m away), but not in, patches of natural flowers of those same species. Thus, the local community context (Hersch and Roy 2007) always included the species being tested, and on a larger spatial scale included mainly white and yellow flowers. Each experimental array contained 16 cut flowers (except for the UV manipulations) placed in 2 mL micro-centrifuge tubes filled with water. The tubes were placed against a uniform green background, generally into nonflowering mats of cushion plants such as *Drachophyllum muscoides* or *Psycrophila obtusa*, and arranged in a 4 × 4 square with 10-cm spacing between flowers (Appendix A).

TABLE 1. Experimental arrays observed for the three species pairs.

| Experiment | Species | Experimental manipulation | Hours observed |
|---|---|---|---|
| A) *Ourisia glandulosa* (gullet shape, white petals) and/or *Ranunculus gracilipes* (dish, yellow petals) | | | |
| A1 | *O. glandulosa/R. gracilipes* | none | 6.0 |
| A2 | *O. glandulosa* | color (yellow/white) | 8.5 |
| A3 | *R. gracilipes* | color (yellow/white) | 4.0 |
| A4 | *O. glandulosa/R. gracilipes* | morphology (white) | 6.0 |
| A5 | *O. glandulosa/R. gracilipes* | morphology (yellow) | 4.0 |
| B) *Brachyscome sinclairii* (white rays) and/or *Brachyglottis bellidioides* (yellow rays) | | | |
| B1 | *B. sinclairii/B. bellidioides* | none | 8.0 |
| B2 | *B. sinclairii* | color (yellow/white) | 8.5 |
| B3 | *B. bellidioides* | color (yellow/white) | 3.0 |
| B4 | *B. sinclairii/B. bellidioides* | morphology (white) | 3.5 |
| B5 | *B. sinclairii/B. bellidioides* | morphology (yellow) | 7.5 |
| B6 | *B. sinclairii/B. bellidioides* | factorial | 6.0 |
| B7 | *B. sinclairii* | paint control | 5.0 |
| B8 | *B. bellidioides* and *D. lyalli* | UV block | 7.0 |
| C) *O. glandulosa* and/or *O. caespitosa* | | | |
| C1 | *O. glandulosa/O. caespitosa* | none | 10.5 |
| C2 | *O. glandulosa* | cut petals | 5.5 |
| C3 | *O. glandulosa* | yellow throat | 4.5 |
| C4 | *O. glandulosa* | cut petals/yellow throat | 7.5 |

Tubes were numbered from 1 to 16 for observation, with flowers assigned to tubes at random. An individual array was observed for 0.5–2.5 h, depending on insect activity, except that if no visitors were recorded during the first 30 min, the array was not observed further and was eliminated from the data set. Only visitors that were foraging for nectar and/or pollen on the flower were counted. For each insect that entered an array, we recorded the sequence of flowers visited until the insect left and either visited a flower outside of the array or disappeared from sight. Each such sequence is hereafter called a foraging bout and was treated as the experimental unit in our statistical analysis. As individual insects were not marked, it is possible that some insects made multiple foraging bouts in the same observation session. However, all experiments were replicated with multiple observation sessions (mean of four) using different flowers, and we observed up to six different individuals foraging simultaneously, reducing the extent of this nonindependence. The four most common insect visitors were: *Allograpta* spp. (Diptera: Syrphidae), *Platycheirus* spp. (Diptera: Syrphidae), *Hylaeus matamoko* (Hymenoptera: Colletidae), and *Leioproctus* spp. (Hymenoptera: Halictidae). Four species of *Allograpta*, two species of *Platycheirus*, and two species of *Leioproctus* have been collected on these flower species (Bischoff 2008), but were not distinguished to species during our observations. Other insect species (mostly muscid and tachinid flies) were usually uncommon visitors and were analyzed as a group. All insect species observed at the arrays normally visit those flower species at the site.

For most experiments, we analyzed two variables for each category of insect, the kind of flower first visited upon entering the array, and the proportion of visits made to a given flower species during the foraging bout (only for bouts in which the insect visited a string of $\geq 2$ flowers). The first variable was compared to the null hypothesis that both species were visited equally, using a likelihood ratio test. The second variable was arcsin-square-root-transformed to improve normality and then compared to the null hypothesis of 0.5 using a one-sample $t$ test. For a given insect type, there were always fewer of these foraging strings than the total flowers offered in that type of array, so an insect bout could be used as an independent unit of replication without inflating degrees of freedom. Results are reported only if there were $\geq 10$ foraging bouts. Reported significance values are uncorrected for multiple comparisons (see Moran 2003), but since up to five insect types were tested for each experiment, all values of $P < 0.01$ can be considered significant even with Bonferroni adjustment. In cases where $0.01 < P < 0.05$, we also report whether the result was significant using sequential Bonferroni. For some experiments, we conducted additional statistical analyses from the plant perspective (as noted under individual sections). All analyses employed SAS version 9.1 (SAS Institute 2006).

Observations were made between 21 January–20 February 2008 and 11 January–19 February 2009. We chose times between 10:30 and 17:30 with relatively warm ($>10°C$), sunny weather to maximize insect activity. In total, we observed 2202 insect foraging bouts (including those where the insect visited a single flower) in 105 h of observation at 65 experimental arrays.

### Natural preferences

To assess natural preferences within each species pair, insect visitors were offered arrays containing eight unmanipulated flowers (or inflorescence flower heads in the case of Asteraceae) of each of the two species (Table 1: A1, B1, C1).

*Petal color arrays*

Two of the flower species pairs (*R. gracilipes/O. glandulosa* and *B. sinclairii/B. bellidioides*) differed in petal color, with one species mainly white and the other yellow. To determine if insect visitors respond to this color difference, we constructed arrays in which all flowers were of a single species, with half painted titanium white and half painted yellow (to resemble the yellow-flowered species in their pair; Table 1: A2, A3, B2, B3). We used water-based acrylic paints (Waser and Price 1983, Melendez-Ackerman and Campbell 1998) applied with a fine brush, and checked the accuracy of the color match to the model species using reflectance spectrometry. These paints can be used to match spectra in the visible range, but they block UV reflectance (Appendix D: Fig. D1-A; Pohl 2008). For this reason and because we did not have routine access to a spectrometer capable of UV measurements, for most of our experiments we matched colors over the visible range (Appendix D; Fig. D1-B). For that purpose, we used an Ocean Optics (Ocean Optics, Dunedin, Florida, USA) Red Tide USB650 spectrometer, light source LS-1, and a fiber optic reflection probe R400-7-UV/VIS held at a 45-degree angle to the petal surface. Thus, our color manipulations presented choices between two distinct colors; yellow paint presumably corresponding to green in bee color space, and white paint corresponding to bee blue-green (Chittka et al. 1994). Insect foraging bouts were observed and analyzed as described in the section *General experimental methods*. In addition, we used ANOVA to analyze these results from the plant perspective by testing whether flower color influenced the total number of insect visits to a flower, relative to the mean for other flowers in the same array. Arrays observed for <1 h were not used in this analysis.

In principle, the acrylic paint could have affected floral fragrance, which can also be a pollinator attractant. To explore whether the paint had unintended effects on insect visitation not driven by color, we set up five preliminary control arrays in which insects were offered a choice between eight unmanipulated *B. sinclairii* (normally white colored) and eight *B. sinclarii* painted with white paint (Table 1: B7). At these arrays we observed ≥10 foraging bouts only for *Allograpta* syrphid flies and tachinid flies ($N = 55$ and 29 bouts, respectively). First visits by both types of flies were made approximately equally often to painted and unpainted flowers ($P = 0.2249$ and $P = 0.0947$, respectively).

Two of our species, *R. gracilipes* and *B. bellidioides*, have petals that reflect in the UV, whereas the three white-petaled species do not (Appendix C). To determine whether insect visitors respond to reflectance in the UV as well as the human-visible color range, we used a UV-absorbing solution prepared as in Johnson and Andersson (2002). We used gentle heating to dissolve equal amounts of avobenzone and octinoxate (Fisher Scientific, Pittsburg, Pennsylvania, USA) in duck preen gland fat at a concentration of 40:60 w/w. A small amount was applied with a toothpick to the upper side of the petals on flowers of *B. bellidioides*, with control flowers receiving the same amount of the duck preen gland fat alone. The treatment completely blocked UV reflectance (Appendix D: Fig. D1-C), as has been shown in studies with other plants (Peter and Johnson 2008). We observed two experimental arrays, each containing six flowers with UV-absorbent and six flowers, for five hours. As scarcity of flowers prevented setting up more arrays, we augmented these data with observation at a similar array of flowers of *Dolichoglottis lyallii* (Hook. f.) B. Nord (Asteraceae) (Table 1: B8), which also has yellow-ray flowers and UV reflectance (Bischoff 2008). Prior to analysis of the combined data, we tested for differences across arrays, using contingency analysis on first type of flower visited and ANOVA on transformed proportion of visits made to UV-block flowers.

*Morphology arrays*

To examine responses of insects to differences in flower traits other than color, we set up experimental arrays that contained the two flower species from one of our pairs of species, but with both types painted the same color (Table 1: A4, A5, B4, B5). For each pair of species, we set up two kinds of arrays: one with all flowers painted white and one with all flowers painted yellow, to match the visible color spectra of both species. Although flower types in these arrays could differ in a variety of flower traits, including rewards and scent along with morphology, for simplicity we refer to these arrays hereafter as "morphology" arrays.

*Factorial arrays*

We used the species pair *B. sinclairii* (white-ray flowered composite) and *B. bellidioides* (yellow-ray flowered composite) to test whether effects of color and other flower traits have additive effects on insect visitation. For this experiment, each array contained four treatments, each with four replicate flowers. The four treatments consisted of the two flower species crossed with the two paint colors (white and yellow) in all factorial combinations (Table 1: B6). We analyzed the visit data in two ways. First from the insect perspective, we examined the type of flower first visited in a foraging bout, and used contingency analysis to test whether the frequency of such visits to white vs. yellow depended on the flower species. Second from the plant perspective, we determined for each individual flower the number of visits made by a particular insect type, expressed as a residual from the mean for that array in order to factor out temporal variation in the overall visitation rate. We combined *Hylaeus* and *Leioproctus* bees into one category to yield nonzero visit rates for each array. We then combined the data from all arrays, and analyzed them with a two-way factorial ANOVA on residual visit rate, with the factors of flower species and color. In cases where residuals did not meet the
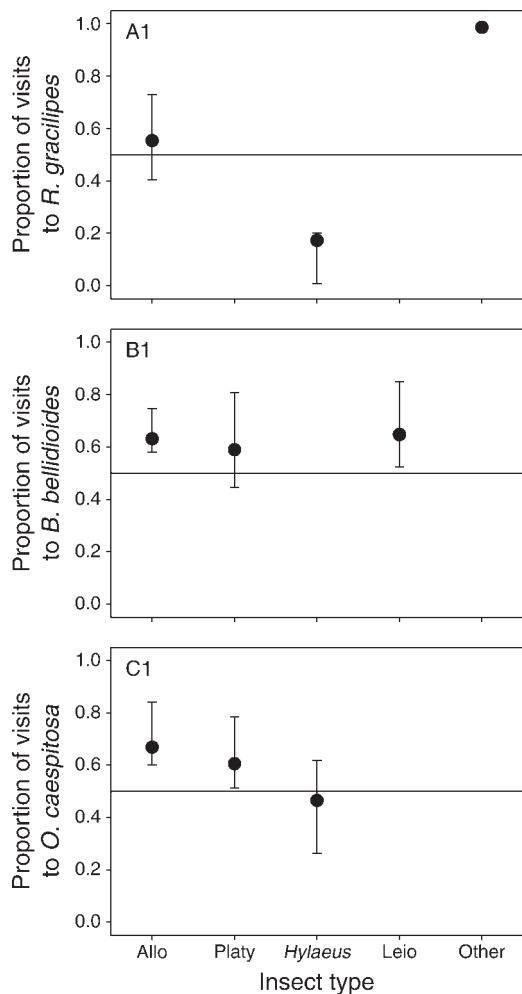
FIG. 1. Natural preferences during foraging bouts with two or more flowers visited. A1, B1, and C1 indicate the respective experiment (see Table 1). Proportion of visits made by each of five insect types to the species with more yellow: (A1) *Ranunculus gracilipes* over *Ourisia glandulosa*, (B1) *Brachyglottis bellidioides* over *B. sinclarii*, and (C1) *Ourisia caespitosa* over *Ourisia glandulosa*. Means and 95% confidence limits based on back-transforming data are plotted for insect types with at least 10 foraging bouts. Nonoverlap between the confidence limits and the null hypothesis line of 0.5 corresponds to significant preference based on a one-sample $t$ test. Abbreviations are: Allo, *Allograpta* spp.; Platy, *Platycheirus* spp.; and Leio, *Leioproctus* spp.

assumption of normality, we ran a generalized linear model on the absolute visit rate, with day as a block factor in addition to crossed factors of flower species and color. This model used a Poisson distribution, as appropriate for count data and implemented in Proc Genmod (SAS version 9.1).

### Multiple-trait manipulations

The two species of *Ourisia* differed in both the size of the color spot on the throat of the flower and in the relative height of the upper petals (Appendix B). We used three types of arrays to examine responses of insects to each of these traits alone and to both traits combined. One type of array offered a choice between eight unmanipulated flowers of *O. glandulosa* and eight *O. glandulosa* flowers with ~50% of the upper petals cut to resemble more closely the shape of *O. caespitosa* (Table 1: C2). A second type offered a choice between eight *O. glandulosa* flowers with a larger yellow-green floral guide painted onto the throat (to resemble *O. caespitosa*) and eight *O. glandulosa* flowers with the normal white color painted instead in that location (sham paint control; Table 1: C3). The final array type offered a choice between eight *O. glandulosa* flowers with both cut petals and a larger floral guide and eight *O. glandulosa* flowers with only the sham white-paint on the throat (Table 1: C4; Appendix B).

### RESULTS

#### Natural preferences

For all flower species pairs, at least one insect type exhibited a significant preference between unmanipulated flowers of the two species. These results were consistent whether based on analysis of the first flower visited (Appendix E) or the proportion of visits in a string of visits (Fig. 1). Given a choice between *R. gracilipes* and *O. glandulosa*, the small bee *Hylaeus matamoko* strongly preferred to visit *O. glandulosa* (A1 in Fig. 1); it visited that species first 79% of the time ($P < 0.001$; Appendix E). In contrast, muscid flies in the genus *Spilogona*, which accounted for 40 of the 47 foraging bouts in the "other" category, strongly preferred *R. gracilipes*, making 95% of its first visits to that species. Whereas syrphid flies did not show significant preference between yellow *R. gracilipes* and white *O. glandulosa* ($P > 0.05$), *Allograpta* preferred to visit the yellow-rayed *B. bellidioides* over white-rayed *B. sinclairii* (panel B1 in Fig. 1), making 65% of its first visits to the former ($P < 0.01$). Bees in the genus *Leioproctus* also strongly preferred *B. bellidioides* (panel B1 in Fig. 1; Appendix E). At arrays offering a more subtle choice between two species of *Ourisia*, both genera of syrphid flies nevertheless exhibited preferences for *O. caespitosa*, visiting that species first 69–72% of the time (both $P < 0.0001$). *Hylaeus* showed no detectable preference between the two *Ourisia* species (panel C1 in Fig. 1).

#### Petal color manipulations

For 7 out of 11 combinations of flower species and insect type tested with alterations of visible petal color, a significant preference was detected for visiting one color over another (first four columns in Table 2). In most cases, insects that had shown a preference for one flower species over another also showed a preference for the color that was more closely associated with that species. For example, the syrphid *Allograpta* (which had over-visited the yellow-flowered *R. gracilipes* compared to *O. glandulosa*, although not significantly so) preferentially

TABLE 2. Frequencies of times that insects first visited one color rather than another in single-species flower arrays with a choice of two petal colors (yellow vs. white).

| | Flower species | | | | |
| | Ranunculus gracilipes | Ourisia glandulosa | Brachyglottis bellidioides | Brachyscome sinclairii | UV-control vs. block |
| Insect type | | | | | |
|---|---|---|---|---|---|
| *Allograpta* | 20 vs. 7* | 32 vs. 13** | 27 vs. 12* | 30 vs. 31[NS] | 28 vs. 38[NS] |
| *Platycheirus* | ⋯ | 51 vs. 25** | 10 vs. 2* | 4 vs. 9[NS] | ⋯ |
| *Hylaeus* | ⋯ | 7 vs. 17*§ | ⋯ | ⋯ | 10 vs. 8[NS] |
| *Leioproctus* | ⋯ | ⋯ | 19 vs. 2*** | ⋯ | ⋯ |
| Other | ⋯ | 13 vs. 13[NS] | ⋯ | 16 vs. 20[NS] | 11 vs. 11[NS] |

*Notes:* Entries give the number of insects that first visited a yellow flower vs. the number of insects that first visited a white flower, except for the last column, which gives the number of insects that first visited a UV-control vs. the number of insects that first visited a UV-block flower. Insect types with $N < 10$ are indicated with ellipses. Total $N$ for all visitors = 529. NS signifies nonsignificance at $P > 0.05$ in a likelihood ratio test against the null hypothesis of equal frequencies.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.
§ Signifies NS after sequential Bonferroni correction.

visited flowers painted yellow over those painted white, regardless of which of the two flower species was presented (panels A2 and A3 in Fig. 2). At *R. gracilipes* color arrays, these flies visited the yellow-painted flowers first 74% of the time and at *O. glandulosa* arrays 71% of the time (Table 2). Similarly, the bee *Hylaeus* (which had preferred the white-flowered *O. glandulosa*) preferentially visited *O. glandulosa* flowers painted their normal white over those painted yellow ($P = 0.0008$; panel A2 in Fig. 2), and did not visit the *R. gracilipes* color arrays at all. Although only six muscid flies entered the *R. gracilipes* color arrays, all six visited a yellow flower first, consistent with their preference for that species.

Color preferences were not necessarily consistent across flower species. For example, *Allograpta* preferred yellow to white when visiting three of the four flower species, but had no such preference when visiting *B. sinclarii* which normally has white petals (panel B2 in Fig. 2).

Summing visits by all insect types, at *R. gracilipes* arrays, flowers painted yellow had a three-fold higher relative visit rate than flowers painted white (averages = 1.59 and 0.42 times the mean; $F_{1,30} = 8.24$, $P = 0.0075$). A similar result was obtained for the other flower species that is normally yellow, *B. bellidioides*, in that yellow-painted flowers received more visits than white-painted ones (relative visit rates = 1.46 vs. 0.54; $F_{1,30} = 36.85$, $P < 0.0001$). However, neither of the naturally white flowers had a significantly higher visit rate when flowers were painted white rather than yellow. Visit rates were similar regardless of color treatments for *B. sinclairii* (relative visit rates = 0.89 and 1.11; $P = 0.1593$). For *O. glandulosa*, flowers painted yellow actually had a higher relative visit rate than flowers painted white (1.24 vs. 0.75; $F_{1,78} = 8.99$, $P = 0.0036$), even though this species is naturally white.

The proportion of visits made by an insect to flowers with UV block did not differ significantly across the three arrays tested (ANOVA, $P > 0.05$ for all insect types; contingency analysis on type of flower first visited, all $P > 0.05$). After combining all arrays to increase sample size, neither the syrphid fly *Allograpta* nor the bee *Hylaeus* made detectably more first visits to flowers with UV-block than to control flowers with the solvent only (Table 2).

### Morphology arrays

These arrays presented a choice of two flower species but both painted an identical color, so that any choice had to be based on other cues (morphology, reward, or scent). When both flower species in a pair were painted white, the syrphid fly *Allograpta* over-visited the normally white-flowered species, either *O. glandulosa* or *B. sinclairii* (Table 3, Fig. 2). Based on the first type of flower visited, *Allograpta* switched from preferring *R. gracilipes* to preferring *O. glandulosa* when both flowers were painted white instead of yellow (Table 3A, latter test nonsignificant with Bonferroni adjustment). Similarly, painting both of the composite flowers white elicited a preference in *Allograpta* for the normally white-flowered *B. sinclarii* (B4 in Table 3B, Fig. 2), even though this insect had preferred *B. bellidioides* when flowers were unmanipulated (Fig. 1), and showed no detectable "morphology" preference when all flowers were painted yellow (B5 in Table 3B, Fig. 2). *Leioproctus* remained consistent in its preference for *B. bellidioides* over *B. sinclairii* even when both were painted yellow (Table 3B).

### Factorial arrays

The effect of other floral traits on responses to color were assessed using arrays with all four combinations of *B. sinclairii* and *B. bellidioides* crossed with painting petals white vs. yellow. *Allograpta* flies, *Platycheirus* flies, and native bees all visited *B. bellidioides* painted yellow the most frequently (Fig. 3). Responses of *Allograpta* and native bees to color depended significantly on flower species, as judged by significant interaction terms between color and flower species in the generalized linear model ($\chi^2 = 10.62$, $P = 0.0011$ and
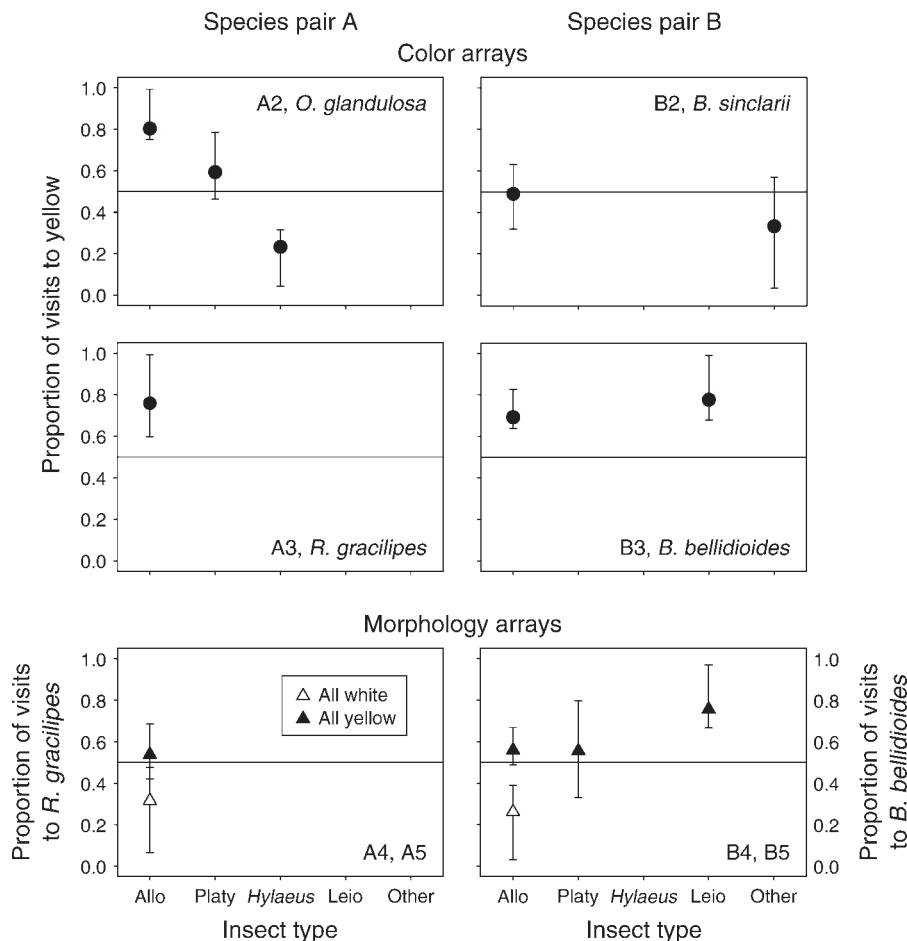
FIG. 2. Preferences at color and morphology arrays. The top two rows show the proportion of visits made to yellow-painted flowers over white-painted flowers in single-species color arrays (A2, A3, B2, B3). The bottom row shows the proportion of visits made to the naturally yellow-flowered species in morphology arrays, in which both species were painted the same color (white arrays A4 and B4 and yellow arrays A5 and B5). Conventions are as in Fig. 1.

$\chi^2 = 30.11$, $P < 0.0001$, respectively). Syrphid flies in the genus *Platycheirus* were more variable in visitation, and the interaction was not significant in this case ($\chi^2 = 1.37$, $P = 0.2411$). Combining visits by all insect types, there was a highly significant interaction between color and flower species on residual visit rate (two-way ANOVA; $F_{1,60} = 16.78$, $P < 0.0001$), as insects discriminated in favor of yellow for *B. bellidioides* but not for *B. sinclairii*. Contingency analyses of the type of flower first visited gave similar results for all insects, except that the interaction between color and flower species was also significant ($P < 0.01$) for *Platycheirus*. Total numbers of first visits were 19, 92, 40, and 32 for white *B. bellidioides*, yellow *B. bellidioides*, white *B. sinclairii*, and yellow *B. sinclairii*, respectively, with the proportion of visits to yellow depending on flower species.

### Multiple-trait manipulations

In arrays offering a choice of unmanipulated *O. glandulosa* flowers and flowers with the upper petal cut approximately in half, the syrphid fly *Platycheirus*

TABLE 3. Frequencies of times that insects first visited one species rather than another in morphology arrays with both species painted the same color.

| Insect type | Both white | Both yellow |
|---|---|---|
| A) *R. gracilipes/O. glandulosa* | | |
| *Allograpta* | 15 vs. 34** | 18 vs. 7*§ |
| *Hylaeus* | 1 vs. 13** | ... |
| Other | 12 vs. 6[NS] | 12 vs. 0*** |
| B) *B. bellidioides/B. sinclairii* | | |
| *Allograpta* | 3 vs. 24**** | 35 vs. 26[NS] |
| *Platycheirus* | ... | 10 vs. 10[NS] |
| *Leioproctus* | ... | 16 vs. 5* |
| Other | ... | 14 vs. 7[NS] |

*Notes:* Total $N$ for all visitors = 292. (A) Arrays A4 ("Both white" column) and A5 ("Both yellow" column): the number of insects that first visited *R. gracilipes* vs. the number of insects that first visited *O. glandulosa*. (B) Arrays B4 ("Both white" column) and B5 ("Both yellow" column): the number of insects that first visited *B. bellidioides* vs. the number of insects that first visited *B. sinclairii*. Statistical conventions are as in Table 2.
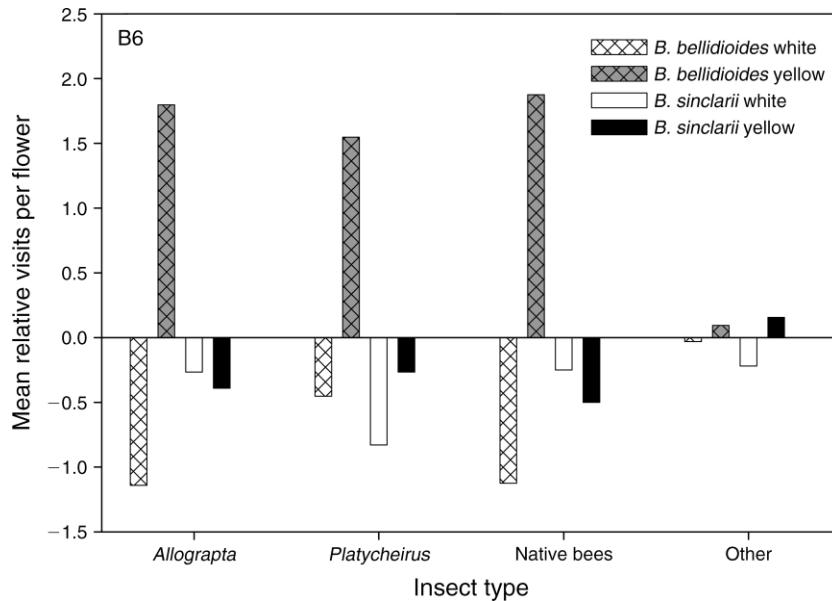
Fig. 3. Visitation rates to four types of flowers in factorial arrays (B6) presenting all combinations of *B. bellidioides* and *B. sinclarii* crossed with white vs. yellow-painted petals. Bars indicate the mean value of the number of visits expressed as a residual from the average for that flower's array.

showed a significant preference for the smaller flowers, choosing to visit them first 69% of the time ($P < 0.01$; C2 in Table 4), although in longer bouts they did not visit these flowers a significantly higher proportion of the time (mean proportion of visit to manipulated flowers = 0.54). Their initial preference for the manipulated flowers is consistent with their preference for *O. caespitosa* over *O. glandulosa* in unmanipulated arrays (Appendix E, Fig. 1). In arrays offering a choice between *O. glandulosa* with a larger yellow spot painted on the throat (to resemble the floral guide of *O. caespitosa*) and control *O. glandulosa* with a similarly-sized white spot, *Platycheirus* again chose more often the flowers resembling *O. caespitosa*, visiting them first 71% of the time ($P < 0.01$; C3 in Table 4) and visiting them a higher proportion of the time in longer bouts ($t_{21} = 2.83$, $P = 0.0100$). Doubly manipulated flowers with both smaller upper petals and a larger floral guide were also over-visited compared to the control by *Platycheirus*, receiving 68% of the first visits (C4 in Table 4).

Interestingly, the double manipulation did not result in stronger preference than either of the single manipulations alone. Other insect types did not show strong preferences at these arrays (Table 4), with the exception that, in foraging bouts involving ≥2 flowers, *Allograpta* visited a higher proportion of the flowers with the larger floral guide (mean proportion = 0.78; $t_{11} = 3.79$, $P = 0.0030$), consistent with their natural preference for *O. caespitosa*.

### Discussion

#### Natural preferences

The prevailing view of insect pollinators in the New Zealand alpine is that they are highly generalized in flower choice (review by Newstrom and Robertson 2005). In the first direct test for flower species preferences by insect visitors in this habitat, we detected preferences by each type of insect we examined, including the syrphid flies *Allograpta* spp. and *Platycheirus* spp. and the native bees *Hylaeus matamoko* and

TABLE 4. Frequencies of first visits in *Oursisa glandulosa* arrays offering a choice between flowers manipulated to resemble *O. caespitosa* and control flowers.

| | Manipulation | | |
|---|---|---|---|
| Insect type | C2, cut petals vs. control | C3, throat yellow vs. throat white | C4, both manipulations vs. throat white |
| *Allograpta* | 27 vs. 24[NS] | 16 vs. 6*§ | 36 vs. 33[NS] |
| *Platycheirus* | 35 vs. 16** | 37 vs. 15** | 54 vs. 26** |
| *Hylaeus* | 16 vs. 15[NS] | 27 vs. 19[NS] | 24 vs. 24[NS] |
| Other | ... | ... | 6 vs. 5[NS] |

*Notes:* Total $N$ for all visitors = 473. Statistical conventions are as in Table 2. The manipulation codes C2, C3, and C4 are explained in Table 1.

PLATE 1. Close-ups of (left) the colletid bee *Hylaeus matamoko* visiting a flower of *Ourisia glandulosa* and (right) the syrphid fly *Allograpta* sp. visiting a flower head of *Brachyscome sinclarii*. Photo credits: M. Bischoff and A. W. Robertson.

*Leioproctus* spp. Even in a choice between two flower species of the same genus (*Ourisia*), *Platycheirus* spp. consistently chose to visit *O. caespitosa* over *O. glandulosa*. Clearly, generalization is not complete.

### Preferences based on color and other floral traits

Not only do these insects make choices between flower species, they also make choices based at least in part on petal color. In 7 out of 11 tests where insects were presented with flowers that differed only in the color the petals were painted, we detected a preference ($P < 0.05$). This ratio is far higher than the rate of false positives that might be expected by chance, and suggests that choices based on color are common, even when color is so conspicuously lacking in the New Zealand alpine flora. In our color array tests, more of the preferences were for yellow-painted petals than white-painted ones. Moreover, some of these preferences were quite strong. For example, in longer foraging bouts the syrphid *Allograpta* averaged 80% of its visits to yellow rather than white *Ourisia glandulosa* (panel A2 in Fig. 2), even though that flower is normally white. And at color arrays of the normally yellow-colored *B. bellidioides*, the bee *Leioproctus* spp. made 90% of its first visits to a flower painted yellow rather than white.

In most but not all cases, the color preference shown was consistent with the choice the same insect made between flower species, suggesting that color in the visible range was one of the cues involved. We found no evidence that these insects based their choices on UV-reflectance patterns, as has been shown for honey bees (Johnson and Andersson 2002) and halictid bees (Peter and Johnson 2008). Flowers painted with a UV-block and flowers painted with the solvent only were visited similarly often by the syrphid *Allograpta* and the bee *Hylaeus*, although sample sizes for other types of insects were too small to draw conclusions, and more work with other flower and insect species in this habitat is warranted. Whereas yellow paint blocked the UV signal, the apparent lack of response to UV-block by itself suggests that it was the difference in other spectral ranges that mattered to the color responses. This point could be tested further by offering a choice of natural yellow flowers, with and without the yellow paint. Choosing between natural flowers, the syrphid *Allograpta* favored the normally yellow-flowered *B. bellidioides* over *B. sinclarii*. In line with that preference (assuming UV reflectance was not critical), in color arrays it chose yellow over white when tested on three of four species. The exception to this pattern was an absence of color preference with flowers of *B. sinclarii*, which is normally white. The latter result shows that color choice is to some extent dependent on the context of other floral traits. Experiments with artificial flowers have shown innate preference of some syrphid flies for the color yellow (Lunau and Maier 1995), but how often such preferences are expressed in the field is not well understood. Free-flying syrphid flies preferred naturally yellow over white morphs of the radish *Raphanus raphanistrum* (Kay 1976), but pink over yellow morphs of *Raphanus sativus* (Stanton 1987). We do not know the extent to which the preferences seen here are innate vs. learned; only that the context dependence of the color preferences suggests that learning is involved to some extent.

Other floral traits besides color also affected insect visitation. Even when color was equalized by painting flowers in "morphology arrays" the same color, *Allograpta*, *Hylaeus*, and *Leioproctus* showed preference between *R. gracilipes* and *O.glandulosa*, and/or between *B. bellidioides* and *B. sinclarii*. Whereas preference by *Platycheirus* was not detected in those experiments, these syrphid flies preferentially visited *O. glandulosa* flowers with upper petals shortened to resemble flowers of *O.*

*caespitosa*. Thus, even a fairly small morphological difference can influence visitation patterns by at least one type of insect in the New Zealand alpine zone. Since trimming the flowers causes mechanical wounding and could have affected volatiles given off by the flower, it is conceivable that these results are explained by changes in odor as well as shape. However, this explanation seems less likely as these *Ourisia* species have relatively low concentrations of floral scents that are attractive to insects (Bischoff 2008), and two other insect types did not distinguish between cut and control flowers. In the other experiments, we did not manipulate traits other than color and so cannot infer which other floral traits were used as cues.

### Interactions between traits

The effect of color on visitation in some cases depended on other traits of the flowers. The most explicit test for interactions came from our factorial experiment with all combinations of the species *B. bellidioides* and *B. sinclarii*, painted either white or yellow. The syrphid *Allograpta*, native bees, and all insects combined preferred yellow over white *B. bellidioides*, but not yellow over white *B. sinclarii* (which normally has white-ray flowers). These nonadditive effects on visitation suggest that insects can respond to combinations of cues rather than to single cues (Raguso 2008, Campbell 2009). Moreover, these more complex responses were usually in the direction of preferring the familiar, normal combination of traits.

Less direct evidence for interactive effects on visitation was obtained from the floral manipulations of *O. glandulosa* to resemble *O. caespitosa*. Either painting a larger floral guide or cutting the upper petal was sufficient to increase visitation by the syrphid *Platycheirus* as much as could be increased by both manipulations together. In this case, the two cues (color and morphology) appear to be redundant rather than acting synergistically.

### Potential implications for selection on flower color

We can reject the simplistic hypothesis that these insect visitors lack color preferences entirely. Instead, the observed preferences for white or yellow petals suggest they have the potential to select in favor of certain flower colors. If insect color preferences can explain the mix of colors found in the community, we would expect that for each flower species examined, insect visitors would prefer its normal color over alternative mutant colors, leading to higher pollination success. The data we have collected are only a first step in assessing this hypothesis.

An important limitation is that we have observed insect visits only and do not know how visitation translates into pollination. To find out requires either direct information on pollination success, or estimates of the effectiveness of each insect type as a pollinator, so that visits by different insect types can be properly weighted in combining their contributions to pollina-

tion. Information of this sort is currently available only for single visits to *Ourisia glandulosa* by the colletid bee *Hylaeus matamoko*. Such visits result in transfer of 19 pollen grains on average, demonstrating that this bee is an effective pollinator (M. Bischoff, *unpublished data*). For most of the plant–pollinator combinations, however, we have only indirect evidence that they are pollinators, based on the kinds of pollen found on the insect body (Bischoff 2008). Pollen of all five species in this study has been reported on bodies of *Allograpta*, *Platycheirus*, and *Leioproctus*. Pollen loads on *Hylaeus matamoko* did not contain pollen from either of the Asteraceae species, but these flowers were also hardly visited by them in the current study (Appendix E). Thus, the available data suggest that the visitors are also pollinators, but we need more studies of single-visit pollinator effectiveness to settle this question. To assess selection, it will ultimately be necessary to know not just whether these insects are pollinators, but the extent to which differences in visitation translate into plant fitness based on seeds produced and sired.

If different insect pollinators respond differently to flower color, then a diversity of insects would have the potential to select for the white and yellow colors observed. In this study, we did uncover a few differences in behavioral responses to color. Whereas most color preferences were for yellow petals (Table 2), the bee *Hylaeus matamoko* chose white over yellow *O. glandulosa*, a plant with normally white petals. A contrasting situation is illustrated by *Brachyglottis bellidioides*, which is visited primarily by insects that exhibited preferences for yellow, including the syrphid flies *Allograpta* and *Platycheirus* and the bee *Leioproctus*.

Under the simplistic model that fitness reflects visitation, insect preferences could be capable of explaining the natural color if all insects together made significantly more visits to the color similar to the one normally found. For the two yellow-flowered species such a pattern was observed (provided the blocking of UV by the paint was of low importance). Interpretation of the white-flowered species is more straightforward, but neither of these species fit the pattern. Both color morphs of *B. sinclarii* received similar visit rates, and yellow-painted flowers of *O. glandulosa* actually had a significantly higher relative visit rate than white-painted ones even though white is the natural color. This discrepancy could mean that factors other than insect visitation maintain the white color, but it could also be explained in two other ways. First, *Hylaeus*, with its preference for white, could be a sufficiently effective pollinator to overcome its low visitation frequency compared to other insects combined. Second, a mutation for yellow petals may have never appeared. Many species of *Ourisia* elsewhere have pink, red, or purple flowers, but yellow is typically found, as on the species examined here, only as markings inside the corolla tube (Meudt and Simpson 2006). In general, we found few preferences for white over yellow, suggesting that insect

behavior may not be the sole explanation for a preponderance of white flowers in this habitat, and if visitation does translate to pollination differences, it may be necessary to look to alternative explanations.

The data reported here do not address the distribution of other petal colors besides human white or human yellow. To test the hypothesis that insect color preferences explain the current mix of flower colors found, it will be necessary to extend this work to include other colors that are absent from this alpine community: bright reds, blues, and purples. Doing so in a phylogenetic context would allow testing the hypothesis that pollinator behavior explains their scarcity. We are currently exploring this possibility by examining insect choices between the normal white (to pale blue) color of alpine *Wahlenbergia albomarginata* and the bright-blue color characteristic of other closely related *Wahlenbergia* species in Australasia.

## Conclusions

At this alpine site in New Zealand, insect visitors to flowers showed preferences for certain flower species, responded to experimental manipulations of visible petal color, and exhibited color preferences that depended in part on the context of other floral traits. Syrphid flies and colletid bees join halictid bees, honey bees, and bumble bees as insects for which there is experimental evidence of flower color choice, based on phenotypic manipulations of flowers in the field. It is clear that flower-visitation behavior in the New Zealand alpine is not as generalized as once thought. Instead, the insect visitors do show preferences based on color, including colors other than white, along with other floral traits, and these interactions have the potential to be complex. Future studies of insect responses to flower color in general should consider how the effect of color interacts with the effects of other specific floral traits.

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## APPENDIX A

Illustrations of the five flower species and the experimental setup (*Ecological Archives* E091-188-A1).

## APPENDIX B

Floral morphological differences between *Ourisia caespitosa* and *Ourisia glandulosa* (*Ecological Archives* E091-188-A2).

## APPENDIX C

A figure showing colors of upper petal surfaces of the five species used in this study (*Ecological Archives* E091-188-A3).

## APPENDIX D

A figure showing sample reflectance spectra (*Ecological Archives* E091-188-A4).

## APPENDIX E

A table of natural preferences for insects visiting arrays with a choice of two natural unmanipulated flower species (*Ecological Archives* E091-188-A5).