

Functional modularity in a forcible flower mechanism: relationships among morphology, biomechanical features and fitness

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Abstract Flowers may be interpreted as complex combinations of organs functionally coordinated to attract pollinators and to mechanically interact with the pollinator's body, particularly when flower mechanisms are actively handled by pollinators. Thus, a functional modularity of traits in keel flowers (Fabaceae) was expected because of a compartmentalization between attraction and mechanical functions. To test this hypothesis, we used *Collaea argentina*, a Fabaceae that exhibits typical keel flowers. The force needed to open keels, the keel displacement angle and floral morphometric traits in 100 plants from a natural population were measured to detect floral characters correlated with the biomechanical variables. Furthermore, we examined the relationships among this functional module, biomechanical variables and female reproductive success to explore whether these traits are the targets of pollinator-mediated phenotypic selection, and used path analysis to examine the causal relationship among these variables. A functional module formed by two morphometric traits of the petals directly involved in the floral mechanism (keel and wings) was found, but no flag trait was involved in this module. Even though the functional module had a positive effect on force and there were significant relationships between the displacement angle and fruit set, no significant effect of force on female reproductive success was detected. These results question whether selection currently plays a role favouring the integration of this module, but this may be consistent with a past stabilizing selection on the force needed to open the keel.

Keywords Pollination · Floral phenotypic selection · Floral biomechanic traits · Functional module · *Collaea argentina* · Keel flowers

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Introduction

Flowers can be considered as complex combinations of organs that are functionally coordinated to attract pollinators and to mechanically interact with pollinators' bodies in order to achieve successful pollen transfer. Thus, traits involved in pollen deposition and removal are expected to be integrated into a module due to the selective pressures exerted by pollinators (Fenster et al. 2004; Specht and Bartlett 2009; Rosas-Guerrero et al. 2010; Diggle 2014; Gómez et al. 2014). Combinations of functionally coordinated traits are recognized as being a functional module when they exhibit high correlations among themselves and are independent or quasi-independent from other modules (Berg 1960; Cheverud 1982; Wagner 1996; Herrera et al. 2002; Murren et al. 2002; Pigliucci 2003; Pigliucci and Preston 2004; Pérez et al. 2007; Klingenberg 2008; Conner et al. 2014; Diggle 2014).

A flower may consequently be understood as a compartmentalized structure consisting of modules dedicated to different functions. Moreover, flowers with mechanisms that are actively manipulated by pollinators, i.e. those in which the access to rewards requires that pollinators exert a mechanical force, should present a distinctive pattern of modularity among floral traits, because traits associated with the forcible pollination mechanism need to be intercorrelated to ensure flower functionality. Here, we will test this idea for the case of keel flowers (Westerkamp and Weber 1999), in which rewards are concealed by specialized corolla structures (namely, the keel and the wings) and where pollinators have to exert force to manipulate a complex mechanism, in order to obtain these rewards (Faegri and van der Pijl 1966; Córdoba and Cocucci 2011).

A number of studies (Carvallo and Medel 2005; Pérez-Barrales et al. 2007; Tucić et al. 2013) have tested the presence of modules in different floral systems and, in many cases, pollinator-mediated selection has been proposed as being the principal mechanism shaping modular patterns. However, all these studies detected functional modules by exploring the correlations of morphometric variables, without including biomechanical traits. In addition, although other studies have considered biomechanical aspects of flower mechanisms (Brantjes 1981a, b; Brantjes and De Vos 1981; Cocucci 1989; Sérsic 1991; Whitaker et al. 2007; Sprayberry and Suver 2011; Muchhala and Thomson 2009), only a few (Brantjes 1981a, b; Claßen-Bockhoff et al. 2004; Reith et al. 2007; Córdoba and Cocucci 2011) have quantified the force needed to operate a floral mechanism. In the case of keel flowers, it was possible to correlate the force necessary to open the keel with morphometric variables to detect the group of floral traits that constitute a functional module (see Córdoba and Cocucci 2011). This methodology is not based on the analysis of covariation patterns among morphological traits, but instead on their functional association with the biomechanical performance.

The force needed to open the flowers may determinate the interaction among flowers and pollinators due to pollinators facing a trade-off between the energy obtained from nectar and the energy consumed to obtain the hidden rewards. Consequently, pollinators should avoid flowers that are too hard to open and select more rewarding ones. On the other hand, flowers that are very weak allow most visitors to access rewards (Córdoba and Cocucci 2011), thereby increasing the risk that some of them behave as nectar or pollen robbers. Consequently, it has been proposed that keel flowers (Westerkamp 1997) and other mechanisms of nectar concealment (Rodríguez-Gironés and Santamaría 2004, 2005) have evolved to exclude undesired visitors.

In this study, we first tested in keel flowers of *Collaea argentina* (Fabaceae) the existence of a functional module consisting of morphological traits associated with the force needed to open the keel. Then, we examined the patterns and strength of phenotypic selection for the morphometric traits involved in the functional module and biomechanical variables. Additionally, we examined the causal relationships among these variables and reproductive success using path analysis to examine the previous hypothesis of contemporary pollinator-mediated phenotypic selection.

Materials and methods

Plant species and study site

Collaea argentina Griseb. (Faboidea: Fabaceae) exhibits a typical keel flower architecture (Faegri and van der Pijl 1966), with each petal consisting of a flat, broad distal part, the lamina, and a basal claw hidden inside the calyx (Fig. 1a, b). This species differs from other keel flowers at the level of association among petals, as its wings and keel are interlocked by folds that fit together in a wing-keel complex that works as a single unit. While the flag's claw is hard and fixed inside the calyx, the wings and the keel are hinged at an open part of the calyx, thus allowing the whole wing-keel complex to be pushed down with respect to the flag. The flag's claw forms a channel that provides access to the nectar chamber at the base of the ovary, but it remains blocked by wing and keel's claws. Thus, pollinators must rotate the wing-keel complex in order to access nectar (Westerkamp and Weber 1999) and, in the same action, the anthers and stigma are exposed and pollen is placed on or removed from the ventral part of the pollinator's abdomen. After visitation,

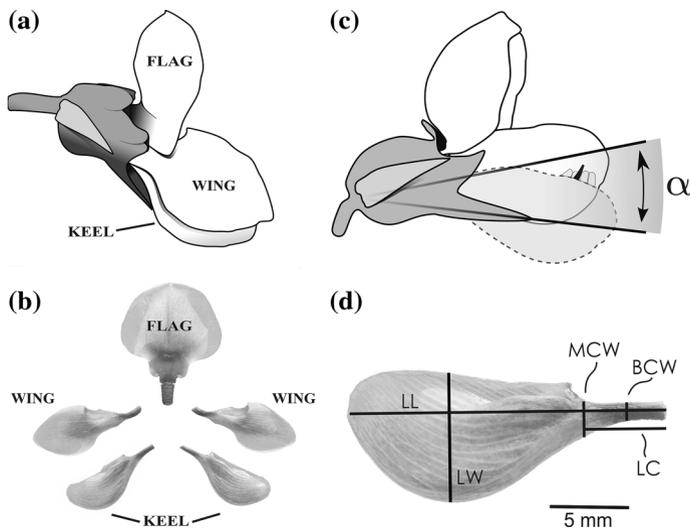


Fig. 1 Morphometric and biomechanical variables from *C. argentina*: **a**, **b** flower diagram and petal arrangement under natural conditions, **c** technique used to measure displacement angle which was obtained by overlapping photographs before and after keel displacement, **d** petal nomenclature and morphometric variables measured in each petal. *LL* length of the lamina, *LW* width of the lamina, *MCW* width of the claw at middle, *BCW* width of the claw at the base, *LC* length of the claw

the keel and wings return to their original position. Flowers produce a mean of 8 μ l of fairly highly concentrated nectar –40 % Brix approximately (pers. obs., $n = 100$ individuals).

Seven species of bees pollinate *C. argentina* flowers: *Megachile* sp. and *Anthidium* sp. (Megachilidae, Megachilinae); *Xylocopa* sp. and *Xylocopa ordinaria* (Apidae, Xylocopinae); *Bombus bellicosus* and *Bombus opifex* (Apidae, Bombinae); and *Centris tricolor* (Apidae, Apinae). Of these, only *Megachile* sp. and *Anthidium* sp. bees actively collect pollen in addition to nectar. In addition, *Apis mellifera* bees are considered to act as nectar thieves, because they access nectar from the space between the wings and keel, without ever exposing the anthers or the stigma of the flower (pers. obs.).

Flowering in the studied population occurs from September to December, with a peak occurring in October, when most individuals are simultaneously in bloom. The flowers open only during sunny days, in a gradual way, beginning at 9.00 a.m. and each individual produces hundreds of inflorescences and 4–7 flowers per inflorescence. Plants are self-incompatible and pollinator-dependant for fruit set (Lagoutte et al. 2001), with fruit production beginning in November. Data collection was carried out from September to October 2009 in a population of *C. argentina* in Cuesta Blanca village (31°28'49"S, 64°34'26"W, 744 m), situated 51 km west of Córdoba city, Argentina.

Biomechanical variables

Three previously bagged virgin flowers per plant were sampled out of a total of 100 individuals of *C. argentina*. The biomechanical variables were measured in each virgin flower and the mean per individual plant was calculated ($n = 100$). The force necessary to open the keel, expressed in mN, was measured using a dynamometer with a measurement range from 0.1 to 10 g (PESOLA, Baar, Switzerland model 20010), which was put on a vertical frame provided with a vertically sliding carriage and a metal tool holder. The flower whose force was to be measured was fixed on the holder at the dynamometer. In this device, the metal tool simulates the flower visitor, while the carriage can be moved up or down by turning a threaded rod with a wheel to emulate the downward pressing movement of the pollinator (for more details see Córdoba and Cocucci 2011). The floral mechanism does not suffer fatigue, so the flower force does not significantly change over successive trials (Córdoba and Cocucci 2011), and for this reason only a single measurement was obtained for each flower.

The displacement angle α of the wing-keel complex was measured by taking two photographs, one in the original position of the flower and other when the keel was opened just to expose the fertile parts (Fig. 1c). These pictures were then overlapped using Gimp 2.8 software (<http://www.gimp.org/>) and the angle was measured in radians using ImageJ software (<http://rsbweb.nih.gov/ij/>).

Morphometric variables

Morphometric variables were obtained from the same flowers used to measure the biomechanical variables i.e. three flowers per individual from a total of 100 individuals. The preserved flowers were dissected, and each petal was placed flat on a Petri dish with a reference scale and photographed with a digital camera (Nikon D80, Tokio, Japan). For each petal (flag, wings and keel), the following measures were taken from the photographs using the ImageJ software (<http://rsb.info.nih.gov/ij/>): length (LL) and width (LW) of the lamina, width of the claw at the base (BCW) and at the middle (MCW), and length of each

petal claw (LC) (Fig. 1d). The mean value of the left and right petals was taken for the laterally symmetrical sets (wings and keel petals). Petal length and width were taken as measures of petal size, while petal claw measurements were particularly important from the biomechanical point of view, because this part enables rotation of the wings and keel and should therefore be related to the stiffness of the mechanism.

Female reproductive success

To estimate female fitness, a single branch from each individual ($n = 100$) was randomly chosen and fruits as well as the scars left by unfertilized flowers or aborted fruits were counted for 15 inflorescences. In addition, three fruits per individual were collected and the number of viable and non-viable seeds was counted. Using these data, four different female fitness measures were calculated: *fruit set*, estimated as the number of fruits/total number of flowers produced; *mean number of viable seeds per fruit*, measured in three fruits; *proportion of matured fertile ovules*, obtained as the proportion of viable seeds in three fruits; and *mean number of viable seeds per flower*, estimated as fruit set \times mean number of viable seeds per fruit.

The fruit set estimates pollination intensity as a proportion of pollinated flowers, which is useful for investigating patterns of selection acting at the level of the individual flower, whereas the proportion of matured fertile ovules assesses the quality of the mating (Benitez-Vieyra et al. 2006). In addition to these female fitness measures, the number of ovules was determined because it affects the mean number of viable seeds per fruit.

Data analyses

Identification of functional modules

To identify the functional module related to the force to open the keel, we used the Bayesian generalized linear mixed models (GLMM) with a bivariate response from the MCMCglmm package of R software (Hadfield 2010). In these models, the morphometric traits as well as strength are treated as a joint response, and individual identity was introduced as a random effect. The following posterior distributions of two variance–covariance matrices were estimated from this model: (a) the variance–covariance matrix for the individual random effect, in which the diagonal corresponds to the variances of the consistent individual effects of force and morphometric traits, with the off-diagonal value being the covariance between them; and (b) the residual variance–covariance matrix, which estimates the equivalent variances and covariances within individuals.

Using the first matrix, the among-individual correlation between force and morphometric variables was estimated as the Pearson correlation coefficient. Bayesian GLMMs were run for 130,000 iterations, with a burnin of 30,000, a thinning interval of 100 and flat gamma-inverse priors. For each correlation, we obtained the 95 % highest posterior density (HPD) intervals and correlations were considered significant if the 95 % HPD did not include zero.

Statistical correlation does not necessarily imply causality among variables. However, given that experimental manipulation of the flower biomechanical traits in living plants was difficult, the measurement of *statistical integration* (understood as patterns of strong phenotypic correlations, see Armbruster et al. 2014) was used as an inference methodology to explore the patterns of correlations among morphometric traits and force.

Pollinator-mediated selection and causal relationships

To evaluate the potential of traits to evolve under pollinator-mediated phenotypic selection, the linear and nonlinear selection coefficients (Brodie et al. 1995) were estimated by using a multiple regression methodology proposed by Lande and Arnold (1983). We performed a phenotypic selection analysis on the morphometric traits that conform the functional module, the force needed to open the keels and the displacement angle in order to assess the patterns and strength of pollinator-mediated selection. Gradients and p values of significance were estimated through multiple regression models except when female fitness was estimated as a proportion, in which case p values were obtained using generalized linear models with a binomial error structure and logit link (Brodie and Janzen 1994).

Additionally, one path model was formulated (Fig. 2) according to the expected relationships among the biomechanical, morphological and fitness measures. The main advantage of path analysis compared with the traditional Lande and Arnold (1983) selection analysis is that it gives the possibility of quantifying the relationship among variables with a priori models that specify the causal and non-causal paths among

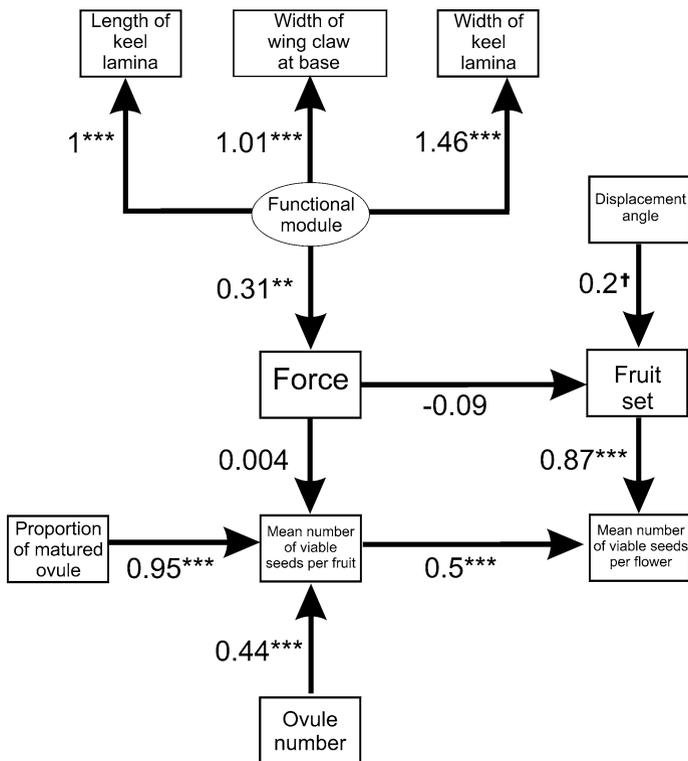


Fig. 2 Path model representing causal relationships among biomechanical traits, morphometric variables and relative female fitness in *C. argentina*. Indicator variables are in boxes, latent variables are in circles. Significance of path coefficients: *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$; † $p \leq 0.1$

Table 1 Pearson correlation coefficients estimated through Bayesian generalized linear mixed models (GLMM) with bivariate response

WING	WING										KEEL						FLAG			Mean ± SD				
	LL		LC		MCW		BCW		LW		LL	LC	MCW	BCW	LW	LL	LC	MCW	BCW					
	LW	LL	LC	LL	LC	MCW	BCW	LW	LL	LC	MCW	BCW	LW	LL	LC	MCW	BCW	LW	LL		LC	MCW	BCW	
WING LW																								10.29 ± 1.35
LL	0.61*																							23.08 ± 1.77
LC	0.3	0.7*																						4.12 ± 0.52
MCW	0.55*	0.51*	0.2																					1.33 ± 0.22
BCW	0.64*	0.52*	0.33*	0.64*																				2.17 ± 0.37
KEEL LW	0.55*	0.36*	0.06	0.28	0.42*																			8.67 ± 1.26
LL	0.46*	0.87*	0.7*	0.34*	0.33*	0.36*																		21.31 ± 1.43
LC	0.36*	0.65*	0.82*	0.28*	0.13	0.12	0.72*																	4.76 ± 0.59
MCW	0.14	0.21	0.28	0.36*	0.29	-0.06	0.1	0.21																1.15 ± 0.18
BCW	0.34*	0.32*	0.28	0.43*	0.49*	0.27	0.34*	0.46*	0.54*															2.19 ± 0.27
FLAG LW	0.19	0.59*	0.3	0.41*	0.31*	-0.44	0.49*	-0.05	0.03	-0.07														21.17 ± 2.58
LL	0.62*	0.82*	0.6*	0.48*	0.54*	0.5*	0.63*	0.59*	0.08	0.18	0.64*													26.53 ± 2.03
LC	0.13*	0.3*	0.67*	0.17	-0.01	-0.01	0.36*	0.69*	0.27	0.2	0.06	0.39*												4.66 ± 0.67
MCW	0.48*	0.51*	0.27*	0.71*	0.56*	0.37*	0.35*	0.33*	0.43*	0.54*	0.54*	0.51*	0.09											2.53 ± 0.33
BCW	0.24	0.5*	0.37*	0.59*	0.39*	-0.04	0.47*	0.42*	0.24*	0.28	0.51*	0.25*	0.23	0.74*										4.01 ± 0.51
Force (mN)	0.13	0.27*	0.07	0.15	0.34*	0.10	0.36*	0.06	0.12	0.27	0.15	0.15	-0.16	0.16	0.09									35.88 ± 12.37

Correlations were obtained from among-individual variance-covariance matrices. For each correlation, the 95 % highest posterior density (HPD) intervals were obtained. *LL* length of the lamina, *LW* width of the lamina, *BCW* width of the claw at the base, *MCW* width of the claw at middle, *LC* length of the claw

* Significant correlations when the 95 % HPD did not include zero. n = 100 plants, three flowers per individual

Table 2 Multivariate phenotypic selection in *C. argentina* for the biomechanical (force and displacement angle) and morphometric variables that form the functional module

	Trait i	$\beta_i \pm 1 \text{ SE}$			$\gamma_{ij} \pm 1 \text{ SE}$		
		Force	Angle	wwcb	Force	Angle	wwcb
Female fitness	Force	-0.003 ± 0.012	0.009 ± 0.027	0.01 ± 0.018	-0.018 ± 0.016	-0.014 ± 0.015°	0.009 ± 0.016
	Angle	0.014 ± 0.012*	-0.026 ± 0.023	0.019 ± 0.023	-0.026 ± 0.014	0.01 ± 0.019	0.01 ± 0.019
	kill	-0.002 ± 0.012	-0.012 ± 0.021	-0.012 ± 0.021	0.49 ± 0.399	0.208 ± 0.39	0.152 ± 0.415
Mean number of viable seeds per fruit	wwcb	-0.017 ± 0.012	-1.145 ± 0.685°	0.555 ± 0.449	-0.381 ± 0.367	0.398 ± 0.485	
	Force	0.381 ± 1.11	0.167 ± 0.592				
	Angle	-0.255 ± 1.095	-0.329 ± 0.601				
Proportion of matured ovules	kill	-0.763 ± 1.092	-0.571 ± 0.539	0.048 ± 0.026*	0.012 ± 0.023	0.026 ± 0.023	0.011 ± 0.024
	Force	-0.005 ± 0.019	-0.092 ± 0.04*	0.006 ± 0.035	-0.036 ± 0.021°	0.029 ± 0.028	
	Angle	-0.023 ± 0.018	0.006 ± 0.035				
Mean number of viable seeds per flower	kill	-0.031 ± 0.018°	-0.003 ± 0.035	0.139 ± 0.16	-0.006 ± 0.142	0.099 ± 0.147	0.173 ± 0.173
	wwcb	0.005 ± 0.019	-0.05 ± 0.031°				
	Force	0.069 ± 0.107	-0.116 ± 0.243				
	Angle	0.026 ± 0.106	-0.231 ± 0.211				
	kill	-0.049 ± 0.106	0.042 ± 0.214				
	wwcb	-0.177 ± 0.11	-0.207 ± 0.192				

Standardized linear selection gradients (β_i), non-linear selection gradients (γ_{ij}), correlational selection gradients (γ_{ij}) and standard errors (SE) are indicated. Significant selection coefficients are in bold

* $p \leq 0.05$; $p \leq 0.1$. n = 100 individuals, three flowers per individual

independent, dependent and intermediary variables, and can even include unobserved or latent variables (Kingsolver and Schemske 1991; Mitchell 1993; Gómez 2000).

In this model, the association among all female relative fitness measures was established including an additional exogenous variable (ovule number). A latent variable (*functional module*) involving the morphometric variables that had shown significant associations with the force was included in the model. A path from this latent variable to the force was set to represent their causal relationship. Additionally, paths between the force with fruit set and with the mean number of viable seeds per fruit were established considering that the force may be an important factor affecting these fitness measures due to deposition of pollen occurring only if the pollinator can exert the required minimal force needed to open the keel. Finally, the model included the effect of displacement angle on the fruit set, because the rotation of the keel may imply an energetic cost to pollinators, thus causing them to avoid flowers that require more energy to open the keel and to deposit less pollen. All statistical analyses were performed using the R software (R Development Core Team 2015) and path analysis was carried out using the *lavaan* package (Rosseeel 2012) of R Version 3.1-3 (R Development Core Team 2015).

Results

Functional module detection

The descriptive statistics of all measurement traits are shown in Table 1. The GLMM showed that the following three morphometric variables were significantly associated with the force: the width of the wing claw at the base (wing BCW) and the length of keel and wing lamina (keel LL and wing LL, respectively). All these variables were associated with the floral mechanism belong to the wing-keel complex (Table 1). Nevertheless, Bayesian GLMM showed a strong correlation between wing LL and keel LL (Table 1) and we assume that this high correlation could be the result of an ontogenetic relationship. Thus, taking into account that the length of the keel lamina is the lever arm and may have an important effect on the mechanical system, we included this morphometric variable instead of wing lamina length in subsequent phenotypic selection analyses.

Pollinator-mediated selection and causal relationships

The pattern and strength of selection are shown in Table 2. There was a significant positive directional selection on displacement angle, using fruit set as the fitness estimation ($\beta = 0.014 \pm 0.012$; $p = 0.04$). Additionally, when the proportion of matured ovules was used as the female fitness measurement, the analysis indicated a stabilizing selection on force ($\gamma_{ii} = -0.092 \pm 0.04$; $p = 0.03$) and a correlational selection between force and displacement angle ($\gamma_{ij} = -0.048 \pm 0.026$; $p = 0.05$). However, non-significant coefficients were found using the mean number of viable seeds per fruit or per flower. The path model revealed that the relationships among female fitness measures were significant and strong. The latent variable *functional module* included in the model had a positive and significant effect on force but the indirect effect of the latent variable on fitness measures was almost negligible. The coefficient between force and the fruit set was weak and negative, suggesting that plants with flowers requiring more force produce fewer fruits.

Due to the high correlation between fruit set and mean number of viable seeds per flower, force had a weak albeit negative indirect effect on the number of fertilized ovules, while the alternative effect on mean number of seeds per fruit was negligible. This means that force may affect fruit set as fitness measure more through the proportion of pollinated flowers than through the number of ovules fertilized, once a flower has been pollinated. In contrast, the displacement angle had a positive effect on fruit set indicating that individuals in which pollinators were able to open the flowers with a large angle also produced more fruits.

Discussion

Functional module

Berg (1960) was among the first to propose and describe modularity in plants by considering “pleiades” as being an all-encompassing floral module which should be integrated separately from vegetative parts. However, the idea that a flower consists of a globally integrated unit overlooks the fact that traits belonging to the same floral whorl may represent intrafloral developmental modules themselves (Herrera et al. 2002). Additionally, it is possible that traits from the same or different whorls cooperate in a distinct function, thus promoting compartmentalization and functional integration (Herrera et al. 2002; Pérez et al. 2007; Córdoba and Cocucci 2011). Consequently, although modularity has been tested in a number of floral systems (e.g. Carvallo and Medel 2005; Bissell and Diggle 2010), the study of functional modularity merits novel approaches that can address the difficulty of separating ontogenetic or functional relationships among the traits.

Here we identified a floral functional module that involves traits from the keel and wings directly involved in the active mechanism of keel flowers. Notably, this functional module involves only a fraction of the corolla whorl, suggesting that its integration is not constrained by ontogeny. In addition, no trait from the flag was part of the functional module, probably because the flag is usually associated to attractiveness, and in only a few Fabaceae genera does the flag function as a landing platform for visitors (Faegri and van der Pijl 1966).

All the traits of the functional module are important from a mechanical perspective. The length of the lever arm is given by the length of the keel and wing lamina, with this length determining the mechanical work needed to open the keel. On the other hand, wider claws could offer a high resistance, reflecting the morphological sturdiness of this part of the petals.

Patterns of phenotypic selection

The pattern of phenotypic selection reflected by Lande and Arnold’s methodology and path analyses are congruent. Thus, the significant and positive effect found of displacement angle on fruit set suggests that the number of fruits increases with the displacement angle. Nevertheless it is possible that this angle could have functions unrelated to mechanical aspects, because when fertile organs are in a deeper position in the keel, greater displacement angles are needed to allow their exposure. Thus, greater angles could be associated with a better protection of fertile organs against detrimental visitors. Accordingly, Westerkamp and Claßen-Bockhoff (2007) suggested that complex keel flowers as

well as bilabiate morphology have evolved as a means to protect pollen. In addition, florivory has been shown to be an important factor affecting reproductive success in legumes such as *Centrosema virginianum* (Cardel and Koptur 2010). To our knowledge, however, no study has tested the hypothesis of protection through the position of the fertile organs inside the keel.

Greater displacement angles may also ensure a more effective contact with pollinators' bodies, thereby improving pollen deposition and removal. Related to this, among pollinator species in *C. argentina* (pers.obs) this contact varied. Most visits of leaf cutter bees (*Megachile* sp. and *Anthidium* sp) involved contact with the stigma when collecting pollen which requires the anthers to be fully exposed, involving greater displacement angles. In contrast, other pollinators did not make contact with the stigma in all visits and the flower did not fully open.

The functional module included in the path analyses as the latent variable was significantly related to force, revealing that the traits included in the module represent a functionally coherent mechanical unit. However, a variation in force did not directly translate to fitness, thus casting doubts on whether selection currently plays a role favouring the integration of this module. A forcible mechanism is present in most Fabaceae with keel flowers, suggesting that this did not evolved very early in evolutionary history and is currently not strongly affected by macroevolutionary process. Consequently, we suggest that functional module might have been the target of selection in the past (Cresswell 1998) and that selection could currently be relaxed because they have attained an adaptive optimum.

The force needed to open the keel might be modulated by two factors: the accessibility to rewards, which promotes weak floral mechanisms; and the secrecy which promotes higher forces to access rewards. Consequently, a possible tradeoff exists between these factors, and for this reason the force may be the target of stabilizing selection currently attaining an adaptive optimum. Indeed, the results of the phenotypic selection analyses reflect a stabilizing selection for force, supporting the idea of an adaptive optimum.

Pollinator-mediated selection has been proposed to be a major factor in the evolution of floral modularity (Gómez et al. 2006; Ordano et al. 2008; Diggle 2014). Because *C. argentina* is self-incompatible, the female reproductive success measures used in this work may be dependent on pollinator activity. However, this study was carried out in a single population of *C. argentina* which is visited by a varied group of pollinators (see “[Materials and methods](#)” section), so it is possible that the pattern of selection for morphometric and biomechanical traits would become clearer if the group of visitors were reduced, because then the selective pressures exerted by the pollinators would change, although environmental differences can also lead to plastic changes in correlations among traits.

The female reproductive success can also be influenced by other factors, such as resource availability, resource allocation among seeds inside the fruit and seed predation (Cariveau et al. 2004). Additionally, floral traits might also be target of selection imposed by non-pollinator agents (Strauss 1997; Gómez 2003; Strauss and Whittall 2006; Rausher 2008), with floral phenotype possibly being constrained by genetic or developmental integration (Ashman and Majetic 2006). Given the low variation showed by most floral traits in *C. argentina*, artificial flower phenotypes that modify the resistance of the floral mechanism could be used to test current pollinator-mediated selection (Campbell 2009; Cuartas-Domínguez and Medel 2010). In such a case, we would expected to find an opposite relationship between higher visitation rates and the amount of force needed to open the flowers, thereby leading to higher fitness returns for individuals with weaker flowers. Additionally, a study of the heritability of the biomechanical variables would

allow the potential of the population to respond to selection to be examined (Ashman and Majetic 2006).

Main contributions

From a broad perspective, this research contributes to the understanding of how selection acts to integrate groups of floral traits into intrafloral functional modules (Herrera et al. 2002; Carvallo and Medel 2005; Ordano et al. 2008; Fornoni et al. 2008; Bissell and Diggle 2010; Rosas-Guerrero et al. 2010). Furthermore, the present study is, to our knowledge, the first to examine biomechanical variables in the context of flower functional modularity. In the case of *C. argentina*, variation in force did not directly translate to fitness. However, to test pollinator-mediated selection on biomechanical traits, further research using phenotypic manipulations is required with the aim of generating phenotypic variations on which pollinators can exert selective pressures.

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