

SHORT COMMUNICATION

Selection on signal–reward correlation: limits and opportunities to the evolution of deceit in *Turnera ulmifolia* L.

S. BENITEZ-VIEYRA*†, M. ORDANO*¹, J. FORNONI*, K. BOEGE* & C. A. DOMÍNGUEZ*

*Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, Distrito Federal, México

†Instituto Multidisciplinario de Biología Vegetal (CONICET – Universidad Nacional de Córdoba), Ciudad de Córdoba, Córdoba, Argentina

Keywords:

deception;
 honesty;
 pollinator-mediated selection;
 reward;
 signal accuracy.

Abstract

Because pollinators are unable to directly assess the amount of rewards offered by flowers, they rely on the information provided by advertising floral traits. Thus, having a lower intra-individual correlation between signal and reward (signal accuracy) than other plants in the population provides the opportunity to reduce investment in rewards and cheat pollinators. However, pollinators' cognitive capacities can impose a limit to the evolution of this plant cheating strategy if they can punish those plants with low signal accuracy. In this study, we examined the opportunity for cheating in the perennial weed *Turnera ulmifolia* L. evaluating the selective value of signal accuracy, floral display and reward production in a natural population. We found that plant reproductive success was positively related to signal accuracy and floral display, but not to nectar production. The intensity of selection on floral display was more than three times higher than on signal accuracy. The pattern of selection indicated that pollinators can select for signal accuracy provided by plants and suggests that learning abilities of pollinators can limit the evolution of deceptive strategies in *T. ulmifolia*.

Introduction

In general, the interaction between plants and their pollinators is mediated by the expression of signals that advertise rewards. Because reward production commonly represents an energetic cost for plants (Pleasants & Chaplin, 1983; Southwick, 1984; Pyke, 1991; Ordano & Ornelas, 2005; but see Leiss *et al.*, 2004), any strategy decreasing this cost without reducing pollinator visitation rates should be favoured by selection. Nectar, the most common floral reward, (Cruden *et al.*, 1983; Simpson & Neff, 1983), is often concealed within the flower and cannot be directly perceived by pollinators. Hence, they must rely on other floral traits (e.g. flower number,

shape, size, odour and colour) to indirectly assess the quantity/quality of nectar offered by plants (Blarer *et al.*, 2002; Schaefer *et al.*, 2004; Armbruster *et al.*, 2005; Chittka & Raine, 2006; Fenster *et al.*, 2006; Gómez *et al.*, 2008; Raguso, 2008). Because a strong component of the foraging decisions of pollinators rely on advertising traits, plants have the opportunity to reduce the costs of reward production by decreasing the strength of their intra-individual covariance between floral signals and rewards (Dafni, 1984; Renner, 2006), to 'misinform' or, in other words, cheat pollinators.

Most studies addressing cheating in plants have focused in rewardless species, which represent an extreme among cheating strategies. Nectarless species are common among Angiosperms and widely spread in the diversified orchid family (Dafni, 1984; Schiestl, 2005; Renner, 2006). These cases are good examples of how manipulation of pollinators' sensory capabilities can be a successful strategy of plants to save resources. Nevertheless, reducing the relationship between signals and the amount of rewards can represent another way of

Correspondence: César A. Domínguez, Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 70-275, Ciudad Universitaria, México, Distrito Federal, CP 04510, México. Tel.: +52 55 5622 9039; fax: +52 55 5616 1976; e-mail: tejada@servidor.unam.mx

¹Present address: Fundación Miguel Lillo, Miguel Lillo 251, T4000JFE, San Miguel de Tucumán, Tucumán, Argentina.

cheating. In this case, cheating plants can be characterized as those with low levels of signal accuracy (i.e. low association between signal and reward) and a reduced reward production relative to the population average. This cheating strategy could be visualized as a subtle, more continuous deceptive mechanism than those involving bonanza/blank displays (Feinsinger, 1983), reward polymorphisms (Golubov *et al.*, 1999; Castillo *et al.*, 2002) or the complete absence of rewards (Dafni, 1984; Schiestl, 2005; Renner, 2006).

Pollinators prefer plants providing high-energy returns, but they commonly choose among plants on the basis of floral cues correlated with the quantity/quality of reward production (Cresswell & Galen, 1991; Blarer *et al.*, 2002; Raguso, 2004, 2008; Schaefer *et al.*, 2004; Armbruster *et al.*, 2005; Fenster *et al.*, 2006; Gómez *et al.*, 2008). Some studies have explicitly recognized that the association between the magnitude of signals and reward production is a potential explanation for selection on advertising traits (Martin, 2004; Armbruster *et al.*, 2005; Gómez *et al.*, 2008), but as far as we know, no study has yet evaluated if pollinator-mediated selection can favour this correlation at an individual level. This scenario would indicate that pollinators actively favour phenotypes that more accurately convey information about rewards.

Within-individual correlation among floral signals and rewards could be the target of pollinator-mediated selection because pollinators gather information about the quality of a patch and decide whether to continue foraging in that patch or leave after sampling a given number of flowers (Dukas & Real, 1993; Biernaskie *et al.*, 2009). Hence, in the particular case when the foraging patch is a single plant or inflorescence, pollinator choices can be affected by intra-individual variation and covariation between flower traits. In this study, we explored whether plants producing noisy signals (i.e. a decrease in the magnitude of intra-individual covariance between floral signals and rewards) enjoy a reproductive advantage compared to plants producing more accurate signals. We propose a measure of signal accuracy based on the assumption that pollinators follow a Bayesian updating process to assess the degree of correlation between flower signals and rewards (Dall *et al.*, 2005; Biernaskie *et al.*, 2009). This approach assumes that pollinators learn from past experiences and update this information after each visit to a flowering plant (Biernaskie *et al.*, 2009). If nectar production imposes a significant cost for plants and noisy signals allow them to reduce this cost, we expected natural selection to favour plants with a low magnitude of signal accuracy. Hence, plants with low levels of signal accuracy are predicted to show a reduction in reward production. Alternatively, if pollinators are able to discriminate between noisy and reliable signals, strong selective pressures promoting increased levels of signal accuracy and nectar production are expected.

Materials and methods

Study system

Turnera ulmifolia L. is a heterostylous perennial weed native to the Neotropics (Barret, 1978). Although this species exhibits homostylous, self-compatible flowers in the study area, pollinator visitation can duplicate the number of ripe fruits (and seeds per fruit) produced by plants (Cuautle & Rico-Gray, 2003). Each plant produces between 3 and 16 (with a mean of 5.54), yellow flowers/day which last on average 4 h. Anthesis occurs 2–3 h after dawn. Flowers of *T. ulmifolia* have five nectar pockets, which alternate with the corolla lobes. Because the flowers last only few hours, the process of nectar secretion, pollen release and stigmatic receptivity is closely synchronized (Elias *et al.*, 1975), suggesting that nectar reabsorption and response to nectar removal by pollinators may be negligible.

Flower visitors at the study site include butterflies (Lycaenidae, Pieridae and Hesperidae); bees (Apidae, Halictidae and Megachilidae); flies (Bombilidae); and occasional nectar-seeking ants and wasps (Nubia Lara Rodríguez, pers. com.). Bees and skippers are more likely to function as true pollinators because their sizes allow them to touch fertile parts of the flower, whereas Lycaenidae and Pieridae butterflies are probably nectar thieves (S. Benitez-Vieyra, pers. obs.). Ants and wasps concentrate their foraging in extra-floral nectaries (Cuautle & Rico-Gray, 2003).

Study site and data collection

Fieldwork was carried out during July 2007 at the Centro de Investigaciones Costeras La Mancha (CICOLMA), Veracruz, Gulf of México (19°31'17"N, 96°13'29"W). In this area, *T. ulmifolia* is usually present at the border of small patches of vegetation within coastal sand dunes. We sampled 57 reproductive plants in an area of nearly 5 ha during five consecutive days (each plant was sampled once). Geographical coordinates of each plant were recorded with a GPS (Magellan Explorist 200, Santa Clara, CA, USA). Each plant was bagged during 3–4 h to allow nectar accumulation; bags were set-up before pollination activity began (between 8:00–9:00 AM), and they were taken off near the end of flower lifespan. This allowed us to have an estimate of nectar production as a plant trait not being affected by previous pollinator visits. When bags were removed, all the opened flowers were harvested (3–16 flowers per plant) to measure the volume of accumulated nectar using 5- μ L microcaps. Nectar concentration was estimated to the nearest 0.25 Brix using hand refractometers (American Optical 10431 and Reichert 137530LO). These data were then used to estimate the amount of sugar produced per hour by each flower following the procedure outlined by Kearns & Inouye (1993). We used one petal from the same flower

and measured its length with a digital calliper to the nearest 0.01 mm. Flowers used for nectar and petal measurements were not included to assess plant fitness because they were harvested. Instead, to have an estimate of reproductive success, we used the fruits previously produced by the plant corresponding to flowers fertilized under natural conditions before plant bagging. This procedure ensured that nectar quantification did not alter the reproductive success of individual flowers and plants.

We estimated the total number of seeds produced per plant as the mean number of seeds per fruit \times mean number of fruits per branch \times total number of branches. The estimation of the mean number of seeds per fruit was based on the seeds produced by 10 fruits per plant, randomly selected from several branches. Mean number of fruits per branch was assessed by counting the fruits produced in 6–8 branches per plant (which represented around half of the branches produced by the plants). We also estimated the mean number of seeds per flower (i.e. estimated number of seeds/total number of flowers previously produced per plant), which allowed us to evaluate selection patterns at the level of individual flowers, avoiding the numerical effects of flower number on total plant fitness (Benitez-Vieyra *et al.*, 2006). Total number of flowers produced can be readily estimated in *T. ulmifolia* because flower pedicel and bracts remain attached to the plant even if the flower fails to set a fruit. Although *T. ulmifolia* plants flower throughout the year, flowering peak occurs during the rainy season (from June to November, Torres-Hernández *et al.*, 2000). To avoid the effects of environmental fluctuations on our estimates of nectar production (Herrera, 2009), we chose to concentrate our sampling during a short period (early June) of the flowering peak.

Signal accuracy

Because flowers of *T. ulmifolia* have radial symmetry, the length of one petal is a good measure of flower size and of its signalling properties (Blarer *et al.*, 2002; Armbruster *et al.*, 2005; Fenster *et al.*, 2006; Gómez *et al.*, 2008). In fact, petal length and nectar production were significantly correlated at the population level ($r = 0.365$; $P < 0.0001$). Signal accuracy was defined as the intra-individual correlation between signal and reward, relative to this relationship at the population level. Signal accuracy was estimated using the same number of flowers harvested per plant to obtain nectar and petal length measurements (on average 5.54 flowers). To estimate signal accuracy as a relative plant trait that influences plant fitness, we first assumed that pollinators are able to detect the association between the signal and the reward (Blarer *et al.*, 2002) and that they learn this association following a Bayesian foraging behaviour. This behaviour implies that the perception of the mean population signal–reward correlation is likely to be

updated from visit to visit and depends on the sequence of pollinator visits and the number of visited flowers. However, to characterize signal accuracy as a plant trait, we considered that before visiting a given plant, pollinators already had the information of the mean signal–reward correlation in the population. This assumption is reasonable provided the low probability that a plant has to be visited by a naive pollinator in a population with a large number of opened flowers during an extended flowering season. Hence, all plants were treated as equivalents in terms of the order in which they are visited by pollinators. Following this rationale, we were able to estimate a relative value of signal accuracy for each plant. This relative value (signal accuracy; SA) corresponds to the difference between the population signal–reward correlation excluding a given plant (prior population correlation, r_0) and the population signal–reward correlation including that specific plant (posterior population correlation, r_p).

$$SA = r_p - r_0 \quad (1)$$

Thus, SA values near zero represent individual plants with a correlation as accurate as the population mean, which do not modify prior estimations of population signal accuracy. Positive values of SA correspond to individual plants that increase the mean population accuracy, whereas negative values of SA relate to less accurate plants that reduce the posterior mean value of the population signal–reward correlation.

Following the expectation of a Bayesian information updating process, the posterior mean value of the population signal–reward correlation (r_p) as estimated by Box & Tiao (1979) was calculated as

$$r_p = \frac{1}{w_0 + w_1} * (w_0 r_0 + w_1 r_1) \quad (2)$$

where r_p = posterior mean population correlation; r_0 = prior mean population correlation; r_1 = correlation value of i-individual, $w_0 = 1/\sigma_0^2$ (the reciprocal of the prior variance); $w_1 = n/\sigma_i^2$ (the reciprocal of the individual variance multiplied by the number of flowers of i-individual). Although plants with larger floral display are more attractive to pollinators (Ohashi & Yahara, 2002; Harder & Johnson, 2009 and references therein), we assumed that after visiting a reduced number of flowers, pollinators leave plants with low signal accuracy.

Statistical analyses

Before conducting the phenotypic selection analyses, we performed a spatial autocorrelation analysis to examine whether environmental variation affected fitness. We estimated Moran's *I* index of spatial autocorrelation and assessed its significance using 1000 permutations (Haining, 2003). We did not find a significant spatial autocorrelation for total seed production per plant ($I = -0.062$; $P = 0.564$) or mean seed production per flower

($I = 0.169$; $P = 0.135$), suggesting that variation in local environmental conditions had no effect on plant reproduction within the study site.

Two phenotypic selection analyses were carried out following the methodology proposed by Lande & Arnold (1983), one for flower reproductive success (seeds per flower) and the other for total plant fitness (number of seeds). Before conducting these analyses, relative fitness was calculated by dividing each plant's fitness value by the population mean. Predictor variables were standardized. Mean nectar production per flower, mean petal length and SA were included as independent variables in both analyses. The number of flowers was also included to control for differences in size among plants because floral display is directly related with the number of branches per plant ($r = 0.732$, $P < 0.0001$, $n = 57$). Selection models were checked for multicollinearity using variance inflation factors (VIFs). In all cases, VIFs were smaller than 1.26, indicating the absence of multicollinearity. Phenotypic correlations among characters were also calculated. The results of selection analyses are likely to be conservative given the reduced chances of pollinator-mediated selection because of the proportion of seeds of *T. ulmifolia* produced by self-pollination.

Because residuals from regression analyses departed from normality, standard errors for selection gradients were calculated using bootstrap methods (Dixon, 1993; Gross *et al.*, 1998; Maad & Alexandersson, 2004). We generated 10 000 bootstrap samples from the original data set. Each sample consisted of the same number of observations as the original data set and was obtained by resampling with replacement. Selection gradients estimated after each bootstrap were used to obtain their frequency distribution. A selection gradient was considered significant if the bias-corrected confidence percentile interval did not include zero (Gross *et al.*, 1998). We used the *boot* package of R software (version 2.8.1, available online at <http://www.r-project.org/>) to perform the bootstrapping and to estimate the 90%, 95%, 99% and 99.9% confidence intervals. Because multiple regression is constrained to adjust the best linear or quadratic approximation to the relationship between fitness and trait variation (or combination of traits) (Brodie *et al.*, 1995), we applied cubic splines nonparametric regressions to avoid *a priori* assumptions about the shape of the relationship (Schluter, 1988). Cubic splines were calculated using D. Schluter's GLMS software version 4.0 (available at <http://www.zoology.ubc.ca/~schluter/software.html>) for traits that were shown to be significantly affected by phenotypic selection. A smoothing parameter for each spline was chosen by generalized cross-validation.

Results

Mean (\pm SD) sugar production rate per flower was $153.78 \pm 142.86 \mu\text{g h}^{-1}$, showing important levels of variation among plants. We also found higher levels of

Table 1 Pearson's correlation coefficients for *Turnera ulmifolia* traits.

	Mean nectar production per flower	Signal accuracy†	Flower number
Mean petal length	0.433*	−0.136	−0.102
Mean nectar production per flower		−0.072	−0.076
Signal accuracy†			−0.046

$n = 57$. * $P < 0.001$.

†Signal accuracy was estimated as the difference between prior population correlation between petal length (signal) and nectar production (reward) and posterior correlation between these traits after pollinator visitation to each individual plant (see Methods).

within plant variation in nectar production (mean CV \pm SD) ($81.82 \pm 50.54\%$) than in petal length ($9.37 \pm 4.87\%$), suggesting that most of the variation in signal accuracy is because of variation in nectar production rather than in flower size.

Except for the correlation between mean petal length and mean nectar production, none of the phenotypic correlations (at the population level) was significant (Table 1). Mean seed production per flower was 29.49 (range: 8–53 seeds), whereas mean seed production per plant was 2857.63 (range: 513–14743 seeds). Phenotypic selection analysis for total number of seeds per plant detected directional selection favouring individuals with both high number of flowers and signal accuracy (Table 2). These findings were supported by cubic splines analyses (Fig. 1d, e). The selection analysis using seeds per flower as the fitness component showed almost identical results (Table 2, Fig. 1a, b), although in this case directional selection on nectar production was also detected (Fig. 1c). Inspection of cubic splines indicated that only those plants with values of signal accuracy above the population average were favoured by selection (Fig. 1b, e). In all cases, selection on flower number was more intense than on nectar production or signal accuracy (Table 2). No significant selection on mean petal length was detected neither for total number of seeds per plant nor for seeds per flower (Table 2). We found no evidence of significant nonlinear (quadratic or correlative) selection (Table 2).

Discussion

Results from this study suggest that natural selection favouring signal accuracy can constrain the evolution of cheating. Accordingly, our findings did not support the hypothesis that plants producing noisy signals enjoy a reproductive advantage compared to plants producing more accurate signals. Interestingly, when total fitness was considered (seed production), we did not find significant selection for nectar investment. In contrast, we found selection on signal accuracy (which was

Table 2 Multivariate phenotypic selection analyses on *Turnera ulmifolia* traits. Standardized linear selection gradients (β_i), non-linear selection gradient (γ_{ii}), and correlational selection gradients (γ_{ij}), and standard errors (SE) are indicated†.

Fitness measure	Character _i	β_i (SE)	γ_{ii} (SE)	γ_{ij} (SE)		
				Mean nectar production	Mean petal length	Signal accuracy‡
Seeds per plant	Flower number	0.629 (0.074)***	0.038 (0.148)	-0.031 (0.102)	0.105 (0.136)	0.228 (0.074)
	Mean nectar production	0.076 (0.082)	0.100 (0.134)		0.067 (0.129)	0.042 (0.114)
	Mean petal length	0.086 (0.082)	-0.076 (0.137)			-0.233 (0.120)
	Signal accuracy‡	0.185 (0.074)*	0.100 (0.085)			
Seeds per flower	Flower number	0.100 (0.033)***	-0.021 (0.076)	-0.008 (0.053)	0.041 (0.070)	< 0.001 (0.038)
	Mean nectar production	0.080 (0.037)**	0.024 (0.069)		0.073 (0.066)	-0.010 (0.059)
	Mean petal length	-0.042 (0.037)	-0.065 (0.070)			-0.022 (0.062)
	Signal accuracy‡	0.050 (0.033)**	0.060 (0.044)			

$n = 57$. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

†Significance of selection gradients was estimated using 10 000 bootstrap iterations and testing if bias-corrected accelerated intervals include zero, with confidences of 0.10, 0.05, 0.01 and 0.001.

‡Signal accuracy was estimated as the difference between prior population correlation between petal length (signal) and nectar production (reward) and posterior correlation between these traits after pollinator visitation to each individual plant (see Methods).

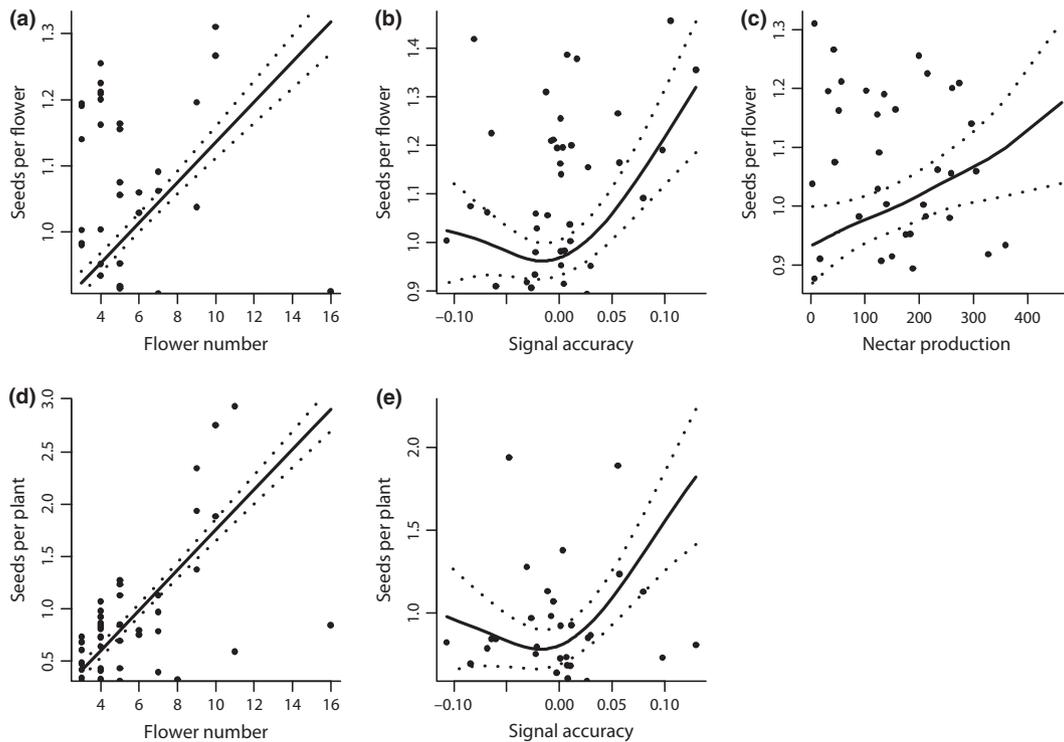


Fig. 1 Cubic spline regressions between floral traits and reproductive success. Dashed lines represent ± 1 SE estimated from 5000 bootstrap replications. Values of relative fitness (a–c) and total fitness (d–e) are standardized to population mean.

independent of nectar investment), thus indicating that plants producing honest signals do not necessarily incur in higher costs of reward production. This is a counter-intuitive result because pollinators are thought to preferentially forage among those plants with higher amounts of nectar (Rathcke, 1992). Nonetheless, because

nectar is concealed within the flower, the only way for pollinators to assess the amount/quality of the rewards is throughout the signals flowers convey. Although there is abundant evidence that floral signals are usually correlated with rewards (Armbruster *et al.*, 2005; Cresswell & Galen, 1991; Fenster *et al.*, 2006; Gómez *et al.*, 2008; this

study), this association is far from being high, indicating some extent of uncertainty around the amount and quality of rewards announced by the flowers. Under this scenario of limited information, signal accuracy may be the pollinators' best source of information regarding the expected amount or quality of the reward a plant offers, as we have demonstrated in this study.

Although we found significant selection for signal accuracy, flower display was the most important variable determining both individual fitness (total seed production) and flower success (seeds per flower) during the reproduction period we measured. This is consistent with a previous review indicating that the intensity of selection on floral display is usually greater than on other reproductive traits (Harder & Johnson, 2009). Given that the total amount of nectar produced by an individual plant increases with the number of flowers, this could be the first and easiest way to evaluate the quality of a plant as a source of resources. In other words, selecting plants with large number of flowers warrants the acquisition of more nectar per foraging bout. From the plant's perspective however, the fitness benefits of floral display also depends on future episodes of selection during fruit dispersal (Cuautle *et al.*, 2005, Garrido *et al.*, 2009). The higher intensity of selection on floral display vs. signal accuracy indicates that the success of individual flowers depended on the reproductive phenotype of the whole plant. In *T. ulmifolia*, nectar production per flower had a significant effect on the number of seeds per flower, although the intensity of selection indicates that quantity (and probably the quality) of nectar is a less important cue that pollinators consider in their foraging decisions. Our results suggest that flowers' reproductive success depends on a two-step hierarchical foraging decision-making process. First, pollinators choose plants with the larger floral display, and second, they choose plants with higher levels of floral accuracy. As long as the variation in floral display among plants plays a major role in determining total fitness than nectar production, the opportunities for cheating remain open. In short, we think we have produced the first explicit demonstration that natural selection can act on the quality of the information provided by plants. However, whether the consequences of signal accuracy on reproductive success are mediated by differential pollinator visitation is a question that remains to be answered.

Associative learning capacity among pollinators has been extensively demonstrated in bees (Blarer *et al.*, 2002), hummingbirds (Healy & Hurly, 2001) and lepidopterans (Weiss, 2001). Biernaskie *et al.* (2009) showed that bumblebees rapidly learn the distribution of resources among patches and use this information to decide when to leave a patch. This kind of Bayesian updating is commonly incorporated into models of animal decision-making processes, and many studies have shown that animals actually combine prior knowledge with current information to assess patch quality (Dall *et al.*,

2005; Valone, 2006). As indicated by the significance of the linear selection gradient on signal accuracy, pollinators of *T. ulmifolia* were able to assess the reliability of the information plants provide. Interestingly, this result does not mean they are choosing directly more rewarding plants because no association between signal accuracy and nectar production per plant was found and we did not detect selection on total nectar production (at the individual level). Accordingly, once pollinators have chosen the plants with a larger display, their preferences seem to be biased towards the quality of the information. This is consistent with the fact that the amount of nectar can only be examined through signal accuracy.

Two sources of variation may have affected our estimation of natural selection. First, pollinators are likely to encounter flowers that were already harvested by previous visitors thus altering the information for pollinators (i.e. increasing the intra-individual variance in reward traits) (Herrera, 2009). This ecological constraint is likely to reduce the floral accuracy perceived by pollinators, thus decreasing the opportunities to detect selection. However, the significant selection gradient on floral accuracy found in this study indicates that the variance induced by pollinator foraging was not enough to eliminate the adaptive value of this trait. In addition, it is well known that folivorous insects, defensive ants and wasps influence fitness of *T. ulmifolia* (Cuautle & Rico-Gray, 2003). It is unlikely, however, that species of these guilds were responsible for the selection patterns observed in this study, given that their activity on flowers during data collection was rather low (e.g. less than 10% of the flowers were visited by ants, S. Benitez-Vieyra, pers. obs.). Moreover, as far as we know, the foraging of these animals is independent of the variation in floral traits, but how they indirectly affect the patterns of selection on floral traits is an open question that remains to be investigated. Second, by concentrating our measurements during a short period of time, we were able to avoid the influence of environmental variation in plant fitness and nectar production. However, we acknowledge that, if other factors (e.g. pollinator identity, resource availability, weather conditions, etc.) change throughout the rainy season, selection patterns on flower display and signal accuracy could be different and this warrants further investigation.

Some studies have suggested that selection on advertisement traits rely on the correlation between such traits and reward production (Armbruster *et al.*, 2005; Fenster *et al.*, 2006; Gómez *et al.*, 2008). However, no study had explicitly found selection acting upon the within-individual correlation between flower signals and rewards. Our findings indicate that pollinators could be favouring the maintenance and reinforcement of the signal–reward correlation through a preference for those plants displaying more accurate signals. This result also indicates that this kind of preference should promote the evolution of higher levels of covariation between signals and

rewards (i.e. increasing flower integration). Obviously, the existence of genetically based differences in signal accuracy among plants is a necessary condition for an evolutionary response mediated by pollinator selection. A review by Ashman & Majetic (2006) suggests that most floral traits maintain significant amounts of genetic variation, but the genetic basis of signal–reward correlations still remain to be assessed.

Determining the extent to which rewards are honestly signalled to mutualists is an exciting new area of research (Schaefer *et al.*, 2004). Although signal honesty is a common topic in animal communication studies (Maynard Smith & Harper, 2003), it has been barely studied in plant–pollinator systems (but see Cresswell & Galen, 1991; Blarer *et al.*, 2002; Armbruster *et al.*, 2005; Fenster *et al.*, 2006; Gómez *et al.*, 2008; Raguso, 2008). In this study, we proposed to disentangle the effects of reward production and signalling quality to understand signal evolution in plants. Cheating may be a successful strategy when pollinators show fixed preferences for some signals (Blarer *et al.*, 2002) (e.g. by mimicking sexual signals) or when they experience speed and accuracy trade-offs in decision-making (Chittka *et al.*, 2009) given the costs associated with learning. In addition, the value of the information of signals can be higher in uncertain or poor environments (McLinn & Stephens, 2006; Biernaskie *et al.*, 2009) than in conditions where foragers find constant and abundant rewards.

Overall, our findings suggest that pollinators' foraging decisions depend on several cues that are assessed in a hierarchical fashion (flowering display > signal accuracy) determined by the feasibility to assess the amount of reward a plant offers. This hierarchy is also characterized by increased gains of information, but also by increased costs of foraging. Under these circumstances, pollinators must rely on an imperfect assessment of the expected reward (flowering display and signal accuracy), and hence opening the opportunity for plants to save some resources through reduced investments on reward production. Whether our results represent a more general form of a continuous cheating strategy than the production of rewardless flowers remains as an open question for future research.

Acknowledgments

We thank Rubén Pérez-Ishiwara, who helped us with logistical support, technical improvements and fieldwork. Hugo López Rosas, Verónica Espejel and Anastasio P. García Rodríguez that encouraged and helped us in several ways during fieldwork at CICOLMA. Nubia Lara Rodríguez kindly provided pollinator data. Rodrigo Méndez facilitated us GPS equipment. SBV was supported by a graduate fellowship from the Red de Macrouiversidades Públicas de América Latina y el Caribe at the Instituto de Ecología, UNAM. SBV and MO were supported by postdoctoral fellowships from the

Universidad Nacional Autónoma de México at the Instituto de Ecología, UNAM. This study was partially funded by the PAPIIT-UNAM IN228907 grant to CAD and PAPIIT-UNAM IN221310 to JF.

References

- Armbruster, W.S., Antonsen, L. & Pélabon, C. 2005. Phenotypic selection on *Dalechampia* blossoms: honest signaling affects pollination success. *Ecology* **86**: 3323–3333.
- Ashman, T.-L. & Majetic, C.J. 2006. Genetic constraints on floral evolution: a review and evaluation of patterns. *Heredity* **96**: 343–352.
- Barret, S.C.H. 1978. Heterostyly in a tropical weed: the reproductive biology of *Turnera ulmifolia* complex (Turneraceae). *Can. J. Bot.* **56**: 1713–1725.
- Benitez-Vieyra, S., Medina, A.M., Glinos, E. & Cocucci, A.A. 2006. Pollinator-mediated selection on floral traits and size of floral display in *Cyclopogon elatus*, a sweat bee-pollinated orchid. *Funct. Ecol.* **20**: 948–957.
- Biernaskie, J.M., Walker, S.C. & Geegar, R.J. 2009. Bumblebees learn to forage like bayesians. *Am. Nat.* **174**: 413–423.
- Blarer, A., Keasar, T. & Shmida, A. 2002. Possible mechanisms for the formation of flower size preferences by foraging bumblebees. *Ethology* **108**: 341–351.
- Box, G.E.P. & Tiao, G.C. 1979. *Bayesian Inference in Statistical Analysis*. John Wiley & Sons, New York.
- Brodie III, E.D., Moore, A.J. & Janzen, F.J. 1995. Visualizing and quantifying natural selection. *Trends Ecol. Evol.* **10**: 313–318.
- Castillo, R.A., Cordero, C. & Domínguez, C.A. 2002. Are reward polymorphisms subject to frequency- and density-dependent selection? Evidence from a monoecious species pollinated by deceit. *J. Evol. Biol.* **15**: 544–552.
- Chittka, L. & Raine, N.E. 2006. Recognition of flowers by pollinators. *Curr. Opin. Plant Biol.* **9**: 428–435.
- Chittka, L., Skorupski, P. & Raine, N.E. 2009. Speed–accuracy tradeoffs in animal decision making. *Trends Ecol. Evol.* **24**: 400–407.
- Cresswell, J.E. & Galen, C. 1991. Frequency-dependent selection and adaptive surfaces for floral character combinations: the pollination of *Polemonium viscosum*. *Am. Nat.* **138**: 1342–1353.
- Cruden, R.W., Hermann-Parker, S.M. & Peterson, S. 1983. Patterns of nectar production and plant pollinator coevolution. In: *Biology of Nectaries* (T.S. Elias & B.A. Bentley, eds), pp. 80–125. Columbia University Press, New York.
- Cuautle, M. & Rico-Gray, V. 2003. The effect of wasps and ants on the reproductive success of the extrafloral nectaried plant *Turnera ulmifolia* (Turneraceae). *Funct. Ecol.* **17**: 417–423.
- Cuautle, M., Rico-Gray, V. & Diaz-Castelazo, C. 2005. Effects of ant behaviour and presence of extrafloral nectarines on seed dispersal of the Neotropical myrmecochore *Turnera ulmifolia* L. (Turneraceae). *Biol. J. Linn. Soc.* **86**: 67–77.
- Dafni, A. 1984. Mimicry and deception in pollination. *Annu. Rev. Ecol. Syst.* **15**: 259–278.
- Dall, S.R.X., Giraldeau, L.-A., Olsson, O., McNamara, J.M. & Stephens, D.W. 2005. Information and its use by animals in evolutionary ecology. *Trends Ecol. Evol.* **20**: 187–196.
- Dixon, P.M. 1993. Population sampling and bootstrapping in complex designs. In: *Design and Analysis of Ecological Experiments* (S.M. Scheiner & J. Gurevitch, eds), pp. 290–318. Chapman & Hall, New York.

- Dukas, R. & Real, L.A. 1993. Effects of recent experience on foraging decisions by bumble bees. *Oecologia* **94**: 244–246.
- Elias, T.S., Rozich, W.R. & Newcombe, L. 1975. The foliar and floral nectaries of *Turnera ulmifolia* L. *Am. J. Bot.* **62**: 570–576.
- Feinsinger, P. 1983. Variable nectar secretion in a *Heliconia* species pollinated by hermit hummingbirds. *Biotropica* **15**: 48–52.
- Fenster, C.B., Cheely, G., Dudash, M.R. & Reynolds, R.J. 2006. Nectar reward and advertisement in hummingbird pollinated *Silene virginica* (Caryophyllaceae). *Am. J. Bot.* **93**: 1800–1807.
- Garrido, J.L., Rey, P.J. & Herrera, C.M. 2009. Influence of elaiosome on postdispersal dynamics of an ant-dispersed plant. *Acta Oecol.* **35**: 393–399.
- Golubov, J., Eguiarte, L.E., Mandujano, M.C., López-Portillo, J. & Montaña, C. 1999. Why be a honeyless honey mesquite? Reproduction and mating system of nectarful and nectarless individuals. *Am. J. Bot.* **86**: 955–963.
- Gómez, J.M., Bosch, J., Perfectti, F., Fernández, J.D., Abdelaziz, M. & Camacho, J.P.M. 2008. Association between floral traits and rewards in *Erysimum mediohispanicum* (Brassicaceae). *Ann. Bot.* **101**: 1413–1420.
- Gross, J., Husband, B.C. & Stewart, S.C. 1998. Phenotypic selection in a natural population of *Impatiens pallida* Nutt. (Balsaminaceae). *J. Evol. Biol.* **11**: 589–609.
- Haining, R. 2003. *Spatial Data Analysis. Theory and Practice*. Cambridge University Press, Cambridge.
- Harder, L.D. & Johnson, S.D. 2009. Darwin's beautiful contrivances: evolutionary and functional evidence for floral adaptation. *New Phytol.* **183**: 530–545.
- Healy, S.D. & Hurly, T.A. 2001. Foraging and spatial learning in hummingbirds. In: *Cognitive Ecology of Pollination* (L. Chittka & J.D. Thomson, eds), pp. 127–147. Cambridge University Press, Cambridge.
- Herrera, C.M. 2009. *Multiplicity in Unity. Plant Subindividual Variation & Interactions with Animals*. The University of Chicago Press, Chicago.
- Kearns, C.A. & Inouye, D.W. 1993. *Techniques for Pollination Biologists*. University Press of Colorado, Niwot.
- Lande, R. & Arnold, S.J. 1983. The measurement of selection on correlated characters. *Evolution* **37**: 1210–1226.
- Leiss, K.A., Vrieling, K. & Klinkhamer, P.G.L. 2004. Heritability of nectar production in *Echium vulgare*. *Heredity* **92**: 446–451.
- Maad, J. & Alexandersson, R. 2004. Variable selection in *Platanthera bifolia* (Orchidaceae): phenotypic selection differed between sex functions in a drought year. *J. Evol. Biol.* **17**: 642–650.
- Martin, N.H. 2004. Flower size preferences of the honeybee (*Apis mellifera*) foraging on *Mimulus guttatus* (Scrophulariaceae). *Evol. Ecol. Res.* **6**: 777–782.
- Maynard Smith, J. & Harper, D.G.C. 2003. *Animal Signals*. Oxford University Press, Oxford.
- McLinn, C.M. & Stephens, D.W. 2006. What makes information valuable: signal reliability and environmental uncertainty. *Anim. Behav.* **71**: 1119–1129.
- Ohashi, K. & Yahara, T. 2002. Visit larger displays but probe proportionally fewer flowers: counterintuitive behaviour of nectar-collecting bumble bees achieves an ideal free distribution. *Funct. Ecol.* **16**: 492–503.
- Ordano, M. & Ornelas, J.F. 2005. The cost of nectar replenishment in two epiphytic bromeliads. *J. Trop. Ecol.* **21**: 541–547.
- Pleasants, J.M. & Chaplin, S.J. 1983. Nectar production rates of *Asclepias quadrifolia*: causes and consequences of individual variation. *Oecologia* **59**: 232–238.
- Pyke, G.H. 1991. What does it cost a plant to produce floral nectar? *Nature* **350**: 58–59.
- Raguso, R.A. 2004. Flowers as sensory billboards: progress towards an integrated understanding of floral advertisement. *Curr. Opin. Plant Biol.* **7**: 434–440.
- Raguso, R.A. 2008. Wake up and smell the roses: the ecology and evolution of floral scent. *Ann. Rev. Ecol. Evol. Syst.* **36**: 549–569.
- Rathcke, B.J. 1992. Nectar distribution, pollinator behaviour, and plant reproductive success. In: *Effects of Resource Distribution on Animal-Plant Interactions* (M.D. Hunter, T. Ohgushi & P.W. Price, eds), pp. 113–118. Academic Press, San Diego.
- Renner, S.S. 2006. Rewardless flowers in Angiosperms and the role of insect cognition in their evolution. In: *Plant-Pollinator Interactions. From Specialization to Generalization* (N.M. Waser & J. Ollerton, eds), pp. 123–144. University of Chicago Press, Chicago.
- Schaefer, H.M., Schaefer, V. & Levey, D.J. 2004. How plant–animal interactions signal new insights in communication. *Trends Ecol. Evol.* **19**: 577–584.
- Schiestl, F.P. 2005. On the success of a swindle: pollination by deception in orchids. *Naturwissenschaften* **92**: 255–264.
- Schluter, D. 1988. Estimating the form of natural selection on a quantitative trait. *Evolution* **42**: 849–861.
- Simpson, B.B. & Neff, J.L. 1983. Evolution and diversity of floral rewards. In: *Handbook of Experimental Pollination Biology* (C.E. Jones & R.J. Little, eds), pp. 142–153. Van Nostrand-Reinhold, New York.
- Southwick, E.E. 1984. Photosynthate allocation to floral nectar: a neglected energy investment. *Ecology* **65**: 1775–1779.
- Torres-Hernández, L., Rico-Gray, V., Castillo-Guevara, C. & Vergara, J.A. 2000. Effect of nectar-foraging ants on the reproductive fitness of *Turnera ulmifolia* (Turneraceae) in a coastal sand dune in Mexico. *Acta Zool. Mex.* **81**: 13–21.
- Valone, T.J. 2006. Are animals capable of Bayesian updating? An empirical review. *Oikos* **112**: 252–259.
- Weiss, M.R. 2001. Vision and learning in some neglected pollinators: beetles, flies, moths, and butterflies. In: *Cognitive Ecology of Pollination* (L. Chittka & J.D. Thomson, eds), pp. 171–190. Cambridge University Press, Cambridge.

Received 29 January 2010; revised 3 September 2010; accepted 9 September 2010