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RESEARCH ARTICLE

Floral syndromes predict the most effective pollinators in five species of *Salvia*

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Abstract

Premise: Pollination syndromes are suites of floral traits associated with the most effective functional group of pollinators. Particular floral traits may not necessarily preclude visitation by different visitor guilds that could also contribute to plant reproduction. The genus *Salvia* comprises ca. 1000 species with floral traits almost exclusively associated with bee or hummingbird pollination syndromes. Nevertheless, the effectiveness of their floral visitors has rarely been evaluated. Here, the pollination effectiveness of floral visitors in five *Salvia* species was analyzed to assess the reliability of floral syndromes.

Methods: The contribution to seed production of the most frequent floral visitors of *Salvia* species with melittophilous, ornithophilous, and mixed phenotypes was evaluated through single-visit experiments. In addition, the relationship between floral morphological traits and pollinator effectiveness was explored using principal component analysis to test the reliability of floral syndromes.

Results: Despite multiple floral visitor guilds to the plants, bees and hummingbirds were the most effective pollinators of plants with melittophilous and ornithophilous pollination syndrome, respectively. These two functional groups effectively pollinated the plant species with mixed floral traits. *Salvia* species pollinated by the same functional group were closer in the multivariate ordination space.

Conclusions: Even when floral syndromes precisely predict the most effective pollinators in *Salvia*, secondary pollinators also play a crucial role in plant sexual reproduction, especially for plant species with mixed floral phenotypes. This study highlights the importance of evaluating the effectiveness of floral visitors to assess the reliability of floral syndromes.

KEYWORDS

Lamiaceae, Mexico, pollinator effectiveness, pollinator efficiency, pollination syndromes

Biotic pollination is crucial for sexual reproduction in approximately 90% of angiosperms (Tong et al., 2023) and has been proposed as the main driver of floral diversification (Bronstein et al., 2006; Kay and Sargent, 2009; Armbruster, 2014; Van der Niet et al., 2014). Thus, floral phenotypes are expected to reflect adaptations to the most effective pollinators (Stebbins, 1970). Pollination syndromes, defined as particular floral phenotypes associated with specific pollinator functional groups (sensu Faegri and Van der Pijl, 1979; Fenster et al., 2004) have been widely used to infer the pollinator group of several flowering plant species when empirical evidence is scarce (Dellinger, 2020) and to explain trait convergence among phylogenetically distant angiosperm taxa (Rosas-Guerrero et al., 2014; Ashworth et al., 2015; Dellinger, 2020).

Nevertheless, the floral syndrome concept has been questioned due to the apparent generalization of pollination systems (i.e., plant species visited by multiple animal guilds regardless of their floral phenotypes; Waser et al., 1996; Kingston and McQuillan, 2000; Ollerton et al., 2009; Wang et al., 2020). It is important to note that different pollinators may vary in their ability to contribute to plant reproductive success (Armbruster et al., 2000; Fenster et al., 2004; Rosas-Guerrero et al., 2014) and that the association between floral phenotypes and specific pollinators should be stronger in pollinator-dependent plants (e.g., self-incompatible,

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dichogamous, monoecious, and dioecious species) than in plants less dependent on pollinators (e.g., self-compatible, autogamous). Therefore, it is crucial to determine the extent to which plant species depend on biotic pollination and to differentiate between true pollinators and floral visitors to evaluate accurately the validity of floral syndromes (Rosas-Guerrero et al., 2014).

Different studies have demonstrated that considering only those floral visitors that contribute to pollen deposition, fruit, or seed production considerably reduces the generalization of pollination systems (e.g., Lopezaraiza-Mikel et al., 2007; Benevides et al., 2013; King et al., 2013; Popic et al., 2013; Rosas-Guerrero et al., 2014; Ashworth et al., 2015; de Santiago-Hernández et al., 2019). Moreover, primary pollinators (i.e., those with the greatest contribution to plant fitness) consistently correspond to those expected by pollination syndromes, even when secondary pollinators (i.e., those that contribute less to plant fitness) are present (Rosas-Guerrero et al., 2014; Ashworth et al., 2015; de Santiago-Hernández et al., 2019).

Some authors have proposed that the assessment of the efficiency (i.e., per-visit contribution of a floral visitor to plant fitness) and the effectiveness (i.e., combined effect of efficiency and visitation frequency; Ne'eman et al., 2010; Willmer, 2011; Armbruster, 2014) of floral visitors is crucial to determine which visitors may act as potential selective agents and hence could shape floral phenotypes (Rosas-Guerrero et al., 2014). Nevertheless, only a few studies have quantitatively assessed pollinator effectiveness under natural conditions to test the reliability of floral syndromes (e.g., Wilson et al., 2004; Martén-Rodríguez et al., 2009; Johnson, 2013; Murúa and Espíndola, 2015; Abrahamczyk et al., 2017; de Santiago-Hernández et al., 2019).

The plant genus *Salvia* (Lamiaceae) is an excellent model to test the predictive accuracy of pollination syndromes since it comprises around 1000 cosmopolitan species (Kriebel et al., 2019; Moein et al., 2023) of impressive phenotypic diversity (e.g., Claßen-Bockhoff et al., 2004; Walker and Sytsma, 2007; Wester and Claßen-Bockhoff, 2007; Fragoso-Martínez et al., 2018; Benitez-Vieyra et al., 2019; Kriebel et al., 2019, 2020; Drew, 2020; Wester et al., 2020) that largely depend on pollinators for reproductive success (e.g., Cairampoma et al., 2020; Barrionuevo et al., 2021; Xiao et al., 2023a). Indeed, some studies have explored the relationship between different floral attributes and particular pollinator guilds in *Salvia* through phylogenetic comparative approaches, providing strong support for pollinator-mediated floral evolution (Fragoso-Martínez et al., 2018; Benitez-Vieyra et al., 2019; Kriebel et al., 2019, 2020).

Although bees and hummingbirds have been reported as the main visitors for melittophilous and ornithophilous species, respectively (e.g., Dieringer et al., 1991; Wester and Claßen-Bockhoff, 2011; Celep et al., 2014, 2020), many sage species are not exclusively visited by such groups. For instance, in melittophilous species there is evidence of frequent floral visitation by butterflies (Tavera et al., 2023), flies (Celep et al., 2014, 2020; Saravia-Nava et al., 2023), hawkmoths (Xiao et al., 2023b), and hummingbirds (Espino-Espino et al., 2014; Barrionuevo et al., 2021), while in ornithophilous species, visitation by bees (Espino-Espino et al., 2014; Saravia-Nava et al., 2023) and butterflies (Grases and Ramírez, 1998) has been reported.

Only a few studies have assessed pollinator efficiency in *Salvia*. For instance, Cairampoma et al. (2020) found that the bee *Caupolicana* cf. *piurensis* and the hummingbird *Thaumastura cora* contributed similarly to seed production in *S. rhombifolia*, a melittophilous sage of Peru. In China, Xiao et al. (2023b), based on the number of pollen grains deposited on the stigma of *S. daiguii*, concluded that *Apis cerana* and the hawkmoth *Macroglossum bombylans* acted, respectively, as primary and secondary pollinators. Finally, the only study that evaluated pollinator effectiveness (in terms of visitation frequency and germinated pollen grains) in four Andean species of *Salvia* found that bees acted as primary or secondary pollinators in ornithophilous species, whereas syrphid flies acted as primary pollinators in melittophilous species (Saravia-Nava et al., 2023).

While these findings are valuable, detailed studies considering pollinator efficiency and effectiveness for seed production may provide a more insightful view of the role of particular pollinator guilds in plant sexual reproduction and floral trait selection (de Santiago-Hernández et al., 2019). Here, we assessed the reliability of floral syndromes in five species of *Salvia* with different floral phenotypes through a set of manipulative pollination experiments combined with multivariate analysis of floral traits. Specifically, we formulated the following questions: (1) Do plant species depend on pollinators for reproductive success? (2) Do the most effective pollinators match those expected by the floral syndrome? (3) Are plant species with mixed phenotypes effectively pollinated by more than one functional group? (4) Are plant species pollinated by the same functional group clustered in a multivariate ordination based on floral morphology?

Accordingly, we stated the following predictions: (1) *Salvia* species will be highly dependent on pollinators for fruit and seed production; (2) floral syndromes will be strongly associated with the most effective floral visitors, with bees as the primary pollinators for melittophilous species and hummingbirds for ornithophilous species; (3) multiple functional groups will effectively pollinate plant species with mixed phenotypes; and (4) melittophilous and ornithophilous species will be clearly separated in the multivariate ordination space, with the mixed-pollinated species placed between them.

MATERIALS AND METHODS

Study site

Fieldwork was conducted during the flowering seasons (July–March) of 2022–2023 and 2023–2024 at three sites north of Michoacan, Mexico, within the Trans-Mexican

Volcanic Belt, between 2130 and 2700 m a.s.l. (Appendix S1). The predominant vegetation consists of pine–oak forest. The sites have a mean annual temperature of 18.6°C, a mean annual precipitation of ~2500 mm with a rainy season from July to September (data from the National Meteorological Service of Mexico).

Study species

According to the most comprehensive floral syndrome classification for the subgenus *Calosphace*, which includes ca. 600 species restricted to America (González-Gallegos et al., 2020), 58% of these species are classified as melittophilous and 31% as ornithophilous, whereas 11% have mixed phenotypes with two or more floral syndromes (Wester and Claßen-Bockhoff, 2011). Melittophilous species usually have blue-purplish flowers with a short corolla tube, wide lower lip, and an active lever mechanism. In contrast, ornithophilous species generally possess reddish flowers with a longer corolla tube, narrower lower lip, and an inactive lever mechanism (Wester and Claßen-Bockhoff, 2011). Species with mixed phenotypes have floral trait combinations not assignable to a single pollination syndrome (Wester and Claßen-Bockhoff, 2011).

Five species from *Salvia* subgenus *Calosphace* were selected for study. According to Wester and Claßen-Bockhoff (2011), two are bee-pollinated (i.e., *Salvia assurgens* Kunth and *S. lavanduloides* Kunth), two are bird-pollinated (i.e., *S. elegans* Vahl and *S. iodantha* Fernald), and one has a mixed phenotype (i.e., *S. mexicana* var. *minor* Benth; Figure 1).

Salvia assurgens displays white flowers with a mean length (±SE) of 13.41 ± 0.82 mm and is visited by small, medium, and large bees (Cultid-Medina et al., 2021), bee-flies, and butterflies (Tavera et al., 2023; Figure 1A–C). *Salvia lavanduloides* displays blue-violet flowers (6.16 ± 0.47 mm) and is visited by small, medium, and large bees (Dieringer et al., 1991; Figure 1D–F). Flowers of both species have a pronounced lower lip that serves as a landing platform for insect visitors, lack nectar guides, and have an active lever mechanism (Wester and Claßen-Bockhoff, 2011).

Salvia elegans displays red tubular flowers (33.62 ± 2.75 mm) and is visited by several species of hummingbirds and medium and large bees (Cuevas et al., 2018; López-Segoviano et al., 2021; Figure 1G–I). *Salvia iodantha* displays intense-pink tubular flowers (20.83 ± 2.19 mm) and is visited by several species of hummingbirds (Lara and Ornelas, 2001; López-Segoviano et al., 2021; Figure 1J–L). Both species have reduced lower lips and exserted stamens and stigma and lack nectar guides and the staminal lever mechanism (Wester and Claßen-Bockhoff, 2011).

Salvia mexicana var. *minor* displays bluish-purple flowers (22.92 ± 2.64 mm) and is primarily visited by several hummingbird species (Arizmendi et al., 1996; Strelin et al., 2017; López-Segoviano et al., 2021). Large bees (Dieringer et al., 1991) and diurnal butterflies (Tavera et al., 2023; Figure 1M–P) have also been reported. Some of its floral traits such as color, bilateral symmetry, and active lever mechanism correspond to the melittophilous syndrome, while others, including corolla length, tubular shape, and nectar volume, correspond to the ornithophilous syndrome (Wester and Claßen-Bockhoff, 2011).

Pollinators' contributions

To determine whether *Salvia* species depend on pollinators to produce seeds, one inflorescence per plant from 30 plants per species was selected for the following treatments: (1) Autonomous self-pollination—open flowers were removed from each inflorescence, leaving only floral buds on the inflorescence, which was then covered with a fine mesh bag; (2) open pollination—inflorescences were tagged, left untreated and not covered. For both treatments, floral life span was monitored, and approximately 3 weeks after flower abscission, fruit set (i.e., number of fruits divided by total number of flowers per species) and seed set (i.e., number of viable seeds divided by the number of ovules per flower) were quantified. Nonviable seeds were smaller and had a wrinkled surface, and only fruits containing at least one viable seed were counted. All *Salvia* species have four ovules per flower.

Two generalized linear models (GLM) using a binomial distribution and a logit link function were performed to test the effect of pollination treatments (independent variable) on fruit and seed set (dependent variables on separate models) for each plant species. All comparisons were performed using the glm function in R version 4.4.2 (R Core Team, 2024).

Floral visitors and pollination performance

Floral visitors

To describe the assemblage of floral visitors, we observed and videorecorded 10–50 flowers (depending on plant species) at 30-min intervals between 08:00 and 15:00 hours (the peak floral visitation period of both hummingbirds and insects based on previous observations) twice a week during the entire flowering season of each *Salvia* species in both years of study. Only legitimate floral visits were recorded (i.e., when visitors contacted anthers and/or stigma). Because the length of the flowering season varied among studied species, the total observation time was different for each plant species. Total observation time and number of observed flowers per species were 51 h and 879 flowers for *Salvia assurgens*, 41 h and 1787 flowers for *S. lavanduloides*, 46.5 h and 2843 flowers for *S. mexicana* var. *minor*, 47 h and 1222 flowers for *S. elegans*, and 50 h and 3052 flowers for *S. iodantha*.

The visitation rate of each floral visitor species was calculated as the mean number of visits per hour and per

FIGURE 1 Floral visitors and pollinators of the *Salvia* species studied. (A–C) *S. assurgens*. (A) *Deltoptila* sp. right before activating the lever mechanism; (B) *Lasioglossum pharum* activating the staminal lever mechanism; (C) *Lon zabulon* probing nectar; (D–F) *S. lavanduloides*. (D) *Bombus ephippiatus* inserting its tongue into the flower; (E) *Bombus weisi* probing nectar and carrying pollen on its legs; (F) *Pygodasis ephippium* with pollen on its mouthparts; (G–I) *S. elegans*. (G) *Archilochus colubris* visiting a flower with pollen on its head; (H) *Basilinna leucotis* hovering in front of an inflorescence; (I) *Lasioglossum* sp. collecting pollen; (J–L) *Salvia iodantha*. (J) *Basilina leucotis* contacting anthers and stigma with its head while visiting a flower; (K) *Aellopos clavipes* probing nectar; (L) *Lasioglossum costale* collecting pollen; (M–O) *Salvia mexicana* var. *minor*. (M) *Bombus sonorus* probing nectar while being dusted with pollen; (N) *Selasphorus rufus* visiting a flower with pollen on its bill; (O) *Megachile* sp. visiting a flower with pollen on its abdomen; (P) *Piruna* sp. probing nectar. Photos: A. Tavera.

flower. A standardized visitation rate was obtained by dividing each visitation rate by the sum of the visitation rates of all the floral visitors recorded for each plant species.

Pollinator efficiency and effectiveness

To evaluate the efficiency of different legitimate floral visitors, we quantified the mean number of seeds resulting from single visits to virgin flowers of each plant species. Several inflorescences per plant (17–32), with open flowers removed, were tagged and covered with fine mesh bags until anthesis. Because incomplete protandry has been reported for some *Salvia* species (e.g., *S. verbenaca*, Navarro, 1997; *S. elegans*, Rosas-Guerrero et al., 2017; *S. stachydifolia*, Barrionuevo et al., 2021), only 2- or 3-day-old virgin flowers were exposed to floral visitors to ensure high stigmatic receptivity. The observer stood between 1 and 3 m from one inflorescence, waiting for floral visitors and carefully recording which flowers were visited only once. The single-visit flowers were tagged, and the floral visitor was identified or captured with an entomological net for later identification. Once all or most virgin flowers were visited, the inflorescence was bagged again to prevent further pollination. Approximately 3 weeks later, the inflorescences were collected, and the viable seeds from tagged flowers were quantified. Nonvisited flowers and flowers visited more than once were discarded. Due to differences in visitation rates, the sample size per floral visitor species varied. In the 2 years of study, the single-visit experiments were conducted twice a week throughout the flowering season of each *Salvia* species. Pollination efficiency was estimated as the mean number of viable seeds produced per visitor, while pollination effectiveness was estimated as the product of pollinator efficiency and the standardized visitation rate (Ne'eman et al., 2010; Willmer, 2011; Armbruster, 2014).

To compare pollination effectiveness among functional groups, we calculated a standardized pollinator effectiveness index (PEI) by pooling the data from the single-visit experiments according to pollinator functional groups (Fenster et al., 2004; Hoehn et al., 2008; Martén-Rodríguez et al., 2009; Willmer, 2011; Blitzer et al., 2016). For each *Salvia* species, PEI was estimated by dividing the pollination effectiveness value of each functional group by the sum of the pollination effectiveness value of all the functional groups recorded for each plant species (modified from Martén-Rodríguez et al., 2009).

Floral morphology and pollinator effectiveness

Three flowers per plant were randomly selected in 30 individuals per species ($N = 90$ flowers per species, except for *S. assurgens*, $N = 75$ flowers due to population size) to measure 11 floral morphological traits, including corolla length, floral tube length, upper lip length, lower lip length, floral tube width, anthers exsertion, stigma exsertion, corolla tube aperture, upper lip width, floral tube aperture, and lower lip width (Appendix S2). These floral traits have been suggested to play a relevant role in favoring legitimate floral visitation by their effective pollinators or in restricting non-legitimate floral visitors (Wester and Claßen-Bockhoff, 2011; Strelin et al., 2017; Benitez-Vieyra et al., 2019; Wester et al., 2020). Fresh flowers were photographed (laterally and frontally) against 1-mm-grid graph paper, and floral traits were measured to the nearest millimeter using ImageJ version 1.54 g (Schneider et al., 2012).

To determine whether floral morphology can be used to group *Salvia* species according to their most effective functional group of pollinators, a principal component analysis (PCA) was conducted using the average measurements per individual per species (max-transformed) of the 11 floral morphological traits. Additionally, the PEI values of each pollinator functional group for each plant species were plotted as vectors on top of the PCA ordination. To assess the significance of the observed clustering patterns, a permutational multivariate analysis of variance (PERMANOVA) with 9999 permutations was performed. The PCA ordination, the plotting of the vectors, and the PERMANOVA test were performed using the functions prcomp, envfit and adonis2 in the R package vegan (Oksanen et al., 2024) (R version 4.4.2; R Core Team, 2024).

RESULTS

Pollinators' contributions

Reproductive success, measured as fruit and seed set, was significantly higher under open pollination than under autonomous self-pollination in the five *Salvia* species (Table 1; Appendix S3). Among plant species, both fruit and seed set per flower resulting from autonomous self-pollination was <0.1 (Figure 2). In the open-pollination treatment, fruit and seed set per flower ranged from 0.27 to 0.65 and from 0.11 to 0.38, respectively (Figure 2). Thus, our results indicate that pollinators contribute substantially to the reproductive success of the five *Salvia* species.

Floral visitors and pollination performance

Floral visitors

During 237.5 h of observations, 12,435 legitimate floral visits were recorded; the 73 visitor taxa included 28 bees, 25 butterflies, eight flies, four hummingbirds, three beetles, two wasps, one hempiteran, one spider, and one thrip species (Appendix S4). The number of floral visitor guilds per plant species ranged from four to six. Only bees, butterflies, and flies were present in the floral visitor assemblages of all *Salvia* species (Figure 3A). During the two flowering seasons studied, the most frequent legitimate floral visitor guilds were bees for melittophilous species (>60% of visits in *S. assurgens* and >80% in *S. lavanduloides*), hummingbirds for ornithophilous species (>80% of visits in *S. elegans* and >75% in *S. iodantha*), and both bees and hummingbirds for

TABLE 1 Comparisons of fruit and seed set between the autonomous self-pollination and the open pollination treatments, resulting from the generalized linear models for each *Salvia* species.

	Fruit set			Seed set		
Plant species	χ^2	**df**	***P***	χ^2	**df**	***P***
S. assurgens	349.79	1, 1257	<0.001	454.68	1, 1257	<0.001
S. lavanduloides	1311.4	1, 3557	<0.001	2556.6	1, 3557	<0.001
S. mexicana var. *minor*	1342.4	1, 3801	<0.001	2682.9	1, 3801	<0.001
S. elegans	202.53	1, 641	<0.001	441.66	1, 641	<0.001
S. iodantha	196.29	1, 1730	<0.001	251.27	1, 1730	<0.001

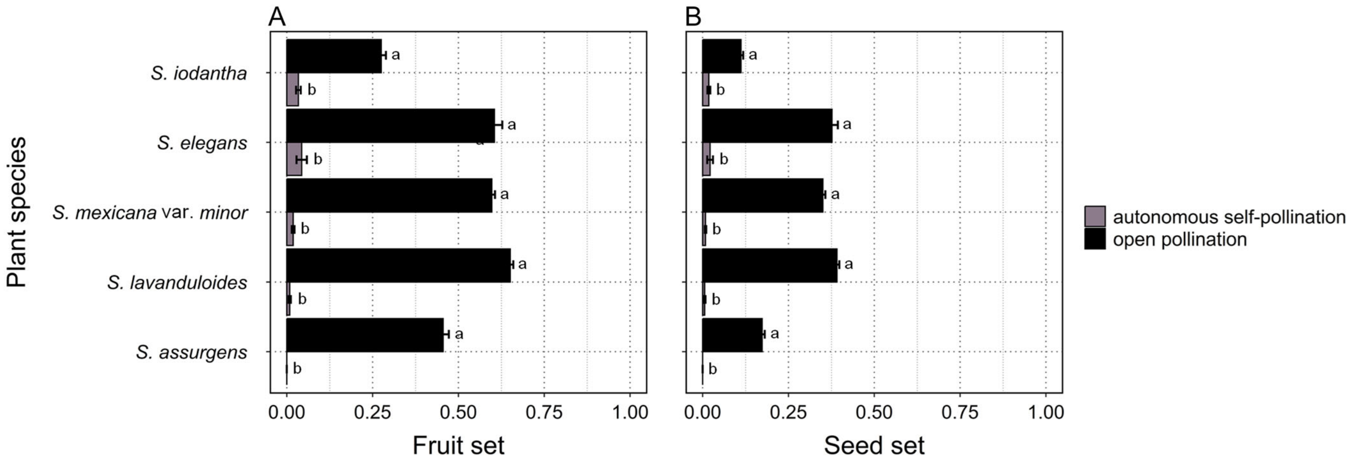

FIGURE 2 Reproductive success of the five *Salvia* species under different pollination treatments. Mean (±SE) (A) fruit and (B) seed set. Different letters next to error bars indicate significant differences between treatments.

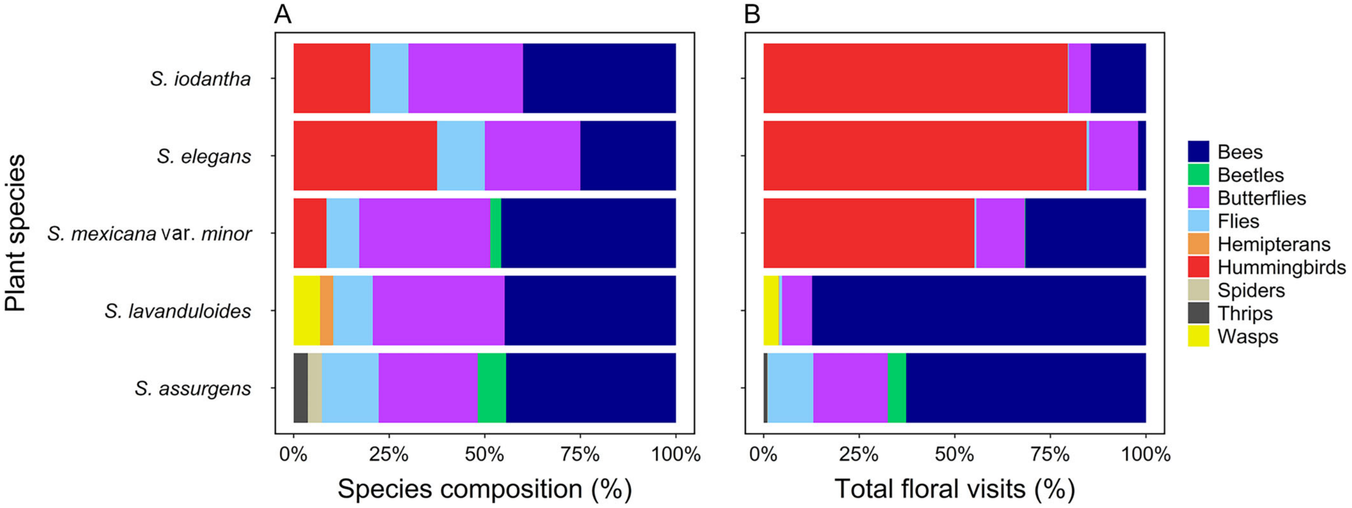

FIGURE 3 Floral visitor diversity recorded in each *Salvia* species during the 2 years of study. (A) Relative contribution of each floral visitor guild to the visitor assemblage composition of each plant species. (B) Relative contribution of each floral visitor guild to the total floral visits recorded in each plant species.

the mixed species *S. mexicana* var. *minor* (>30% and >50% of visits, respectively; Figure 3B).

For *S. assurgens*, the main floral visitors were two *Deltoptila* bee species and one *Exoprosopa* bee-fly species. For *S. lavanduloides*, two bumblebee species (*Bombus ephippiatus* and *B. weisi*), and the honeybee *Apis mellifera* were the most abundant floral visitors. The hummingbirds *Basilinna leucotis* and *Archilochus colubris* were the main floral visitors for *S. elegans* and *S. iodantha*. For *S. mexicana* var. *minor*, *Selasphorus rufus* and *Bombus sonorus* were the most frequent visitors (Appendix S4).

Except for *Salvia lavanduloides*, illegitimate floral visitors were observed in all *Salvia* species. Mainly, large and medium bees (e.g., *Bombus* spp., *Xylocopa* spp., *Apis mellifera*) were observed robbing nectar in both ornithophilous species and the mixed species. However, sometimes they visited the flowers legitimately. Different butterfly species were also observed behaving as nectar thieves in *S. assurgens* (Appendix S4).

Pollinator efficiency and effectiveness

Pollination performance differed among functional groups in all *Salvia* species. In *S. assurgens*, the butterfly *Lon zabulon* had an efficiency of 1.33 ($N = 3$), while the bee *Deltoptila badia* had 0.60 ($N = 48$; Table 2). However, considering the standardized visitation rate of both pollinator species, *D. badia* had an effectiveness of 0.20, while *L. zabulon* had 0.02 (Table 2). Similarly, for *S. lavanduloides*, the wasp *Pygodasis ephippium* was the most efficient, but *B. ephippiatus* was more effective due to its higher visitation rate (Table 2).

TABLE 2 Efficiency and effectiveness of each pollinator recorded for each *Salvia* species.

Plant species	Pollinator species	Single visits (*N*)	Standardized visitation rate	Efficiency	Effectiveness
S. assurgens	*Deltoptila badia* (MB)	48	0.33	0.6	0.20
	Deltoptila sp. (MB)	55	0.05	0.62	0.03
	Thygater sp. (MB)	6	0.04	0.17	0.01
	Lon zabulon (B)	3	0.02	1.33	0.02
	Lasioglossum pharum (SB)	11	0.03	0.09	<0.01
	*Exoprosopa rostrifera** (LTF)	20	0.11	0.00	0.00
	*Thorybes dorantes** (B)	22	0.10	0.00	0.00
S. lavanduloides	*Bombus ephippiatus* (LB)	133	0.57	1.59	0.91
	Apis mellifera (MB)	170	0.15	1.12	0.17
	Pygodasis ephippium (W)	11	0.04	3.64	0.13
	Bombus weisi (LB)	131	0.07	1.29	0.09
	Largus sp.* (BU)	11	<0.01	0.00	0.00
	Lasioglossum sp.* (SB)	5	0.01	0.00	0.00
S. mexicana var. *minor*	*Bombus sonorus* (LB)	11	0.17	1.55	0.27
	Selasphorus rufus (H)	31	0.24	0.68	0.16
	Megachile sp. (MB)	4	0.02	1.5	0.03
	Apis mellifera (MB)	13	0.03	0.69	0.02
	Halictidae 2 (SB)	10	0.01	0.8	0.01
	Deltoptila elefas (LB)	2	<0.01	0.5	<0.01
	Halictidae 1 (SB)	10	<0.01	0.3	<0.01
	Lasioglossum sp. (SB)	4	0.01	0.25	<0.01
	Piruna sp. (B)	3	<0.01	0.33	<0.01
S. elegans	*Basilinna leucotis* (H)	29	0.51	0.83	0.42
	Archilochus colubris (H)	19	0.33	1.26	0.41
	Phoebis philea (B)	12	0.12	0.25	0.03
	Lasioglossum sp.* (SB)	7	0.01	0.00	0.00
S. iodantha	*Basilinna leucotis* (H)	95	0.77	1.21	0.93
	Deltoptila elefas (LB)	14	0.13	0.14	0.02
	Halictidae 2* (SB)	5	<0.01	0.00	0.00
	*Lasioglossum costale** (SB)	5	<0.01	0.00	0.00

Notes: Codes for pollinator functional group is in parentheses after the taxon. Pollinator species: butterfly, B; bug, BU; hummingbird, H; large bee, LB; medium bee, MB; small bee, SB; long-tongued fly, LTF; wasp, W. Asterisks indicate that the floral visitor species failed to produce seeds.

Both ornithophilous species shared the hummingbird *Basilinna leucotis* as their primary pollinator but differed in their secondary pollinators. The secondary pollinator for *Salvia elegans* was the hummingbird *Archilochus colubris*; for *S. iodantha*, it was the bee *Deltoptila elefas*. The primary and secondary pollinators of *S. elegans* had similar effectiveness (0.42 and 0.41, respectively), whereas those of *S. iodantha* had contrasting values (0.93 and 0.02, respectively; Table 2). The lowest diversity in pollinator species and functional groups was recorded in ornithophilous species.

The highest pollinator diversity was recorded for *S. mexicana* var. *minor*, which was pollinated by hummingbirds, butterflies, and small, medium, and large bees (Table 2). The primary and secondary pollinators of this species were, respectively, the bumblebee *Bombus sonorus* (efficiency = 1.55; effectiveness = 0.27; $N = 11$) and the hummingbird *Selasphorus rufus* (efficiency = 0.68; effectiveness = 0.16; $N = 31$; Table 2).

In all but the mixed *Salvia* species, some other floral visitor species belonging to different functional groups were recorded in the single-visit experiments; however, their visits did not contribute to seed production (Table 2). According to the pollinator effectiveness index, the most effective functional groups were large and medium bees for melittophilous species, hummingbirds for ornithophilous species, and large bees and hummingbirds for the mixed species (Table 3).

Floral morphology and pollinator effectiveness

The PCA showed that the first and second principal components explained 52.42% and 35.02% of the observed variance, respectively (Figure 4; Appendix S5), which together explained 87.44% of the total variance. The floral traits with the highest loadings for PC1 were upper lip length (ULL), corolla length (CL) and corolla aperture (CA; Appendices S6, S7). For PC2, the traits with the highest loadings were anther exsertion (AE), stigma exsertion (SE) and lower lip length (LLL; Appendices S6, S7). Species with longer upper lips and corollas were placed along the positive PC1 axis, whereas species with more exserted reproductive structures and shorter lower lips were placed along the negative PC2 axis (Figure 4).

TABLE 3 Values for the pollinator effectiveness index (PEI) of each functional group for each *Salvia* species.

Plant species	Functional group	PEI
S. assurgens	Medium bee	0.92
	Butterfly	0.08
	Small bee	0.01
S. lavanduloides	Large bee	0.75
	Medium bee	0.14
	Wasp	0.11
S. mexicana var. *minor*	Large bee	0.52
	Hummingbird	0.35
	Medium bee	0.1
	Small bee	0.03
	Butterfly	0.004
S. elegans	Hummingbird	0.98
	Butterfly	0.02
S. iodantha	Hummingbird	0.98
	Large bee	0.02

Note: Only the pollinator functional groups contributing to seed production are reported.

The PCA plot showed a clear separation among floral syndromes, and *Salvia mexicana* var. *minor*, the species with the mixed phenotype, was placed in an intermediate position between the melittophilous species *S. assurgens* and the ornithophilous species *S. elegans*, with most of its individuals closer to *S. elegans* (Figure 4). The PEI vectors indicated that medium bees, large bees and wasps pollinators were associated with negative values of PC1 axis and positive values of PC2 axis. Butterflies and small bees were associated with positive values of PC1 and PC2 axes, and hummingbirds were associated with positive values of PC1 axis and negative values of PC2 axis (Figure 4). According to the PERMANOVA test, the floral morphology of each *Salvia* species differed significantly from the others ($F_{4,140} = 718.83$; $R = 0.953$, $P < 0.001$).

DISCUSSION

This study is one of the few to quantify the contribution of several floral visitors to plant reproductive success and to explore whether floral syndromes match the most effective pollinators in *Salvia*. Overall, our results showed that pollinators contributed substantially to the reproductive success of the five species studied and highlight the importance of considering pollinator effectiveness. Moreover, our findings support the idea that the most-effective pollinators exert selective pressure on floral characters, which is the foundation of the floral syndrome concept.

Pollinators' contributions

According to our first prediction, we found higher reproductive success with open pollination than with autonomous self-pollination. These results were congruent with other studies indicating that *Salvia* species are pollinator-dependent (e.g., Arizmendi et al., 1996; Grases and Ramírez, 1998; Aximoff and Freitas, 2010; Cuevas et al., 2013; Jorge et al., 2015; Cairampoma et al., 2020; Barrionuevo et al., 2021; Xiao et al., 2023a). However, considering the fruit and seed set in all *Salvia* species, the reproductive success observed in natural conditions was rather low, which suggests pollen limitation. For pollinator-dependent plant species, the number and quality of floral visits directly affect plant fitness (Aizen and Harder, 2007). Pollen limitation caused by a depauperate pollinator fauna, or plant–plant antagonistic interactions such as heterospecific pollen deposition and pollinator competition could explain the low reproductive success observed here because it has been found for other sage species (e.g., *S. sclareoides*, Jorge et al., 2015; *S. daiguii*, Xiao et al., 2024). The evaluation of the impact of plant–plant antagonistic interactions on the reproductive success of the five *Salvia* species was beyond the scope of this study; nevertheless, it deserves further attention.

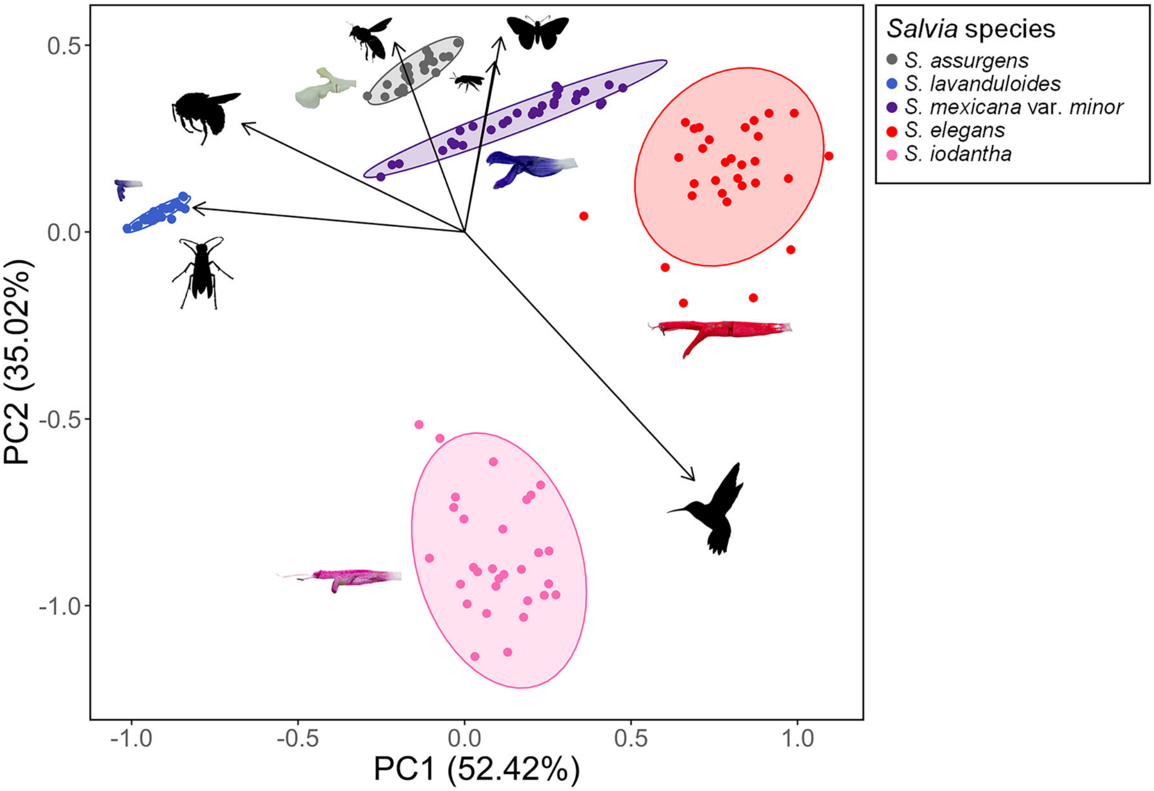

FIGURE 4 PCA ordination based on the max-transformed data of the 11 floral traits of each *Salvia* species. Drawings at the end of each vector represent the effective pollinator functional groups recorded: butterfly, hummingbird, large bee, medium bee, small bee, and wasp.

Floral visitors and pollination performance

Consistent with our second prediction, pollinator assemblages were mainly composed of insects and birds that matched the floral syndrome of each *Salvia* species; however, the floral visitor assemblages of each *Salvia* species were more diverse than expected by the floral syndrome concept. Nevertheless, this concept implies that only the floral visitors with the greatest contribution to plant reproduction will be the principal agents of selection on floral traits. Moreover, since selection has a quantitative basis (Stebbins, 1970), it is crucial to determine the most effective pollinators (i.e., the most frequent and efficient ones). Therefore, pollinator effectiveness should be used as the most comprehensive metric to test the concept of floral syndrome accurately.

Salvia assurgens was primarily visited and pollinated by the bee *Deltoptila badia* (Apidae). Similarly, other species from this bee genus have also been reported as frequent visitors of *S. assurgens*, but their contribution to plant reproduction had only been assessed via pollen load analysis (Cultid-Medina et al., 2021). The bee-fly *Exoprosopa rostrifera* (Bombyliidae) was the second most frequent visitor, but none of its visits resulted in seed production. Even when flies have been reported as frequent pollinators for some melittophilous species of *Salvia* (Celep et al., 2014, 2020; Saravia-Nava et al., 2023), our findings suggest they cannot be considered pollinators of the studied species, given their null efficiency.

Although the bumblebee *Bombus sonorus* was the most-effective pollinator of *S. lavanduloides*, the most-efficient pollinator was the wasp *Pygodasis ephippium* (Scoliidae). To our knowledge, this is the first evidence of the contribution of wasps to reproductive success in *Salvia* and one of the few studies that have reported the interaction between wasps and melittophilous sages (e.g., Grant and Grant, 1964; Tavera et al., 2023).

Salvia elegans was mainly visited by hummingbirds, as indicated by previous records from different localities in Mexico (Espino-Espino et al., 2014; Cuevas et al., 2018; López-Segoviano et al., 2021). *Basilinna leucotis* and *Archilochus colubris* were the main pollinators for this species, and they were almost equally effective. Bees were also recorded, but they represented less than 2.5% of the total visits, and none of their visits resulted in fruit or seed production. These results contrast with the observations of Espino-Espino et al. (2014), who found that up to 23% of the total floral visits during one flowering season were by bees. Instead, we found that butterflies were the second most frequent and effective pollinator functional group, where *Phoebis philea* (Pieridae) was the only non-hummingbird pollinator of this species.

For *Salvia iodantha*, we found that *Basilinna leucotis* was the only hummingbird pollinator. This result differs from previous studies in different locations of Mexico that have found that this sage species interacts with several hummingbird species (Lara and Ornelas, 2001; López-Segoviano et al., 2021). Even though Dieringer et al. (1991) suggested that the bee *Deltoptila elefas* was a nectar robber in *S. iodantha*, all the floral visits by this species were legitimate. Nevertheless, our single-visit experiments revealed that *D. elefas* barely contributed to seed production and should be considered as a secondary pollinator.

Although both ornithophilous species have similar floral morphology (tubular corolla and exserted stamens and stigma), the hummingbird *Basilinna leucotis* performed better in *Salvia iodantha* than in *S. elegans*. Morphological matching promotes high interaction frequencies between plant species and their hummingbird pollinators and is strongly related to plant fitness (Maruyama et al., 2014; Bustos et al., 2023). The differences in efficiency and effectiveness of the primary pollinator of the ornithophilous species studied suggest a higher degree of trait matching between *S. iodantha* and *B. leucotis*; however, this hypothesis needs to be tested. It is important to note that *S. iodantha* was way more abundant than *S. elegans* at our study site. Therefore, the asymmetrical contribution of *B. leucotis* could also reflect a higher likelihood of conspecific pollen deposition among *S. iodantha* individuals, and heterospecific pollen deposition among *S. elegans* individuals. Interestingly, the negative effects of reproductive interference were greater for *S. iodantha* than for *S. elegans*, likely because the latter relied on different hummingbird species for pollination.

As expected, *Salvia mexicana* var. *minor* was mainly pollinated by bees and hummingbirds. However, contrary to previous observations (Strelin et al., 2017), bees served as its main pollinators. Additionally, unlike the other sage species studied, *S. mexicana* var. *minor* depended on different functional groups for pollination each year. In 2022, the primary pollinator was the bumblebee *Bombus sonorus*, while in 2023 it was the hummingbird *Selasphorus rufus*. This strategy may be beneficial in the context of global change and pollinator decline.

Secondary pollinators may be crucial for plant reproduction if primary pollinators are absent or scarce (Rosas-Guerrero et al., 2014; Ashworth et al., 2015; de Santiago-Hernández et al., 2019). Moreover, it has been suggested that secondary pollinators usually correspond to the ancestral primary pollinators (Rosas-Guerrero et al., 2014). Accordingly, the secondary pollinators reported here are consistent with some of the major pollinator shifts documented in the literature (e.g., bees for ornithophilous flowers, and butterflies for melittophilous and ornithophilous flowers; Van der Niet and Johnson, 2012; Rosas-Guerrero et al., 2014). Nonetheless, recent studies indicate that only evolutionary transitions between bee and hummingbird pollination have occurred in *Salvia* (Fragoso-Martínez et al., 2018; Benitez-Vieyra et al., 2019; Kriebel et al., 2019, 2020). We propose that future phylogenetic comparative studies aimed to elucidate ancestral pollination systems should include studies like ours for a more insightful view on pollinator transitions.

Floral morphology and pollinator effectiveness

According to the PCA plot, there was greater morphological affinity among melittophilous species than among ornithophilous species, despite the evident morphological differences between *Salvia assurgens* and *S. lavanduloides* (e.g., flower size and corolla aperture) and the similarities between *S. elegans* and *S. iodantha* (e.g., long corollas and exserted reproductive structures). In another study on the floral morphology of 18 sage species, Benitez-Vieyra et al. (2019) found a very similar pattern, with melittophilous and ornithophilous species occupying opposite regions of the multivariate space.

The match between clustering patterns and pollinator vectors in the PCA plot was more evident for melittophilous than for ornithophilous sage species, which could be due to our pollinator functional group classification. That is, *S. assurgens* and *S. lavanduloides* were primarily pollinated by bees of different sizes, representing different functional groups and vectors in the plot, whereas *S. elegans* and *S. iodantha* were primarily pollinated by hummingbirds, a single vector in the plot representing a broad functional group that does not necessarily capture the great intraspecific morphological variation across hummingbird species (Leimberger et al., 2022; Barreto et al., 2024).

On the other hand, despite the higher effectiveness of large bees, *S. mexicana* var. *minor* remained closer to *S. elegans* and *S. assurgens* due to morphological resemblance (e.g., corolla length and floral tube length) and was pollinated by the same functional groups recorded for these two species. Previous studies relating floral morphology and visitation in *Salvia* have found *S. mexicana* var. *minor* to be more similar to ornithophilous species than to melittophilous species (Strelin et al., 2017), and some have even considered it to be bird-pollinated (Benitez-Vieyra et al., 2019).

Mixed-pollinated *Salvia* species are part of a continuum in which floral phenotypes may be mainly melittophilous and partially ornithophilous or vice versa (Wester and Claßen-Bockhoff, 2011). Our findings suggest that *S. mexicana* var. *minor* could represent a stable intermediate phenotype between melittophily and ornithophily, that enables reproductive success in pollinator-changing environments, however, long-term studies are needed to test this idea. Alternatively, other mixed-pollinated sage species have been regarded as transitional states in pollinator shifts (e.g., *S. chiapensis*, *S. clevelandii*, and *S.discolor*; Wester et al., 2020); hence, the idea that *S. mexicana* var. *minor* represents a transition from bee to hummingbird pollination cannot be excluded.

Geographic variation in pollinator fauna may drive intraspecific floral divergence and originate different pollination ecotypes (e.g., Newman et al., 2014; Van der Niet et al., 2014). Except for *Salvia assurgens*, the rest of the sages studied here are widely distributed in Mexico; thus, the major caveat of this study is the limited geographical area it encompassed. Recent work on the Andean *S. stachydifolia* revealed the existence of three pollination ecotypes adapted to bee, hummingbird, and mixed pollination (Izquierdo et al., 2023). Therefore, future studies on *Salvia* should assess how geographic variation in the identity of effective pollinators relates to intraspecific floral variation.

Except for *Salvia mexicana* var. *minor*, the species with a mixed phenotype, our results support the floral syndrome concept and agree with the most-effective pollinator principle (Stebbins, 1970), which states that floral phenotypes should be adapted to the most-effective (i.e., most frequent and efficient) pollinator. Furthermore, secondary pollinators not expected by the syndrome could reflect the evolutionary transitions among pollination systems. Finally, our findings emphasize the importance of considering pollination effectiveness as a proxy to assess the reliability of pollination syndromes, and for a better comprehension of the ecology and evolution of plant–pollinator interactions.

AUTHOR CONTRIBUTIONS

A.T.: conceptualization, data curation, formal analysis, investigation, methodology, supervision, validation, visualization, writing original draft, review and editing. M.H.S.-H.: conceptualization, data curation, formal analysis, investigation, methodology, supervision, validation, writing original draft, review and editing. V.R.-G.: conceptualization, methodology, supervision, validation, writing original draft, review and editing. C.G.: conceptualization, methodology, supervision, validation, writing original draft, review and editing. E.C.: conceptualization, investigation, methodology, project administration, resources, supervision, validation, writing original draft, review and editing.

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DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available from the Figshare open repository: https://doi.org/10.6084/m9.figshare.28935098.v1.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Geographic location of the five *Salvia* species studied.

Appendix S2. Floral morphological traits measured in each *Salvia* species.

Appendix S3. Reproductive success of each *Salvia* species under different pollination treatments.

Appendix S4. Visitation rate of each floral visitor across plant species.

Appendix S5. Summary table of the PCA using the average per plant max-transformed measurements of the 11 floral morphological traits.

Appendix S6. Loadings of each floral morphological trait for the first and second principal components.

Appendix S7. Average measurements of the 11 morphological floral traits for all the studied *Salvia* species.

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