



# Association of flower color with pollen reward may explain increased bumblebee visitation to the Scotch broom yellow morph

Andrés M. Devegili · Alejandro G. Farji-Brener

Received: 10 December 2019 / Accepted: 5 September 2021  
© The Author(s), under exclusive licence to Springer Nature B.V. 2021

**Abstract** Given that pollinators usually visit flowers for hidden rewards, they need to rely on floral traits that indicate reward status (“honest signals”). However, the relationship between pollination, honest signals, and floral rewards is little documented in natural conditions. The Scotch broom (*Cytisus scoparius*) is an invasive shrub with polymorphism in the color of its flowers that can be yellow, orange, or red. In three areas dominated by the Scotch broom, we described the abundance of the floral morphs and estimated bumblebee (*Bombus terrestris*) visitation rate. We examined whether bumblebee visitation to the floral morphs was related to pollen reward. We collected flowers and classified their stamens according to their function: reward or pollen export. Then, we measured anther size and estimated pollen quantity. The yellow morph was more abundant and more visited by bumblebees than the orange and red morphs. The yellow flowers did indeed offer more pollen than the other morphs and this occurred only for rewarding anthers, suggesting that bumblebees could use yellow color as an honest signal to visit the most rewarding flowers. We discuss whether innate and/or learned

preferences of bumblebees can explain why the yellow morph is more visited, pollinated, and abundant, while the other morphs are maintained at a lower frequency. This is one of the few field works that shows that variation in intra-specific floral traits is associated with variation in floral reward and pollinator visitation rate, helping to understand the foraging preferences of pollinators and the coexistence of floral morphs in nature.

Clinical trials registration: Not applicable.

**Keywords** Scotch broom · Floral polymorphism · Honest signal · Patagonia · Pollination

## Introduction

Pollinators often visit flowers in search of hidden rewards, therefore, they must rely on floral traits that indicate reward status, commonly named “honest signals” (Knauer and Schiestl 2015). To attract pollinators, flowering plants use rewards (e.g., nectar and pollen) and/or attractants (e.g., flower color, size, symmetry) (Wester and Lunau 2017). Attractants are easily detectable by pollinators and serve as a guide to the rewards (Wester and Lunau 2017). When using attractants, plants can benefit by sending dishonest or honest signals. Dishonest signals are highly beneficial to plants, as flowers are pollinated while pollinators are not rewarded (Urru et al. 2011; Lunau and Wester

---

Communicated by Shayne Martin Jacobs.

---

A. M. Devegili (✉) · A. G. Farji-Brener  
LIHO (Lab. de Investigaciones en Hormigas), INIBIOMA  
(CONICET - UNCOMA), Pasaje Gutiérrez 1125,  
Bariloche, Argentina  
e-mail: andresdevegili@gmail.com

2017). Conversely, honest signals are beneficial to both plants and pollinators, as flowers are pollinated and pollinators are rewarded (Knauer and Schiestl 2015). The validity of the honest signals depends on the variability of flower signals and rewards, the association of floral signals with the quality or quantity of rewards, and the accuracy by which pollinators can detect the signals (McLinn and Stephens 2006). Many works documented the variability of floral traits and rewards and the diversity of floral visitors associated with floral traits (Pleasants and Chaplin 1983; Rosas-Guerrero et al. 2014; Chartier et al. 2016). There is also evidence that pollinators have the sensory ability to perceive the variability of floral traits (Chittka 1998). However, few studies have tested whether the floral signals selected by pollinators are honest indicators of reward status in field situations (Benitez-Vieyra et al. 2010). Knowing which floral signals can honestly indicate reward status and how they are used by pollinators in nature is key to understanding the ecology and evolution of plant–pollinator interactions (Knauer and Schiestl 2015).

Floral traits such as size, scent, symmetry, and color can function as honest signals. For example, the floral visitors of the wild radish (*Raphanus sativus* L.) prefer to visit large flowers because this floral trait is positively associated with a greater amount of nectar and pollen reward (Stanton and Preston 1988). A similar pattern was found in *Turnera ulmifolia* L. and some *Salvia* spp., where bigger flowers are preferred by floral visitors because they offer more nectar (Benitez-Vieyra et al. 2010, 2014). Pollinators also show a preference for flowers with scents that indicate better rewards (Knauer and Schiestl 2015). Also, the symmetrical flowers of *Epilobium angustifolium* L., which produce more nectar than asymmetrical ones, were preferred by bumblebees (Møller 1995). Among these traits, intra-specific variation in flower color (hereafter flower color polymorphism) is an ideal scenario to study whether color can function as an honest signal for pollinators. First, flower color is easily detected by pollinators and acts as a selective target for floral visitors (Nuttman et al. 2006; Papiorek et al. 2013). Second, color variation occurs within a population, so there is no need for phylogenetic adjustment. Third, floral morphs are usually found growing very close sharing similar abiotic and biotic conditions. Thus, the potential effect of confounding factors is reduced. For all these reasons, plants with

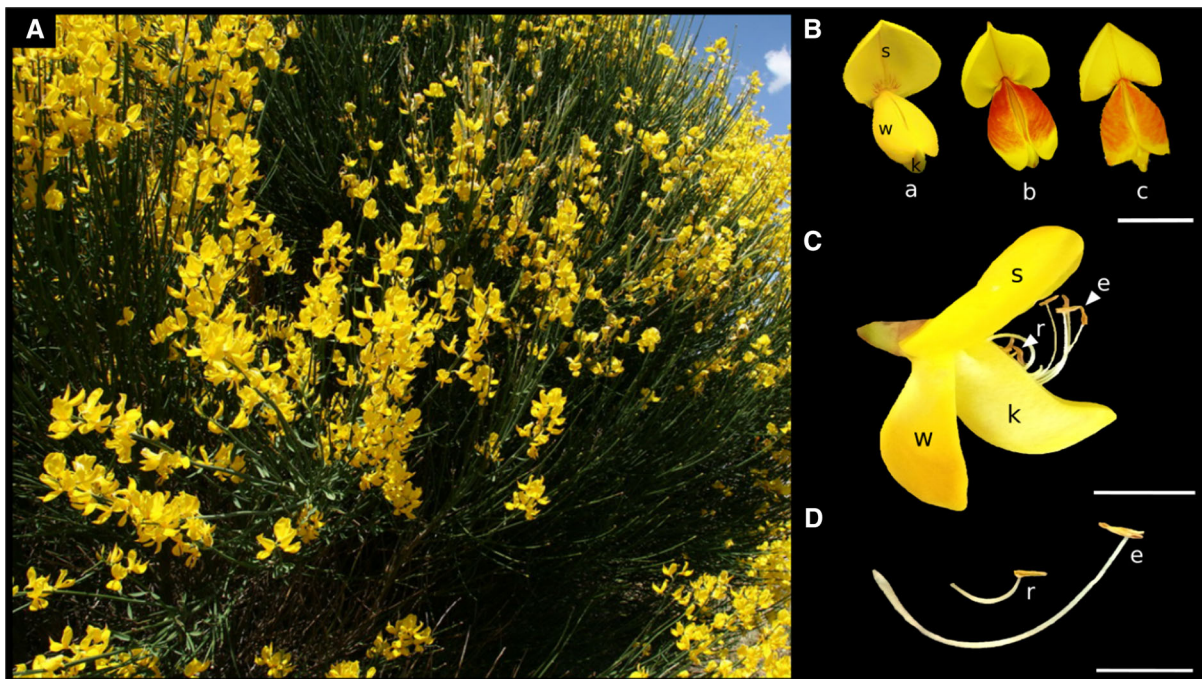
flower color polymorphism are excellent models for studying the importance of flower color in plant pollination.

Understanding why pollinators show a preference for certain flower colors is relevant to understand both the evolution of flower traits and the maintenance of the flower color polymorphism (Waser and Price 1981; Nuttman et al. 2006). Pollinator preferences for floral traits can be summarized in two mechanisms: innate and learned preferences (Lunau and Maier 1995; Haverkamp et al. 2016). On the one hand, innate preferences are phylogenetic adaptations of pollinators that operate before the individual experience with flowers. In this regard, many insects use their visual system to locate flowers (Chittka and Menzel 1992) and show an innate preference for certain flower colors (Lunau and Maier 1995; Kelber 1997; Goyret et al. 2008; Rohde et al. 2013). This innate bias to flower colors is important for inexperienced foragers to locate their first food reward (Giurfa et al. 1995; Kelber 1997; Ings et al. 2009). However, the innate preference of pollinators to particular floral traits is independent of the actual reward conditions (Lunau and Maier 1995). On the other hand, learned preferences are temporary specializations of pollinators toward particular floral traits, and these preferences are dependent on the learning abilities of the species and the reward conditions of food sources (Russell et al. 2016; Giurfa 2007 and reference therein). In a system comprising plants with flower color polymorphism associated with variation in floral reward, the maintenance of the floral color polymorphism could be explained by a learned preference. Although experienced pollinators should target the color morph with the highest reward, which would cause its prevalence in the population, naive and inexperienced individuals may initially pollinate flowers of all colors. Therefore, the continuous production of new individuals would ensure the presence of the less rewarding color morphs in the population. Furthermore, as memorization is not always perfect in pollinators (Keasar et al. 1996), experienced individuals may still contribute to ensuring that less rewarding morphs do not disappear from the population. Apart from pollination-related mechanisms, other factors can also be relevant to explain the maintenance of flower color polymorphism in nature, such as edaphic factors (Horovitz 1976), moisture availability (Godoy et al. 1975), shade (Zhao et al. 2012), herbivore pressure (Irwin et al. 2003), and

mutations affecting pigment biosynthesis (Clegg and Durbin 2003).

*Cytisus scoparius* (L.) Link (Scotch broom) is a shrub that presents yellow, orange, or red flowers (hereafter floral morphs; Fig. 1A, B). In northwestern Patagonia (Argentina), these floral morphs show a clear pattern in their relative abundances and pollinator visitation rates: (i) the yellow morph is more abundant than the orange and red morphs and (ii) the yellow morph is the most visited by the bumblebee *Bombus terrestris*, the main *C. scoparius* pollinator in the area (Gavini and Farji-Brener 2015). *C. scoparius* flowers only provide pollen as a reward to floral visitors (Paulino et al. 2016). We hypothesize that yellow flowers are more visited than orange and red flowers because the yellow color acts as an honest signal for a greater pollen reward. The validity of an honest floral signal depends on three conditions: (i) pollinators must be able to detect the floral signal, (ii) the floral reward should differ according to the

floral signal, and (iii) there must be consistency between floral traits and reward. Condition (i) was tested in previous research in which flower color was manipulated evidencing that bumblebees are attracted to the yellow morph because of its color and not because of its higher abundance relative to the other morphs (Gavini and Farji-Brener 2015). Conditions (ii) and (iii) remain unknown in this system. If our hypothesis is valid, we expect to find higher pollen rewards in the yellow flowers compared to the orange and red morphs [i.e., support for condition (ii)]. Further, since *C. scoparius* flowers present division of labor in their male sexual parts, with rewarding and pollen-export stamens (Paulino et al. 2016), we expect to find greater pollen content solely in the rewarding stamens of the yellow morph [i.e., support for condition (iii)].



**Fig. 1** **A** Scotch broom shrub [*C. scoparius* (L.) Link, Fabaceae] and **B** flower color morphs. **C** Triggered flower showing the position of stamens. **D** Comparison of reward and pollen-export stamens. a = yellow, b = red, and c = orange

morphs; s = banner, w = wing, and k = keel petals; e = pollen-export stamens and r = rewarding stamens. Scale bars: **B**, **C** = 1 cm; **D** = 0.5 cm. (Color figure online)

## Methods

Fieldwork was conducted in three sites ( $\sim 1000 \text{ m}^2$  each) dominated by *C. scoparius* in Bariloche, Argentina ( $71^\circ 18'0''\text{W}$ ,  $41^\circ 9'0'' \text{S}$ ). Sites were at least 500 m from each other. The annual rainfall in the area is  $\sim 1800 \text{ mm}$  and the average temperature ranges from  $2^\circ \text{C}$  in winter to  $13^\circ \text{C}$  in summer.

*C. scoparius* is a European invasive plant that is widely distributed in northwestern Patagonia (Argentina). Its flowers are zygomorphic and exhibit flower color polymorphism (Fig. 1B, C). Since all the flowers of an individual plant corresponded to a unique color morph, hereafter, when we speak of yellow, orange, or red flowers, we are referring to plants with that color morph. The floral morphs grow intermingled throughout the study area. Flowers have ten stamens, of which (a) four have a long filament and large anther, (b) five have a short filament and small anther, and (c) one has an intermediate-length filament (respect to the others) and a small anther. Flowers only produce pollen as a reward for floral visitors. There is strong evidence that *C. scoparius* flowers have a division of labor in the male sexual parts: pollen from long-filament stamens serves male function while pollen from short-filament stamens serves as a reward (Paulino et al. 2016). Therefore, we grouped anthers into two categories: (i) pollen-export anthers (from the four long-filament stamens) and (ii) rewarding anthers (from the five short- + intermediate-filament stamens) (Fig. 1C, D).

The mechanism of pollen dehiscence in *C. scoparius* is explosive (Suzuki 2003). Initially, the stamens and pistil remain subjected to great tension inside the keel, and when a pollinator lands on a flower its weight and buzz cause the keel to open, releasing the stamens and pistil which impact the body of the animal. *Bombus dahlbomii* (native), *Apis mellifera* (exotic), and, more recently, *B. terrestris* (exotic) have been reported as effective *C. scoparius* pollinators (Morales and Aizen 2002; Aizen et al. 2008; Gavini and Farji-Brener 2015). Many bees (Halictidae, Colletidae), Diptera (Syrphidae, Nemeletridae), and Coleoptera have been also reported as frequent flower visitors (Aizen et al. 2008). In the three study sites (surroundings of S. C. de Bariloche city), *B. dahlbomii* and *A. mellifera* were not present, while the invasive *B. terrestris* was highly abundant (A. M. Devegili pers. observations). In NW Patagonia (Argentina), *B.*

*terrestris* has rapidly increased in abundance since 1997 (Aizen et al. 2018) and it is highly successful in opening *C. scoparius*' flowers (Stout 2000; Gavini and Farji-Brener 2015). The importance of *B. terrestris* in *C. scoparius* pollination was evidenced after a volcanic eruption in 2011 that led to a short-term drop of *B. terrestris* and resulted in a reduction in the proportion of *C. scoparius*' triggered flowers (Morales et al. 2014). The explosive mechanism of pollen release means that a closed keel is indicative of an unvisited flower, while an open keel can be used as a proxy of successful floral visitation (Parker 1997). Unopened flowers produce no fruit, implying that autonomous selfing and apomixis do not occur and that flower opening by bees is critical to reproductive success (Suzuki 2003; Simpson et al. 2005).

We first corroborated the floral morph abundance and the floral visit patterns described by Gavini and Farji-Brener (2015). To estimate the abundance of the floral morphs in the population, in each of the three patches we randomly located 3 plots of  $10 \text{ m}^2$  and counted all the individuals of each floral morph. To estimate the floral visitation rate of bumblebees, we marked branches with closed flowers of the three floral morphs (yellow:  $N = 35$ , orange:  $N = 33$ , and red:  $N = 35$ ). We waited a week for the pollinators to visit the flowers and then we selected the first 25 flowers from the apex and counted the number of open flowers. Branches from different plants were located at similar heights from the ground ( $\sim 1.5 \text{ m}$ ) and freely exposed to potential visits (i.e., they were not hidden behind the vegetation). Plants with flowers of each color morph were interspersed in space. The floral visit rate was expressed as the percentage of open flowers on a branch:

$$\text{Floral visit rate} = \frac{\text{Open flowers}}{25} \times 100.$$

To compare the amount of pollen between the floral morphs and stamen types, we used the anther area which is a character strongly associated with the number of pollen grains (see Results). We selected 15 plants per floral morph and collected 3 flowers per plant (i.e.,  $N = 45$  flowers per floral morph). We separated and categorized the stamens and attached their anthers with scotch tape to glass slides ( $N = 450$  anthers per floral morph, of which 270 were rewarding anthers and 180 pollen-export anthers); the anther area was then measured using ImageJ Software (Schneider



et al. 2012). To examine whether the anther area is a good predictor of the number of pollen grains in a subset of anthers we estimated the number of pollen grains following Paulino et al. (2016). We collected ten flowers of each floral morph and from different plants and then we carefully dissected the petals (without triggering the anthers) and cut one pollen export and one rewarding anther from each flower. We took photographs of the anthers and measured their size using ImageJ Software. To release the pollen grains from the anther, we put each anther in an Eppendorf tube with 200  $\mu\text{L}$  of water + 1  $\mu\text{L}$  of detergent (surfactant that helps to release pollen) and then we vortexed the mixture during 60 s. We placed a droplet of 10  $\mu\text{L}$  of the mixture on a glass slide and, using a stereoscopic loupe, we took a photograph of the droplet. We repeated this last procedure three times for each mixture and then counted the pollen grains in the three droplets using ImageJ. Finally, to estimate the total number of pollen grains within the anther, we multiplied the average number of pollen grains in the droplets by its dilution factor.

Since flower number, flower size, and plant size could influence floral rewards and then floral visitation rates (Pleasants and Chaplin 1983), we estimated and included these co-variables in the analyses. To estimate the number of flowers per plant, we selected three branches and counted the flowers between the apex and 50 cm below. For the flower size, we measured flower height (distance between standard and keel) and width (distance between wings). Finally, to estimate plant size, we measured plant height and width (average from three measures) and used the formula of a cylinder (volume = height  $\times$   $\Pi$   $\times$  radius<sup>2</sup>).

To compare the floral visitation rate of bumblebees to the three floral morphs and for the three study sites, we used a general linear model with negative binomial error distribution (Zuur et al. 2009). To compare the anther areas between the floral morphs, we used generalized linear mixed models, with the floral morph as a fixed effect, plant size, flower size, and flower number as co-variables and plant as a random effect (Zuur et al. 2009). Both homoscedasticity and normality were tested. Chi-squares and  $p$  values were obtained by likelihood ratio tests of the full model with the effect in question against the model without the effect. GLM, GLMMs, and multiple comparisons

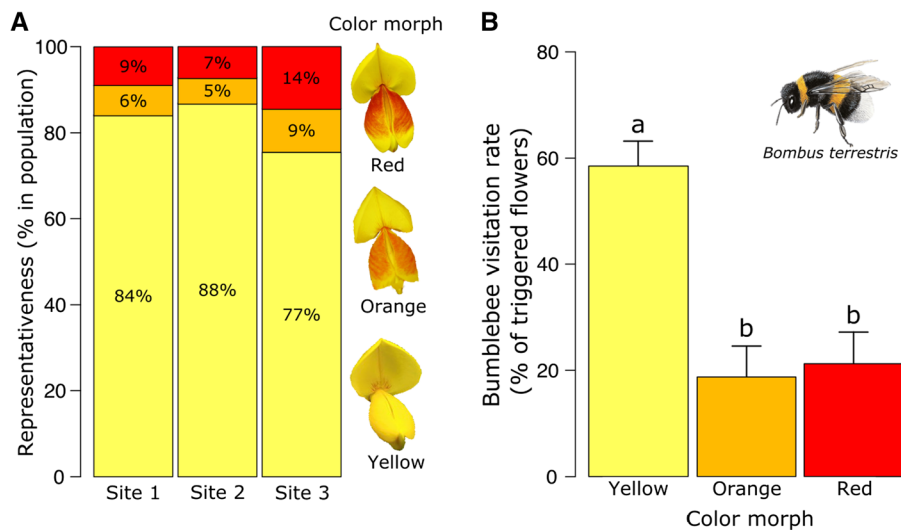
were performed with *glm.nb*, *lmer*, and *lsmeans* function from MASS, lme4, and lsmeans packages, respectively (Bates et al. 2007; Ripley et al. 2013; Lenth 2015). All the analyses were done in R (R Core Team 2015).

## Results

The yellow morph was more abundant than the orange and red morphs (Fig. 2A). Field observations allowed us to confirm that the opening of the flowers was actively carried out by the bumblebee *B. terrestris* and that these pollinators rarely visit open flowers. Consequently, the percentage of open flowers in a branch is a reliable estimator of the bumblebee visitation rate to the floral morphs. Bumblebee visitation rates differed among floral morphs and this difference did not vary across the study sites (GLM, floral morphs:  $\chi^2 = 17.19$ ,  $df = 2$ ,  $p < 0.001$ ; sites:  $\chi^2 = 1.16$ ,  $df = 2$ ,  $p = 0.56$ ); the yellow morph showed  $59 \pm 5\%$  (mean  $\pm$  SE) of flowers visited, the orange morph  $19 \pm 4\%$ , and the red morph  $21 \pm 4\%$  (Fig. 2B).

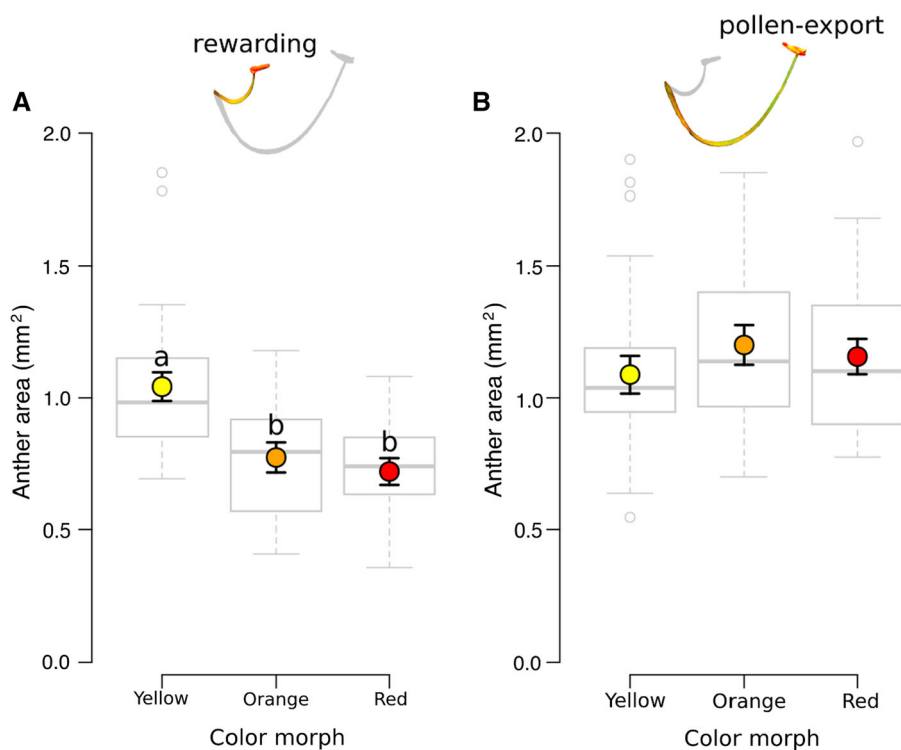
The number of flowers per plant, flower size, plant size, and the study sites did not influence the area of the rewarding anthers (GLMM,  $\chi^2 = 2.45$ ,  $df = 2$ ,  $p = 0.29$ ) and pollen-export anthers (GLMM,  $\chi^2 = 1.78$ ,  $df = 2$ ,  $p = 0.62$ ). The area of the rewarding anthers differed among the floral morphs (GLMM,  $\chi^2 = 18.26$ ,  $df = 5$ ,  $p = 0.003$ ; Fig. 3A); with yellow flowers having a bigger rewarding anther area than the other morphs. The areas of the pollen-export anthers did not vary between the morphs (GLMM,  $\chi^2 = 0.25$ ,  $df = 2$ ,  $p = 0.88$ ; Fig. 3B).

The areas of the rewarding and pollen-export anthers were positively associated with the number of pollen grains, explaining 78% and 63% of its variation, respectively (Fig. 4A, B). The number of pollen grains in rewarding anthers was much higher for yellow flowers ( $3518 \pm 253$  pollen grains, mean  $\pm$  SE) than the orange and red morphs (orange:  $1743 \pm 325$ , red:  $1753 \pm 328$ ). Conversely, the number of pollen grains in pollen-export anthers was similar between the morphs (yellow:  $9125 \pm 1001$ , orange:  $8286 \pm 1061$ , red:  $7597 \pm 828$ ).



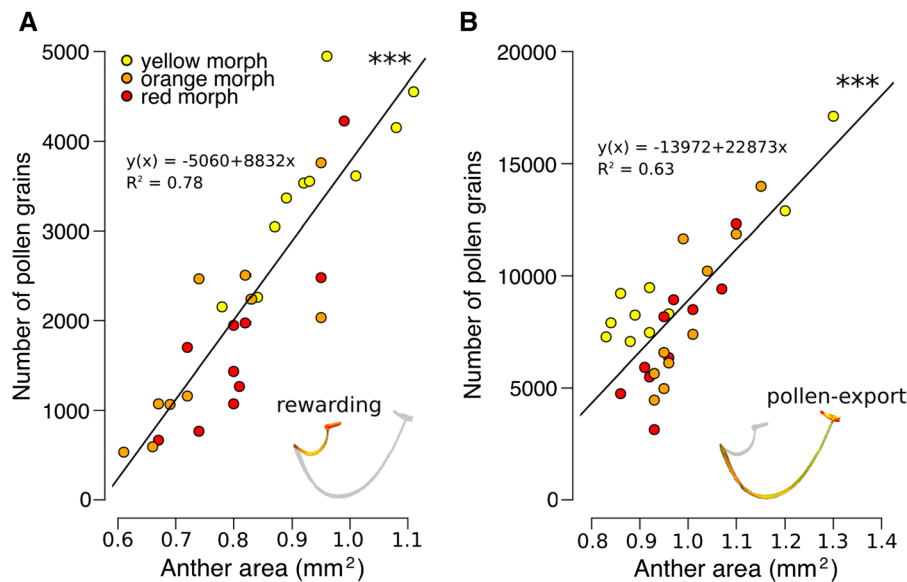
**Fig. 2** **A** Representativeness of Scotch broom floral morphs in the three study sites. Representativeness is expressed in percentages obtained with the abundances of the morphs at each site. **B** Comparison of bumblebee visitation rate for the Scotch broom color morphs. Visitation rate is the percentage of

open flowers per 25 flowers counted from the branch apex. Sites are not differentiated because the bumblebee visitation rate was not different among the sites for each floral morph. Bars and whiskers represent mean  $\pm$  SE. Different letters depict significantly different groups



**Fig. 3** Comparison of the anther area of the Scotch broom floral morphs. Morphs are further separated by **A** rewarding and **B** pollen-export stamens. Sites are not differentiated because the

anther area did not vary among the sites for each floral morph. Dots and whiskers represent mean  $\pm$  SE. Different letters depict significantly different groups



**Fig. 4** Relationship between the anther area and the number of pollen grains for **A** rewarding stamens and **B** pollen-export stamens of the Scotch broom shrub. The floral morphs are represented by colors. Asterisks depict significant linear regressions. \*\*\* $p < 0.001$

## Discussion

Floral traits that indicate reward status (“honest signals”) are critical to plant fitness because seed output depends on the ability of pollinators to deliver pollen while searching for food rewards (Knauer and Schiestl 2015). Evidence for a positive association between floral trait variation and associated rewards is scarce for polymorphic flowers. We found that flower color may function as an honest signal of increased pollen reward for Scotch broom pollinators. The yellow flowers of the polymorphic Scotch broom give more pollen to pollinators than the orange and red flowers. This is supported by characteristics of the rewarding anthers, which were 18% larger and had 101% more pollen in the yellow morph than the orange and red morphs, while pollen-export anthers sustained a similar amount of pollen among the three floral morphs. Collectively, results suggest that flower color acts as an honest signal in the Scotch broom, explaining the higher rate of pollinator visits to yellow flowers and the higher abundance of the yellow morph in the population.

In plant–pollinator interactions, the validity of an honest floral signal depends at least on three conditions: (i) a variable reward amount offered by flowers that differ in certain traits, (ii) a consistency between floral traits and reward, and (iii) the accuracy by which

pollinators can detect those traits. Here we found a high variability of pollen supply among the floral morphs [i.e., support for condition (i)] and a consistent relationship between flower color and reward status [i.e., support for condition (ii)]. Concerning condition (iii), it is well known that bumblebees can readily distinguish the flower colors studied herein (Niesenbaum et al. 1999; Dyer and Chittka 2004) and can learn to associate particular colors with rewards to discriminate flowers with different reward qualities (Waddington 2001; Raine and Chittka 2007). Moreover, flower color is the most reliable attractant signal, even under variable and changing light environments (Dyer and Chittka 2004). In our study system, color appears to be a key signal for the bumblebee *B. terrestris* (Gavini and Farji-Brener 2015) to choose among the more rewarding yellow flowers. Floral scents can also vary between floral morphs (Majetic et al. 2007; Salzmann and Schiestl 2007) and floral scent variation can influence the choice of naïve bumblebees such as *B. terrestris* (Suchet et al. 2011). Although it is clear that the three conditions that validate flower color as an honest signal are met in the Scotch broom, it is less clear whether bumblebees have an innate preference for yellow flowers or they learn to associate yellow color with a greater pollen reward. Knowing which foraging preference operates

in pollinators can be important for understanding the maintenance of color polymorphism in nature.

Foraging preferences can be influenced by innate biases, flower constancy, previous individual experience, and/or social information acquired from conspecifics. Bumblebees in general and our target species in particular do not appear to show an innate preference for a single color (Gumbert 2000; Tastard et al. 2008). For instance, in the study area, *B. terrestris* is the main pollinator of *Alstroemeria aurea*, which has orange flowers (Aizen and Raffaele 1998). Moreover, variability of a single floral trait as in flower color polymorphism does not necessarily translate in flower constancy in bumblebees (Gegear and Laverty 2005). Alternatively, previous experience and/or social learning may also explain the foraging preferences of bumblebees in nature (Goulson 2010). We hypothesize that a learned preference of *B. terrestris* to the most rewarding yellow flowers can explain the higher visitation rate to yellow flowers, the higher abundance of the yellow morph in nature, and the persistence of the orange and red morphs in lower abundances. First, naïve individuals may visit all the floral morphs before learning to associate flower color with reward status (Raine and Chittka 2007). Second, as memorization not always persists for the whole lifetime of bumblebees (Keasar et al. 1996), experienced individuals would still occasionally visit red and orange flowers. And third, experienced bumblebees would still visit red and orange flowers when they are close to the yellow ones (Gavini and Farji-Brener 2015). These mechanisms, which are related to the learning abilities of pollinators, could explain why the orange and red morphs are maintained in the Scotch broom population. Finally, other mechanisms different from pollination have been proposed to explain the existence of flower color polymorphism, such as edaphic factors, moisture availability, sunny vs. shady habitats, or herbivory (Hannan 1981; Irwin et al. 2003). However, these latter mechanisms may not be relevant for explaining Scotch broom polymorphism, as the floral morphs of *C. scoparius* grow very close to each other sharing the same abiotic and biotic conditions.

Pollinators visit flowers in search of rewards and, therefore, to maximize their foraging efficiency they should select floral cues that indicate reward status, so called ‘honest signals’ (Knauer and Schiestl 2015). We presented evidence suggesting that the yellow

flowers of the Scotch broom may function as an honest signal to a higher pollen reward to bumblebees. The link between flower color and pollen reward may explain the higher visitation rate of the bumblebee *B. terrestris* to the yellow morph and the higher abundance of the yellow morph in the Scotch broom population. This is one of the few works that shows in field conditions that variation in intra-specific floral traits can be related to variation in floral reward, helping to understand the foraging preferences of pollinators and the coexistence of floral morphs in nature.

**Acknowledgements** We thank two anonymous reviewers for their valuable suggestions and recommendations on an earlier version of the manuscript. We thank MA Aizen for his suggestions on an earlier version of the manuscript. We thank K Huberman for her help with English. AM Devegili is supported by a doctoral fellowship from the Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina. This research was partially funded by Agencia Nacional de Promoción Científica y Tecnológica (PICT FARJI-BRENER 2015-1319).

**Author contributions** AMD and AGFB conceived and designed the experiments; AMD collected the data and performed the experiments; AMD analyzed the data; AMD and AGFB wrote the manuscript.

**Funding** This research was partially funded by Agencia Nacional de Promoción Científica y Tecnológica (PICT FARJI-BRENER 2015–1319).

**Data availability** The research data are available in the figshare repository (<https://doi.org/10.6084/m9.figshare.16567539>).

#### Declarations

**Conflict of interest** AMD and AGFB declare that they have no conflict of interest.

**Ethical approval** Not applicable.

**Consent to participate** AMD and AGFB participated in the research.

**Consent to publish** AMD and AGFB give their consent to publish the research.

**Plant reproducibility** Not applicable.



## References

- Aizen MA, Raffaele E (1998) Flowering-shoot defoliation affects pollen grain size and postpollination pollen performance in *Alstroemeria aurea*. *Ecology* 79:2133–2142. <https://doi.org/10.2307/176716>
- Aizen MA, Morales CL, Morales JM (2008) Invasive mutualists erode native pollination webs. *PLoS Biol* 6:e31. <https://doi.org/10.1371/journal.pbio.0060031>
- Aizen MA, Smith-Ramírez C, Morales CL, Vieli L, Sáez A, Barahona-Segovia RM et al (2018) Coordinated global species-importation policies are needed to reduce serious invasions globally: the case of alien bumble bees in South America. *J Appl Ecol* 00:1–7. <https://doi.org/10.1111/1365-2664.13121>
- Bates D, Sarkar D, Bates MD, Matrix L (2007) Package ‘lme4’. R package version 1.1–21. Available at <https://cran.r-project.org/web/packages/lme4/index.html>. Accessed June 1, 2018
- Benitez-Vieyra S, Ordano M, Fornoni J, Boege K, Domínguez CA (2010) Selection on signal–reward correlation: limits and opportunities to the evolution of deceit in *Turnera ulmifolia* L. *J Evolution Biol* 23:2760–2767. <https://doi.org/10.1111/j.1420-9101.2010.02132.x>
- Benitez-Vieyra S, Fornoni J, Pérez-Alquicira J, Boege K, Domínguez CA (2014) The evolution of signal–reward correlations in bee- and hummingbird-pollinated species of *Salvia*. *Proc Roy Soc Lond B Biol* 281:20132934. <https://doi.org/10.1098/rspb.2013.2934>
- Chartier M, Liagre S, Weiss-Schneeweiss H, Kolano B, Bessièr JM, Schönenberger J et al (2016) Floral traits and pollination ecology of European *Arum* hybrids. *Oecologia* 180:439–451. <https://doi.org/10.1007/s00442-015-3498-9>
- Chittka B (1998) Sensorimotor learning in bumblebees: long-term retention and reversal training. *J Exp Biol* 201:515–524
- Chittka L, Menzel R (1992) The evolutionary adaptation of flower colors and the insect pollinators color-vision. *J Comp Physiol A* 171:171–181. <https://doi.org/10.1007/BF00188925>
- Clegg MT, Durbin ML (2003) Tracing floral adaptations from ecology to molecules. *Nat Rev Genet* 4:206–215. <https://doi.org/10.1038/nrg1023>
- Dyer AG, Chittka L (2004) Biological significance of distinguishing between similar colours in spectrally variable illumination: bumblebees (*Bombus terrestris*) as a case study. *J Comp Physiol A* 190:105–114. <https://doi.org/10.1007/s00359-003-0475-2>
- Gavini SS, Farji-Brener AG (2015) La importancia del color: morfos florales, tasas de visita y éxito reproductivo en el arbusto *Sarothamnus scoparius*. *Ecologia Austral* 25:204–211. <https://doi.org/10.25260/EA.15.25.3.0.82>
- Gegear RJ, Laverty TM (2005) Flower constancy in bumblebees: a test of the trait variability hypothesis. *Anim Behav* 69:939–949. <https://doi.org/10.1016/j.anbehav.2004.06.029>
- Giurfa M (2007) Behavioral and neural analysis of associative learning in the honeybee: a taste from the magic well. *J Comp Physiol A* 193:801–824. <https://doi.org/10.1007/s00359-007-0235-9>
- Giurfa M, Nunez J, Chittka L, Menzel R (1995) Color preferences of flower-naive honeybees. *J Comp Physiol A* 177:247–259. <https://doi.org/10.1007/BF00192415>
- Godoy R, Iturra P, Koref-Santibáñez S, Navarro J, Pacheco N, Stebbins GL (1975) Polymorphism and geographic variation of flower color in Chilean populations of *Eschscholzia californica*. *Plant Syst Evol* 123:185–198. <https://doi.org/10.1007/BF00989403>
- Goulson D (2010) Bumblebees: behaviour, ecology, and conservation. Oxford University Press on Demand, Oxford
- Goyret J, Pfaff M, Raguso RA, Kelber A (2008) Why do *Manduca sexta* feed from white flowers? Innate and learnt colour preferences in a hawkmoth. *Naturwissenschaften* 95:569–576. <https://doi.org/10.1007/s00114-008-0350-7>
- Gumbert A (2000) Color choices by bumble bees (*Bombus terrestris*): innate preferences and generalization after learning. *Behav Ecol Sociobiol* 48:36–43. <https://doi.org/10.1007/s002650000213>
- Hannan GL (1981) Flower color polymorphism and pollination biology of *Platystemon californicus* Benth. (Papaveraceae). *Am J Bot* 68:233–243. <https://doi.org/10.1002/j.1537-2197.1981.tb12383.x>
- Haverkamp A, Bing J, Badeke E, Hansson BS, Knaden M (2016) Innate olfactory preferences for flowers matching proboscis length ensure optimal energy gain in a hawkmoth. *Nat Commun* 7:1–9. <https://doi.org/10.1038/ncomms11644>
- Horovitz A (1976) Edaphic factors and flower colour distribution in the *Anemoneae* (Ranunculaceae). *Plant Syst Evol* 126:239–242. <https://doi.org/10.1007/BF00983363>
- Ings TC, Raine NE, Chittka L (2009) A population comparison of the strength and persistence of innate colour preference and learning speed in the bumblebee *Bombus terrestris*. *Behav Ecol Sociobiol* 63:1207–1218. <https://doi.org/10.1007/s00265-009-0731-8>
- Irwin RE, Strauss SY, Storz S, Emerson A, Guibert G (2003) The role of herbivores in the maintenance of a flower color polymorphism in wild radish. *Ecology* 84:1733–1743. [https://doi.org/10.1890/0012-9658\(2003\)084\[1733:TROHIT\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2003)084[1733:TROHIT]2.0.CO;2)
- Keasar T, Motro U, Shur Y, Shmida A (1996) Overnight memory retention of foraging skills by bumblebees is imperfect. *Anim Behav* 52:95–104. <https://doi.org/10.1006/anbe.1996.0155>
- Kelber A (1997) Innate preferences for flower features in the hawkmoth *Macroglossum stellatarum*. *J Exp Biol* 200:827–836
- Knauer AC, Schiestl FP (2015) Bees use honest floral signals as indicators of reward when visiting flowers. *Ecol Lett* 18:135–143. <https://doi.org/10.1111/ele.12386>
- Lenth R (2015) Package ‘lsmeans’. R package version 2.30-0. Available at <https://cran.r-project.org/web/packages/lsmeans/index.html>. Accessed May 5, 2018
- Lunau K, Maier EJ (1995) Innate colour preferences of flower visitors. *J Comp Physiol A* 177:1–19. <https://doi.org/10.1007/BF00243394>
- Lunau K, Wester P (2017) Mimicry and deception in pollination, vol 82. Academic Press, New York, pp 259–279
- Majetic CJ, Raguso RA, Tonsor SJ, Ashman TL (2007) Flower color–flower scent associations in polymorphic *Hesperis matronalis* (Brassicaceae). *Phytochemistry* 68:865–874. <https://doi.org/10.1016/j.phytochem.2006.12.009>

- McLinn CM, Stephens DW (2006) What makes information valuable: signal reliability and environmental uncertainty. *Anim Behav* 71:1119–1129. <https://doi.org/10.1016/j.anbehav.2005.09.006>
- Møller AP (1995) Bumblebee preference for symmetrical flowers. *Proc Natl Acad Sci USA* 92:2288–2292. <https://doi.org/10.1073/pnas.92.6.2288>
- Morales CL, Aizen MA (2002) Does invasion of exotic plants promote invasion of exotic flower visitors? A case study from the temperate forests of the Southern Andes. *Biol Invasions* 4:87–100. <https://doi.org/10.1023/A:1020513012689>
- Morales CL, Saez A, Arbetman MP, Cavallero L, Aizen MA (2014) Detrimental effects of volcanic ash deposition on bee fauna and plant-pollinator interactions. *Ecol Austral* 24:42–50. <https://doi.org/10.25260/EA.14.24.1.0.36>
- Niesenbaum RA, Patselas MG, Weiner SD (1999) Does flower color change in *Aster vimineus* cue pollinators? *Am Midl Nat* 141:59–68. [https://doi.org/10.1674/0003-0031\(1999\)141\[0059:DFCCIA\]2.0.CO;2](https://doi.org/10.1674/0003-0031(1999)141[0059:DFCCIA]2.0.CO;2)
- Nuttman CV, Semida FM, Zalut S, Willmer PG (2006) Visual cues and foraging choices: bee visits to floral colour phases in *Alkanna orientalis* (Boraginaceae). *Biol J Linn Soc* 87:427–435. <https://doi.org/10.1111/j.1095-8312.2006.00582.x>
- Papiorek S, Rohde K, Lunau K (2013) Bees' subtle colour preferences: how bees respond to small changes in pigment concentration. *Naturwissenschaften* 100:633–643. <https://doi.org/10.1007/s00114-013-1060-3>
- Parker IM (1997) Pollinator limitation of *Cytisus scoparius* (Scotch broom), an invasive exotic shrub. *Ecology* 78:1457–1470. [https://doi.org/10.1890/0012-9658\(1997\)078\[1457:PLOCSS\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1997)078[1457:PLOCSS]2.0.CO;2)
- Paulino JV, de Freitas MV, Prenter G (2016) Evidence for division of labor and division of function related to the pollen release in *Papilionoideae* (Leguminosae) with a heteromorphic androecium. *Int J Plant Sci* 177:590–607. <https://doi.org/10.1086/687351>
- Pleasant JM, Chaplin SJ (1983) Nectar production rates of *Asclepias quadrifolia*: causes and consequences of individual variation. *Oecologia* 59:232–238. <https://doi.org/10.1007/bf00378842>
- R Core Team (2015) R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Available at <https://www.R-project.org/>
- Raine NE, Chittka L (2007) The adaptive significance of sensory bias in a foraging context: floral colour preferences in the bumblebee *Bombus terrestris*. *PLoS ONE* 2:e556. <https://doi.org/10.1371/journal.pone.0000556>
- Ripley B, Venables B, Bates DM, Hornik K, Gebhardt A, Firth D et al. (2013) Package 'MASS'. R package version 7.3-51.4. Available at <https://cran.r-project.org/web/packages/MASS/index.html>
- Rohde K, Papiorek S, Lunau K (2013) Bumblebees (*Bombus terrestris*) and honeybees (*Apis mellifera*) prefer similar colours of higher spectral purity over trained colours. *J Comp Physiol A* 199:197–210. <https://doi.org/10.1007/s00359-012-0783-5>
- Rosas-Guerrero V, Aguilar R, Martín-Rodríguez S, Ashworth L, Lopezaraiza-Mikel M, Bastida JM et al (2014) A quantitative review of pollination syndromes: do floral traits predict effective pollinators? *Ecol Lett* 17:388–400. <https://doi.org/10.1111/ele.12224>
- Russell AL, Golden RE, Leonard AS, Papaj DR (2016) Bees learn preferences for plant species that offer only pollen as a reward. *Behav Ecol* 27:731–740. <https://doi.org/10.1093/beheco/arv213>
- Salzmann CC, Schiestl FP (2007) Odour and colour polymorphism in the food-deceptive orchid *Dactylorhiza romana*. *Plant Syst Evol* 267:37–45. <https://doi.org/10.1007/s00606-007-0560-z>
- Schneider CA, Rasband WS, Eliceiri KW (2012) NIH image to ImageJ: 25 years of image analysis. *Nat Methods* 9:671–675. <https://doi.org/10.1038/nmeth.2089>
- Simpson SR, Gross CL, Silberbauer LX (2005) Broom and honeybees in Australia: an alien liaison. *Plant Biol* 7:541–548. <https://doi.org/10.1055/s-2005-865855>
- Stanton ML, Preston RE (1988) Ecological consequences and phenotypic correlates of petal size variation in wild radish, *Raphanus sativus* (Brassicaceae). *Am J Bot* 75:528–539. <https://doi.org/10.2307/2444218>
- Stout JC (2000) Does size matter? Bumblebee behaviour and the pollination of *Cytisus scoparius* L. (Fabaceae). *Apidologie* 31:129–139. <https://doi.org/10.1051/apido:2000111>
- Suchet C, Dormont L, Schatz B, Giurfa M, Simon V, Raynaud C et al (2011) Floral scent variation in two *Antirrhinum majus* subspecies influences the choice of naïve bumblebees. *Behav Ecol Sociobiol* 65:1015–1027. <https://doi.org/10.1007/s00265-010-1106-x>
- Suzuki N (2003) Significance of flower exploding pollination on the reproduction of the Scotch broom, *Cytisus scoparius* (Leguminosae). *Ecol Res* 18:523–532. <https://doi.org/10.1046/j.1440-1703.2003.00575.x>
- Tastard E, Andalo C, Giurfa M, Burrus M, Thébaud C (2008) Flower colour variation across a hybrid zone in *Antirrhinum* as perceived by bumblebee pollinators. *Arthropod-Plant Inte* 2:237. <https://doi.org/10.1007/s11829-008-9046-3>
- Urru I, Stensmyr MC, Hansson BS (2011) Pollination by brood-site deception. *Phytochemistry* 72:1655–1666. <https://doi.org/10.1016/j.phytochem.2011.02.014>
- Waddington KD (2001) Subjective evaluation and choice behavior by nectar-and pollen-collecting bees. In: Chittka L, Thomson JD (eds) *Cognitive ecology of pollination*. Cambridge University Press, Cambridge, pp 41–60
- Waser NM, Price MV (1981) Pollinator choice and stabilizing selection for flower color in *Delphinium nelsonii*. *Evolution* 35:376–390. <https://doi.org/10.2307/2407846>
- Wester P, Lunau K (2017) Plant–pollinator communication. In: Becard G (ed) *How plants communicate with their biotic environment, advances in botanical research*, vol 82. Academic Press, Elsevier, London, pp 225–257
- Zhao D, Hao Z, Tao J (2012) Effects of shade on plant growth and flower quality in the herbaceous peony (*Paeonia lactiflora* Pall.). *Plant Physiol Biochem* 61:187–196. <https://doi.org/10.1016/j.plaphy.2012.10.005>
- Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) *Mixed effects models and extensions in ecology with R*. Springer Science & Business Media, New York

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.