

## Nectar guide fluctuating asymmetry does not relate to female fitness in *Mimulus luteus*

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### Abstract

Most studies assessing the importance of developmental instability of floral characters for pollinator visits and plant fitness have focused on the fluctuating asymmetry (FA) of the corolla phenotype. The importance of stability process for nectar guide characters that signal floral reward has not been considered in the literature. In principle, flowers with symmetrical guides should be more successful at attracting pollinators, therefore increasing their reproductive success in comparison to asymmetrical flowers. In this paper we test this hypothesis in a population of 171 individuals of the Andean monkey flower, *Mimulus luteus* in northern Chile. This species shows a conspicuous red spot in the landing yellow petal, which permits assessment of the functional relationship between nectar guide FA and female fitness. Our results did not reveal a significant linear nor nonlinear relationship between nectar guide FA and fitness. This result was consistent after controlling the level of FA by guide and corolla size. Because the corolla of *M. luteus* did not show evidence of UV wavelength reflectance, our negative result could not be attributable to a confounding effect of UV guides. Even though we can not rule out that nectar guide FA correlates better with male than female fitness, the low fraction of the variance in female fitness accounted for nectar guide FA, suggests that other components of the floral phenotype as well as environmental factors may be more important to predict pollinator preference and reproductive success in this species.

### Introduction

Fluctuating asymmetry (FA) is a random deviation of morphometric characters from perfect symmetry, especially from bilateral symmetry, that occurs as a consequence of genetic or environmental factors affecting the stability of the developmental process (Van Valen 1962). Individuals may show asymmetrical phenotypes because they experienced poor environmental conditions during development, or because they are inbred or have poorly co-adapted gene complexes (see reviews in Palmer and Strobeck 1986; Palmer 1994; Møller and Swaddle 1997). While theoretical expectations predict that individuals with high levels of FA will tend to be less fit in sexual se-

lection, less efficient in terms of resource acquisition for growth, survival, and reproduction, or more susceptible to natural enemies such as parasites and predators, than their symmetrical counterparts, the exact relationship of FA and fitness continues to being a source of controversy (e.g., Møller 1997; Clarke 1998; Kruuk et al. 2003).

There have been relatively few attempts to examine whether FA is a robust predictor of fitness in natural plant populations as compared to animal models (see reviews in Møller and Swaddle 1997; Møller and Shykoff 1999). This tendency is unfortunate because flower FA may be a reliable indicator of reproductive success in outcrossing plants through the behavior of pollinators (e.g., Jennions 1996; Neal et



Figure 1. Symmetric and asymmetric nectar guides of *Mimulus luteus*. The position of the nectar guide in the landing petal is represented in the photograph.

al. 1998; Evans and Marshall 1996; Salonen and Lammi 2001; Rao et al. 2002; but see Andalo et al. 2000; Waldmann 2001). It is known that pollinators show preferences for symmetrical corollas over asymmetrical alternatives (e.g., Kelber 1997; Møller and Sorci 1998; Giurfa et al. 1999), probably because symmetrical tend to produce more nectar than asymmetrical ones (e.g., Møller 1995; Møller and Eriksson 1995; Møller 2000). Recent studies, however, find no relationship among corolla asymmetry and seed set, germination percentage, seed mass, and seedling mass in the perennial herb *Lychnis viscaria* (Siikamäki et al. 2002). One possible confounding factor is an inappropriate choice of floral characters relevant to pollinators on which to measure FA. For instance, in addition to corolla shape and symmetry, spots, lines, and blotches of contrasting color or UV wavelength on petals provide visual cues for short-distance orientation and landing on flowers (e.g., Lehrer et al. 1995; Lehrer 1997; Johnson and Dafni 1998; Lehrer 1999). Bees and butterflies use floral pigments as cues that help them to reduce searching and handling time as a way of minimizing the high energetic cost of flight (e.g., Heinrich 1975; Waser and Price 1981). As pollinators gain knowledge of these cues, they can use them to guide their searches for pollen or nectar reward, and improve their foraging performance on subsequent bouts. In this study we attempt to answer the following questions (1) Is nectar guide a character that shows fluctuating asymmetry? (2) What is the functional relationship of the nectar guide FA with female fitness?

### Materials and methods

*Mimulus luteus* (Scrophulariaceae) is a hydrophilic species that inhabits streamsides and boggy areas through the South American Andes from 29° to 45° S (Von Bohlen 1995). In Chile, flowering and fruiting occur from December to February. Plants are 53 cm tall on the average and most individuals produce 1 – 4 zygomorphic flowers that last 3.6 and 5.9 days on average if pollinated and unpollinated, respectively. Flowers have inserted anthers and a bilobed stigma that closes upon contact. The five-lobed yellow corolla has an average area of 960.2 mm<sup>2</sup> (measured from scaled frontal-viewed digital photographs, range: 487 – 1458 mm<sup>2</sup>, CV = 14.8%), and presents a conspicuous red nectar guide spot on the lower lobe (mean = 56.9 mm<sup>2</sup>, range: 0 – 206.6 mm<sup>2</sup>, CV = 76.4%, Figure 1). Guide size is more variable between than within plants (one way ANOVA,  $F_{46, 72} = 20.34$ ,  $P < 0.001$ ). A previous study revealed that bees and hummingbirds differ in their floral choice, with bees preferring small flowers with large nectar guides, the reverse being true for hummingbirds (Medel et al. 2003). Even though the outline of the nectar guide was also important for pollinators, the extent to which the symmetry of nectar guides influences female fitness in *M. luteus* is unknown.

This study was conducted during the summer season of 1999 and 2000 in a protected area of the mining company Los Pelambres (31°45'40.8" S, 70°30'33.1" W, 2730 m above the sea level), located 55 km east Salamanca, IV Región, Chile. In the study site, *M. luteus* is distributed in conspicuous monospe-

cific patches, surrounded by a vegetation matrix that includes *Montiopsis sericea* (Portulacaceae), *Calceolaria biflora* (Scrophulariaceae), *Cerastium montioides* (Caryophyllaceae), *Chuquiraga oppositifolia* (Compositae), *Vicia graminea* (Fabaceae), *Senecio polygaloides* (Compositae), and *Astragalus looseri* (Fabaceae) as the most common species. Pollinator species consist of the bees *Centris nigerrima* (Anthophoridae), *Megachile semirufa* (Megachilidae), and *Melissoptila* sp. (Anthophoridae), the butterfly *Tatochila mercedis* (Pierinae), and the Andean hummingbird *Oreotrochilus leucopleurus* (Trochilidae). Nighttime observations indicate that nocturnal pollination is absent in this species (Medel, personal observation).

We tagged and took digital pictures of 300 first-day opened flowers, each from a different individual, and collected their capsules after 22 days, when no evidence of additional fruit formation was observed. Seeds were counted in the laboratory under a binocular microscope. Relative fitness values were calculated by dividing the number of seeds of each flower by the mean number of seeds in the population. Flowers were recorded as digital pictures using a scaled FD-7 Sony digital Mavica, taking into account the perspective of approaching pollinators, in a plane 90° to the long axis of the corolla. Distance from the objective to the flower was kept constant by using a rigid support. We captured the details of the nectar guide shape with an additional close up on the lower lobe, taking care to minimize potential damage to flowers due to handling. Only pictures with sufficient resolution to perform unbiased quantification of fluctuating asymmetry were included in analyses (N = 171). Images were analyzed with UTHSCSA Image-Tool for Windows, version 2.0 (Wilcox et al. 2000). We digitized images by recording the x, y-coordinates of the nectar guide outline in a 1280 × 1224 pixel screen, and rotated pictures until a 0° alignment by visual inspection. After that, we computed the middle position from the right and left sides of the nectar guide at every row from a bidimensional array developed in MATLAB 5.0, and estimated the bilateral plane of symmetry as the straight line that minimized the squared deviations from the middle pixels. The nectar guide size was calculated by applying conversion factors relating pixels to area in mm<sup>2</sup>. The asymmetry of the nectar guide was calculated from the absolute difference between the nectar guide area at the right and left sides, |R-L|, of the estimated plane of symmetry. We estimated the level of measurement

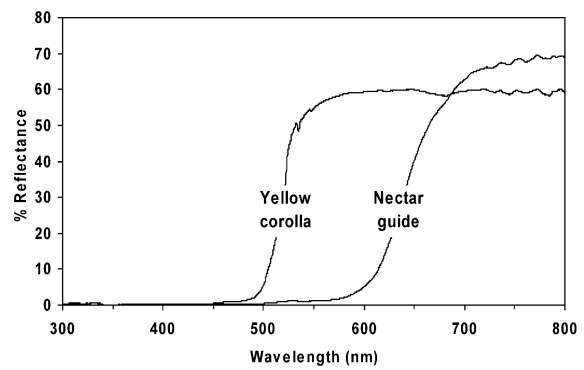


Figure 2. Flower reflectance pattern of *Mimulus luteus*. The mean reflectance percentage at 0.3 – 0.5 nm wavelength intervals is shown. Reflectance at each interval was sampled six times.

error by using Merilä and Björklund's (1995) method on the amount of variation of nectar guide FA measures after 3 independent alignments of pictures for each flower.

Because both insects as birds may detect UV light, a potential confounding factor for nectar guide perception, it was necessary to assure that flowers did not reflect UV wavelengths. We quantified the reflectance pattern of 100 randomly sampled flowers in a S2000 Miniature Fiber Optic Spectrometer (Ocean Optics®). Reflectance measures quantify the percentage of light reflected by the sample at 0.3 – 0.5 nm wavelength intervals. These percentages are commonly related to a calibrated standard white that reflects 100% of the light emitted by the spectrometer.

## Results

The corolla of *M. luteus* did not reflect ultraviolet wavelengths (320-400 nanometers) (Figure 2). The yellow portion of the corolla acted as a high pass filter, allowing reflection of all wavelengths above 500 nm, which corresponds to colors perceived by humans as green, yellow, orange and red. Nectar guides reflected all wavelengths above 600 nm, corresponding to human orange and red. These results agree with previous suggestion of Guldberg and Atsatt (1975) that irrespective of color, nectar guides have a low probability of UV reflection. Our findings suggest that even though insects and hummingbirds involved in this study probably can detect UV light within their visible spectrum, perception of nectar guides of *M. luteus* is most likely the same by pollinators and human beings.

Table 1. Functional relationship of nectar guide fluctuating asymmetry (NGFA) with female fitness (seed production) in *Mimulus luteus*. Correction of estimates were made on an individual flower basis. Df = 169 in linear models, df = 168 in quadratic models. Sequential Bonferroni techniques were applied to linear and non-linear analyses separately.

| Independent variable             | b (SE)         | t     | P     | r <sup>2</sup> |
|----------------------------------|----------------|-------|-------|----------------|
| NGFA                             | -0.076 (0.077) | 0.998 | 0.320 | 0.006          |
| NGFA/guide size                  | -0.047 (0.077) | 0.608 | 0.544 | 0.002          |
| NGFA/corolla size                | -0.098 (0.076) | 1.288 | 0.200 | 0.010          |
| (NGFA) <sup>2</sup>              | 0.048 (0.201)  | 0.243 | 0.808 | 0.006          |
| (NGFA/guide size) <sup>2</sup>   | 0.406 (0.179)  | 2.267 | 0.025 | 0.032          |
| (NGFA/corolla size) <sup>2</sup> | 0.045 (0.214)  | 0.210 | 0.834 | 0.010          |

The distribution of guide asymmetry did not differ from a normal distribution as revealed by the Shapiro-Wilk's test ( $W = 0.993$ ,  $P = 0.987$ ). The average signed right – left character values did not deviate significantly from zero (one-sample  $t$ -test,  $t = 0.08$ ,  $P = 0.987$ ), therefore satisfying two necessary conditions for characters under fluctuating asymmetry (Palmer and Strobeck 1986). Measurement error of nectar guide FA was low (3.3%), indicating this source did not introduce a significant bias in analyses. Linear regression analysis did not provide evidence of a significant relationship between nectar guide asymmetry and fitness (Table 1), indicating that guide size asymmetry had a low importance in accounting for female fitness variation in *Mimulus luteus*. These results were consistent after correcting the individual asymmetry data by guide size, and corolla size (Table 1). Quadratic models did not reveal significant curvilinear relationships between guide asymmetry and fitness, except for values of asymmetry corrected by guide size (Table 1). No coefficient was significant after adjusting the  $\alpha$ -values in a sequential Bonferroni technique (Rice 1989). Overall, our results reject the hypothesis that flowers with symmetrical nectar guides have a higher reproductive success than flowers with asymmetrical guides.

## Discussion

The question why nectar guide asymmetry did not correlate with female fitness in *M. luteus* may relate to four non-mutually exclusive factors. First, it is possible that guide asymmetry correlates better with male than female fitness. It has been suggested that the strongest selective force on hermaphroditic flow-

ers appears to be for the male function of dispersing pollen (Bell 1985). For instance, variation in flower morphology relates with the amount or rate of pollen transfer in several species (Gori 1989; Campbell 1989; Devlin et al. 1992). Because we measured female fitness only, we cannot rule out this possibility. Second, current issues regarding the importance of floral asymmetry for pollination success have invoked mainly insect pollinators, assuming that non-insect pollinators use similar cues to make their foraging decisions. This is not necessarily true. For instance, the insect and hummingbird species involved in the pollination of *M. luteus* differ significantly in the corolla and nectar guide size of the flowers they visit (Medel et al. 2003). In the context of this study, if the hummingbird species, *O. leucopleurus*, does not use guide symmetry to orientate its foraging decisions, the resulting analysis may include a source of variation attributable to foragers to which guide symmetry is unimportant to their foraging decisions. Our sampling design did not allow us to disentangle the relative contribution of insects and hummingbirds to female fitness. Future studies designed to assess the relationship of floral asymmetry and fitness in multi-specific pollinator assemblages need to take into account the way different pollinator species respond to floral symmetry variation. Third, for pollinators respond to the cues provided by guide symmetry, it is necessary that signals provide honest information about floral reward (Giurfa et al. 1999, but see Jansson et al. 2002). Although there is good support to the idea that discrete changes in flower coloration can signal presence or absence of pollinator rewards (Weiss 1991), evidence that continuous variation in floral traits is positively related to pollinator rewards is lacking (Jennions 1996). For instance, corolla and guide size in *M. luteus* are neither associated with nectar volume nor sugar concentration, probably precluding the evolution of pattern recognition in pollinator species (Medel et al. 2003). Perhaps a more parsimonious explanation for the lack of functional relationship described in this study is that genetic factors (e.g., homozygosity, hybridization, mutation, and quantitative genetic differences among individuals), as well as environmental factors (e.g., substrate, radiation, ultraviolet light, pollutants, herbivory, and competition), are more important in accounting for developmental instability in the nectar guide of *M. luteus*. Because other studies designed to test a negative relationship between flower FA and female fitness have also rejected the hypothesis (e.g., Siika-

mäki et al. 2003), it is likely that lack of relationship is the rule rather than the exception in pollination studies. More research is needed to evaluate the real importance of floral FA on pollinator preference and female fitness before extracting useful generalizations for the role of floral developmental instability on plant reproductive success.

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