

Bee, hummingbird, or mixed-pollinated *Salvia* species mirror pathways to pollination optimization: a morphometric analysis based on the Pareto front concept

Marina M. Strelin, Federico Sazatornil, Santiago Benitez-Vieyra, and Mariano Ordano

Abstract: Optimization of flower phenotypes to ensure pollination by agents differing in their match with fertile flower structures can involve fitness trade-offs if the aspects of the phenotype that enhance the fitness contribution of one pollinator are detrimental for pollination by the other agents. If these trade-offs are substantial, flower optimization for specialized pollination is expected. However, optimization for generalized pollination may also take place in trade-off scenarios, as long as the joint contribution of two or more types of pollinators to global pollination fitness is greater than each individual contribution. We used an observational approach to evaluate the role of pollination fitness trade-offs in flower trait optimization, a matter seldom addressed because of the difficulties in conducting experiments. A pattern-searching tool based on the Pareto front concept, borrowed from the fields of economics and engineering, was used to test for fitness trade-off patterns in the flower shape of four *Salvia* (Lamiaceae) species. Two are pollinated exclusively either by bees or by hummingbirds; the remaining species have mixed-pollination systems, with varying contributions of bee and hummingbird pollination. The patterning of flower shape in this study suggests a bee–hummingbird pollination trade-off in *Salvia*, and the optimization of generalized flower shapes.

Key words: fitness trade-off, pollination syndrome, flower shape, Pareto front, geometric morphometrics.

Résumé : L'optimisation des phénotypes floraux pour assurer la pollinisation par des agents qui diffèrent dans leur appariement aux structures fertiles de la fleur peut impliquer des compromis de la valeur d'adaptation (fitness) si les aspects du phénotype qui accroissent la contribution de la valeur d'adaptation d'un pollinisateur sont néfastes à la pollinisation par d'autres agents. Si ces compromis sont substantiels, l'optimisation de la fleur pour une pollinisation spécialisée est attendue. Cependant, l'optimisation pour une pollinisation généralisée peut aussi prendre place dans des scénarios de compromis, tant que la contribution conjointe de deux types de pollinisateurs ou plus à la valeur d'adaptation globale de la pollinisation est plus grande que chaque contribution individuelle. Les auteurs ont utilisé une approche observationnelle pour évaluer le rôle des compromis de la valeur d'adaptation de la pollinisation dans l'optimisation des traits floraux, un sujet rarement traité à cause des difficultés à réaliser les expériences. Un outil de recherche de patrons basé sur le concept du front de Pareto, emprunté aux domaines de l'économie et du génie, a été utilisé pour tester les patrons de compromis de la valeur d'adaptation dans la forme de la fleur de quatre espèces de *Salvia* (Lamiaceae). Deux sont pollinisées exclusivement par les abeilles ou par les colibris, les autres présentent des systèmes de pollinisation mixtes, avec des contributions variables de la pollinisation par les abeilles et les colibris. La structuration de la forme de la fleur dans cette étude suggère l'existence d'un compromis de pollinisation abeille–colibri chez *Salvia* et l'optimisation des formes de fleurs généralistes. [Traduit par la Rédaction]

Mots-clés : compromis de la valeur d'adaptation, syndrome de pollinisation, forme de la fleur, front de Pareto, morphométrie géométrique.

Received 3 June 2016. Accepted 7 September 2016.

M.M. Strelin. Laboratorio Ecotono, INIBIOMA (Universidad Nacional del Comahue-CONICET), Pasaje Gutierrez 1125, 8400 Bariloche, Rio Negro, Argentina.

F. Sazatornil and S. Benitez-Vieyra. Laboratorio de Ecología Evolutiva y Biología Floral, Instituto Multidisciplinario de Biología Vegetal (IMBIV), CONICET-Universidad Nacional de Córdoba, Casilla de Correo 495, 5000 Córdoba, Argentina.

M. Ordano. Unidad Ejecutora Lillo (UEL), Fundación Miguel Lillo (FML) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Miguel Lillo 251, T4000JFE San Miguel de Tucumán, Tucumán, Argentina.

Corresponding author: Marina M. Strelin (email: marina.strelin85@gmail.com).

Copyright remains with the author(s) or their institution(s). Permission for reuse (free in most cases) can be obtained from [RightsLink](https://www.nrcresearchpress.com/cjb).

Introduction

The principle of the most efficient pollinator states that flowering plants should evolve specializations to their most frequent and effective pollinator in a given environment (Stebbins 1970). The assumption of this evolutionary process predicts arrays of flower traits that constitute pollination syndromes, which ensure adequate pollen export and receipt (Fenster et al. 2004; Rosas-Guerrero et al. 2014). A particular set of syndrome traits represents optimality in a given pollination strategy, which in its purest state involves a unique pollinator type. However, most flowering plant species are pollinated by more than one type of pollinator (Waser et al. 1996), and these pollinators sometimes differ in how effectively they match flower reproductive structures, which is required for pollen export and receipt (Armbruster et al. 2009). This is expected to drive an optimization process, after which, interspecific variation in floral morphology will mirror the match with different groups of effective pollinators.

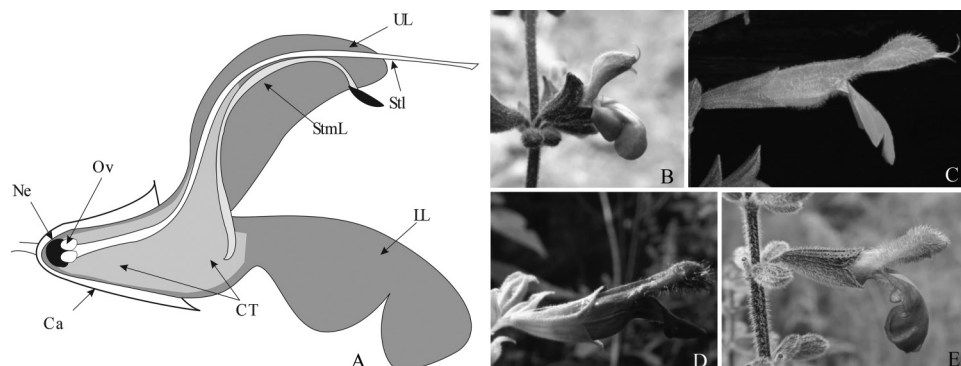
If the flower trait configuration benefiting pollen export and receipt by a particular functional group of pollinators (sensu Ollerton et al. 2007), conflicts with the requirements of efficient pollen transfer by other functional groups of pollinators, trade-offs among the optimal trait configurations will take place (Aigner 2001). In other words, a pollination fitness trade-off arises when the phenotype maximizing the fitness contribution of one functional group of pollinators (e.g., bees) negatively affects the contribution of the other functional group (e.g., birds). For instance, pollination fitness trade-offs can arise when the optimal length of stamens or styles differ in accordance with pollinators varying in size or behavior (Armbruster et al. 2009). Moreover, other flower traits, also related to the match between a flower's fertile structures and the body and behavior of pollinators, e.g., flower traits restricting the access to nectar contained at the bottom of flower tubes or spurs (Thomson and Wilson 2008) or regulating the angle at which a pollinator approaches flower fertile structures (Muchhala 2007), can be affected by fitness trade-offs.

In a scenario of functional generalization, i.e., when different functional groups of pollinators interact with the plant, optimization of a functionally generalized flower will be prevented if the phenotypic aspects that increase the efficiency of one functional group of pollinators simultaneously decrease the efficiency of the second by the equal amount (Waser et al. 1996). Such strong trade-offs can take place when the requirements for pollen transportation involve functional groups of pollinators that are remarkably different with regard to their body sizes and behaviors. Nevertheless, the dichotomous trade-off scenario constituted by two (or more) opposite functional forms of pollinators might, at least in theory and under particular conditions, promote the "optimization" of generalized flower phenotypes (Aigner 2001;

Sargent and Otto 2006). This will take place as long as global pollination fitness becomes maximized in a synergistic fashion (Aigner 2001). Despite the apparent relevance of fitness trade-offs as a mechanism underlying flower trait optimization, whether flower trait optimization is actually driven by fitness trade-offs in plant-pollinator systems and whether optimization of flower phenotypes in response to functional generalization can take place, has seldom been explored. Two exceptional works addressing these issues are investigations by Aigner (2004) and Muchhala (2007). While the study of Muchhala (2007) shows how strong fitness trade-offs can optimize flower shape for specialization on different functional groups of pollinators, Aigner (2004) demonstrated that fitness trade-offs are not necessarily involved in the evolution of functional specialization.

Transitions between bee and hummingbird pollination are the most prevailing in literature (Rosas-Guerrero et al. 2014). Differences in flower morphology between bee and hummingbird pollinated species are proposed to have resulted from optimization responding to different pollinator sizes and behaviors (Thomson and Wilson 2008). The evolution of hummingbird pollination from ancestral bee pollination generally involved the acquisition of longer corolla tubes or nectar spurs (Thomson and Wilson 2008), although other changes to flower shape took place during transitions from bee to hummingbird pollination in angiosperm lineages lacking those floral structures (Strelin et al. 2016). The genus *Salvia* L. subgenus *Calosphace* (Lamiaceae) presents several independent transitions from bee to hummingbird pollination, and flower phenotype is under pollinator selection by bees and hummingbirds in this lineage (Benitez-Vieyra et al. 2014). While most of the species rely exclusively either on bee or on hummingbird pollination, some of them present mixed-pollination systems. Here, we investigated the pathways to the optimization of flower shape in four species of *Salvia* subgenus *Calosphace*. In particular, we investigated whether optimization of flower shape in these four species took place in a context of fitness trade-off. While two of these species rely exclusively on bee and on hummingbird pollination, the two remaining rely on mixed-pollination systems, with varying contributions of bee and hummingbird pollination. We used a pattern-searching tool that allowed us to identify fitness trade-offs, based on the arrangement of quantitative phenotypic data (Shoval et al. 2012a). We expected the approach to reveal that flower shape in these four *Salvia* species runs in pathways of pollination optimization that reflect the functional matching of flower structures with the most efficient pollinators. Depending on the plant species, these pollinators belong to a single or to two functional groups. Finally, since pollination trade-offs are expected to affect populations of species with mixed-pollination systems,

Fig. 1. Flower morphology in *Salvia* (subgenus *Calosphace*, Lamiaceae). (A) Basic structure of a *Salvia* flower. Flower structures are indicated. CT, corolla tube; UL, upper corolla lip; LL, lower corolla lip; StmL, staminal lever; Stl, style; Ov, ovary; Ne, nectary; Ca, calyx. (B–E) Flowers of *Salvia cuspidata* subsp. *gilliesii* (B); *Salvia fulgens* (C); *Salvia mexicana* var. *minor* (D), and *Salvia stachydifolia* (E).



we also explored whether flower shape accommodated to a trade-off pattern at the intra-specific level.

Materials and methods

Studied species and pollinator data

The genus *Salvia*, with more than 900 species, is the most diversified in Lamiaceae (Claßen-Bockhoff et al. 2004). Flowers present fused corollas, with nectar accumulating at the bottom of the corolla tubes (Fig. 1A). A pair of stamens is attached to the corolla tube, constituting an anther-lever mechanism that allows pollen to be deposited on the pollinator's body while it manipulates the flower to access nectar (Fig. 1A). This anther-lever mechanism is a unique structure among angiosperms and is considered as a key innovation, since it allowed the genus to radiate into different pollination syndromes (Claßen-Bockhoff et al. 2004).

Within *Salvia* subgenus *Calosphace*, bee-pollination is the ancestral state, whereas bird-pollination is a derived condition (Wester and Claßen-Bockhoff 2011; Benitez-Vieyra et al. 2014). This transition involved changes in several traits and in the correlation between nectar and advertising traits (Benitez-Vieyra et al. 2014). In particular, differences in flower morphology between bee- and hummingbird-pollinated species are proposed to have resulted from optimization responding to different pollinator sizes and behaviours (Thomson and Wilson 2008). When compared with bee-pollinated species, hummingbird-pollinated *Salvia* species tend to have longer corolla tubes and a more reduced lower corolla lip (Wester and Claßen-Bockhoff 2011). Longer corolla tubes in hummingbird-pollinated species enforce the fit between the hummingbird's forehead and fertile flower structures, excluding bees from the interaction. The shorter corolla tubes of bee-pollinated species enable these insects to reach the nectar at the corolla base (Thomson and Wilson 2008; Wester and Claßen-Bockhoff 2011); larger lower corolla lips in bee-pollinated species offer bees a landing platform (Wester and Claßen-Bockhoff 2011).

This study focused on four Neotropical *Salvia* species. As a proxy to the relative contributions of bee and hummingbird pollination in these species, we gathered visitation frequency data for each of them. These data were obtained from the literature and from our own field observations (Table 1). Visitation frequency data was used as a surrogate of pollinator importance for each plant species. This approach is widely used in macroecology, macroevolution, and community ecology, since the importance of a given pollinator to the reproductive fitness of a plant species is highly correlated with its visitation frequency (Vázquez et al. 2005).

The studied species were: *Salvia cuspidata* subsp. *gilliesii* (Benth.) J.R.I. Wood (Fig. 1B), for which only bees were recorded visiting the flowers; *Salvia fulgens* Cav. (Fig. 1C), for which only hummingbird pollination was recorded; *Salvia mexicana* var. *minor* Benth. (Fig. 1D), which is mainly pollinated by hummingbirds but is also pollinated by bees; and *Salvia stachydifolia* Benth., which is mainly pollinated by bees but is also pollinated by hummingbirds (Fig. 1E).

Since mixed-pollinated species are a minority within *Salvia* (Wester and Claßen-Bockhoff 2011), choice of the mixed-pollinated species was based on the availability of accurate pollinator records, to avoid the circularity of inferring the importance of each type of pollinator only from floral traits. Likewise, *S. fulgens* and *S. cuspidata* subsp. *gilliesii* were chosen based on their local abundance and availability of pollinator data. The four species in this study belong to different *Calosphace* clades (Jenks et al. 2013), allowing us to ignore the phylogenetic context.

Floral measurements

We described the shape of the corolla (with the attached stamens) using geometric morphometric variables. As mentioned above, corolla shape is crucial for mediating flower pollinator fit, especially in plant lineages that present transitions between bee and hummingbird pollination (Thomson and Wilson 2008). We

Table 1. Pollinator observations in *Salvia* species.

Plant species and study site	Period of observation (min)	Flower visitors	Visitation rate (visits-flower ⁻¹ .hour ⁻¹)	Visitation rate (%)
<i>S. cuspidata</i> ^a subsp. <i>gilliesii</i> (Argentina; 31°5'26"S, 64°27'39"W)	460	Hymenoptera	0.161	100
		<i>Bombus</i> (3 spp.)	0.062	38.72
		<i>Apis mellifera</i>	0.011	06.50
		Apidae: Eucerinii	0.042	26.12
		Megachillidae	0.029	17.89
		Other hymenoptera	0.017	10.77
<i>S. fulgens</i> ^b (Mexico; 19°16'00"N, 99°12'00"W)	2880	Hummingbirds	NA	100
		<i>Hylocaris leucotis</i>	NA	NA
		<i>Eugenes fulgens</i>	NA	NA
		<i>Colibri thalassinus</i>	NA	NA
<i>S. mexicana</i> ^a (Mexico; 19°35'15"N, 104°16'26"W)	120	Hummingbirds (4 species)	0.2803	76.19
		Hymenoptera	0.0876	23.81
		<i>Bombus</i> sp.	0.0861	23.40
		Other hymenoptera	0.0015	00.40
<i>S. mexicana</i> ^b (Mexico; 19°16'00"N, 99°12'00"W)	1440	Hummingbirds	NA	NA
		<i>Hylocaris leucotis</i>	NA	NA
		<i>Colibri thalassinus</i>	NA	NA
		<i>Eugenes fulgens</i>	NA	NA
		Hymenoptera	NA	NA
		<i>Bombus</i> sp.	NA	NA
		Other bees	NA	NA
<i>S. stachydifolia</i> ^a (Argentina; 26°47'48"S, 65°43'22.34"W)	1530	Hymenoptera	0.0031	82.32
		<i>Bombus</i> sp.	0.0028	74.81
		Apidae: Centridini	0.0001	02.25
		Apidae: Eucerini	0.0001	01.97
		Other Hymenoptera	0.0001	03.29
		Diptera	<0.0001	00.28
		Hummingbirds (1 species)	0.0006	17.39

Note: Pollinator guilds are indicated in bold font. NA, no data available.

^aOwn observations.

^bFrom Arizmendi et al. 2007.

used the computer digitalizing program tpsDig (Rohlf 2006) to plot landmarks and semi-landmarks on the corolla with the attached stamens (Supplementary data, Fig. S1¹) and performed a Procrustes fit to these landmarks to control for size-related variation and position using the program MorphoJ (Klingenberg 2011). Average Procrustes coordinates were calculated for each individual, and were later used to explore whether the patterning of flower shape showed a trade-off between bee and hummingbird pollination in the *Salvia* species selected for this study.

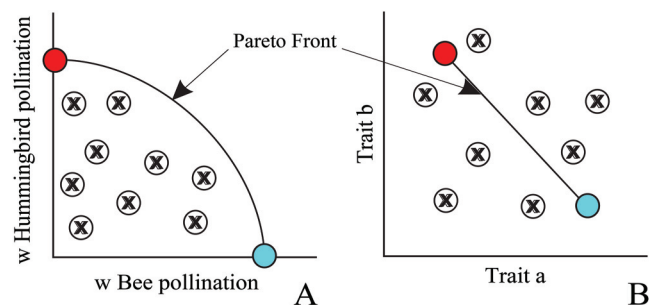
Sample sizes (individuals) were as follows: *S. cuspidata* subsp. *gilliesii*, $n = 31$ (Argentina, 31°5'26"S, 64°27'39"W); *S. fulgens*, $n = 31$ (México, 19°18'47"N, 99°18'41"W); *S. stachydifolia*, $n = 66$ (Argentina, 26°47'48"S, 65°43'22.34"W); *S. mexicana* var. *minor* $n = 29$ (Mexico (19°35'15"N, 104°16'26"W). Three flowers per individual were kept in 70% EtOH and later photographed, in lateral view, using a Nikon D80 digital camera and backlight illumination (Supplementary data, Fig. S1).

The Pareto optimality front concept

The Pareto optimality front concept, borrowed from economics and engineering, is straightforward to use and suitable for exploring the incidence of trade-offs in flower shape optimization. Shoval et al. (2012a) demonstrated that linear trait correlations can result from fitness trade-offs. In fact, this was surprising because trait correlations were traditionally considered to be the result of intrinsic processes such as genetic and developmental constraints (references in Shoval et al. 2012a). The Pareto optimality front concept has two important assumptions. First, it considers the existence of optimized phenotypes, so-called archetypes by Shoval et al. (2012a), which are the phenotypes represented as combinations of character states that maximize the performance in a particular task. In our study, these particular tasks correspond to hummingbird and bee pollination (Fig. 2A). Second, it states that the performance function of the task carried out by each specialized phenotype (e.g., hummingbird pollination) decreases with the distance

¹Supplementary data are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjb-2016-0145>.

Fig. 2. Schematic representation of how, according to Pareto front theory, a trade-off between bee and hummingbird pollination would affect two flower traits (a and b). Filled circles represent specialized, or archetypical, phenotypes: red = hummingbird pollination; blue = bee pollination. Strikethrough circles represent phenotypes that are selected against because of low performance. The Pareto front is represented: (A) in performance (w) space and (B) in phenotypic space. The Pareto front can be conceived as (A) the curve or (B) the line containing the phenotypes that remain after the elimination of phenotypes with a lower global performance. This figure is based on Fig. 1 in Shoval et al. (2012). [Colour online.]



to the corresponding specialized phenotype (e.g., the optimal hummingbird pollinated flower). The phenotypes with lower global performance are selected against (Fig. 2A), and the remaining phenotypes are those that are optimized for generalization, so called trade-off phenotypes by Shoval et al. (2012a). These phenotypes constitute the Pareto front. When viewed in the phenotypic space (Fig. 2B), the phenotypes that were optimized for generalization are expected to occur along the segment connecting the optimal specialized trait configurations (archetypes). Their positions on that segment will depend on the relative importance of each adaptive strategy. Phenotypes not occurring on the segment are further from the two optima than any phenotype on the segment, thus implying a lower global performance. If a trade-off does not exist, trait values can vary independently and will fill an uncorrelated cloud in phenotypic space (Shoval et al. 2012a).

If a pollination fitness trade-off affects flower shape in the studied *Salvia* species, the following are “expected”: (i) individuals of species with specialized and generalized pollination will be significantly aligned along a single axis of shape space; and (ii) individuals of species with generalized pollination will occur in-between individuals of species with specialized pollination, their position matching the relative importance of each pollination strategy. If the shape of species with mixed pollination is not affected by a fitness trade-off, at least one of these conditions will not be fulfilled.

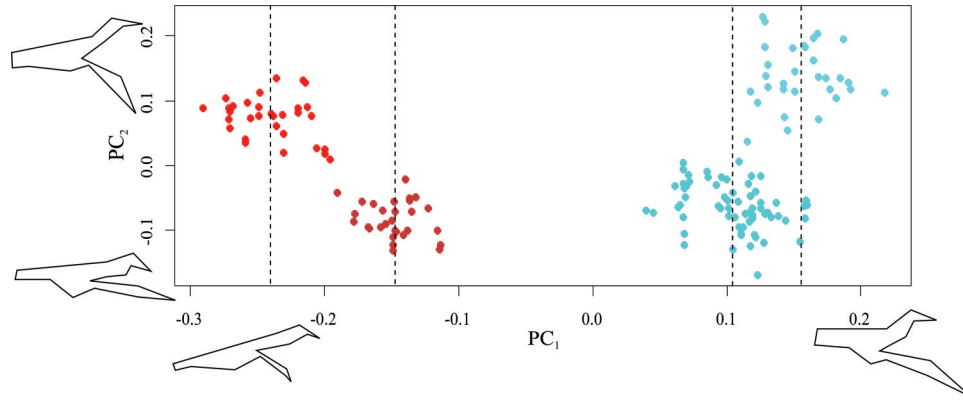
As a final point in this section, it is important to note that optimization-related concepts in this work are used to describe a macroevolutionary scenario, where species traits are being optimized, e.g., Hansen (1997).

Detecting and validating the Pareto front

To determine whether flower shape in *Salvia* accommodates along a single axis of shape space, we followed the method suggested by Shoval et al. (2012b) and performed a principal component analysis (PCA) to test whether the first principal component (PC_1) of phenotypic variables (shape Procrustes coordinates in our study) explains most of the variation in the analyzed sample. Procrustes coordinates from flowers belonging to the same individual were averaged, exported from Morphoj (Klingenberg 2011) and imported into R (R Core Team 2016), where we performed a PCA using the function *prcomp*. The observed ratio between the first eigenvalue and the sum of the remaining eigenvalues of this PCA, $\lambda_{10} / \sum_{i=2}^n \lambda_{i0}$ (where n is the total number of eigenvalues), was calculated. From here on we call this ratio $EV.R_0$. We then obtained a null model distribution of $EV.R_0$ using uncorrelated data sets. These data sets were created by randomizing each variable (Procrustes coordinate) to create individuals with new configurations of variables. In all, 10 000 randomized data sets were generated and $EV.R_0$ computed to generate a random distribution of this ratio. $EV.R_0$ was then compared with this distribution to give its p value. An $EV.R_0$ with a probability $p < 0.05$ of being higher than the ratio expected under the null model was considered to be significant. Once the meaningfulness of PC_1 was determined, we proceeded to check whether the individuals of the bee- and the hummingbird-pollinated species accommodate close to the edges of the data cloud along PC_1 and whether the individuals of species with mixed-pollination systems accommodate in-between, reflecting the relative contributions of each functional group of pollinators.

Two additional randomizations were undertaken to assess whether within-species variation in flower shape, in species with mixed pollination, repeats, at a smaller scale, the global (macroevolutionary) pollination trade-off pattern. We did not expect this for species with specialized pollination. In the first randomization, we assessed whether within-species correlations in species with mixed pollination made a significant contribution to PC_1 . For this, 10 000 data sets including the four species, with uncorrelated values for species with mixed pollination, were generated and a new distribution of $EV.R_0$ generated. An $EV.R_0$ higher than the value expected under this alternative null model was considered to show that within-species correlations in species with mixed pollination contribute to PC_1 and therefore repeat the global trade-off pattern. To rule out the possibility of within-species correlations in specialized species also presenting the trade-off pattern, we generated 10 000 uncorrelated data sets including the four species, with uncorrelated values for species with specialized pollination. $EV.R_0$ was then compared with this last null model. An $EV.R_0$ higher than the value expected under this last null model was considered to show that within-

Fig. 3. Output of the PCA analysis performed to evaluate the presence of a Pareto front in flower shape data. The colour gradient indicates different degrees of reliance on bee (cyan) and hummingbird (red) pollination. Shape changes along PC₁ and PC₂ are shown. The average value of each species along PC₁ is indicated with a broken line. Distances between the average value along PC₁ of each species with mixed pollination and the average value of each archetype are as follows: *Salvia stachydifolia* and *S. cuspidata* subsp. *gilliesii* (bee-pollinated archetype) = 0.0497; *S. stachydifolia* and *S. fulgens* (hummingbird-pollinated archetype) = 0.3449; *S. mexicana* var. *minor* and *S. cuspidata* subsp. *gilliesii* (bee-pollinated archetype) = 0.3031; *S. mexicana* var. *minor* and *S. fulgens* (hummingbird-pollinated archetype) = 0.0915. [Colour online.]



species correlations in species with specialized pollination also contribute to PC₁, repeating the global trade-off pattern.

Results

PC₁ explained 67.19% of variability in flower shape and was significantly meaningful when compared with the sum of the remaining principal components ($p < 0.0001$). PC₂ explained 24.3% of variability in flower shape. Further details of the output of the PCA analysis are presented in the Supplementary data, Table S1. Increasing values along PC₁ correspond to a proportionally shorter and more open corolla tube and to a proportionally larger lower corolla lip. Increasing values along PC₂ correspond to more open corollas. Individuals of different species occurred in distinct clusters along PC₁ (Fig. 3). Individuals of the hummingbird-pollinated species, *S. fulgens*, and the bee pollinated species, *S. cuspidata* subsp. *gilliesii*, occurred at the edges, corresponding with low and high PC₁ values, respectively; individuals of the two species with mixed pollination, *S. stachydifolia*, and *S. mexicana* var. *minor*, occurred at intermediate positions. Individuals of *S. stachydifolia*, which is mainly bee-pollinated, occupy positions that are closer to the bee-pollinated archetype; individuals of *S. mexicana* var. *minor*, which are mainly hummingbird-pollinated, occupy positions that are closer to the hummingbird-pollinated archetype (Fig. 3). Within-species correlations both in species with mixed and specialized pollination significantly contribute to PC₁ ($p < 0.0001$, $p < 0.001$ in each case, respectively), which means that variation of flower shape along PC₁ includes a significant component of within-species variation.

Discussion

The results of this study suggest that the optimization of flower shape in *Salvia* takes place in a fitness trade-off

context. This is the case under functional specialization but also under functional generalization. In other words, flower shape in the studied *Salvia* species matches the importance of each functional group of pollinators (inferred through pollinator visitation frequency), in agreement with the prediction of the Pareto front theory. In turn, within-species flower shape variation in specialized and in mixed-pollinated species contributes significantly to PC₁, which suggest that the patterning of within-species variation matches, to some extent, the interspecific variation pattern.

Typically, optimality is deemed to be in the context of functional specialization, depicted by a dichotomous scenario (e.g., bees versus hummingbirds) in which pollination syndromes correspond to a pure pollination strategy. We have shown that flower trait optimization in *Salvia* might arise in the context of a trade-off between adapting to hummingbird and bee pollination, and that the optimization of generalized flower shapes in species with mixed pollination systems can take place. Although the evolution of generalized pollination has been associated with an increase in flower trait variance, which ensures the match with different types of pollinators (Armbruster et al. 1999), generalized pollination in *S. stachydifolia* and in *S. mexicana* var. *minor* seems to have involved optimization of flower shape configurations that are weighted averages of the specialized phenotypes (archetypes) and maximize the contribution of each type of pollinator (Shoval et al. 2012a). Since these averages are weighted by the importance of bee and hummingbird pollination in each plant lineage (Shoval et al. 2012a), *S. stachydifolia*, and *S. mexicana* var. *minor* differ in their optimal flower shapes.

Flower shape configurations at the edges of the data cloud along PC₁ correspond to previous descriptions of pollination syndromes in *Salvia* (Wester and Claßen-Bockhoff

2011). Increasing values along PC₁ (increasing importance of bee pollination) correspond to a proportionally shorter and more open corolla tube and to a more conspicuous lower corolla lip; decreasing values along PC₁ (increasing importance of hummingbird pollination) correspond to a proportionally longer and narrower corolla tube, and to a less conspicuous lower corolla lip. Differences between the shape configurations at the edges of the data cloud along PC₁ make sense in a pollination fitness trade-off context. While long corolla tubes containing nectar at their bottom may play an important role in promoting the fit between the hummingbird's forehead and the fertile structures of the flower, they may at the same time isolate, or at least partially block, bees from the interaction (Thomson and Wilson 2008; Ashworth et al. 2015). Comparatively shorter corolla tubes may instead allow nectar access to bees but lower the efficiency of hummingbird pollination, since pollen would be deposited on the beak, where it is more likely to get lost than from feathered foreheads (Thomson and Wilson 2008). While a narrower corolla may subtract degrees of freedom to the hovering movement of hummingbirds, thereby forcing the contact between the hummingbird's forehead and the fertile structures of the flower (Muchhala 2007), this may at the same time impede bees from accessing nectar. Conversely, wider corollas may enable bees to access nectar, but may not be very effective in promoting the contact between the bird's forehead and the fertile structures of the flower. Finally, the size of the lower lip may also be subject to a trade-off, more related to flower advertisement than to flower-pollinator fit. Pollinators that have to land on the flower to forage, e.g., bees, tend to use frontal advertisement traits such as the size of the lower lip; whereas pollinators that hover and easily change their flight direction, e.g., hummingbirds, are more likely to use side advertisement traits (Dafni 1994). This was in fact demonstrated for bee- and hummingbird-pollinated species of *Salvia* (Benitez-Vieyra et al. 2014), where large lower lips make an important contribution to frontal flower display. Since the resources that can be invested in flower production are limited (Holland et al. 2004), a trade-off may affect the amount of resources that can be allocated to producing tissue participating in frontal and side flower display.

Although almost 70% of flower shape variation was explained by PC₁, more than 20% of shape variation is orthogonal to this component. Individuals in species with mixed pollination depart towards lower values of PC₂, corresponding with narrower corollas, which confers a slightly curved shape to the accommodation of flower shape data. Pareto fronts with straight-line shapes are expected in idealized scenarios, where the performance functions of different phenotypic aspects; e.g., corolla tube length, corolla tube narrowness, and size of the lower corolla lip, decay at the same rate when depart-

ing from the two archetypical trait configurations (Sheftel et al. 2013). However the contribution of each phenotypic aspect to the performance in each task may vary, e.g., corolla narrowness may be more limiting for effective hummingbird than for effective bee pollination, which can result in curved Pareto fronts (see Sheftel et al. 2013).

Finally, our results suggest that within-species variation also mirrors the global fitness trade-off pattern. Although we expected this to be the case only in species with mixed-pollination systems, we also found this for functionally specialized species, where this pattern does not make sense from a functional (pollination) perspective. This leads us to two speculations: (i) maybe bees and hummingbirds contribute, to some extent, to pollination in *S. fulgens* and *S. cuspidata* subsp. *gilliesii*, respectively; (ii) the patterning of flower shape in the four studied *Salvia* species, as well as the global Pareto front pattern, are developmentally determined. This last point does not exclude the possibility of developmental shape correlations in specialized species to have been moulded by pollination fitness trade-off in their ancestors. In fact, variation expressed by developmental systems is expected to be imprinted by past selection (West-Eberhard 2005).

Summing up, the optimization of flower shape in response to different pollination strategies in *Salvia* subgenus *Calosphace* seems to take place in a context of fitness trade-off. The optimization of flower shape responding to functional generalization can also take place in this lineage. Although we demonstrated the straightforwardness of the Pareto front approach for detecting trade-off patterns applicable to field studies, complementing this exploratory approach with experiments, e.g., experimentally assessing the efficiency of pollen transportation by different agents in each species, is still required for a deeper understanding of fitness trade-offs and flower trait optimization. This approach also affords a novel visualization of plant-pollinator interactions, where these give rise to a whole system, namely the Pareto front, which includes the optimization of flower shape for functionally specialized pollination, but also for functionally generalized pollination.

Acknowledgements

We thank M.A. Aizen, A.A. Cocucci, and the two anonymous reviewers for contributing with their comments and suggestions to the quality of this manuscript. We also thank A.A. Cocucci, A.C. Ibáñez, C.C. Maubecin, R. Pérez-Ishiwara, J. Izquierdo and A.N. Sérsic for assistance during fieldwork. Funds were provided by Alexander von Humboldt Foundation (Germany), Fondo Nacional de Ciencia y Tecnología (FONCYT, Argentina, PICT 2012 2603), Consejo Nacional de Ciencia y Tecnología (CONICET, Argentina, PIP 11220110100245), and Universidad Nacional de Córdoba (SECyT-UNC, Argentina). M.M.S has a scholarship at the Lab. Ecotono, INIBIOMA (Universidad

Nacional del Comahue–CONICET) funded by CONICET; F.S. has a scholarship at the Instituto Multidisciplinario de Biología Vegetal, funded by CONICET; and M.O. and S.B.V are staff researchers at CONICET.

References

- Aigner, P.A. 2001. Optimality modelling and fitness trade-offs: when should plants become pollinator specialists? *Oikos*, **95**: 177–184. doi:10.1034/j.1600-0706.2001.950121.x.
- Aigner, P.A. 2004. Floral specialization without trade-offs: optimal corolla flare in contrasting pollination environments. *Ecology*, **85**: 2560–2569. doi:10.1890/03-0815.
- Arizmendi, M.C., Monterrubio-Solis, C., Juárez, L., Flores-Moreno, I., and López-Saut, E. 2007. Effect of the presence of nectar feeders on the breeding success of *Salvia mexicana* and *Salvia fulgens* in a suburban park near Mexico City. *Biol. Conserv.* **136**: 155–158. doi:10.1016/j.biocon.2006.11.016.
- Armbruster, W.S., Di Stilio, V.S., Tuxill, J.D., Flores, T.C., and Runk, J.L.V. 1999. Covariance and decoupling of floral and vegetative traits in nine neotropical plants: a re-evaluation of Berg's correlation-pleiades concept. *Am. J. Bot.* **86**: 39–55. doi:10.2307/2656953.
- Armbruster, W.S., Hansen, T.F., Pélabon, C., Pérez-Barrales, R., and Maad, J. 2009. The adaptive accuracy of flowers: measurement and microevolutionary patterns. *Ann. Bot.* **103**: 1529–1545. doi:10.1093/aob/mcp095.
- Ashworth, L., Aguilar, R., Martén-Rodríguez, S., Lopezarazola-Mikel, M., Avila-Sakar, G., Rosas-Guerrero, V., and Quesada, M. 2015. Pollination syndromes: a global pattern of convergent evolution driven by the most effective pollinator. In *Evolutionary biology: biodiversification from genotype to phenotype*. Edited by P. Pontarotti. Springer, Marseille. pp. 203–224.
- Benitez-Vieyra, S., Fornoni, J., Pérez-Alquicira, K., Boege, and Domínguez, C.A. 2014. The evolution of signal-reward correlations in bee- and hummingbird-pollinated species of *Salvia*. *Proc. R. Soc. B Biol. Sci.* **281**: 1471–2954. doi:10.1098/rspb.2013.2934.
- Claßen-Bockhoff, R., Speck, T., Tweraser, E., Wester, P., Thimm, S., and Reith, M. 2004. The staminal lever mechanism in *Salvia* L. (Lamiaceae): a key innovation for adaptive radiation? *Org. Div. Evol.* **4**: 189–205. doi:10.1016/j.ode.2004.01.004.
- Dafni, A. 1994. Note on side advertisement in flowers. *Funct. Ecol.* **8**: 136–138.
- Fenster, C.B., Armbruster, W.S., Wilson, P., Dudash, M.R., and Thomson, J.D. 2004. Pollination syndromes and floral specialization. *Annu. Rev. Ecol. Evol. Syst.* **35**: 375–403. doi:10.1146/annurev.ecolsys.34.011802.132347.
- Hansen, T.F. 1997. Stabilizing selection and the comparative analysis of adaptation. *Evolution*, **51**: 1341–1351. doi:10.2307/2411186.
- Holland, J.N., Bronstein, J.L., and DeAngelis, D. 2004. Testing hypotheses for excess flower production and low fruit-to-flower ratios in a pollinating seed-consuming mutualism. *Oikos*, **105**: 633–640. doi:10.1111/j.0030-1299.2004.13058.x.
- Jenks, A.A., Walker, J.B., and Kim, S.C. 2013. Phylogeny of New World *Salvia* subgenus *Calosphace* (Lamiaceae) based on cpDNA (psbA-trnH) and nrDNA (ITS) sequence data. *J. Plant. Res.* **126**: 483–496. doi:10.1007/s10265-012-0543-1.
- Klingenberg, C.P. 2011. MorphoJ: an integrated software package for geometric morphometrics. *Mol. Ecol. Res.* **11**: 353–357. doi:10.1111/j.1755-0998.2010.02924.x.
- Muchhala, N. 2007. Adaptive trade-off in flower morphology mediates specialization for flowers pollinated by bats and hummingbirds. *Am. Nat.* **169**: 494–504. doi:10.1086/512047.
- Ollerton, J., Killick, A., Lamborn, E., Watts, S., and Whiston, M. 2007. Multiple meanings and modes: on the many ways to be a generalist flower. *Taxon*, **56**: 717–728. doi:10.2307/25065856.
- R Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Rohlf, F.J. 2006. TPS series software. Available at: <http://life.bio.sunysb.edu/morph/>.
- Rosas-Guerrero, V., Aguilar, R., Martén-Rodríguez, S., Ashworth, L., Lopezarazola-Mikel, M., Bastida, J.M., and Quesada, M. 2014. A quantitative review of pollination syndromes: do floral traits predict effective pollinators? *Ecol. Lett.* **17**: 388–400. doi:10.1111/ele.12224.
- Sargent, R., and Otto, S.P. 2006. The role of local species abundance in the evolution of pollinator attraction in flowering plants. *Am. Nat.* **167**: 67–80. doi:10.1086/498433.
- Sheftel, H., Shoval, O., Mayo, A., and Alon, U. 2013. The geometry of the Pareto front in biological phenotype space. *Ecol. Evol.* **3**: 1471–1483. doi:10.1002/ece3.528.
- Shoval, O., Sheftel, H., Shinar, G., Hart, Y., Ramote, O., Mayo, A., Dekel, E., Kavanagh, H., and Alon, U. 2012a. Evolutionary trade-offs, Pareto optimality, and the geometry of phenotype space. *Science*, **336**: 1157–1160. doi:10.1126/science.1217405. PMID:22539553.
- Shoval, O., Sheftel, H., Shinar, G., Hart, Y., Ramote, O., Mayo, A., Dekel, E., Kavanagh, H., and Alon, U. 2012b. Supplementary materials for Evolutionary trade-offs, Pareto optimality, and the geometry of phenotype space. *Science*, **336**: 1157–1160.
- Stebbins, G.L. 1970. Adaptive radiation of reproductive characteristics in angiosperms. I: Pollination mechanisms. *Annu. Rev. Ecol. Evol. Syst.* **1**: 307–326. doi:10.1146/annurev.es.01.110170.001515.
- Strelin, M.M., Benitez-Vieyra, S., Ackermann, M., and Cocucci, A.A. 2016. Flower reshaping in the transition to hummingbird pollination in Loasaceae subfam. Loasoideae despite absence of corolla tubes or spurs. *Evol. Ecol.* **30**: 401–417. doi:10.1007/s10682-016-9826-7.
- Thomson, J.D., and Wilson, P. 2008. Explaining evolutionary shifts between bee and hummingbird pollination: convergence, divergence and directionality. *Int. J. Plant Sci.* **169**: 23–38. doi:10.1086/523361.
- Vázquez, D.P., Morris, W.F., and Jordano, P. 2005. Interaction frequency as a surrogate for the total effect of animal mutualists on plants. *Ecol. Lett.* **8**: 1088–1094. doi:10.1111/j.1461-0248.2005.00810.x.
- Waser, N.M., Chittka, L., Price, M.V., Williams, N.M., and Ollerton, J. 1996. Generalization in pollination systems and why it matters. *Ecology*, **77**: 1043–1060. doi:10.2307/2265575.
- West-Eberhard, M.J. 2005. Developmental plasticity and the origin of species differences. *Proc. Natl. Acad. Sci.* **102**: 6543–6549. doi:10.1073/pnas.0501844102.
- Wester, P., and Claßen-Bockhoff, R. 2011. Pollination syndromes of New World *Salvia* species with special reference to bird pollination. *Ann. Mo. Bot. Gard.* **98**: 101–155. doi:10.3417/2007035.