

SHORT COMMUNICATION

Variable selection patterns on the labellum shape of *Geoblasta pennicillata*, a sexually deceptive orchid

S. BENITEZ-VIEYRA, A. M. MEDINA & A. A. COCUCCHI

*Instituto Multidisciplinario de Biología Vegetal (CONICET - Universidad Nacional de Córdoba), Ciudad de Córdoba, Córdoba, Argentina**Keywords:*

geometric morphometrics;
labellum;
orchids;
phenotypic selection;
pollination;
pseudocopulation;
sexual deception.

Abstract

By mimicking shape and female mating pheromones, flowers of sexually deceptive orchids attract sexually excited males which pollinate them while trying to copulate. Although many studies have demonstrated the crucial importance of odour signals in these systems, most flowers pollinated by pseudocopulation resemble, at least superficially, an insect body and these visual cues may be important to cheat pollinators. In this 2-year study, we show that the shape of the labellum of *Geoblasta pennicillata* is a target of pollinator-mediated natural selection. Contrary to our expectations, plants with a labellum shape more similar to female wasps were not favoured. The strength and pattern of phenotypic selection varied between study years and sexual functions. Although selection through female success was probably associated to the fine-tuning of the mechanical fit between flower form and male wasp, shape was the target of natural selection through male success in both study years indicating that male wasps use this trait when choosing flowers. The imperfect mimicry and patterns of selection observed indicated that an exact imitation is not needed to attract and deceive the pollinators and they suggested a receiver perceptual bias towards uncommon phenotypes.

Introduction

Since discovered by Pouyanne (1917), pollination by sexual mimicry has been reported in several orchid species. Orchids from phylogenetically independent clades pollinated by pseudocopulation can be found in the Mediterranean basin, South Africa, Australia, Central and South America (Schiestl, 2005; Ayasse, 2006; Ciotek *et al.*, 2006). Their flowers, by mimicking the shape and mating pheromones of female insects, attract sexually excited males which, by attempting to copulate, carry out pollination, a phenomenon known as pseudocopulation (Ayasse, 2006). Odour signals have been the subject of most studies on these systems and they have demonstrated their crucial importance in pollinator attraction (Borg-Karolson, 1990; Schiestl *et al.*, 1997, 1999, 2003, 2004; Ayasse *et al.*, 2003; Ayasse, 2006) and the chemical similarity of some of their components to female insect

pheromones (Schiestl *et al.*, 1999; Ayasse *et al.*, 2003) that elicit the sexual behavioural response of pollinating males (Dafni, 1984). In addition, as sexual pheromone blends are exclusive to each insect species, pollination systems involving sexual deceit are highly specialized (Schiestl, 2005). Most flowers pollinated by pseudocopulation also resemble, at least superficially, the female insect body (van der Pijl & Dodson, 1966; Borg-Karolson, 1990), and early works have shown similarities between models and mimics in visual signals as shape, colour and hairiness (Kulleberg, 1961; Ågren *et al.*, 1984). However, the importance of these signals in adaptation and evolution has not been addressed yet. In particular, no study has demonstrated whether these cues have any impact in pollinator-mediated selection. The difficulty in quantifying floral shapes is probably one of the reasons explaining this lack of studies.

Visual cues may be crucial in short distance attraction and together with tactile stimuli are necessary to complete deception of pollinators (Dafni, 1984; Schiestl, 2004). The importance of insect-like structures in pollinator attraction is supported by experiments showing that certain pollinators only attempt to copulate with

Correspondence: Santiago Benitez-Vieyra, Instituto Multidisciplinario de Biología Vegetal (CONICET - Universidad Nacional de Córdoba) C.C. 495, C.P. 5000, Ciudad de Córdoba, Córdoba, Argentina.
Tel.: ++54 + 351 4331096 ext. 2; fax: ++54 + 351 4331056;
e-mail: santiagombv@gmail.com

flowers if visual and olfactory stimuli are present together (Peakall, 1990). This should be expected since the ability of hymenopterans to discriminate shapes has been demonstrated in behavioural experiments (Hempel de Ibarra & Giurfa, 2003; Lehrer & Campan, 2004). Only one study has addressed the importance of coloured perigon in attracting pollinators in sexually deceptive orchids, but in this case colour increases the visual contrast between labellum and background or mimics food plants where females are usually found (Spaethe *et al.*, 2007). Sexual deceit in pollination takes advantage of animals' innate behaviour. 'Sensory exploitation' hypothesis has been invoked only to explain the evolution of animal traits through sensorial biases, in particular, the use of pre-existing preferences (e.g. related to feeding preferences) for sexual advertisement is well known for animals (Schaefer *et al.*, 2004). But this hypothesis can also be used to explain the evolution of some plant traits related to the attraction of animals. Such sensory biases of the signal receiver may determine the direction of an adaptive process (Ryan, 1990, 1998; Schaefer *et al.*, 2004). We expect that this perspective will particularly be useful to explain the evolution of flower form in orchids pollinated by sexual swindle.

Size, colour, texture and shape of insect-like structures can be analysed to establish their similarity with the female insect model. In particular, shape can be studied and quantified using geometric morphometric techniques (Bookstein, 1991; Rohlf & Marcus, 1993; Zelditch *et al.*, 2004). Better than individual measures, this relatively recent methodology, which was very little used in plant morphology until now, allows studying an object directly as a cohesive whole. In addition, floral features of specialized plants, including shape, have often been shown to be the targets of natural selection (Johnson & Steiner, 2000). Procedures for phenotypic selection analysis (Lande & Arnold, 1983; Arnold & Wade, 1984; Brodie *et al.*, 1995) are now standardly used to reveal the relationship between plant traits and plant reproductive success and have been successful, for example, to study the selection on the shape of nectar guides (Medel *et al.*, 2003) and the evolution of flower symmetry (Gómez *et al.*, 2006).

A new case of pseudocopulation has recently been reported for the orchid *Geoblasta pennicillata* (Rchb. f.) Hoehne ex Correa (Ciotek *et al.*, 2006), which is the first record of this pollination mode among terrestrial orchids of southern South America. The flowers of *G. pennicillata*, which are pollinated by male *Campsomeris bistrimaculata* (Lepelletier) wasps (Hymenoptera, Scoliidae), have a clearly insect-like labellum (Ciotek *et al.*, 2006) with features resembling body parts such as head, wings and abdomen. Most studies of phenotypic selection on flower traits have focused on display size or on structures associated to the match with the pollinator's morphology, whereas studies about selection on the general aspect of flower shape are comparatively few (Kingsolver

et al., 2001, see database). The aim of this study was to establish if the labellum of *G. pennicillata* is a target of natural selection mediated by pollinator preferences. According to mimicry theory (Kulleberg, 1961; Dafni, 1984; Schiestl, 2005) labella of sexually deceptive orchids show adaptations to resemble an insect model. Hence, we first tested if *G. pennicillata* individuals whose labella are more similar to the *C. bistrimaculata* female attained higher reproductive success. Secondly, we explored other patterns of selection on flower shape and size, considering that male wasps' perceptive biases could result in preferences to labellum features not necessarily similar to female wasps. Preferences of male wasps may stem either from a high response to exaggerated traits, i.e. for 'supernormal stimuli' (Timbergen, 1951; Bergström, 1978; Schiestl *et al.*, 2004; Ayasse, 2006), or from a 'receiver bias' towards novel signals (Vereecken & Schiestl, 2008). In the first case, we expect that pollinator-mediated selection will favour plants with large labella, that produce more prominent visual and, possibly, olfactory stimuli, the latter due to larger areas of odour diffusion. If pollinators' preferences are focused on labellum features other than size, we expect to find phenotypic selection acting on specific shape traits. Finally, in the presence of pollinator's biased preferences towards novel stimuli, we expect to find patterns of disruptive selection, favouring phenotypes that are less-common in the population.

Materials and methods

Study system

Geoblasta pennicillata is a terrestrial orchid with tuberous roots and a basal rosette of 2–5 leaves that produces a single flower at the end of a short scape (Correa, 1968). Its flowers lack nectar and remain open during 5 days if not pollinated, but close immediately after pollination (Correa, 1968; Ciotek *et al.*, 2006). In the study area, flowering takes place between 10 November and 20 November and is synchronic in most individuals. *G. pennicillata* is self-incompatible and hence requires pollinators to set fruits (Ciotek *et al.*, 2006). This species, present in the Argentine provinces of Entre Ríos and Buenos Aires, the Brazilian State of Rio Grande do Sul, and in the Republic of Uruguay, is exclusively pollinated by *C. bistrimaculata* (Scoliidae) male wasps that attempt to copulate with flower labellum by directing their genitalia towards the labellum base, leaving their head outwards. While the wasps attempt copulation the pollinaria are attached to the dorsal surface of their metasoma (Ciotek *et al.*, 2006).

We labelled 141 flowering plants during November 2006 and 197 during November 2007, in a population located in Sierras de Tandil, Argentina (37°27'55''S; 59°05'27''W, 411 m). Plants grew in open grasslands and were sometimes protected under bushes. We

photographed the labellum of each plant *in situ* next to a reference scale using a Nikon D50 digital camera. Each photograph was taken following a standardized protocol, with the camera's axis perpendicular to the labellum surface. Twenty days after flowering, we recorded whether plants had set fruits and if the pollinaria had been exported. As *G. pennicillata* entirely depends on pollinators for pollen export and receipt, these measures should accurately reflect pollinator choices. As we consider that this species could be endangered due to habitat lost, we did not harvest nor harm the plants in any way that might affect their natural reproduction.

To study the similarity between *G. pennicillata* and its model *C. bistrimaculata*, we captured 29 female wasps and took photographs of each one next to a reference scale as explained above.

Morphometric analysis

We used landmark methodology to study the labellum shape of *G. pennicillata* and the shape of *C. bistrimaculata* females. To choose the set of analogous landmarks, we superposed a number of scaled images of labella and wasps. Considering these superimpositions and taking

into account the pollination mechanism of this orchid, we followed the criterion that the distal part of the labellum covered with lustrous projections mimics the head and most of the thorax of the wasps (Fig. 1a,b). Thus, landmark 1 defines the distal tip of the wasp's head and of the orchid's labellum; landmarks 2 and 9 correspond with the maximum width of the orchid's head-like distal projection, analogous to the maximum distance between the wasp's compound eyes. Landmarks 3 and 8 are placed at the wasps' tegulae, which are the insertion points of first wing pair. In the orchid labella, these landmarks correspond to the beginning of two clearly distinguishable glossy lateral black plates. Landmark 4 points a change in texture in the labellum, which we assume corresponds to the division of the thorax in two clearly distinguishable aspects, i.e. the scutum and scutellum. Although not evident in a dorsal view, the lateral view of both labella and wasps indicate that this point represents the boundary between convex and flat sections (Fig. 1c,d). The convex section corresponds to the distal part of the orchids' labellum and to the wasp's head and thorax. The flat sections are represented in the labellum by the plane of the lateral plates and in the wasp by the plane of the wings. In average, the length of these

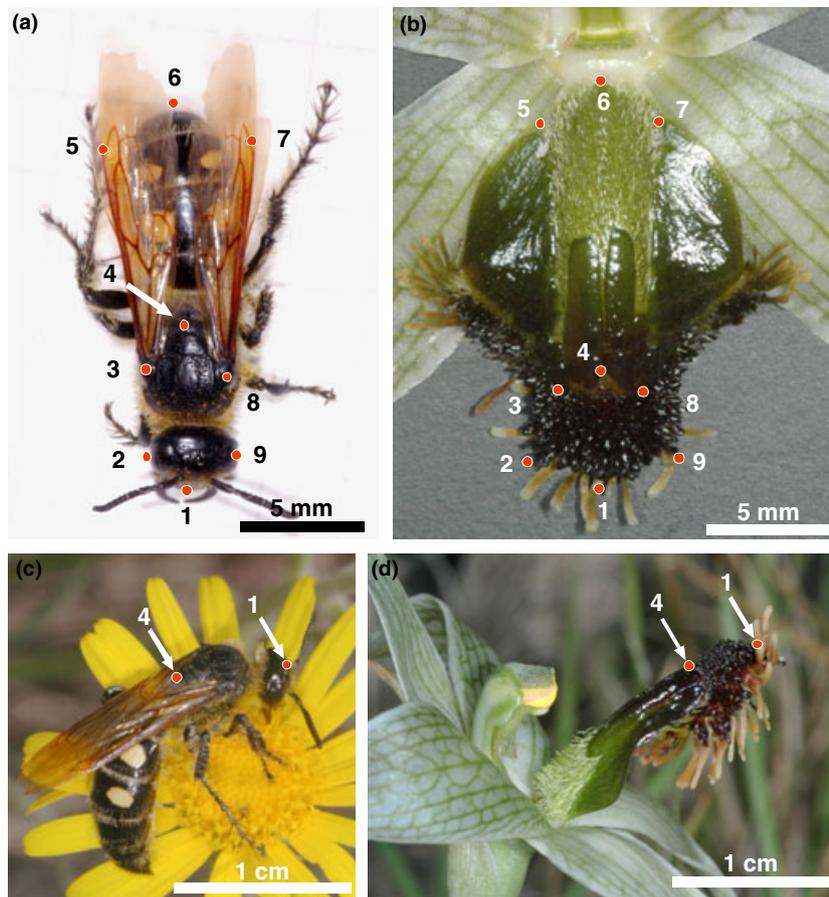


Fig. 1 Location of the nine landmarks used in geometric morphometric analysis of *Capsoomeris bistrimaculata* body shape (a) and *Geoblasta pennicillata* labellum shape (b). Lateral view of *C. bistrimaculata* (c) and *G. pennicillata* (d), showing the position of landmarks 1 and 4.

lateral plates is similar to that of the wasp's wings, but their shape is evidently different. Notwithstanding, the position of the wings in live wasps is variable. Considering the position of wasps while attempting copulation (Ciotek *et al.*, 2006), we chose the wasp's resting position, i.e. wings covering the abdomen, to place landmarks 5 and 7 that indicate the end of lateral plates in the labellum and the end of the marginal cell in the wasp's first pair of wings. Finally, landmark 6 indicates the end of the abdomen in wasps and the insertion point of labellum in the orchid flower.

Landmarks 3, 4, 5, 7, and 8 can be classified as type I (Bookstein, 1991; Zelditch *et al.*, 2004), because they represent the limits between different kinds of tissues in the orchid and different anatomical structures in the wasp. In turn, landmarks 1, 2, 6 and 9 are points of maximum curvature, and can be classified as type II as they are supported mainly by geometric evidence.

Landmark coordinates were captured using `TPSDIG` version 2.04 software (F.J. Rohit, Department of Ecology and Evolution, SUNY, Stony Brook, NY, USA). These coordinates were used to compute average or consensus configurations for both labella and wasps using Generalized Procrustes Analysis as a superimposition method. This analysis was carried out using the `TPSRELW` software, version 1.42. The unit centroid size was used as the alignment-scaling method and the orthogonal as the alignment-projection method. Bookstein's (1996) estimate of uniform component was used. We considered the differences in shape between each individual labellum and the wasp consensus configuration using `TPSPLIN` 1.20 software that allows comparing pairs of landmarks (tps series software is available at <http://life.bio.sunysb.edu/morph/index.html>).

To describe variations in labella shape, we used the orchid consensus configuration as a reference for the morphometric analysis, as suggested by Rohlf (1998). We thus computed the partial warps that describe individual landmark configurations as deformations of the average or consensus configuration. Principal component analysis on partial warps scores was used to obtain $2p - 4$ orthogonal relative warps (where p is the number of landmarks). Relative warps (RW) summarize shape information and can be used in subsequent statistical analyses (Gómez *et al.*, 2006). Morphometric analyses were performed merging data from both years because marked plants occurring in the same population did not flower in consecutive years. This multiannual flowering cycle was also confirmed in potted plants.

We obtained the Procrustes distance between each labellum and the wasp's consensus used as reference specimen. This distance can be used for quantifying overall differences in shape (Dryden & Mardia, 1998). We then tested for significant differences among labella and insect shapes with a generalized Goodall F -test (Zelditch *et al.*, 2004). Finally, as the variables obtained by geometric morphometric methods were not informa-

tive of size, we measured the labellum area, a trait probably related to pollinator attraction (Schiestl, 2004).

Adaptive value of floral traits

Female fitness was measured as fruit set (presence/absence) and male fitness as pollinarium exportation (yes/no), the latter because *G. pennicillata*, as most orchids, disperses all the pollen in the anther as a packed unit called pollinarium, which is picked up by pollinators in one visit. However, a single pollinarium is able to pollinate several flowers because it shreds in pollen clumps when touching stigma. We analysed whether male and female fitness was independent applying a χ^2 test. We first tested if orchids whose labella were more similar in shape to *C. bistrimacula* females attained higher reproductive success. We applied a logistic regression to relate plant fitness measures to the Procrustes distance between each individual plant and the consensus configuration of female wasps. Then, we followed Lande and Arnold's model (1983) to estimate multivariate selection gradients on flower size and shape. We performed the analysis using a model that included labellum size and a set of relative warps describing deformations of the labellum consensus configuration. As relative warps are descriptors of shape, significant selection coefficients related to them could indicate which specific deformations relative to the consensus flower shape are being favoured by natural selection disregarding similarity with the insect model. However, the complete set of 14 relative warps produced a model with too many parameters, most of them accounting for minimum changes in shape variance. Hence, we analysed only the relative warps explaining more than the 10% of the variation in shape. In this way, we retained for selection analysis the first three relative warps, which together accounted for the 65.02% of the total variance in shape.

We used logistic regressions instead of multiple least-square regressions because fitness values were binary. Logistic regressions produce better significance tests for discrete dependent data (Janzen & Stern, 1998), but logistic coefficients are not directly comparable with the selection gradients obtained by least-square regressions. Consequently, we applied the transformation of the logistic coefficients suggested by Janzen & Stern (1998) such that they were equivalent to common selection gradients. Before any analysis, all trait values were standardized to zero mean and unit variance. All these statistics calculations were made using the `R` 2.7.0 software (The R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria. Available <http://www.R-project.org/index.html>). To explore univariate associations between phenotypic traits and fitness significantly affected by phenotypic selection we applied cubic spline nonparametric regressions, a helpful method that avoids *a priori* assumptions on the shape of the relationship (Schluter, 1988). Cubic splines were

calculated using Schluter's *gls* software version 4.0 (available at <http://www.zoology.ubc.ca/~schluter/software.html>). To visualize the correlational selection acting on two traits we plotted fitness surfaces using thin-plate spline fit, a three-dimensional analogue of cubic spline (Blows *et al.*, 2003; McGlothlin *et al.*, 2005). A smoothing parameter for each spline was chosen by generalized cross-validation. We used *fields* package of *R* software version 2.7.0 to estimate the thin-plate splines.

Results

Morphometric analysis

The Goodall *F*-test indicates that the average shape of *G. pennicillata* labellum is significantly different from the average shape of *C. bistrimaculata* female wasp ($F_{14,5110} = 27.120$, $P < 0.0001$).

From the first three relative warps used for selection analysis, the first one is associated to changes in the relative proportion between the distal and basal portions of the labellum. Thus, this variable attains maximum

values when the relative distance between landmarks 1 and 4 is greater, and the separation between landmarks 4 and 6 smaller, than the consensus. Minimum values of this variable are represented by the opposite configuration, i.e. when the basal portion of labellum is proportionally greater and the distal portion is proportionally smaller than the consensus (Fig. 2). The second RW represents changes in the distal portion of labellum (analogous to the wasp's head and thorax). The maximum score is represented here by labella with a maximum separation between landmarks 1 and 4, and a minimum separation between landmarks 2 and 9, which results in a narrower but more prominent distal portion of the labellum. The opposite is represented by a minimum score of RW 2 (Fig. 2). The third RW is associated with changes in the distal and basal portions of the labellum (Fig. 2), such that a maximum score is attained when landmarks 5, 6, and 7 are distant from each other (making a more prominent basal portion), and landmarks 1, 2, and 9 tend to be closer together and closer to the 3-4-8 landmark group (making a less prominent and relatively smaller distal portion).

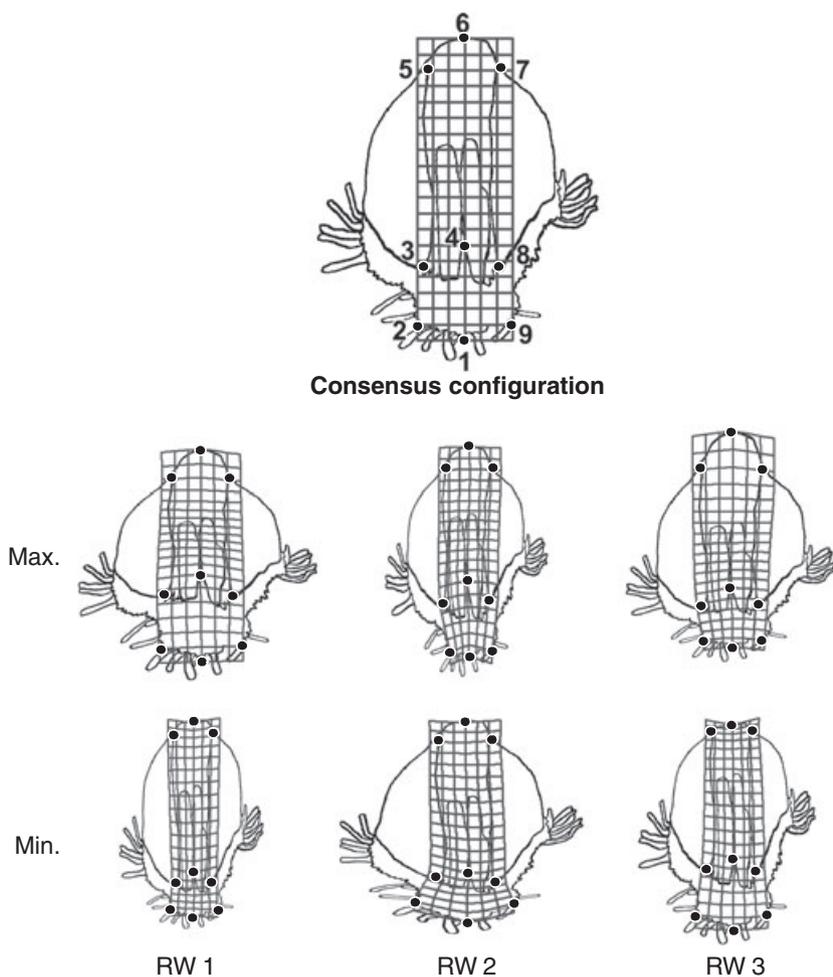


Fig. 2 Consensus configuration and thin-plate splines of the minimum and maximum scores of the first three relative warps (RWs) yielded by geometric morphometric analysis on the labella of 338 *Geoblasta pennicillata* plants. These relative warps account for 65.02% of the total variance in shape. The outline of an individual labellum was adjusted to each landmark configuration.

Table 1 Mean values, standard deviations, and Pearson's correlation coefficients of *Geoblatta pennicillata* floral traits.

Year	Trait	Mean \pm 1 SD	Pearson's correlation coefficients			
			RW 1	RW 2	RW 3	Labellum area
2006 $n = 141$	Procrustes distance	0.093 \pm 0.020	0.341***	-0.534***	-0.060	-0.170*
	RW 1	-0.001 \pm 0.040		0.075	0.075	-0.038
	RW 2	0.010 \pm 0.026			-0.208*	0.218**
	RW 3	-0.003 \pm 0.022				-0.093
	Labellum area (mm ²)	163.781 \pm 27.82				
2007 $n = 197$	Procrustes distance	0.111 \pm 0.029	0.429***	-0.707***	-0.256***	-0.140
	RW 1	0.001 \pm 0.052		-0.025	-0.042	0.017
	RW 2	-0.007 \pm 0.034			0.157*	0.192**
	RW 3	0.002 \pm 0.023				0.051
	Labellum area (mm ²)	177.110 \pm 90.08				

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.01$.

Adaptive value of floral traits

We examined if the probabilities of exporting pollinaria and setting fruits were dependant. χ^2 test indicated that sexual functions were independent in 2006 ($\chi^2 = 0.043$, $P = 0.836$). However, dependence between sexual functions was marginally significant in 2007 ($\chi^2 = 3.528$, $P = 0.060$) which is accounted by the low number of plants that set fruits and exported pollinaria. The proportion of exported pollinaria was 29.79% in 2006 and 22.84% in 2007, while fruiting success was 38.29% in 2006 and 26.39% in 2007.

We used Procrustes distance as an overall measure of differences in shape between each *G. pennicillata* labellum and the average configuration of landmarks of *C. bistrimaculata* female wasps. We did not find in any of

study years association neither between this measure and exported pollinaria (year 2006: $\beta = -4.85$, $Dev_{1,140} = 0.27$, $P = 0.603$; year 2007: $\beta = 2.95$, $Dev_{1,196} = 0.29$, $P = 0.593$), nor between it and fruiting success (year 2006: $\beta = 8.15$, $Dev_{1,140} = 0.87$, $P = 0.350$; year 2007: $\beta = 1.46$, $Dev_{1,196} = 0.06$, $P = 0.802$).

Descriptive statistics of measured floral traits are shown in Table 1. In the phenotypic selection analysis, we found different selection patterns acting via both sexual functions (Table 2). Lineal (2006) and disruptive (2007) selection was significantly acting through male fitness on RW2 as was shown by the cubic splines (Fig. 3a,b). Directional selection favoured plants with high values of RW2 in 2006; i.e. plants having labella with a relatively prominent distal portion. In contrast, during 2007 the phenotypic range for RW2 was larger and disruptive

Table 2 Multivariate phenotypic selection on labellum size and shape†.

Year	Sexual function	Trait i	$\beta_i \pm 1$ SE	$\gamma_{ii} \pm 1$ SE	$\gamma_{ij} \pm 1$ SE		
					RW 1	RW 2	RW 3
2006 $n = 141$	Male	Labellum area	-0.144 \pm 0.130	0.048 \pm 0.190	0.106 \pm 0.158	0.063 \pm 0.179	0.006 \pm 0.157
		RW 1	-0.194 \pm 0.130	0.003 \pm 0.199		-0.267 \pm 0.181	0.145 \pm 0.128
		RW 2	0.322 \pm 0.139*	-0.640 \pm 0.342#			-0.228 \pm 0.183
	Female	Labellum area	0.063 \pm 0.102	0.235 \pm 0.156	0.325 \pm 0.143*	-0.037 \pm .108	-0.081 \pm 0.132
		RW 1	0.299 \pm 0.109**	0.064 \pm 0.186		.022 \pm .118	0.020 \pm 0.111
		RW 2	0.028 \pm 0.106	-0.123 \pm 0.169			0.043 \pm 0.125
2007 $n = 197$	Male	Labellum area	-0.169 \pm 0.125	-0.545 \pm 0.272	-0.330 \pm 0.159	0.153 \pm 0.142	-0.089 \pm 0.161
		RW 1	-0.021 \pm 0.119	-0.320 \pm 0.229		0.067 \pm 0.120	0.139 \pm 0.146
		RW 2	-0.024 \pm 0.121	0.468 \pm 0.181**			-0.008 \pm 0.129
	Female	Labellum area	0.167 \pm 0.130	-0.351 \pm 0.271	0.098 \pm 0.161	-0.151 \pm 0.192	-0.100 \pm 0.162
		RW 1	0.099 \pm 0.130	-0.027 \pm 0.248		-0.308 \pm 0.195	0.143 \pm 0.145
		RW 2	-0.067 \pm 0.131	-0.604 \pm 0.332#			0.095 \pm 0.185
	RW 3	-0.018 \pm 0.130	-0.056 \pm 0.184				

†Shape was quantified using the first three relative warp scores (RWs). Standardized linear selection gradients (β_i), non-linear selection gradients (γ_{ii}), correlational selection gradients (γ_{ij}) and standard errors (SE) are indicated.

$P < 0.10$; * $P < 0.05$; ** $P < 0.01$.

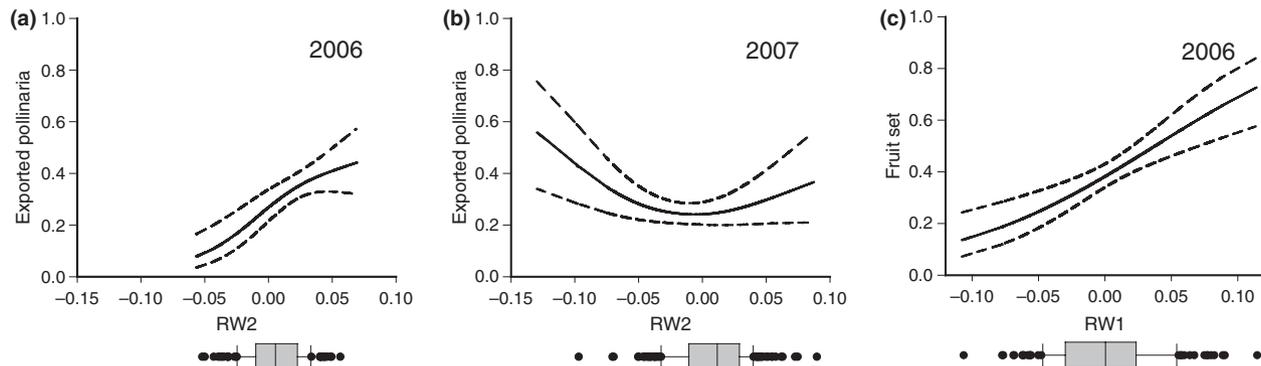


Fig. 3 Cubic spline regressions between floral traits and reproductive success during the years 2006 and 2007. Dashed lines show ± 1 SE, estimated from 1000 bootstrap replications. Box-plots indicate the distribution of each variable. Natural logarithms of the smoothing parameter λ are as follows: (a) $\lambda = -6.4$; (b) $\lambda = -5.5$; (c) $\lambda = -8.8$.

selection pattern found indicate that plants with shape phenotypes near the population mean attained lower reproductive success. Through female function we only found evidence of selection in the year 2006. Positive directional selection acted on RW1 (Fig. 3c), favouring plants having labella with proportionally larger distal portions. In the same year, correlational selection between labellum area and RW1 (Fig. 4) was found. Inspection of the thin-plate spline indicates that plants with higher scores of RW1 and larger labella attained higher fruit set.

Discussion

This study is the first to evaluate if floral form is a target of pollinator-mediated selection in an orchid pollinated by sexual deceit applying a landmark methodology to

quantify labellum shape and the similarity between labella and female wasps. Although landmarks were analogous between orchids and wasps, they represent the main features of labella and wasps shape and thus should reveal general deviations and coincidences in shape. Natural selection driven by male wasp preferences is expected to favour orchids whose labella are more similar to its animal model (Kulleberg, 1961; Dafni, 1984). However, *G. pennicillata* plants with a closer similarity in labellum shape to the female wasps did not attain better reproductive success. This result could be attributed to the low visual spatial resolution of hymenoptera, at least at medium and long distances (Giurfa & Leher, 2001; Land, 2005; Chittka & Raine, 2006), implying that information on flower shape would be quite reduced if the decision to visit a flower was made at a long distance (Vorobyev *et al.*, 1997). If a superficial similarity with the model were enough to promote pollinator approach, no strong selection on shape would be detected. However, we found that shape and size were indeed under selection. This can indicate that male wasps had biased preferences to particular features of the labellum.

Preference of male wasps for supernormal stimuli, as was found by Schiestl (2004) in the sexually deceptive Australian orchid *Chiloglottis trapeziformis*, would explain selection on labellum size. In this case, selection would favour plants with large labella, because they would display better visual stimuli and also greater amounts of olfactory attractants, the latter due to wider areas of odour diffusion. However, our findings did not support this hypothesis, because in *G. pennicillata* selection for larger labella was only found in one study year and through female function alone.

Another possible explanation about patterns of selection on shape is that male wasps show preference or 'receiver bias' towards novel signals. Recently, Vereecken & Schiestl (2008) have found that the imperfect match between the odour of *Ophrys exaltata* and the sexual pheromones of *Colletes cunicularis* might have an adaptive

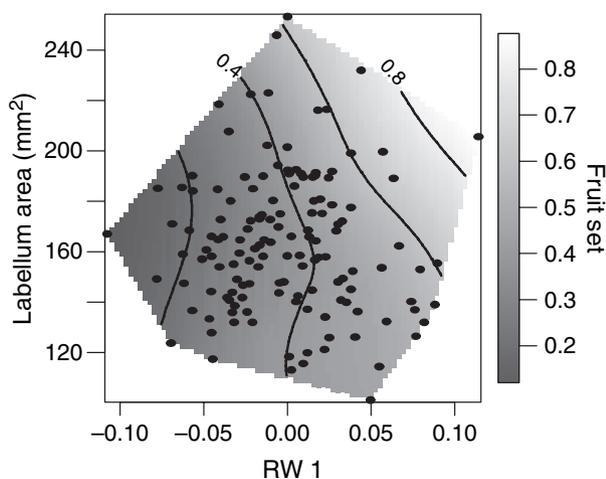


Fig. 4 Selection surface (thin-plate spline) for fruit set as a function of relative warp 1 (RW1) and labellum area. Fruit set is shown as grey scale and as continuous lines on the selection surface.

value, since male bees show preferences for similar but novel scent stimuli. Hence, this receiver bias towards novel signals can be an important force driving signal evolution in mimicry (Vereecken & Schiestl, 2008). Although RW2 was consistently the target of phenotypic selection in both study years, which indicates that male wasps use this trait when selecting flowers, the patterns of selection varied among years and sexual functions. Thus, the hypothesis of receiver bias towards novel signals can only enlighten the patterns of selection on RW2 in 2007 when pollinator-mediated selection favoured the less-common phenotypes and would explain how the broad variability in this trait is maintained. As the RW2 shape component was mainly related to changes of the distal portion of the labellum, this trait may be a key signal for pollinators to distinguish between basal and distal portions of the labellum when approaching and landing on the flower.

Although evolution of flower form can be driven by total fitness, we found consistent differences in selection patterns and a decoupling between sexual functions. This decoupling made it more difficult to predict the outcome of selection based on total fitness. Because fruit set is a final component of female success while pollinarium exportation is the first component of male success and may not reflect final siring success (given that it does not take into account that pollinaria can be lost during transportation), we considered that simple estimates of total fitness (e.g. the addition of sexual functions) can lead to misleading interpretations. Thus, we restrict our discussion to the analysis of selection based on separate sexual functions. The specific requirements for pollinator matching by either male or female sexual functions may explain differences in selection through sexual functions. For instance, axially compressed labella (as those with high values of RW1) may facilitate wasps contact the stigma, being selected through female function. But the same shapes may not affect contact with viscidium, consequently not influencing pollinarium exportation. Sexual function may also be decoupled because pollinarium depositions on the stigma occur as result of a second or subsequent visit by the same insect, and selection through female fitness may depend from shifts in sensorial preferences of sexually excited males after a first unsuccessful mating attempt, as in other deceptive systems (Ayasse *et al.*, 2000).

Conclusions

Selection on flower shape was evident, although the interpretation of the selection outcomes is complex due to the variation between years and sexual functions. However, several interesting explanatory possibilities arise from this study, which are promising for future work such as sensory exploitation patterns and decoupling between male and female functions. Finally, our results deviate from the expectations of the mimicry

theory (Kulleberg, 1961; Dafni, 1984), as we did not detect a reproductive advantage of plants carrying labella more similar to the female wasps. We stress the importance of selection mediated by receiver bias in signal evolution.

Acknowledgments

We thank Lucrecia Díaz, Evangelina Glinos, Sergio Matiz, Daniel Morales, Alicia Sérsic and Joseph Patt for their assistance in fieldwork. Manfred Ayasse, Rolando González-José, Alfredo Peretti and two anonymous reviewers provided helpful comments on the manuscript and Adriana Zapata provided suggestions on morphometric analysis. This work was partially financed by grants PIP 5174 from CONICET, PICT 01-10952, 01-14606 and 01-33755 from SECYT-FONCYT and by Idea Wild Foundation. SBV and AAC are postdoctoral fellowship and researcher of CONICET respectively.

References

- Ågren, L., Kulleberg, B. & Sensenbaugh, T. 1984. Congruences in pilosity between three species of *Ophrys* (Orchidaceae) and their Hymenopteran pollinators. *Nov. Act. R. Soc. Sci. Ups.* **5**: 15–25.
- Arnold, S.J. & Wade, M.J. 1984. On the measurement of natural and sexual selection: applications. *Evolution* **38**: 734.
- Ayasse, M. 2006. Floral scent and pollinator attraction in sexually deceptive orchids. In: *Biology of Floral Scent* (N. Dudareva & E. Pichersky, eds), pp. 219–241. CRS Press, Boca Raton, FL.
- Ayasse, M., Schiestl, F.P., Paulus, H.F., Löfstedt, C., Hansson, B., Ibarra, F. & Francke, W. 2000. Evolution of reproductive strategies in the sexually deceptive orchid *Ophrys sphegodes*: how does flower-specific variation of odor signals influence reproductive success. *Evolution* **54**: 1995–2006.
- Ayasse, M., Schiestl, F.P., Paulus, H.F., Ibarra, C. & Francke, W. 2003. Pollinator attraction in a sexually deceptive orchid by means of unconventional chemicals. *P. Roy. Soc. B-Biol. Sci.* **270**: 517–522.
- Bergström, G. 1978. Role of volatile chemicals in *Ophrys*-pollinator interactions. In: *Biochemical Aspects of Plant and Animal Co-evolution* (J.B. Harborne, Ed.), pp. 207–232. Academic Press, London.
- Blows, M.W., Brooks, R. & Krafts, P.G. 2003. Exploring complex fitness surfaces: multiple ornamentation and polymorphism in male guppies. *Evolution* **57**: 1622–1630.
- Bookstein, F.L. 1991. *Morphometric Tools for Landmark Data: Geometry and Biology*. Cambridge University Press, Cambridge, MA.
- Bookstein, F.L. 1996. Standard formula for the uniform shape component in landmark data. In: *Advances in Morphometrics. Proceedings of the 1993 NATO Advanced Studies Institute on Morphometrics in Il Ciocco, Italy* (L.F. Marcus, M. Corti, A. Loy, G. Naylor & D.E. Slice, eds), pp. 153–168. Plenum Publishing Corp., New York.
- Borg-Karlson, A.-K. 1990. Chemical and ecological studies of pollination in the genus *Ophrys* (Orchidaceae). *Phytochemistry* **29**: 1359–1387.

- Brodie, E.D. III, Moore, A.J. & Janzen, F.J. 1995. Visualizing and quantifying natural selection. *Trends Ecol. Evol.* **10**: 313–318.
- Chittka, L. & Raine, N.E. 2006. Recognition of flowers by pollinators. *Curr. Opin. Plant Biol.* **9**: 428–435.
- Ciotek, L., Giorgis, P., Benitez-Vieyra, S. & Cocucci, A.A. 2006. First confirmed case of pseudocopulation in terrestrial orchids of South America: pollination of *Geoblasta pennicillata* (Orchidaceae) by *Campomeris bistrimaculata* (Hymenoptera, Scoliidae). *Flora* **201**: 365–369.
- Correa, M.N. 1968. Rehabilitación del género “*Geoblasta*” Barb. *Rodr. Rev. Mus. La Plata n.s.* **11**: 69–74.
- Dafni, A. 1984. Mimicry and deception in pollination. *Annu. Rev. Ecol. Syst.* **15**: 259–278.
- Dryden, I.L. & Mardia, K.V. 1998. *Statistical Analysis of Shape*. Wiley, Chichester, UK.
- Giurfa, M. & Leher, M. 2001. Honeybee vision and floral displays: from detection to close-up recognition. In: *Cognitive Ecology of Pollination: Animal Behavior and Floral Evolution* (L. Chittka & J.D. Thomson, eds), pp. 61–82. Cambridge University Press, Cambridge, MA.
- Gómez, J.M., Perfectti, F. & Camacho, P.M. 2006. Natural selection on *Erysimum mediohispanicum* flower shape: insights into the evolution of zygomorphy. *Am. Nat.* **168**: 531–545.
- Hempel de Ibarra, N. & Giurfa, M. 2003. Discrimination of closed colored shapes by honeybees requires only contrast to the long wavelength receptor type. *Anim. Behav.* **66**: 903–910.
- Janzen, F.J. & Stern, H.S. 1998. Logistic regression for empirical studies of multivariate selection. *Evolution* **52**: 1564–1571.
- Johnson, S.D. & Steiner, K.E. 2000. Generalization versus specialization in plant pollination systems. *Trends Ecol. Evol.* **15**: 140–143.
- Kingsolver, J.G., Hoekstra, H.E., Hoekstra, J.M., Berrigan, D., Vignieri, S.N., Hills, C.E., Hoang, A., Gibert, P. & Beerli, P. 2001. The strength of phenotypic selection in natural populations. *Am. Nat.* **157**: 245–261.
- Kulleberg, B. 1961. Studies on *Ophrys* pollination. *Zoolog. Bidr. Ups.* **34**: 340.
- Land, M.F. 2005. The optical structures of animal eyes. *Curr. Biol.* **15**: R319–R323.
- Lande, R. & Arnold, S.J. 1983. The measurement of selection on correlated characters. *Evolution* **37**: 1210–1226.
- Lehrer, M. & Campan, R. 2004. Shape discrimination by wasps (*Paravespula germanica*) at the food source: generalization among various types of contrast. *J. Comp. Phys. A* **190**: 651–663.
- McGlothlin, J.W., Parker, P.G., Nolan, V. Jr & Ketterson, E.D. 2005. Correlational selection leads to genetic integration of body size and an attractive plumage trait in dark-eyed juncos. *Evolution* **59**: 658–671.
- Medel, R., Botto-Mahan, C. & Kalin-Arroyo, M. 2003. Pollinator-mediated selection on the nectar guide phenotype in the Andean monkey flower, *Mimulus luteus*. *Ecology* **84**: 1721–1732.
- Peakall, R. 1990. Responses of male *Zaspilothynnus trilobatus* Turner wasps to females and the sexually deceptive orchid it pollinates. *Func. Ecol.* **4**: 159–167.
- van der Pijl, L. & Dodson, C.H. 1966. *Orchid Flowers: Their Pollination and Evolution*. University of Miami Press, Coral Gables, FL.
- Pouyanne, A. 1917. La fécondation des *Ophrys* par les insectes. *Bull. Soc. Hist. Nat. Afriq. Nord* **8**: 6–7.
- Rohlf, F.J. 1998. On applications of geometric morphometrics to studies of ontogeny and phylogeny. *Syst. Biol.* **47**: 147–158.
- Rohlf, F.J. & Marcus, L.F. 1993. A revolution in morphometrics. *Trends Ecol. Evol.* **8**: 129–132.
- Ryan, M.J. 1990. Sexual selection, sensory systems and sensory exploitation. *Oxford Surv. Evol. Biol.* **7**: 157–195.
- Ryan, M.J. 1998. Sexual selection, receiver biases, and the evolution of sex differences. *Science* **281**: 1999–2003.
- 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. 2004. Floral evolution and pollinator mate choice in a sexually deceptive orchid. *J. Evol. Biol.* **17**: 67–75.
- Schiestl, F.P. 2005. On the success of a swindle: pollination by deception in orchids. *Naturwissenschaften* **92**: 255–264.
- Schiestl, F.P., Ayasse, M., Paulus, H.F., Erdmann, D. & Francke, W. 1997. Variation of floral scent emission and post pollination changes in individual flowers of *Ophrys sphegodes* subsp. *sphogodes* (Miller). *J. Chem. Ecol.* **23**: 2881–2895.
- Schiestl, F.P., Ayasse, M., Paulus, H.F., Löfstedt, C., Hansson, B.S., Ibarra, F. & Francke, W. 1999. Orchid pollination by sexual swindle. *Nature* **399**: 422.
- Schiestl, F.P., Peakall, R., Mant, J.G., Ibarra, F., Schulz, C., Franke, S. & Francke, W. 2003. The chemistry of sexual deception in an orchid-wasp pollination system. *Science* **302**: 437–438.
- Schiestl, F.P., Peakall, R. & Mant, J.G. 2004. Chemical communication in the sexually deceptive orchid genus *Cryptostylis*. *Bot. J. Linn. Soc.* **144**: 199–205.
- Schluter, D. 1988. Estimating the form of natural selection on a quantitative trait. *Evolution* **42**: 849–861.
- Spaethe, J., Moser, W.H. & Paulus, H.F. 2007. Increase of pollinator attraction by means of a visual signal in the sexually deceptive orchid, *Ophrys heldreichii* (Orchidaceae). *Plant. Syst. Evol.* **264**: 31–40.
- Timbergen, N. 1951. *The Study of Instinct*. Oxford University Press, Oxford.
- Vereecken, N.J. & Schiestl, F.P. 2008. The evolution of imperfect floral mimicry. *P. Natl. Acad. Sci. USA* **105**: 7484–7488.
- Vorobyev, M., Kunze, J., Gumbert, A., Giurfa, M. & Menzel, R. 1997. Flowers through the insect eyes. *Israel J. Plant Sci.* **45**: 93–102.
- Zelditch, M.L., Swiderski, D.L., Sheets, H.D. & Fink, W.L. 2004. *Geometric Morphometrics for Biologists. A Primer*. Elsevier, London.

Received 17 April 2009; revised 6 August 2009; accepted 17 August 2009