Annals of Botany **108**: 919–931, 2011
doi:10.1093/aob/mcr196, available online at www.aob.oxfordjournals.org

ANNALS OF
BOTANY
Founded 1887

Flower power: its association with bee power and floral functional morphology in papilionate legumes

Silvina A. Córdoba and Andrea A. Cocucci*

Laboratorio de Biología Floral y Ecología Evolutiva, Instituto Multidisciplinario de Biología Vegetal (IMBiV-CONICET UNCba), Casilla de Correo 495, CP 5000, Córdoba, Argentina
** For correspondence. E-mail aacocucci@gmail.com*

Received: 22 March 2011 Returned for revision: 6 May 2011 Accepted: 14 June 2011 Published electronically: 5 August 2011

- *Background and Aims* A test was made of the hypothesis that papilionate legume flowers filter pollinators according to their ability to exert strength to open flowers to access rewards. In addition, interactions with pollen vectors were expected to explain the structural complexity of the architecture of these flowers since operative flower strength may be determined by a combination of morphological traits which form part of an intrafloral functional module.
- *Methods* Six papilionate species were studied: *Collaea argentina*, *Desmodium uncinatum*, *Galactia latisiliqua*, *Lathyrus odoratus*, *Spartium junceum* and *Tipuana tipu*. Measurements were made of the strength needed to open keels and the strength that pollinators were capable of exerting. Morphological traits of all petals were also measured to determine which of them could be either mutually correlated or correlated with operative strength and moment of strength and participated in a functional module.
- *Key Results* It was observed that pollinators were capable in all cases of exerting forces higher and often several times higher than that needed to access floral rewards, and no association could be detected between floral operative strength and strength exerted by the corresponding pollinators. On the other hand, strong and significant correlations were found among morphometric traits and, of these, with operative strength and moment. This was particularly evident among traits of the keel and the wings, presumably involved in the functioning of the floral moveable mechanism.
- *Conclusions* Though visitors are often many times stronger than the operative strength of the flowers they pollinate, exceptionally weak bees such as *Apis mellifera* cannot open the strongest flowers. On the other hand, strong correlations among certain petal morphometric traits (particularly between the keel and wings) give support to the idea that an intrafloral module is associated with the functioning of the mechanism of these legume flowers. In addition, the highly significant correlations found across petals support the view of functional phenotypic integration transcending the ontogenetic organization of flower structure.

Key words: Fabaceae, legume, papilionate flowers, pollination, biomechanic, filtering mechanism, floral phenotypic integration, bee, strength, functional module.

INTRODUCTION

A considerable number of plant species are known whose flowers have moveable parts that have to be actively handled by insects for pollination to take place. Such is the case, among others, of species of *Antirrhinum*, *Salvia*, *Linaria*, *Cornus*, *Schizanthus*, *Calceolaria*, *Phlomis*, *Plectranthus*, *Polygala* (Brantjes, 1981*a*, *b*; Brantjes and De Vos, 1981; Cocucci, 1989; Sérsic, 1991; Edwards *et al.*, 2005; Claßen-Bockhoff *et al.*, 2004*b*) and, most notably, of papilionoid Fabaceae. It has been suggested previously that the weight of pollinators is not enough to trigger such a mechanism and that additional muscular power exerted by them is needed (Faegri and van der Pijl, 1966). Herein, a flower mechanism will be termed 'forcible' when an obstacle has to be actively moved by a visitor to access the flower rewards and, consequently, to perform pollination. We will call the mechanical strength needed to open such a forcible floral mechanism the 'operative strength'. The above-mentioned plants all have flowers with forcible mechanims but can differ greatly in operative strength. For example, operative strength measured for the forcible mechanisms of *Salvia pratensis* and *Phlomis fruticosa* are 2·98 and 49 mN, respectively (Brantjes, 1981*a*; Claßen-Bockhoff *et al.*, 2004*a*). In most legumes with papilionate flowers, i.e. those with the corolla architecture consisting of one dorsal petal representing the flag, two lateral petals representing the wings and two ventrally attached petals representing the keel, fertile organs are hidden by the keel and pollinators mostly access rewards by pushing down the lateral and ventral petals, which are moveable, causing them to descend. Only in that way do rewards and fertile organs become exposed and make contact with the ventral side of the body of the pollinator.

Though the study of the strength needed to operate moveable parts should allow us to shed light on relevant aspects of the functional morphology of flowers, very little has been published in this respect after seminal contributions (Brantjes, 1981*a*, *b*; Brantjes and De Vos, 1981), and nothing on legumes, a plant family where forcible flower mechanisms are widespread. Two relevant ecological and evolutionary aspects immediately arise with regard to the relationship of floral operative strength with either functional specialization in plant–pollinator interaction or morphological specialization in floral phenotype.

In regard to functional specialization, similar to operative length (Moré *et al.*, 2007), a flower's operative strength may serve to filter out visitors that are unsuitable for efficient pollination. Operative strength would prevent weak visitors gaining access to rewards and admit stronger ones which, for some reason correlated with strength, could be better pollinators. This is suggested by the finding of a correlation between a flower's operative strength and pollinator mass (Brantjes, 1981*b*) among several species of Lamiaceae, Polygalaceae and Orchidaceae, in addition to the observations by Edwards *et al.* (2005) showing that only large insects could trip the flower mechanism of *Cornus*. Both observations assume that pollinator mass or size is correlated with the strength they can exert when operating a floral mechanism, which is known only for very few flower visitors (see, however, Claßen-Bockhoff *et al.*, 2004*a*, *b*; Muchhala and Thomson, 2009). In addition, since recent studies in sage do not support the previous view (Claßen-Bockhoff *et al.*, 2004*b*) the pollinator-filtering explanation remains only weakly supported.

In regards to the morphological specialization in floral phenotype, the interaction through a floral mechanism of plants with animal pollen vectors should explain a great part of this structural complexity, since effective pollen delivery and reception requires that several traits individually, or in an integrated manner, adjust to the pollinator morphology and behaviour (Stebbins, 1970). There has been recent effort to determine whether or not combinations of traits explain the association of plants with different kinds of pollinators (Martén-Rodríguez *et al.*, 2009; Ollerton *et al.*, 2009). Also information is beginning to accumulate showing that trait combinations may respond to pollinator-driven natural selection (O'Connell and Johnston, 1998; Maad, 2000; Benítez-Vieyra *et al.*, 2006, 2009; Nattero *et al.*, 2010). However, the premise that flower phenotype should work and evolve as an integrated whole has received very limited support (Herrera *et al.*, 2002; Ordano *et al.*, 2008). It has been argued that flowers actually have a parcellated phenotype and are, consequently, not integrated as a whole because several functional modules are nested within a single flower with strong intramodular and weak intermodular correlations (Ordano *et al.*, 2008). In spite of this interesting pattern, there have been difficulties in identifying which combinations of traits are expected to act coordinately for a given flower function.

In this study, we address these above aspects by testing the following hypotheses: (1) a flower's operative strength has a visitor-filtering property by allowing access to floral rewards to pollinators strong enough to trip the floral forcible mechanism; and (2) the operative strength of a flower is given by a combination of traits that form a functional flower module.

The first hypothesis predicts that plant species with forcible floral mechanisms will differ in the operative strength of their flowers and this will be correlated to the strength that pollinators are capable of exerting. Previously (Brantjes, 1981*b*), correlations were studied with body mass, whose correlation with strength is not certain. Consequently, in addition to explicitly testing the operative strength and pollinator strength, we also studied correlation between body mass and the strength of the pollinators to determine how well body mass predicts pollinator strength. In addition, and relative to this aspect, we wanted to prove if the forcible mechanism was stable during successive visits or if it was stronger or weaker at different visits.

The second hypothesis predicts that morphological traits that are significantly correlated with operative strength should have higher and more significant correlations between them than with traits not related to operative strength and that this correlation could involve traits belonging to different developmental modules, i.e. the types of petal (flag, wing or keel).

MATERIALS AND METHODS

Study species and locations

The following six species of papilionoid Fabaceae were studied: *Collaea argentina*, *Desmodium uncinatum*, *Galactia latisiliqua*, *Lathyrus odoratus*, *Spartium junceum* and *Tipuana tipu*. Sampling was performed in September and October 2008 in the following localities of Argentina (all in Córdoba Province): *L. odoratus* in Río Ceballos (31°09′12″S, 64°19′31″W), *T. tipu* in Córdoba City (31°25′09″S, 64°13′02″W), *G. latisiliqua* in La Serranita (31°44′05″S, 64°27′41″W) and *D. uncinatum*, *C. argentina* and *S. junceum* in Cuesta Blanca (31°28′49″S, 64°34′26″W). Two species, *L. odoratus* and *S. junceum*, are adventitious while the others are native to Argentina.

There are differences in the way the floral mechanisms of these species work. In two species (*C. argentina* and *G. latisiliqua*) the mechanism is relatively simple; the keel and wings pivot elastically on the respective petal claws when pressed downward, recovering their initial position when released. In these two species, anthers presenting pollen and the style are exposed when the keel is lowered. The mechanism of *L. odoratus*, though also reversible, differs from the latter two species in that pollen is not presented directly by the anthers but by the style on a brush-like pollen presenter (Yeo, 1993). In the remaining two species, *D. uncinatum* and *S. junceum*, the mechanism is explosive and can only by tripped once. Here, the keel is prevented from downward movement by the interlocking of the wings with the flag which, thus, retain the tension between them. When this interlocking is released by additional strength exerted on the wings and keel, pollen is dusted violently onto the visitor's body (Faegri and van der Pijl, 1966).

Measurement of the flowers' operative strength

An apparatus was built to measure operative strength (F). A dynamometer with measurement ranges from 0·1 to 10 g and from 0·25 to 30 g (PESOLA, Baar, Switzerland models 20010 and 20031, respectively) was set on a vertical frame which has a vertically sliding carriage provided with a tool holder. The flower whose operative strength was to be measured was fixed through a flower holder on the dynamometer. The metal tool simulates the flower visitor while the carriage can

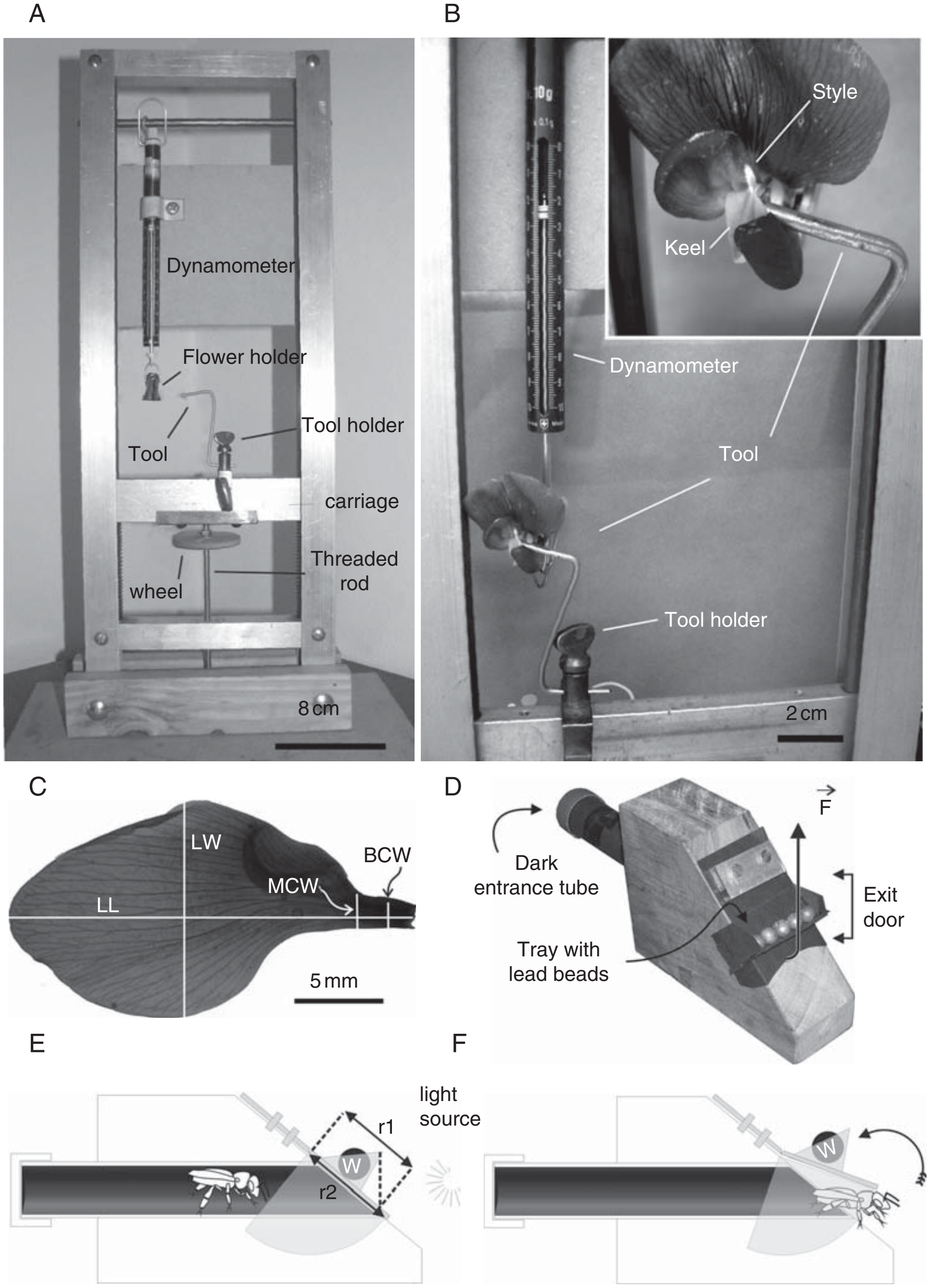


FIG. 1. Measurements of strength and morphometric traits. (A) The apparatus constructed to measure operative strength in flowers. (B) Detail showing how flowers are handled to measure operative strength. Downward sliding of the carriage with the tool moves down the wings and the keel exposing the fertile flower parts. (C) Morphometric traits measured on the petals (LL, length of the lamina; LW, width of the lamina; BCW, width of the claw at the base; MCW, width of the claw at the middle). (D) Apparatus constructed to measure the strength exerted by pollinators; the force measured to open the trap door is indicated with the vector F applied at the exit window. (E, F) Schematic showing how the bee strength measuring apparatus is operated by a bee (see also Supplementary Data video, online). Distances from the door hinge (r_1 and r_2) are shown.

be moved upwards and downwards by turning a threaded rod with a wheel to emulate the downward pressing movement of the pollinator (Fig. 1A, B). We expressed F in mN.

Large flowers could be directly held in the flower holder by the calyx without disrupting the functioning of the floral mechanism. Smaller flowers had first to be fixed with cyanoacrylate glue (La Gotita®) by the calyx to a plastic piece before clipping them to the flower holder on the dynamometer. The passive weight of the flower plus the flower holder was taken as the tare weight. By sliding the carriage down with the metallic tool the wings and the keel could be pressed down and the corresponding strength recorded on the dynamometer when the fertile parts were visible in the keel (Fig. 1B).

For flowers with reversible mechanisms, the operative strength was measured up to three consecutive times to determine if the mechanism was subject to short-term fatigue. Measurements were made in the field on a levelled surface only with freshly picked virgin flowers.

Fifteen flowers from five individuals were chosen randomly from each species. Only exceptionally, due to reduced size of the populations studied, was the number of individuals and flowers smaller.

Since several hierarchical sources of variation could explain our measurements of strength and because our main focus was on differences among species, we wanted to know how much of the total variation was attributable to differences among species. For this reason we performed nested analyses of variance (ANOVAs) of the data without phylogenetic correction. This allowed us to distinguish the contributions to variation in operative strength that are attributable to differences among species, individuals of a species, flowers of an individual, and successive trials on one flower. One nested ANOVA model was carried out with all species using the following hierarchical structure: species > individuals > flower; all levels were taken as random factors, and the response variable was operative strength. For species with reversible mechanisms, only the measurements of the first trial were considered. To test differences in operative strength between trials among species with reversible mechanisms, another nested ANOVA was carried out with the following hierarchical structure: species > individuals > flower > trial; individuals and flowers were random factors and trial was a fixed factor. A significant departure from normality of the raw operative strength data as revealed through Shapiro–Wilks test was corrected through a $\log_{10}$ transformation. Analyses were performed using R statistical software (R Development Core Team, 2010).

Operative strength is a measure of the effective strength a pollinator must exert to open a flower. Since this is not useful to ascertain the strength actually exerted on the hinges of the mechanism, we also calculated the moment of strength, a physical variable that reflects the effect of strength on causing a rotational movement of a body on its axis, in this case the rotation of the keel on the claw. The moment of strength, τ, is given by $\tau = rF\sin\Theta$, were r is the distance between the pivoting point of the moveable flower parts and the point of application of the strength, and Θ is the angle of application of the F with respect to r. When the keel is about to move and the maximum F is attained $\Theta \approx 90^\circ$ and $\sin\Theta \approx 1$, consequently $\tau \approx rF$.

Flower morphometry

Five flowers of each species were randomly chosen and fixed in 70 % ethanol after the operative strength was measured. These were later dissected and each petal placed flat and photographed with a stereomicroscope (Leica M420, Wetzlar, Germany) using LAS software (Leica, Wetzlar, Germany) which allows calibration of the photographs to the corresponding magnification. For each petal, the following measurements were taken from the photographs using ImageJ software (http://rsb.info.nih.gov/ij/): length (LL) and width (LW) of the lamina, and width of the claw at the base (BCW) and at the middle (MCW) (Fig. 1C). For the laterally symmetrical sets (wings and keel petals) the means between left and right petals were taken for further analysis. Untransformed data did not show significant departures from normality with the Shapiro–Wilks test. To visualize species differences in petal morphology we performed a principal component analysis (PCA) across species for each type of petal and plotted the samples in a two-dimensional phenospace defined by the two first PCA axes.

Studies across species are better than within-population studies to assess the degree to which traits are correlated as a result of evolutionary divergence and hence may provide insights into the roles of genetic constraints and adaptation (Armbruster *et al.*, 2004). Thus, to perform a correlational analysis across species on the morphometric and biomechanic variables, we used, in addition to standard Pearson's correlation, a phylogenetic independent contrast analysis to evaluate if the patterns of variation among species was influenced by phylogenetic associations (Felsenstein, 1985). For this purpose, all the possible pairwise relationships among observed morphometric and biomechanic values were analysed using standardized linear phylogenetically independent contrasts in the PDTREE program, as a module of Mesquite (Maddison and Maddison, 2004). The latter was based on the phylogeny of the six study species obtained from the existing phylogenetic trees of the pertinent Fabaceae (Wojciechowski *et al.*, 2004; Sede *et al.*, 2009) trimmed to include just the species of interest. Branch lengths were assigned according to Pagel's arbitrary method (Pagel, 1992). The variance homogeneity of contrasts was verified by examining the lack of fit ($P > 0{\cdot}05$) of the linear relationship between the absolute value of the standardized contrasts and the sum of the squares of branch lengths (Garland *et al.*, 1999).

We analysed correlation patterns among flower traits bearing in mind that correlations could be attributable either to their adaptive cooperation in a mechanical function or to (not necessarily adaptive) developmental constraints which are attributable to pleiotopy and linkage disequilibrium (Armbruster *et al.*, 2004). We did this by asking if either developmental or functional hypotheses better explained our empirical data. Since traits of different developmental units, i.e. types of petal, were expected to co-operate to constitute a floral mechanism, correlations of traits across types of petal should not be lower than correlations of traits within types of petal. We addressed this expectation by pursuing the rejection of a theoretical developmental hypothesis of higher expected correlations within than between types of petal. For this purpose the association between a theoretical and an empirical matrix was tested with the Mantel test. In the theoretical matrix, a value of 1 was assigned to expected correlations within types of petal and value of 0 to correlations between types of petal. Correlation analyses were carried out with InfoStat software (Di Rienzo *et al.*, 2000) and Mantel tests using R statistical software (R Development Core Team, 2010) with the number of permutations set to 1000.

A hypothesis that considers all correlations between types of petal as potentionally functional is overlooking the fact that a flower may have several functions and that only some of the correlations between developmental units could be engaged in the mechanical functioning. To reveal the existence of a functional module within the flower related to mechanical aspects of the tripping mechanism, we studied the correlations of the flower morphometric traits with operative strength and moment of strength. If some floral traits were co-operating in an integrated mechanism, morphometric traits that had high correlations with F or τ should also have relatively high correlation among them. To test if correlations within the functional module were significantly higher than correlations outside this module, a Mantel test was performed between the empirical trait correlation matrix and a hypothetical matrix where the value of 1 was assigned to intramodular correlations and a value of 0 to extramodular correlations. The significance of association between these two matrixes was calculated as above. Three different functional hypotheses were tested according to the physical variable considered and the significance level of the correlation coefficients among traits.

Measurement of the mass and strength of pollinators

Pollinators, which were always bees, were trapped in the same localities where plants were studied and were kept alive with water and sucrose provided *ad libitum* to measure their mass and strength. Live biomass was measured with a Sartorius (model CP224S, Data Weighing Systems Co., Illinois, USA) analytical scale.

To measure the strength exerted by pollinators, an apparatus was constructed by drilling in a wooden block a small tunnel that could be sealed at one end and had a moveable obstacle at the other end which captive insects must lift to find their way out (Fig. 1D). The tunnel can be darkened once closed and the exit is shut by an inclined black paper door with a small light window at the base. The exit door has a tray on the outside where lead weights (W) can be placed. Once inside, the insects moved to the window and pushed the door. The weight of the door can be progressively increased by adding lead weights until the door can no longer be opened (Supplementary Data video; Fig. 1E, F). Each time the door was opened the test insect was removed, fed and allowed to rest. The highest strength exerted by insects to open the door was recorded with the same dynamometers used above to measure a flower's operative strength. To do this, the dynamometer clip was fastened to the door at a distance from the door hinge (r_1) which allowed us to measure the force (F_1) associated with the respective rotational moment (τ_{door}), i.e. $\tau_{\text{door}} = r_1 F_1 \sin\Theta$. Since the bees push the door and exert a rotational moment at a distance from the door hinge (r_2), the force (F_2) associated with the same rotational moment ($\tau_{\text{door}} = r_2 F_2 \sin\Theta$) is calculated as $F_2 = F_1 \times r_1/r_2$. Although the door opens upwards, differing from the tripping of a flower, which requires a downward force, we observed that the same body parts and movements of the insects are utilized in both cases, i.e. turning up of the head and counteraction with the legs. Therefore, we are confident that the measured strength is comparable with the strength exerted when tripping the forcible mechanism.

To determine which insects were visiting the flowers, we dedicated a total of 52 h of observation (mean 8·7 h per species) distributed among all focal plants. The strength of all visiting insect species was measured. The number of insects tested was from seven to 20 individuals per species, except for the rare *Xylocopa augusti* of which we could capture only a single individual.

Differences between species in mass and strength of the pollinators were tested with ANOVA after checking for normality. For some species we have only one measurement, in which case we tested whether the single measurement was within the variation range of the other species with Student's *t*-test (Sokal and Rohlf, 2000). To study the relationship between body weight and strength of the bees, a regression analysis was carried out with strength as response variable and mass as independent variable. For this analysis, InfoStat software was utilized (Di Rienzo *et al.*, 2000).

Finally the relationship between a flower's operative strength and bee strength was analysed graphically and with Pearson's correlations.

RESULTS

Flower operative strength

The distribution of the raw operative strength data departed significantly from normality. This departure is attributable to the skewed distribution of operative strength measurements of the two species with explosive mechanisms, i.e. *D. uncinatum* and *S. junceum* (Fig. 2). The nested ANOVA of the log-transformed data with the hierarchical structure species > individuals > flower showed strong and significant differences in operative strength at the three levels (Table 1). The variation between species accounted for 90·1 % of the total variation in operative strength. The ANOVAs with hierarchical structure species > individuals > flower > trial also showed significant and strong differences at all levels tested (Table 1). As in the previous case, the variation between species determined 90·5 % of the total variation. Most species, except for *C. argentina* and *L. odoratus*, differed significantly in mean operative strength according to Tukey's *a posteriori* test (Fig. 3).

Separate analysis of short-term mechanical fatigue for each species showed significant differences between trials only for *T. tipu* and *L. odoratus* (Fig. 4), showing a progressive weakening of the mechanism.

Correlations among morphometric and biomechanic traits

Every morphometric trait showed strong and significant differences among species according to the ANOVA (Table 2), and species could be separated in most cases according to the dispersion of their petals in the two-dimensional phenospace (Fig. 5).

The phylogenetic correction performed on the data resulted in an increase in the number and strength of the associations among morphometric traits and between these and both biomechanic traits.

The correlation analysis showed strong associations between traits of different petals (particularly between keel and wings);

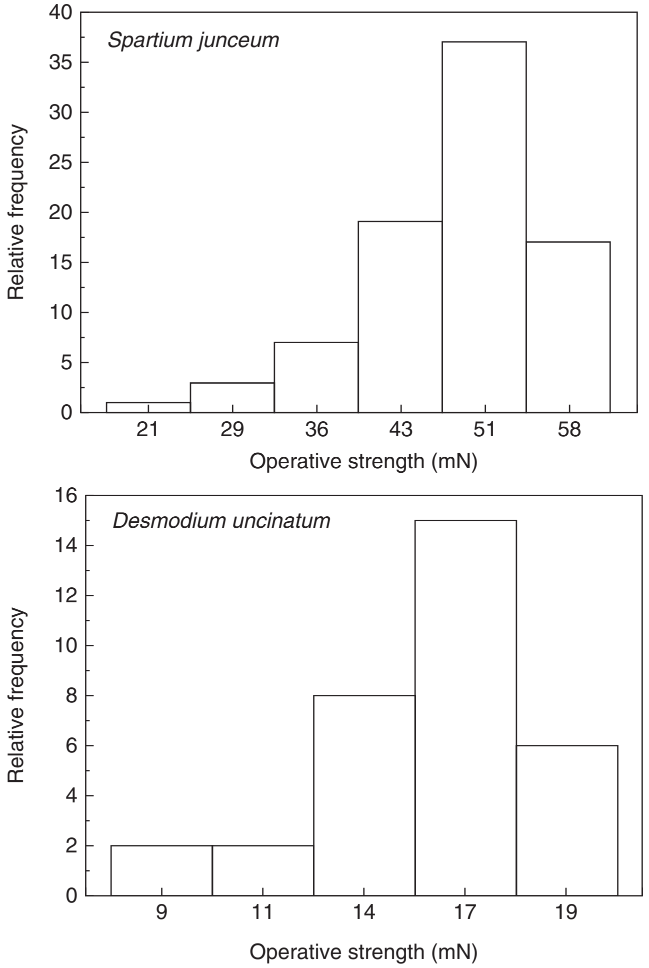


FIG. 2. Relative frequency of operative strength of flowers of two legume species with an explosive pollination mechanism.

approx. 75 % of all phylogenetically corrected correlations between morphometric traits (66) were significant (<0·05) and 100 % of the correlations between keel and wing were strong and significant, with $r > 0{\cdot}85$ (Table 3). This suggests a strong functional relationship between keel and wings.

Operative strength showed strong and significant correlations with some morphological measurements, such as both widths of the wing claw, all the keel traits and flag length. When the moment of strength is taken as a measure of flower strength, the number and intensity of correlations increased encompassing all except the flag claw widths (Table 3).

The results of the Mantel test for functional and developmental hypothesis are shown in (Fig. 6). The developmental hypothesis was rejected while the functional hypothesis with the highest statistical support was the one that relates morphometric traits to the moment of the strength.

Body mass and strength of the bees

Nine species of bees belonging to two families (Megachilidae and Apidae) were observed visiting the species examined (Table 4). There were large and significant differences among the bee species in both mean body mass and strength, with two *Anthidium* species and *Apis melifera* being the lightest and weakest, and *Xylocopa ordinaria* the heaviest and strongest according to Tukey's *a posteriori* test (Table 4). The Student's *t*-test of the single bee captured of *X. augusti* showed a significantly higher body mass ($P <$ 0·01) and strength than *X. ordinaria*, the next heaviest and strongest species. The remaining species had intermediate body masses and could not be clearly assigned either to the light or to a moderately heavy group. In terms of strength,

TABLE 1. *Nested ANOVA and variance components for operative strength (log*$_{10}$ *mN) of data without phylogenetic correction: (A) components between species, individuals within species and flowers within individuals; the first trial of every species is included; (B) components between species, individuals within species, flowers within individuals and trial within flowers; only species with a reversible mechanism are included:* C. argentina, G. latisiliqua, L. odoratus *and* T. tipu

Source	d.f.	*F* ratio	Probability > *F*	Variance components (%)
(A)				
Species	5	1308·42	<0·001	90·1
Individual (species)	17	4·13	<0·001	0·9
Flower (individuals, species)	300	6·65	<0·01	4·7
Residual	740	–	–	4·3
Total		–	–	100
(B)				
Species	3	1833·88	<0·001	90·5
Individual (species)	11	4·99	<0·001	0·9
Flower (individuals, Species)	199	9·31	<0·001	5
Trial (flower, individuals, species)	427	6·85	<0·001	3·6
Residual	625	–	–	–
Total		–	–	100

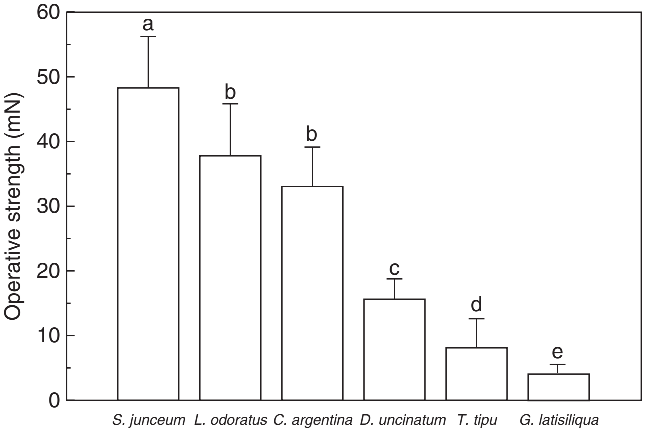


FIG. 3. Mean (± s.d.) operative strength of the flowers of six legume species [$F_{5,300} = 180{\cdot}41$, $P < 0{\cdot}0001$). Different letters indicate significant differences ($P < 0{\cdot}05$, Tukey's test).

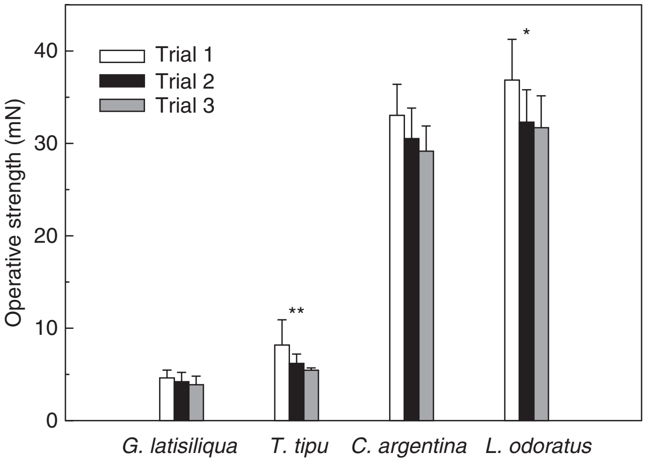


FIG. 4. Test of short-term fatigue in operative strength (mean ± s.d.) in three consecutive trials of the floral mechanism in four legumes species with a reversible mechanism. $**P \leq 0{\cdot}01$; $*P \leq 0{\cdot}05$.

these intermediately heavy species clearly belonged to the intermediately strong group (Table 4).

The regression of weight vs. strength revealed a strong positive and significant association ($r^2 = 0{\cdot}94$, Fig. 7). Considering its own weight as reference, *A. mellifera* was the weakest, being able to exert strength 32 times its own weight, while *Anthidium* sp. is the strongest and can exert a strength equivalent to 122 times its weight. Considering all species, on average, the bees are able to exert a strength equivalent to 71 times their own weight.

Relationship between flower operative strength and bee strength

The dispersion plot shows that the strength of pollinators is always higher, and sometimes several times higher, than the operative strength of flowers of the species they pollinate (Fig. 8). Even relatively small and weak bees such as *Megachile* sp. may trip open and pollinate plant species with the highest operative strength (*S. junceum*). In contrast, *A. melifera*, a bee nearly as large as *Megachile* sp., was too weak to trip flowers other than *T. tipu*. Though this bee was present in every study location, it either did not visit or robbed nectar from other studied legume species (Table 4). The Pearson's correlation between operative strength of flowers and pollinator strength indicated the absence of an association between these variables ($r = 0{\cdot}34$ and $P = 0{\cdot}28$).

DISCUSSION

Flower operative strength

Operative strength has rarely been measured in flowers, so its adaptive and ecological value has been almost completely ignored. Previous studies have been carried out in species belonging to disparate plant families, such as Lamiaceae, Polygalaceae, Plantaginaceae, Solanaceae, Orchidaceae and Cornaceae (Brantjes, 1981*a*, *b*; Brantjes and De Vos, 1981; Cocucci, 1989; Claßen-Bockhoff *et al.*, 2004*b*; Edwards *et al.*, 2005). Approximately 13 plant species belonging to

TABLE 2. *Mean ± s.d. (mm) of linear measurements of the three types of petal (wing, keel and flag) of the flowers of six legume species*

Species	Flowers/ individuals	Wing				Keel				Flag			
		LL	MCW	BCW	LW	LL	MCW	BCW	LW	LL	MCW	BCW	LW
C. argentina	5/5	$23{\cdot}99 \pm 2{\cdot}19^{d}$	$2{\cdot}42 \pm 0{\cdot}41^{e}$	$1{\cdot}50 \pm 0{\cdot}15^{a}$	$11{\cdot}83 \pm 1{\cdot}06^{d}$	$19{\cdot}98 \pm 1{\cdot}57^{d}$	$2{\cdot}35 \pm 0{\cdot}44^{c}$	$1{\cdot}27 \pm 0{\cdot}26^{c}$	$8{\cdot}71 \pm 1{\cdot}11^{c}$	$26{\cdot}75 \pm 2{\cdot}63^{d}$	$3{\cdot}65 \pm 0{\cdot}15^{bc}$	$2{\cdot}34 \pm 0{\cdot}23^{c}$	$22{\cdot}63 \pm 2{\cdot}09^{cd}$
D. uncinatum	5/3	$11{\cdot}12 \pm 0{\cdot}66^{a}$	$0{\cdot}88 \pm 0{\cdot}06^{a}$	$0{\cdot}5 \pm 0{\cdot}05^{b}$	$5{\cdot}36 \pm 0{\cdot}37^{b}$	$11{\cdot}45 \pm 0{\cdot}37^{b}$	$1{\cdot}37 \pm 0{\cdot}09^{b}$	$0{\cdot}46 \pm 0{\cdot}03^{ab}$	$4{\cdot}73 \pm 0{\cdot}21^{b}$	$11{\cdot}83 \pm 0{\cdot}44^{ab}$	$2{\cdot}85 \pm 0{\cdot}15^{b}$	$1{\cdot}43 \pm 0{\cdot}21^{ab}$	$11{\cdot}71 \pm 0{\cdot}68^{b}$
G. latisiliqua	5/3	$8{\cdot}08 \pm 2{\cdot}23^{a}$	$0{\cdot}65 \pm 0{\cdot}04^{a}$	$0{\cdot}27 \pm 0{\cdot}06^{a}$	$2{\cdot}41 \pm 0{\cdot}41^{a}$	$8{\cdot}57 \pm 2{\cdot}21^{a}$	$0{\cdot}70 \pm 0{\cdot}16^{c}$	$0{\cdot}26 \pm 0{\cdot}06^{a}$	$3{\cdot}12 \pm 0{\cdot}1^{a}$	$9{\cdot}2 \pm 0{\cdot}82^{a}$	$0{\cdot}78 \pm 0{\cdot}11^{a}$	$0{\cdot}61 \pm 0{\cdot}09^{a}$	$7{\cdot}48 \pm 0{\cdot}29^{a}$
L. odoratus	5/5	$20{\cdot}47 \pm 0{\cdot}07^{bc}$	$1{\cdot}99 \pm 0{\cdot}15^{bc}$	$1{\cdot}53 \pm 0{\cdot}16^{e}$	$10{\cdot}45 \pm 0{\cdot}37^{c}$	$15{\cdot}18 \pm 0{\cdot}70^{c}$	$2{\cdot}03 \pm 0{\cdot}20^{c}$	$1{\cdot}36 \pm 0{\cdot}20^{c}$	$7{\cdot}69 \pm 0{\cdot}57^{c}$	$23{\cdot}8 \pm 2{\cdot}91^{cd}$	$9{\cdot}71 \pm 0{\cdot}99^{d}$	$7{\cdot}29 \pm 0{\cdot}93^{d}$	$29{\cdot}38 \pm 2{\cdot}3^{e}$
S. junceum	5/5	$18{\cdot}64 \pm 1{\cdot}06^{b}$	$1{\cdot}80 \pm 0{\cdot}29^{d}$	$1{\cdot}04 \pm 0{\cdot}11^{b}$	$10{\cdot}08 \pm 0{\cdot}49^{c}$	$22{\cdot}70 \pm 0{\cdot}55^{e}$	$1{\cdot}28 \pm 0{\cdot}06^{b}$	$0{\cdot}73 \pm 0{\cdot}01^{b}$	$5{\cdot}37 \pm 0{\cdot}19^{b}$	$25{\cdot}08 \pm 0{\cdot}76^{cd}$	$3{\cdot}11 \pm 0{\cdot}65^{bc}$	$2{\cdot}03 \pm 0{\cdot}25^{bc}$	$22{\cdot}22 \pm 0{\cdot}74^{c}$
T. tipu	5/2	$23{\cdot}19 \pm 1{\cdot}56^{cd}$	$1{\cdot}09 \pm 0{\cdot}21^{a}$	$0{\cdot}81 \pm 0{\cdot}06^{c}$	$12{\cdot}92 \pm 0{\cdot}94^{d}$	$14{\cdot}62 \pm 1{\cdot}34^{c}$	$1{\cdot}06 \pm 0{\cdot}06^{ab}$	$0{\cdot}71 \pm 0{\cdot}04^{b}$	$4{\cdot}67 \pm 0{\cdot}16^{b}$	$18{\cdot}79 \pm 8{\cdot}20^{bc}$	$4{\cdot}18 \pm 0{\cdot}96^{c}$	$2{\cdot}29 \pm 0{\cdot}12^{c}$	$25{\cdot}87 \pm 2{\cdot}57^{d}$
ANOVA ($F_{5,24}$)		$86{\cdot}17^{***}$	$46{\cdot}16^{***}$	$116{\cdot}66^{***}$	$185{\cdot}95^{***}$	$81{\cdot}55^{***}$	$40{\cdot}83^{***}$	$49{\cdot}85$ ***	$77{\cdot}74^{***}$	$19{\cdot}06^{***}$	$113{\cdot}57^{***}$	$147{\cdot}63^{***\dagger}$	$124{\cdot}46^{***}$

Symmetrical petals were averaged within a flower. Different letters indicate significant differences (Tukey's test).
BCW, width of the claw at the base; LL, length of the lamina; LW, width of the lamina; MCW, width of the claw at the middle.
$***P \leq 0{\cdot}001$.
$^{\dagger}F_{5,23}$.

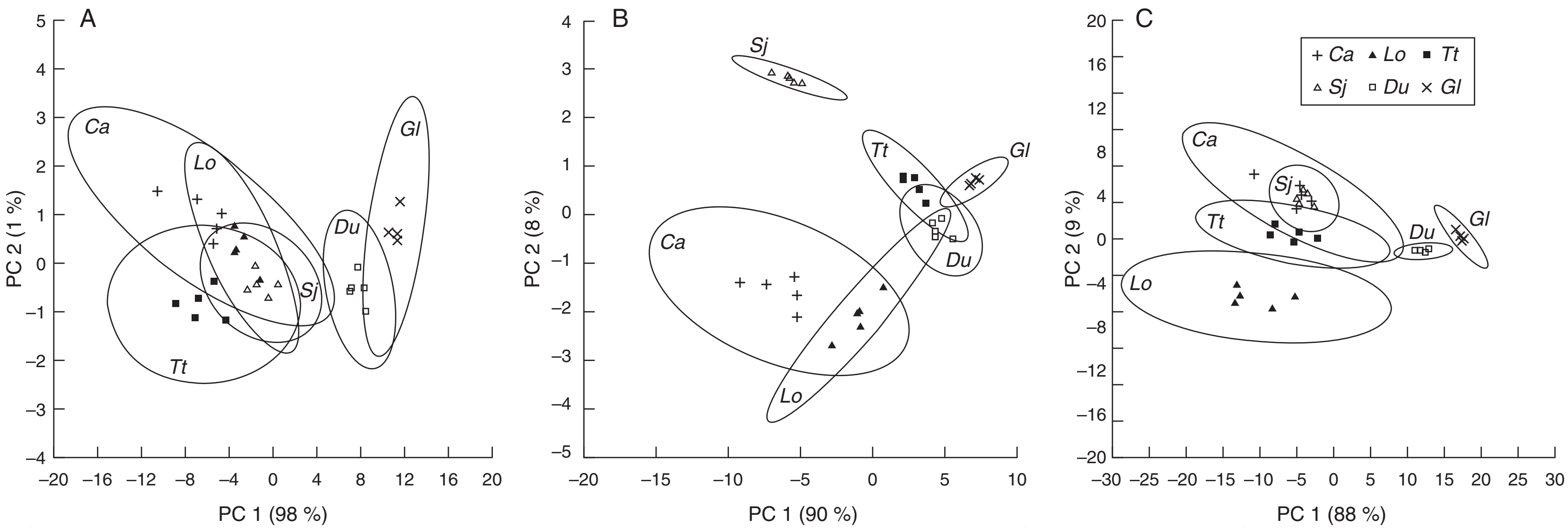


FIG. 5. Differences among species in morphological traits of each type of petal. Samples are plotted on the space given by the two first axes (PC1 and PC2) of a principal component analysis. The contribution of each axis to the total variance is given in brackets. (A) Wings; (B) keel, and (C) flag. Abbreviations: *Ca*, *Collaea argentina*; *Lo*, *Lathyrus odoratus*; *Tt*, *Tipuana tipu*; *Sj*, *Spartium junceum*; *Du*, *Desmodium uncinatum*; *Gl*, *Galactia latisiliqua*.

TABLE 3. *Pearson's correlations among morphometric and biomechanic traits across six legume species*

	Wing				Keel				Flag				
	LL	MCW	BCW	LW	LL	MCW	BCW	LW	LL	MCW	BCW	LW	Strength (mN)
Wing													
LL													
MCW	0·93**												
BCW	0·94**	0·99***											
LW	0·99***	0·90*	0·92*										
Keel													
LL	0·91*	0·94**	0·90*	0·90*									
MCW	0·9*	0·96**	0·96*	0·88*	0·95*								
BCW	0·93**	0·97**	0·99***	0·91*	0·96**	0·96*							
LW	0·93**	0·98***	0·98***	0·90*	0·99***	0·99***	0·98***						
Flag													
LL	0·96*	0·99**	0·98***	0·95**	0·93*	0·93**	0·96**	0·96***					
MCW	0·50	0·53	0·66	0·50	0·58	0·57*	0·69	0·59	0·53				
BCW	0·53	0·58	0·69	0·52	0·60	0·60	0·73	0·62	0·57	0·99***			
LW	0·98***	0·9*	0·93*	0·99***	0·86*	0·86*	0·93**	0·90*	0·95***	0·62	0·64		
Strength (mN)	0·77°	0·91*	0·89*	0·76°	0·85*	0·86*	0·86*	0·87*	0·91*	0·58	0·62	0·79°	
Moment	0·86*	0·97*	0·96*	0·84*	0·91*	0·91*	0·93**	0·94**	0·97**	0·57	0·62	0·86*	0·98***

***$P \le 0{\cdot}001$; **$P \le 0{\cdot}01$; *$P \le 0{\cdot}05$; $^{\circ}P \le 0{\cdot}1$. Abbreviations are the same as in Table 2.

the botanical families mentioned above were examined in different studies taking into account the opening strength required to operate the floral mechanism, but never legumes where the mechanism that require pollinators to exert active strength is widespread.

The above studies gave values which range from the very low 0·1–0·5 mN for *Cornus canadensis* (Edwards *et al.*, 2005) to the relatively high 49 mN of *P. fruticosa* (Lamiaceae) (Brantjes, 1981*a*). Flower operative strength measured in the present study at 8·01–48·21 mN falls within this range, but is higher than most measures obtained from previously studied species: three species of sage are within the range of the present study and only *P. fruticosa* is above it.

In the present study, the highest operative strength was for a species with an explosive mechanism, *S. junceum*, and the second species with such a mechanism had a very high operative strength when compared with other species with flowers of similar size and a reversible mechanism. In addition, the two species with an explosive mechanism had a negatively skewed operative strength distribution, i.e. with a preponderance of high operative strength values. A high operative strength and the skewed distribution in these two species could be explained by two non-mutually exclusive arguments. First, the interlocking of the keel petals and the flag must be stiff, only yielding when a certain threshold strength has been achieved (Faegri and van der Pijl, 1966); secondly, such a population distribution may result from prolonged positive directional selection. Both explanations support an adaptive value of the operative strength of the flower.

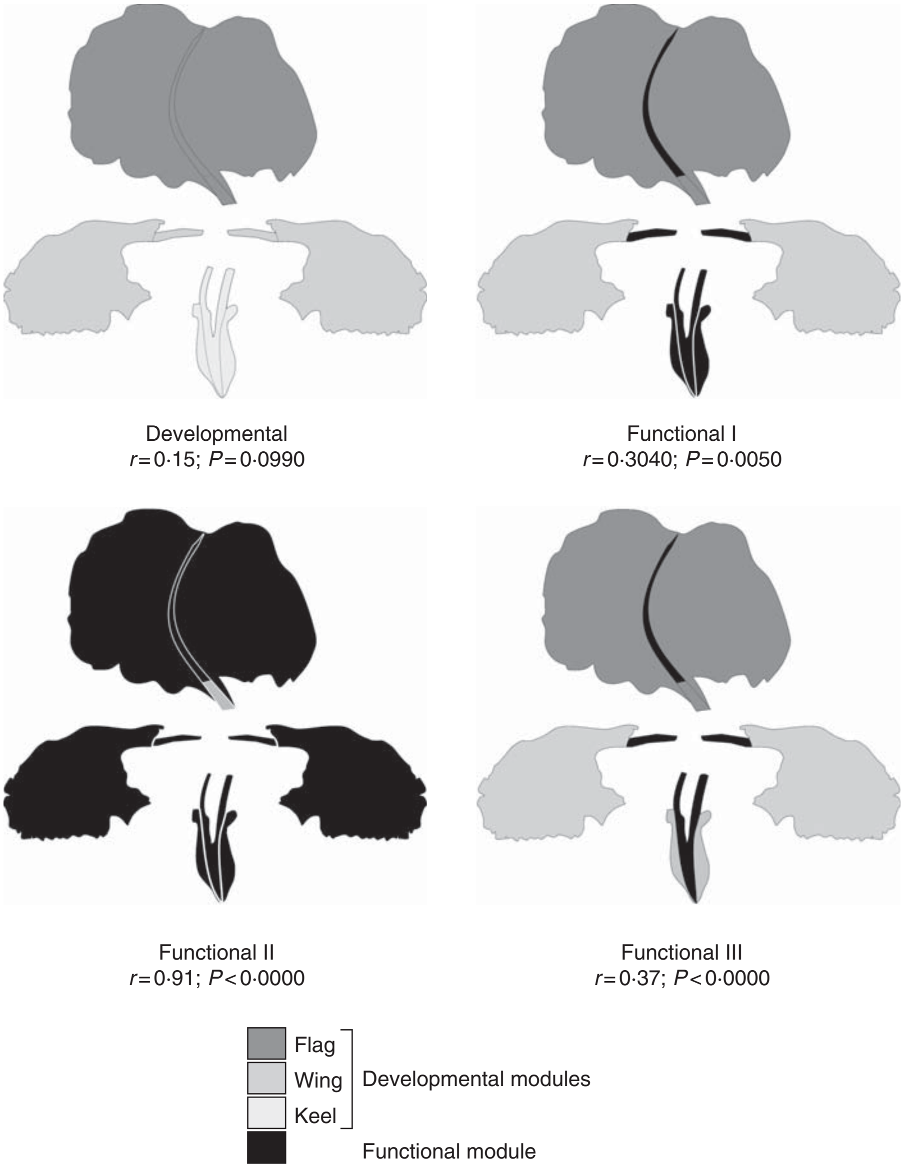
Species with reversible floral mechanisms showed short-term fatigue in some species, indicating that functioning is significantly altered during the first visits. This suggests that under a hypothetical pollinator-filtering mechanism, there would be a strategic progressive relaxation of the filtering strategy.

Correlations among morphometric and biomechanic traits

The strong correlation between operative strength and some morphometric traits showed that parts of different petals have a bearing on the mechanical functioning of the flower and support the idea that the flowers contain modules integrating traits into distinct functions. Similar and more extended associations were found between moment of strength and morphometric traits, further improving the identification of a module related to mechanical flower functioning. In addition, correlations were stronger among traits that constituted this functional module than were extramodular correlations. The fact that high correlations were found between operative strength and morphometric traits from different types of petal – wing, keel and flag – is meaningful from a phenotypic integration perspective because it suggests that functional co-ordination transcends developmental constraints within each type of petal (see Herrera *et al.*, 2002; Armbruster *et al.*, 2004; Pérez *et al.*, 2007; Ordano *et al.*, 2008). The quantification of operative strength allows us to assess flower functionality independently from morphological traits which are affected by developmental constraints, and provides the opportunity to uncover functional aspects of morphological integration in a more robust way than when studying correlations among morphometric traits alone. Functional intrafloral modules that are involved in a specific function can thus be distinguished from others involved in other functions.

The associations among morphometric and biomechanic traits are maintained or are even higher after variation accounted for by phylogenetic relationships was removed from the independent contrast analysis. This supports the idea that pollinator-driven selection on combinations of traits responsible for the functioning of the forcible flower mechanism has taken place to produce these high correlations.

Strong correlations were more often between wings and keel than with the flag. This can be expected when


Developmental
$r = 0{\cdot}15$; $P = 0{\cdot}0990$

Functional I
$r = 0{\cdot}3040$; $P = 0{\cdot}0050$

Functional II
$r = 0{\cdot}91$; $P < 0{\cdot}0000$

Functional III
$r = 0{\cdot}37$; $P < 0{\cdot}0000$

FIG. 6. Test of association across six legume species between the empirical matrix of phylogenetically corrected correlation coefficients and four flower integration hypotheses. The developmental hypothesis presumes a high correlation between traits belonging to the same type of petal, i.e. flag, wing and keel (each in different grey tones), and the functional hypothesis assumes the highest correlation between traits that had the highest correlation with operative strength (functional I) or with moment of strength (functional II and III); among the latter a distinction was between modules that were detected at a low ($P < 0{\cdot}05$) level (functional II) or a very highly significant ($P < 0{\cdot}001$) correlation level (functional III). Black areas superimposed on the developmental areas indicate to which petals and petal parts (claw or lamina) functionally correlated traits belong. On the lamina of petals functional modules are indicated with a black longitudinal strip when only length is correlated with strength, while the whole lamina is filled in black when both length and width are correlated with strength.

considering that wings and keel are simultaneously lowered by pollinators while the flag is engaged more in a visual function (Faegri and van der Pijl, 1966). In legumes that are considered to have a primitive construction, wings and keel have independent movement, while these parts have a structural interlocking in other more derived flowers, the wings contributing to the opening of the keel when down and laterally drawn (Faegri and van der Pijl, 1966; Wojciechowski *et al.*, 2004).

Interspecific variation in the phylogenetically corrected operative strength is more strongly and significantly explained by wing claw width, all keel traits and flag length. This is even stronger and more highly significant with moment of strength, indicating that mechanical resistance of the floral module is mainly responding to the rotational effect exerted by the keel.

It was unexpected that measures of the flag, a petal mainly engaged in visual display, also had a high correlation with operative strength and moment of strength. This would indicate that either functional intrafloral modules are not fully independent – they could be constrained by allometric relationships – or that the flag takes part in the functioning

TABLE 4. *Mean body mass ± s.d. (mg) and strength of the bees ± s.d. (mN).*

Bee species	*n*	Body mass	Strength	Plant species	Status
Megachilidae					
Megachilinae					
Anthidium sp. (A)	10	68·71 ± 14·45^{a} (7)	66·08 ± 14·11^{ab} (7)	*C. argentina*	P
				D. uncinatum	NV
				S. junceum	NV
Anthidium sp. (B)	2	–	–	*G. latisiliqua*	P
Megachile sp.	20	83·27 ± 19·77^{a} (11)	62·01 ± 13·8^{a} (11)	*C. argentina*	P
				D. uncinatum	P
				L. odoratus	P
				S. junceum	P
Apidae					
Apinae					
A. mellifera	10	104·97 ± 8·25^{a} (10)	26·26 ± 3·89^{a} (10)	*C. argentina*	R
				D. uncinatum	NV
				G. latisiliqua	NV
				L. odoratus	NV
				S. junceum	NV
				T. tipu	P
Bombinae					
B. bellicosus	7	436·07 ± 346·48^{bc} (7)	253·03 ± 41·67^{c} (7)	*C. argentina*	P
				D. uncinatum	NV
				S. junceum	P
B. morio	12	254·37 ± 68·49^{ab} (11)	236·52 ± 47·11^{bc} (11)	*T. tipu*	P
B. opifex	12	239·54 ± 129·21^{ab} (11)	243·2 ± 68·9^{c} (11)	*C. argentina*	P
				D. uncinatum	P
Xylocopinae					
×*ordinaria*	10	643·43 ± 227·48^{c} (9)	420·85 ± 190·6^{d} (9)	*C. argentina*	P
				D. uncinatum	NV
				S. junceum	NV
×*augusti*	1	812·4^{d} (1)	582·24^{d} (1)	*C. argentina*	NV
				D. uncinatum	NV
				S. junceum	P
ANOVA		$F_{8,13} = 17{\cdot}3^{***}$	$F_{8,19} = 23{\cdot}71^{***}$		

Different letters indicate significance differences (Tukey's test). Numbers in parentheses indicate the number of individuals used to perform the ANOVA. Status indicates whether the bee species was a pollinator (P), nectar robber (R) or was present in the study area of each legume species and did not visit its flowers (NV).

of the mechanisms in other possibly indirect ways which we were unable to determine. For example, the flag may have a protective function for the keel and the wings by covering them in the bud stage. Consequently its correlation with keel length and, indirectly, claw diameter may be linked to this protective function.

Body mass and strength of bees

Strength exerted by pollinating bees is clearly related to body mass. *Apis melifera* was the weakest bee, though it is somewhat weaker than is expected for its weight. Although this species is well known for its ability to obtain floral resources from many different plants it apparently has a limitation to handle flowers which require a relatively high operative strength. This can be explained by the fact that in the colony of the highly eusocial honey bee, individuals must not specialize in certain flower types but must use whatever plants are available for the colony to be long-lived (Westerkamp, 1991). In addition, the relative weakness may be explained in that *A. melifera* may not exert as much strength as solitary bees and bumble-bees in the task of nest construction (Michener, 2000).

Relationships between operative strength and pollinator strength

All the bees examined in the present study had strengths greater and frequently several times greater than needed to open the floral mechanism of the flowers of the species they visit. However, none may open flowers by their weight alone. *Apis melifera* was only seen tripping the mechanism of *T. tipu* and it was too weak to handle the flowers of other species studied here. This would explain why it was not observed tripping flowers of other species studied, though present in every study site, and was seen occasionally accessing nectar without tripping the flowers.

Though there was no significant correlation between bee strength and flower operative strength, our testing of the filtering hypothesis is not conclusive with a closer look at the data and because of contrasting evidence provided by the observation of bee behaviour on the flowers. On the one hand, native bees were capable of exerting strengths always higher and often several times higher than the operative strengths of flowers and could have easily tripped open strong flowers. On the other hand, the fact that the honey bee avoided or robbed nectar from all but one legume species provides clear evidence that this exceptionally weak bee was not pollinating flowers because it was unable to trip open flowers stronger than

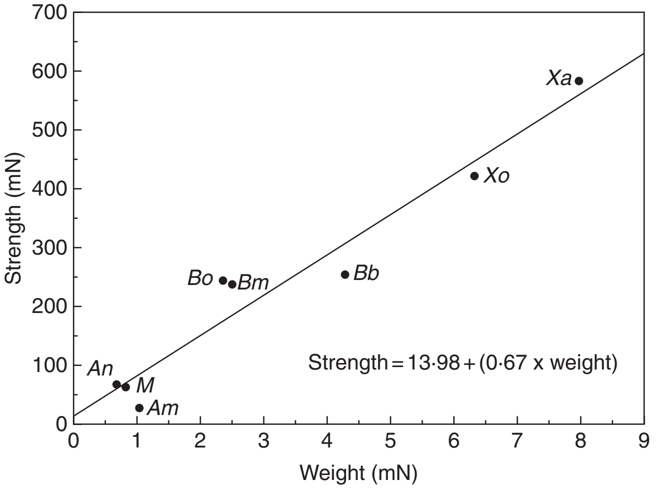


FIG. 7. Linear regression with weight as an independent variable and strength as a dependent variable. $P = 0{\cdot}0001$; $r^2 = 0{\cdot}94$. Abbreviations: *Am*, *A. mellifera*; *An*, *Anthidium* sp.; *M*, *Megachile* sp.; *Bb*, *B. bellicosus*; *Bo*, *B. opifex*; *Bm*, *B. morio*; *Xo*, *X. ordinaria*; *Xa*, *Xylocopa augusti.*

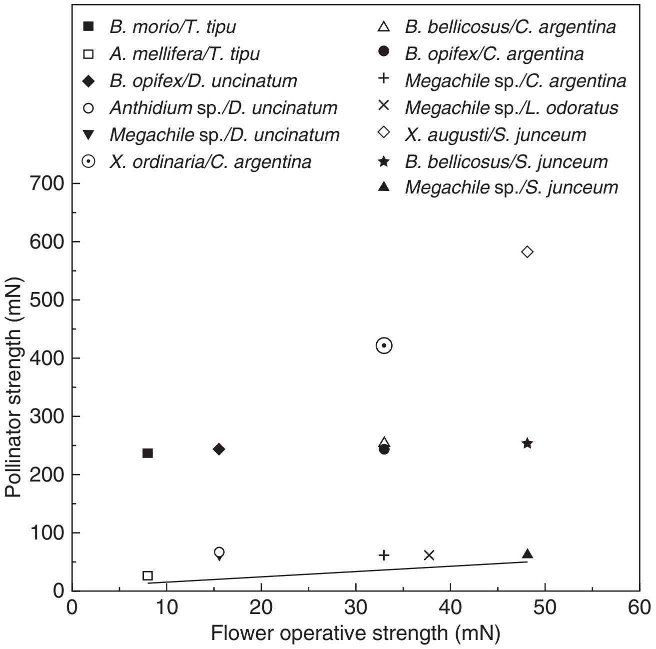


FIG. 8. Scatter-plot of strength exerted by each species of pollinator vs. operative strength of the legume species, as indicated in the key. The solid line is the 1 : 1 relationship.

itself. In addition, native bees smaller and presumably even weaker than the honey bee (which certainly form part of the local bee fauna) may not be interested in legumes precisely as a consequence of the filtering properties of their flowers. To explain differences in the assemblages of strong bees that pollinated the study legumes, factors other than flower strength must be invoked, such as pollinator sensory biases, abilities other than strength to handle flowers or the energetic balance between the cost of visiting (flight plus opening cost) and the energy content of rewards. For similar cases of the honey bees robbing nectar on comparatively large legume flowers, other aspects of the flower structure, such as asymmetry and coiling of the keel, have been associated with the inability of these bees to trip open the flowers (Westerkamp, 1993; Etcheverry *et al.*, 2008). However, this conclusion deserves to be tested again with data on flower operative strength.

The biomechanical approach in the study of flowers has allowed us to address relevant ecological and evolutionary questions of the legume–pollinator interaction. It has proved fruitful to reveal functional modules within the legume flowers that suggest functional coordination transcending developmental constraints. The ability to measure a functional aspect of flower phenotype which can be handled as ‘external’ to the morphometric set should open up the possibility to explore further the functional modules within the flower structure in other organisms. In the present study we could additionally provide evidence showing that the mechanical features of the legume flower have only a very limited capability to act as a pollinator filter. Consequently, bee strength is not sufficient to explain variation in flower strength among legume flowers, and other reasons have to be invoked.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of a video showing how bees operate the apparatus constructed to measure the strength exerted by pollinators.

ACKNOWLEDGEMENTS

The authors are grateful to CONICET for whom A.A.C. is a Researcher and S.A.C. is a Fellowship holder. We are also grateful to Dr Francisco Ludueña-Almeida and Dr. Juan Fornoni for useful input in a preliminary version of this manuscript, and to Dr Claudio Sosa for help in the identification of bees. The study was supported by SECYT (69/08), CONICET (PIP 5174) and FONCYT (PICT 01-33755) grants.

LITERATURE CITED

Armbruster WS, Pélabon C, Hansen TF, Mulder CPH. 2004. Floral integration, modularity, and accuracy. Distinguishing complex adaptations from genetic constraints. In: Pigliucci M, Preston K, eds. *Phenotypic integration*. Oxford: Oxford University Press, 23–49.

Benitez-Vieyra S, Medina M, Glinos E, Cocucci AA. 2006. Pollination mechanism and pollinator mediated selection in *Cyclopogon elatus* (Orchidaceae). *Functional Ecology* **20**: 948–957.

Benitez-Vieyra S, Medina M, Cocucci AA. 2009. Variable selection patterns on the labellum shape of *Geoblasta pennicillata* a sexually deceptive orchid. *Journal of Evolutionary Biology* **22**: 2354–2362.

Brantjes NBM, De Vos OC. 1981. The explosive release of pollen in flowers of *Hyptis* (Lamiaceae). *New Phytologist* **87**: 425–430.

Brantjes NBM. 1981*a*. Floral mechanics in *Phlomis* (Lamiaceae). *Annals of Botany* **47**: 279–282.

Brantjes NBM. 1981*b*. Mechanicals aspects of the corolla, a quantitative and functional approach. *Acta Botanica Neerlandica* **30**: 317.

Claßen-Bockhoff R, Crone M, Baikova E. 2004*a*. Stamen development in *Salvia* L.: homology reinvestigated. *International Journal of Plant Science* **165**: 475–498.

Claßen-Bockhoff R, Speck T, Tweraser E, Wester P, Thimm S, Reith M. 2004*b*. The staminal lever mechanism in Salvia L. (Lamiaceae): a key innovation for adaptive radiation? *Organisms, Diversity and Evolution* **4**: 189–205.

Cocucci AA. 1989. El mecanismo floral de *Schizanthus* (Solanaceae). *Kurtziana* **20**: 113–132.

Di Rienzo J, Robledo W, Casanoves F, Balzarini M. 2000. *InfoStat, Software estadístico*. Córdoba: Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba.

Edwards J, Whitaker D, Klionsky S, Laskowski M. 2005. A record-breaking pollen catapult. *Nature* **435**: 164.

Etcheverry AV, Aleman MM, Figueroa Fleming T. 2008. Flower morphology, pollination biology and mating system of the complex flower of *Vigna caracalla* (Fabaceae: Papilionoideae). *Annals of Botany* **102**: 305–316.

Faegri K, van der Pijl L. 1966. *The principles of pollination ecology*, 3rd edn. Canada: Pergamon Press.

Felsenstein J. 1985. Phylogenies and the comparative method. *American Naturalist* **125**: 1–15.

Garland TJr, Midford PE, Ives AR. 1999. An introduction to phylogenetically based statistical methods, with a new method for confidence intervals on ancestral states. *American Zoologist* **39**: 374–388.

Herrera C, Cerda X, Garcia B *et al*. 2002. Floral integration, phenotipic covariance structure and pollinator variation in bumblebee-pollinated Helleborus foetidus. *Journal of Evolutionary Biology* **15**: 108–121.

Maad J. 2000. Phenotypic selection in hawkmoth-pollinated *Platanthera bifolia*: targets and fitness surfaces. *Evolution* **54**: 112–123.

Maddison WP, Maddison DR. 2004. *Mesquite: a modular system for evolutionary analysis. Version 1·01*. Available at: http://mesquiteproject.org

Martén-Rodríguez S, Almarales-Castro A, Fenster CB. 2009. Evaluation of pollination syndromes in Antillean Gesneriaceae: evidence for bat, hummingbird and generalized flowers. *Journal of Ecology* **97**: 348–359.

Michener CD. 2000. *The bees of the world*. Baltimore, MD: Johns Hopkins University Press.

Moré M, Sérsic AN, Cocucci AA. 2007. Restriction of pollinator assemblage through flower length and width in three long-tongued hawkmoth-pollinated species of Mandevilla (Apocynaceae, Apocynoideae). *Annals of the Missouri Botanical Garden* **94**: 487–506.

Muchhala N, Thomson JD. 2009. Going to great lengths: selection for long corolla tubes in an extremely specialized bat–flower mutualism. *Proceedings of the Royal Society B: Biological Sciences* **276**: 2147–2152.

Nattero J, Sérsic AN, Cocucci AA. 2010. Patterns of contemporary phenotypic selection and flower integration in the hummingbird-pollinated *Nicotiana glauca* between populations with different flower–pollinator combinations. *Oikos* **119**: 852–863.

O'Connell LM, Johnston MO. 1998. Male and female pollination success in a deceptive orchid, a selection study. *Ecology* **79**: 1246–1260.

Ollerton J, Alarcón R, Waser NM *et al*. 2009. A global test of the pollination syndrome hypothesis. *Annals of Botany* **103**: 1471–1480.

Ordano M, Fornoni J, Boege K, Domínguez CA. 2008. The adaptive value of phenotypic floral integration. *New Phytologist* **179**: 1183–1192.

Pagel MD. 1992. A method for the analysis of comparative data. *Journal of Theoretical Biology* **156**: 431–442.

Pérez F, Arroyo MT, Medel RJ. 2007. Phylogenetic analysis of floral integration in *Schizanthus* (Solanaceae): does pollination truly integrate corolla traits? *Journal of Evolutionary Biology* **20**: 1730–1738.

R Development Core Team. 2010. *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. http://www.R-project.org

Sede SM, Tosto D, Talia P, Luckow M, Poggio L, Fortunato R. 2009. Phylogenetic relationships among southern South American species of *Camptosema*, *Galactia* and *Collaea* (Diocleinae: Papilionoideae: Leguminosae) on the basis of molecular and morphological data. *Australian Journal of Botany* **57**: 76–86.

Sérsic AN. 1991. Observaciones sobre el mecanismo floral de *Calceolaria* (Scrophulariaceae). *Kurtziana* **21**: 153–164.

Sokal RR, Rohlf FJ. 2000. *Biometry*. 3rd edn. New York: W.H. Freeman.

Stebbins GL. 1970. Adaptive radiation of reproductive characters in Angiosperms. I. Pollination mechanisms. *Annual Review of Ecology and Systematics* **1**: 307–326.

Westerkamp C. 1991. Honeybees are poor pollinators – why? *Plant Systematics and Evolution* **177**: 71–75.

Westerkamp C. 1993. The co-operation between the asymmetric flower of *Lathyrus latifolius* (Fabaceae-Vicieae) and its visitors. *Phyton* **33**: 121–137.

Wojciechowski MF, Lavin M, Sanderson MJ. 2004. A phylogeny of legumes (Leguminosae) based on analysis of the plastid matK gene resolves many well-supported subclades within the family. *American Journal of Botany* **91**: 1846–1862.

Yeo PF. 1993. Secondary pollen presentation. Form, function and evolution. *Plant Systematics and Evolution* **6** (Suppl.): 1–268.