Flora 330 (2025) 152788

Contents lists available at ScienceDirect

Flora

journal homepage: www.elsevier.com/locate/flora

Ethological and mechanical isolation promotes pollinator partitioning in sympatric Andean *Salvia* species

Lianka Cairampoma [a], Juan A. Tello [b,c], Carlos Martel [c,d], Manfred Ayasse [e], Regine Claßen-Bockhoff [a,*]

[a] Institute of Organismic and Molecular Evolution (iomE), Johannes Gutenberg-University, D-55099, Mainz, Germany
[b] Postgraduate School, Biological Sciences Faculty, Universidad Nacional Mayor de San Marcos, Lima, Peru
[c] Instituto de Ciencias Ómicas y Biotecnología Aplicada, Pontificia Universidad Católica del Perú, Av. Universitaria 1801, San Miguel 15088, Lima, Peru
[d] Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, UK
[e] Institute of Experimental Ecology, University of Ulm, D-89081, Ulm, Germany

ARTICLE INFO

Edited by Timotheus van der Niet

Keywords:
Bee pollination
Bird pollination
Floral colour
Flower construction
Pollinator partitioning
Reproductive isolation
Scent chemistry

ABSTRACT

Plants that co-occur and co-flower may suffer negative fitness consequences through pollinator sharing. However, if they have evolved specific adaptations such as being pollinated by only a subset of the available pollinator spectrum, they may co-exist without losing species integrity. Pollinator partitioning can be achieved by exhibiting specific floral traits, which promote the attraction of certain floral visitors (ethological isolation) and/or by specific flower proportions, which either only fit to part of the possible visitors or contribute to pollinator sharing without pollen mixing (mechanical isolation). In this study, we aimed to identify the floral traits and suite of pollinators associated with the reproductive isolation of three co-occurring and co-flowering *Salvia* species (*S. cruikshanksii, S. tafallae, S. tubiflora*) from the high elevation shrubland in the central Peruvian Andes. We investigated flower morphology, spectral reflectance (colour), and scent chemistry, as well as pollinator behaviour and breeding system. *Salvia cruikshanksii* and S. *tafallae* are adapted for bee pollination, whereas *S. tubiflora* is adapted to hummingbirds. Accordingly, all floral traits of *S. tubiflora* are distinct from those of the other two *Salvia* species. Although *S. cruikshanksii* and *S. tafallae* both attract bees, they do not share a single pollinator species. Reproductive isolation might be predominantly based on species-specific odour bouquets reinforced by visual signals (ethological isolation), and on differences in floral proportions between the *Salvia* species such as flower tube lengths (mechanical isolation). The three *Salvia* species demonstrate the significance of diversified floral traits in a species assemblage causing pollinator partitioning and allowing co-existence

1. Introduction

In highly biodiverse ecosystems such as the Andes, species belonging to species-rich genera like *Salvia* can be found co-occurring at the same locality (sympatry) and co-flowering in the same periods of the year (Benitez-Vieyra et al., 2019). This can risk the integrity of species by creating conditions for interspecific pollen flow mediated by pollinators. Therefore, floral specificity resulting in pollinator partitioning is considered an important prezygotic barrier that prevents interspecific pollen flow among (closely) related species (Grant, 1994; Botes et al., 2008). Floral specificity can be achieved by the preference of pollinators to specific floral signals such as colour and/or scent (ethological isolation), and by morphological traits either restricting the accessibility of floral rewards to a subset of available pollinators or enabling the use of the same pollinators by placing the pollen onto different body sites (mechanical isolation) (Grant, 1994; Rieseberg and Willis, 2007; Schiestl and Schlüter, 2009; Ruchisansakun et al., 2016).

Mechanical isolation can be the consequence of pollinator-mediated selection when changes in floral traits such as flower tube length or width of the flower entrance exclude visitors of certain body proportions (e.g. bee pollinated *Salvia*: Grant, 1994; oil secreting orchids: Pauw, 2006). Moreover, mechanical isolation can also occur among flowers of plants that share the same functional pollinator group (sensu Fenster et al., 2004). For instance, the pollen of different plant species is deposited on different parts of the pollinator's body (e.g., *Pedicularis*: Macior, 1983; *Stylidium*: Armbruster et al., 1994; *Salvia*:

* Corresponding author.
E-mail address: classenb@uni-mainz.de (R. Claßen-Bockhoff).

https://doi.org/10.1016/j.flora.2025.152788
Received 15 December 2024; Received in revised form 17 June 2025; Accepted 19 June 2025
Available online 19 June 2025

Claßen-Bockhoff et al., 2004; Celep et al., 2020; *Impatiens*: Ruchisansakun et al., 2016).

Ethological isolation is caused by sensorial adaptation or learned preferences of the pollinators, which result in pollinator specialisation or foraging constancy. Individual pollinators respond specifically to floral signals and forage preferentially on a certain flower type during a succession of visits (Grant, 1994; Schiestl and Schlüter, 2009). *Mimulus* (Phrymaceae) and *Ophrys* (Orchidaceae) species are classical examples, as the different pollinators visit flowers guided by floral colour and odour (Paulus and Gack, 1990; Schemske and Bradshaw, 1999).

The genus *Salvia* (Lamiaceae) is a highly diverse genus with c. 1000 accepted species, around 600 of them distributed in the Neotropics (Will and Claßen-Bockhoff, 2017; Kriebel et al., 2019, 2020). Species often occur sympatrically and are mainly pollinated by bees or birds (Benitez-Vieyra et al., 2019; Wester and Claßen-Bockhoff, 2011). Hybridisation has been documented among neotropical species (Epling, 1947; Grant and Grant, 1964; Haque and Ghoshal, 1981; Owens and Ubera-Jiménez, 1992; Ramamoorthy and Elliott, 1998), suggesting absence of postzygotic barriers among these species. Co-existence may therefore mainly depend on prezygotic isolation mechanisms. In the present study, we investigate three co-occurring and co-flowering *Salvia* species from the Peruvian Andes, aiming to elucidate whether and how niche partitioning in pollinator use is associated with variation in floral traits. We expect that these coexisting species are adapted to different pollinators and exhibit differences in floral traits, and that species with the same pollinator group are characterized by specific floral traits and/or signals resulting in assortative mating. We specifically aim to elucidate whether these species share pollinators and deposit pollen on a species-specific site of the pollinator's body as known from certain bee-pollinated *Salvia* species (Claßen-Bockhoff et al., 2004; Celep et al., 2020) or have different nectar tube lengths attracting pollinators with different mouthpart lengths. We focus on the identification and specificity of floral volatiles, which are known to play a prominent role in flower-pollinator interactions (Dötterl and Vereecken, 2010), but have, to our knowledge, not yet been considered in a *Salvia* field study.

2. Material and methods

2.1. Study site

The field study was carried out in a natural, c. 4 ha large area located in the Central Peruvian Andes near Pariamarca village (11.494° S, 76.633° W) at 2900–3100 m above sea level (Fig. 1A). This area is characterized by a shrubland vegetation, sub-humid climate, and strong

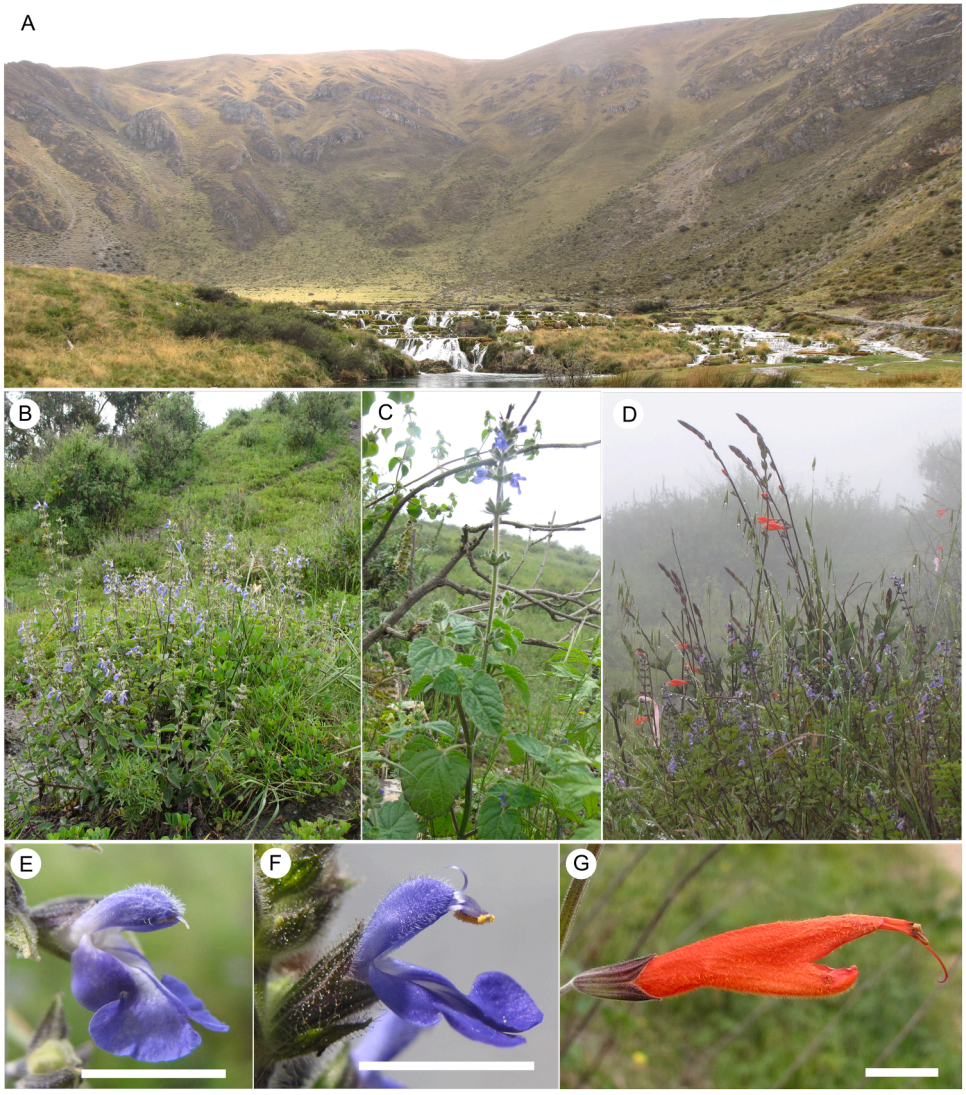

Fig. 1. Habitat, growth form and *Salvia* species included in the study. **A.** High Andean shrubland vegetation at 3000 m above sea level. Growth form of *Salvia cruikshanksii* (**B**), *Salvia tafallae* (**C**), and *Salvia tubiflora* and *S. cruikshanksii* growing side by side (**D**). Flower side view of *S. cruikshanksii* (**E**), *S. tafallae* (**F**) and *S. tubiflora* (**G**). Bars = 1 cm.

seasonality. The wet season lasts from November to April, with dry conditions during the rest of the year. *Salvia cruikshanksii, S. tafallae* and *S. tubiflora* co-occur and co-flower in this area. Plants of the three species were scattered in the area with 4.2 individuals per m^2 for *S. cruikshanksii* and 1.5 individuals per m^2 for the other two species. Individuals of different species were direct neighbours (Fig. 1D) or only few meters (5–10 m) apart from each other. Fieldwork was carried out during February, March and August of 2015, and March and April of 2016.

2.2. Study taxa

The three studied *Salvia* species belong to three different sections of the neotropical subgenus *Calosphace*. Their phylogenetic relationships are not known as only *S. tubiflora* has been included in molecular phylogenies (Fragoso-Martínez et al., 2018; Kriebel et al., 2020). The species overlap in geographical distribution, altitudinal range, habitat preference, and flowering season and differ in growth form and flower colour (Table 1).

2.3. Flower life span and floral anthesis

To determine flower longevity, 15–20 flowers per species were monitored from the bud stage until corolla senescence. Monitored flowers were observed every day early in the morning (between 6:00 and 7:00 h) and in the afternoon (between 16:00 and 17:00 h). To avoid effects of pollination-induced wilting, flowers were covered with net bags (5 × 2.5 cm) during the monitoring period. To determine the period of stigma receptivity ('female' phase), we used the peroxidase test (H_2O_2 solution at 10 %; Dafni et al., 2005); likewise, the pollen donating ('male') phase was identified by recording pollen release from the anthers during the observation period.

2.4. Flower morphology

Open flowers of *S. cruikshanksii* (n = 45), *S. tafallae* (n = 32) and *S. tubiflora* (n = 37) were collected, each flower from a different individual. To quantify differences in the morphology of the *Salvia* species, we measured morphological flower traits related to pollen transfer using a pair of callipers. In *S. cruikshanksii* and *S. tafallae,* 11 traits were selected: a, Flower length. b, Length of the corolla tube. c, Distance from the flower entrance to the nectar chamber. d, Distance from the thecae to the nectar chamber. e, Total length of the connective. f, Length of the upper connective arm. g, Length of the filament. h and i, Horizontal and vertical diameters of the corolla entrance. j, Style length. k, Length of the lower lever arm (Fig. 2). In *S. tubiflora* with exerted styles and thecae, four additional traits were measured: l, Exertion of the lower stigmatic lobe. m, Exertion of the thecae. n, Distance from the lower stigmatic lobe to the distal end of the theca. o, Distance between the distal ends of the thecae. To identify statistical differences among species, a Kruskal-Wallis test was performed followed by Mann-Whitney tests for pairwise comparisons. A Benjamini correction was later performed to identify statistical differences among species.

Special attention was given to the morphometric data of the similar looking *S. cruikshanksii* and *S. tafallae*. To investigate whether the flower morphology as a whole is different between these species, we performed a principal component analysis (PCA) based on non-standardized values. The tests were performed by using SPSS v. 20 (IBM Corp.) and PAST 3.25.

2.5. Floral colour

The spectral reflectance of the outer corolla was measured in flowers of *S. tubiflora* (n = 12), of the inner corollas in *S. cruikshanksii* (n = 11) and *S. tafallae* (n = 11) and of the nectar guide (n = 7) in *S. cruikshanksii.* Measurements were taken by using an Ocean Optics JAZ spectrometer (Ocean Optics, Inc., Florida, USA) equipped with a pulsed xenon light source (JAZ-PX) and attached to a fibre-optic cable (UV/VIS 400 µm; World Precision Instruments, Inc., Florida, USA) held at 45° and ca. 5 mm from the measured spot. A WS-1-SL (Ocean Optics) was used as white standard and a black film cannister as black standard. To characterize the perception of corolla and nectar guide colours by bees, we used the colour hexagon (Chittka, 1992), based on the spectral sensitivities of *Apis mellifera* (L.). In this model, the measured reflections are plotted as loci in the bee colour space (Chittka and Kevan, 2005) with the bee-achromatic area located in the center of the model.

2.6. Floral scent

We collected floral volatile samples of *Salvia* species in situ by using a headspace sampling technique. Inflorescences from seven individuals per species were individually enclosed in a polyester oven bag (12 × 3 cm, Toppits®, Germany) and the floral scent was left to accumulate for 40 min, after which the floral scents were pumped for 5 min to an adsorbent tube at a constant flow rate of 200 mL min^{-1}. The adsorbent tubes consisted of Chromatoprobe quartz glass microvials (Agilent Inc.; length: 15 mm; inner diameter: 2.5 mm) filled with 3 mg of a 1:1 mixture of Tenax™ TA (60/80 mesh; Supelco, Bellefonte, PA, USA) and Carbotrap B (20/40 mesh; Supelco, Bellefonte, PA, USA). In parallel, we collected volatiles from green parts (i.e., inflorescences in development and stems with leaves) of *Salvia* species (n = 1 per each species) and environmental controls (empty bags; n = 3) using the same protocol to be able to exclude floral from non-floral volatiles and volatiles present in the ambient air. Adsorbent tubes were placed in 2 ml screw cap vials, labelled, and stored at −20 °C until the chemical analyses.

In the lab, collected floral scent samples were analysed using a coupled gas chromatograph-mass spectrometer system (7890B GC – 5977A MSD, Agilent Technologies, Germany), equipped with a thermal desorption unit (TDU, Gerstel, Mühlheim a.d. Ruhr) and a cold injection system (CIS 4C, Gerstel, Mühlheim a. d. Ruhr). The GC was equipped with a DB-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 µm film

Table 1
Characteristics of the *Salvia* species investigated. [1], after Fragoso-Martínez et al., 2018; Cairampoma, 2021. [2], after Brako and Zarucchi, 1993; Macbride, 1960; Wood, 2007.

Characteristics	*S. cruikshanksii* (Fig. 1B, E)	*S. tafallae* (Fig. 1C, F)	*S. tubiflora* (Fig. 1D, G)
Section [1]	Flocculosae	Rhombifoliae	Biflorae
Geographical distribution[2]	north Peru to central Peru	central Peru to north Bolivia	central Peru to north Chile
Altitude above sea level (in meters)[2]	1500–3000	2000–3750	0–3000
Vegetation type[2]	shrublands semi-disturbed areas, rocky slopes	shrublands disturbed areas	shrublands disturbed areas, rocky slopes
Flowering season (in months)[2]	XII-IV	I-IV	XII-VII
Growth form [2]	Shrub, 1–1.5 m tall	Annual, 0.4–0.5 tall	Shrub, 1–2 m tall
Flower colour (as perceived by humans)[2]	blue	blue	red

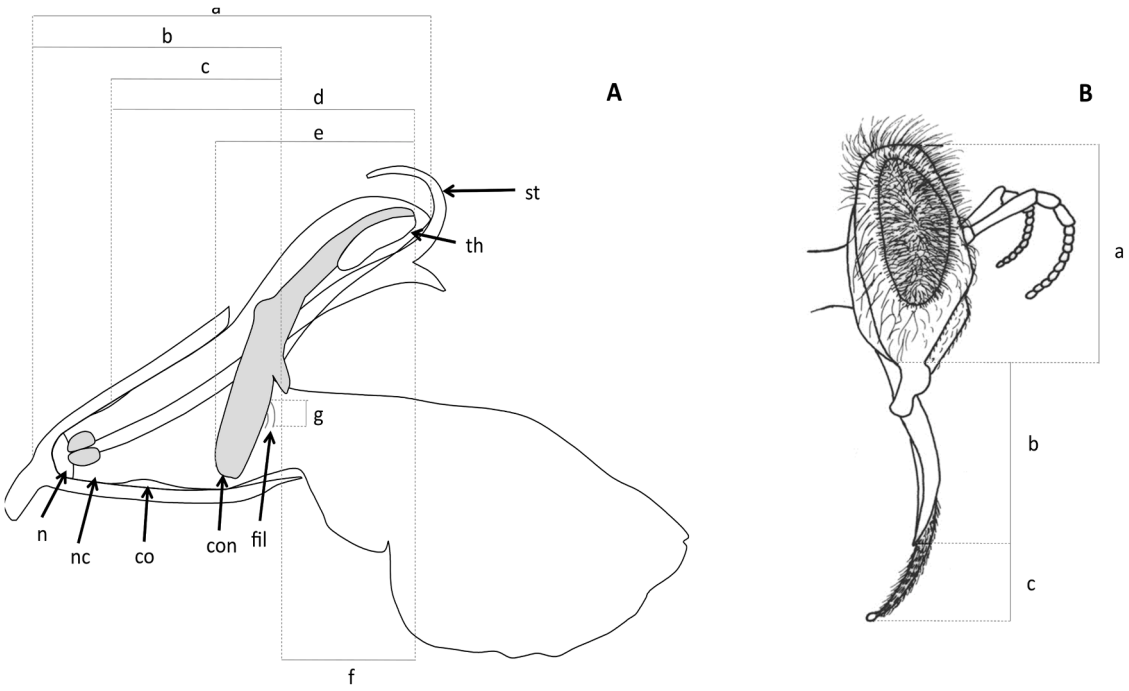

Fig. 2. Morphometric measurements in flowers. Example: longitudinal section of a *Salvia cruikshanksii* flower. co, constriction of the floral tube. con, connective. fil, filament. n, nectary. nc, nectar chamber (lower part of the floral tube where nectar can be stored). st, stigma. th. theca. **a–g**, morphometric data (see Table 3).

thickness, J&W Scientific, Folsom, CA). We used helium as carrier gas with a constant flow of 1 ml/min. Chromatoprobes were thermally desorbed at 300 °C for 10 min and refocused with liquid nitrogen. Oven temperature started at 50 °C for 1 min, was then raised to 310 °C at a rate of 4 °C/min. The mass spectrometer was run in electron ionization (EI) mode (70 eV) and set to a scan range from 35 to 450 m/*z* with a scanning rate of 3.5 scans/*sec*. Obtained mass spectra were processed using the Automated Mass Spectral Deconvolution and Identification System (AMDIS) software (National Institute of Standards and Technology, Gaithersburg, United States). Floral scent compounds were identified based on their mass spectra using a commercial mass spectra library (NIST11), the library of the Institute of Evolutionary Ecology and Conservation Genomics at Ulm University, and the chemicals' retention indices (e.g. Adams, 2017). The peak areas on the mass spectra were integrated and used to determine the relative percentages of each compound. Green parts of *Salvia* species are generally scented, independently of bearing flowers. Therefore, we consider it unlikely that they would play a role in pollinator attraction. As we were interested in floral traits, we excluded chemicals produced by the green parts from our final floral scent list.

Differences in floral scent composition of the three *Salvia* species were analysed by comparing the relative proportions of the compounds identified in our samples. Relative proportions of the floral scent chemicals were fourth-root transformed before calculating the Bray-Curtis similarity index to determine semi-quantitative differences in floral scents. Then, a PERMANOVA with 10,000 permutations was used to test for differences in scents between species and types of functional pollinator groups (bee vs bird pollination). An NMDS was performed to graphically display the (dis)similarities in floral scent patterns among samples. PERMANOVA and NMDS tests were performed using the vegan package and displayed graphically using the `ggplot2 package' of R.

2.7. Nectar volume and sugar concentration

Nectar volume was assessed for each *Salvia* species from 10 to 15 flowers per species, one flower per individual. Prior to the beginning of anthesis, flowers were bagged. On the first day of anthesis, nectar volume was measured using 5 μL microcapillary tubes and sugar concentration was determined using a hand-held refractometer. Differences in nectar production and sugar concentration among species were assessed through Mann-Whitney U tests followed by a Bonferroni correction using the `stats package' of R.

2.8. Pollinator observations

Floral visitors were recorded by direct observations in intervals of 15 min per hour, from 8:00 to 16:00 h, during 10 to 12 days per species. Observations were performed on 26 individuals in *S. cruikshanksii*, 24 in *S. tafallae* and 17 in *S. tubiflora*. A photo camera (Canon Powershot G16) and a video camera (Panasonic HC-X810) were used to document the floral visitors. Photographs of the bird pollinators were used to identify the bird species by using a bird field guide (Schulenberg et al., 2007) and the collection of bird skin material from the Museum of Natural History of San Marcos University (Department of Ornithology). Insect pollinators were captured with an insect net and preserved in ethanol (70 %) for later determination. They were identified by using keys of local insects (Aguilar, 1961, 1965), by comparing them with those from the entomological collection at the Museum of Natural History of San Marcos University, and by consulting specialists. Videos were used to characterize the pollinators based on the frequency of visits, contact with the stigma and anthers, position of pollen placement, and flights performed among con-specific plants. They were produced during the observation times and comprise a total time of 12–14 h per species.

Morphometric data (head height, maxilla length and glossa length; sample size of $n = 10$) were taken from 11 bee species excluding uncommonly recorded bee species (samples per species <10) from the analysis. *Xylocopa viridigastra* was also excluded, since it has markedly larger body proportions than the other pollinators. We applied a Principal Component Analysis (PCA) using SPSS v. 20 (IBM Corp.) and PAST 3.25 to visualize how the bee species group according to their body proportions.

Table 2
Floral biology and nectar characterisation of the studied *Salvia* species. FL, floral longevity in days. Ad, time of anther dehiscence. Sr, time of stigma receptivity. Numbers in Ad and Sr: 1, before corolla opening. 2, during corolla opening. 3, after corolla opening. n, sample size.

Species	Fl (n)	Ad	Sr	Dichogamy (≤ 3 h)	Nectar (µL) Mean ± SD (n)	% Sugar (Brix) Mean ± SD (n)
S. cruikshanksii	5 (27)	2	3	Protandry	0.55 ± 0.33 (22)	33.7 ± 5.9 (14)
S. tafallae	1 (19)	3	1	Protogyny	0.46 ± 0.16 (25)	37.7 ± 8.6 (15)
S. tubiflora	4 (34)	3	1	Protogyny	8.22 ± 3.62 (25)	20.94 ± 2.6 (24)

Table 3
Morphometric data of studied *Salvia* species. SD = standard deviation, *N* = sample size (a-g see Fig. 2A). Characters: a, flower length. b, length of the corolla tube. c, distance from the flower entrance to the nectar chamber. d, distance from the thecae to the nectar chamber. e, total length of the connective. f, length of the upper connective arm. g, length of the filament. h, diameter of the corolla entrance (horizontal). i, diameter of the corolla entrance (vertical). j, style length in natural position. k, length of the lower lever arm.

Character	***Salvia cruikshanksii*** Mean (cm) ± SD (n)	***Salvia tafallae*** Mean (cm) ± SD (n)	***Salvia tubiflora*** Mean (cm) ± SD (n)
a	1.07 ± 0.18 (45)[a]	1.33 ± 0.13 (34)[b]	4.27 ± 0.32 (37)[c]
b	0.70 ± 0.13 (45)[a]	0.78 ± 0.15 (34)[a]	3.13 ± 0.54 (37)[b]
c	0.37 ± 0.07 (45)[a]	0.49 ± 0.13 (34)[b]	2.74 ± 0.41 (37)[c]
d	0.70 ± 0.10 (45)[a]	1.05 ± 0.10 (34)[b]	4.18 ± 0.43 (37)[c]
e	0.52 ± 0.10 (45)[a]	0.84 ± 0.09 (34)[b]	3.37 ± 0.70 (37)[c]
f	0.37 ± 0.09 (45)[a]	0.56 ± 0.09 (34)[b]	2.01 ± 0.60 (37)[c]
g	0.26 ± 0.04 (45)[a]	0.26 ± 0.06 (34)[a]	0.39 ± 0.07 (37)[b]
h	0.22 ± 0.04 (45)[a]	0.22 ± 0.03 (34)[a]	0.85 ± 0.10 (37)[b]
j	0.17 ± 0.04 (45)[a]	0.16 ± 0.03 (34)[a]	0.41 ± 0.14 (37)[b]
j	1.12 ± 0.09 (45)[a]	1.28 ± 0.08 (34)[b]	5.09 ± 0.41 (37)[c]
k	0.36 ± 0.06 (45)[a]	0.36 ± 0.03 (34)[a]	1.62 ± 0.23 (37)[b]
l			1.06 ± 0.29 (37)
m			0.66 ± 0.27 (37)
n			0.43 ± 0.24 (37)
o			0.54 ± 0.16 (37)

Different letters indicate significant differences among means ($p < 0.05$; Mann-Whitney test with Benjamini correction).

2.9. Breeding system

Hand-pollination experiments were used to test for self-compatibility as follows.

- Natural pollination: untreated flowers were marked to determine seed set under natural conditions.
- Geitonogamy: bagged flowers were emasculated and hand pollinated with pollen from the same plant.
- Xenogamy: bagged flowers were emasculated and hand pollinated with conspecific pollen of other genets.

After two to three weeks of the hand pollination events, we collected the fruits and assessed the fruit and seed set. For each species and treatment, the sample size was between 10 and 21 flowers of different individuals. To identify differences in seed set among treatments and species, we performed a non-parametric Kruskal-Wallis-test using SPSS v. 20 (IBM Corp.) and PAST 3.25.

3. Results

3.1. Inflorescence architecture and phenology

The three *Salvia* species have open inflorescences (thyrses) with flowers arranged in cymes. At each node, two cymes form a verticillaster. In *S. cruikshanksii* and *S. tafallae*, inflorescences have <10 nodes each with 3–6 flowers in the first and up to 10 flowers in the second species, respectively. The flowering sequence is acropetal along the main axis and ordinal within the cymes resulting in a mixed flowering pattern of flowers of 1st and 2nd order. Inflorescences of *S. tubiflora* have >10 nodes. The cymes are usually reduced to the primary flowers altering the thyrse into a spike with two flowers per node.

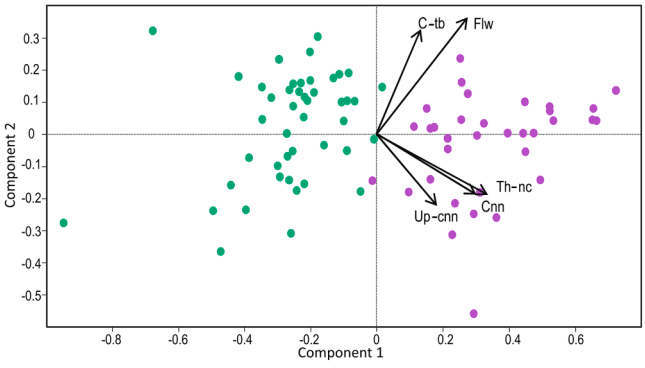

Fig. 3. Floral morphological traits of *Salvia cruikshanksii* (green) and *Salvia tafallae* (violet). The first axis of the principal component analysis accounts for 66.6 % of the observed variance between the individuals, Th–nc, distance between theca and nectar cover. Cnn, connective length. Up–cnn, upper connective arm length. The second axis accounts for 15.6 %. C–tb, corolla tube length. Flw, flower length.

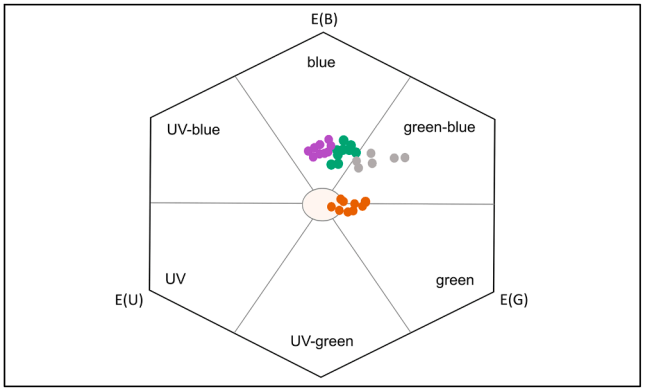

Fig. 4. Floral colour perception by bees. Hexagonal model for bee vision. Corolla colours: *S. cruikshanksii* (green), *S. tafallae* (violet), *S. tubiflora* (orange). Nectar guide reflectance: *S. cruikshanksii* (grey).

Table 4
Floral scent profile (compounds and relative proportions in %) of bouquets emitted by the inflorescences of *Salvia cruikshanksii, Salvia tafallae* and *Salvia tubiflora*. Undetected values are listed as "0". RI = retention index.

RI	Chemical compound	*Salvia cruikshanskii*	*Salvia tafallae*	*Salvia tubiflora*
967.51	Mesitylene			1.06 ± 0.90
972.00	Sabinene		21.01 ± 13.93	
975.42	β-Pinene		6.08 ± 1.72	
985.35	Sulcatone		0.73 ± 0.93	
990.19	β-Myrcene		6.02 ± 1.74	
1003.65	α-Phellandrene		2.90 ± 0.94	
1007.94	3,3-Dimethyl octane			36.54 ± 22.36
1019.18	2,5-Dimethyl nonane			13.97 ± 9.39
1036.31	cis-β-Ocimene	3.56 ± 2.65		
1047.81	trans-β-Ocimene	8.99 ± 9.19	0.30 ± 0.28	
1057.86	γ-Terpinene	17.75 ± 27.18		
1071.04	1-Octanol		0.94 ± 0.66	
1089.84	Terpinolene		0.86 ± 0.42	
1095.08	2,3,6,7-Tetramethyl octane			7.71 ± 6.02
1095.19	Methyl benzoate	7.64 ± 4.96		
1100.95	Linalol		0.15 ± 0.15	
1112.00	p-Mentha-1,3,8-triene		0.29 ± 0.24	
1116.97	β-Thujone		0.76 ± 0.63	
1121.28	trans-p-Mentha-2,8-dienol		0.31 ± 0.23	
1129.95	(E,E)-2,6-Dimethyl-1,3,5,7-octatetraene		0.16 ± 0.11	
1138.23	cis-Limonene oxide		3.44 ± 2.16	
1141.43	trans-Sabinol		1.48 ± 0.92	
1145.45	Camphor			5.41 ± 6.29
1194.88	Methyl salicylate	0.82 ± 0.94		
1211.80	Octyl acetate		7.05 ± 3.03	
1229.36	Unidentified (aliphatic)		4.89 ± 2.09	2.05 ± 3.19
1230.87	Unidentified (monoterpene)		14.54 ± 7.95	
1245.46	D-Carvone		0.54 ± 0.48	
1265.79	cis-carvone oxide		0.39 ± 0.24	
1302.52	Carvacrol		0.38 ± 0.59	
1307.23	Undecanal		0.60 ± 0.47	
1330.97	Unidentified (aliphatic)		3.22 ± 1.56	
1339.22	δ-EIemene	1.21 ± 1.63		
1351.77	α-Cubebene	0.79 ± 0.97		
1374.13	1-Undecanol		0.52 ± 0.53	
1378.39	Copaene	2.61 ± 1.76		
1380.41	α-Bourbonene	3.98 ± 4.97	2.46 ± 1.61	
1389.45	β-Bourbonene	33.18 ± 17.60		
1392.30	Sativene		1.40 ± 2.48	
1412.77	α-Gurjunene		0.59 ± 0.60	
1430.45	Unidentified (aliphatic)		3.40 ± 2.29	
1434.53	β-Gurjunene		4.81 ± 4.12	
1447.39	Unidentified (sesquiterpene)	2.34 ± 1.52		
1452.55	Unidentified (sesquiterpene)			11.03 ± 15.45
1473.66	1-Dodecanol		1.37 ± 0.76	
1480.35	γ-Muurolene		0.76 ± 0.97	
1484.54	Unidentified (sesquiterpene)	7.13 ± 13.07		
1486.76	Unidentified (sesquiterpene)			1.20 ± 2.43
1491.54	Unidentified (sesquiterpene)		1.92 ± 2.32	
1498.69	Unidentified (sesquiterpene)	4.73 ± 3.51		
1501.89	Unidentified (sesquiterpene)	1.34 ± 1.89		
1510.18	β-Bisabolene			21.03 ± 29.80
1516.36	γ-Cadinene	1.28 ± 1.88		
1525.26	trans-Calamenene	2.19 ± 1.55		
1530.93	Unidentified (aliphatic)		4.84 ± 2.53	
1558.76	Unidentified (aliphatic)		0.87 ± 1.29	
1564.88	(E)-Nerolidol	0.48 ± 1.10		

Consequently, flowering sequence is strictly acropetal. After anthesis, the internodes elongate elevating the open flowers out of the shrub (Fig. 1D).

Anthesis of all species started around 7:00 – 8:00 a.m., slightly varying with cold or warm weather conditions. *Salvia cruikshanksii* exhibited protandry for a short time (≤ 3 h), whereas *S. tafallae* and *S. tubiflora* showed a similarly short period of protogyny (Table 2). Anthesis lasted only one day in the annual *S. tafallae* and 4–5 days in the shrubby species (Table 2).

3.2. Flower morphology

S. tubiflora has tubular red flowers with a large distance between flower entrance and nectar chamber. Stamens are exposed (Fig. 1G), but shorter than the exserted style resulting in approach herkogamy (Table 2; Fig. 1G). The flowers of. *S. tubiflora* are larger than those of *S. cruikshanksii* and *S. tafallae* in all floral dimensions measured (Table 3).

Salvia cruikshanksii and *S. tafallae* have bilabiate blue flowers. The first species presents well-developed nectar guides on the lower corolla lip and stamens covered by the upper corolla lip (Fig. 1E); *S. tafallae* has exposed stamens (Fig. 1F). The two species significantly differ in six of the 11 measured characteristics, among them the distance from the flower entrance to the nectar chamber and the distance from the thecae to the nectar chamber (Table 3). The results of the PCA indicate that the first two main axes account for 82.2 % of the observed variance (Fig. 3).

The first axis explains 66.6 % of the variation and has a high positive correlation with the distances between theca and nectar cover, and between connective length and upper connective arm length. Flower length and corolla tube length were positively correlated with the second PCA axis confirming the clear separation between *S. cruikshanksii* and *S. tafallae* individuals (Fig. 3A).

3.3. Floral colour

The corolla of *S. tubiflora* reflects strongly in the orange-red region (600–700 nm), whereas the corollas of both *S. cruikshanksii* and *S. tafallae* reflect strongly in the violet-blue region (400–500 nm) and in the red region (650–700 nm). The nectar guide of *S. cruikshanksii* reflects between 500 and 700 nm. UV signals were not detected in any species. Correspondingly, the flower colour of *S. tubiflora* and the other two *Salvia* species plotted separately in the bee colour vision model. The loci of the colour measurements in *S. cruikshanksii* and *S. tafallae* plotted in the blue sector of the bee hexagon and that of the nectar guide (*S. cruikshanksii*) in the blue-green sector (Fig. 4). Colour loci of *S. tubiflora* plotted in and around the achromatic area (central area) of the bee hexagon. The average distance between the colour loci of the two blue species was $d_E = 0.075$ units, the distance between corolla and nectar guide was 0.16 units.

3.4. Floral scent

A total of 57 compounds were detected in the floral scent of the three *Salvia* species, of which 44 were identified (Table 4). The compounds emitted were predominantly terpenes (i.e., monoterpenes and sesquiterpenes). Among the 9 compounds found in *S. tubiflora,* 3,3-dimethyl octane and β-bisabolene represented 57.57 % of the total blend, among the 17 compounds in *S. cruikshanksii,* γ-Terpinene and β-bourbonene accounted for 50.93 %, and among the 34 compounds in *S. tafallae,* sabinene and an unidentified monoterpene accounted for 35.55 % (Table 4). No single compound was shared among all three species, none was shared between *S. cruikshanksii* and *S. tubiflora*, only two between *S. cruikshanksii* and *S. tafallae* (trans-β-ocimene, α-bourbonene) and one unidentified aliphatic compound between *S. tafallae* and *S. tubiflora.* The floral bouquets were thus for 88.2 % (*S. cruikshanksii*), 88.9 % (*S. tubiflora*) and 92.2 % (*S. tafallae*) specific for the species (Fig. 5). The investigated *Salvia* species emitted significantly different floral scent bouquets overall (ANOSIM: global $R_{4,} = 0.704$; $p < 0.001$). Post-hoc comparisons between species pairs revealed relatively high R values (*Salvia cruikshanksii - S. tafallaeR* = 0.93, *S. cruikshanksii - S. tubifloraR* = 0.81 and *S. tafallae - S. tubifloraR* = 0.724) and $p < 0.0001$ in all combinations, indicating that the floral scents differ significantly among all *Salvia* species, which is further confirmed by distinct grouping of each species in morphospace (stress = 0.04; Fig. 5).

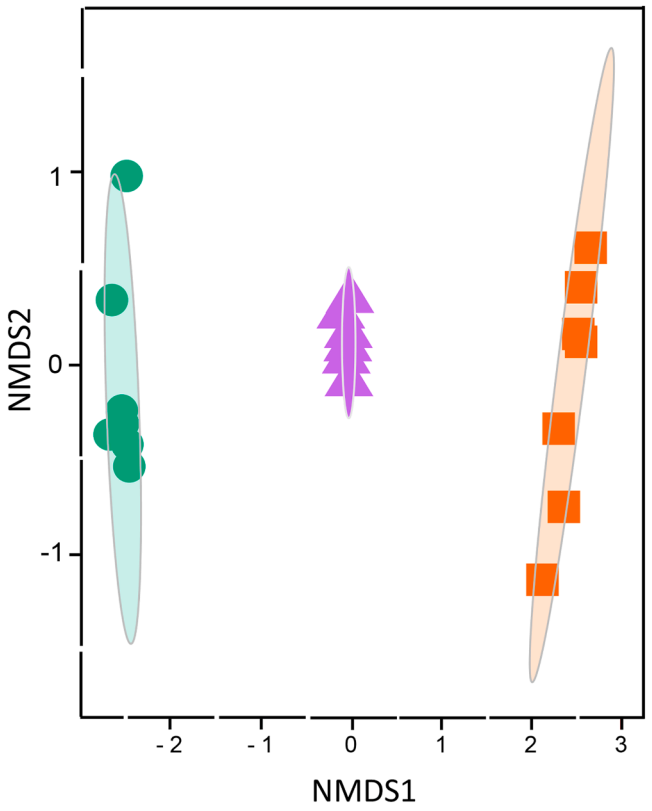

Fig. 5. Floral scent traits of the studied *Salvia* species. Non-metric multidimensional scaling (NMDS) based on semi-quantitative Bray-Curtis similarities (stress = 0.02). *S. cruikshanksii* (green), *S. tafallae* (violet) and *S. tubiflora* (orange). *S. cruikshanksii* (green), *S. tafallae* (violet), *S. tubiflora.*

3.5. Nectar characteristics

Nectar volume of *S. tubiflora* (8.22 μL) differed considerably from the volumes found in *S. cruikshanksii* (0.55 μL) and *S. tafallae* (0.46 μL). Similarly, the latter two species showed a higher percentage of sucrose (33.7 and 37.7) compared to *S. tubiflora* (20.94) (Table 2).

3.6. Pollinators

In total, 21 species of floral visitors were observed on the flowers of the studied *Salvia* species, most of them searching for nectar (Table 5). Sixteen species were regarded as pollinators since they touched both reproductive structures (anthers and the lower stigmatic lobe) during their visits: two hummingbird species for *Salvia tubiflora*, five bee and one fly species for *S. cruikshanksii* and eight bee species for *S. tafallae.*

3.6.1. Salvia tubiflora

Salvia tubiflora was pollinated by *Rhodopis vesper vesper* (Fig. 6L) and *Colibri coruscans.* The pollen-loaded heads of the hummingbirds first touched the lower stigmatic lobes of the visited flowers, before they received the flower's own pollen. *Rhodopis vesper vesper* was observed constantly foraging for nectar thereby visiting several flowers of the same plant in an apparently random pattern. The birds were frequent and visited the flowers for around eight hours per day (ca. 7:30 h - ca. 15:30 h) with a peak at noon (11:00–13:00 h). During its visits, *Rhodopis vesper* displayed territorial behaviour chasing competitors away. *Colibri coruscans* was faster than *R. vesper vesper* exhibiting a typical trapliner behaviour. The birds visited flowers at certain intervals along routes through different patches. As they only visited the flowers in absence of *Rhodopis vesper vesper,* they were less frequent and only visited few flowers per plant (Table 5). In the dry season, when bee-flowers were not available, bees visited *S. tubiflora* as nectar and pollen thieves. *Centris neffi* and *Agapostemon* sp. made and used lateral holes in the corolla tube, while both *Agapostemon* species entered the corolla tube without touching the reproductive surfaces or collected pollen from the exserted thecae (Fig. 6I).

3.6.2. Salvia cruikshanksii and S. tafallae

Flowers of the two blue-coloured species were mainly visited by bees; no single visit by hummingbirds was recorded. Bees usually approached the base of the inflorescences and moved upwards visiting several open flowers. During nectar consumption (which lasted ca. 1–4 s), they first contacted the stigma and then the thecae, thereby transferring pollen. Activity of the bees started early in the morning, between 7:00 h and 8:00 h and ceased around 16:00 h. The peak of visits varied with the species but tended to occur between 11:00 h to 14:00 h.

Salvia cruikshanksii flowers were visited by 12 species, of which five bee species and one bombyliid fly (*Exoprosopa* sp.) acted as pollinators

Table 5
Floral visitors recorded for the three *Salvia* species. p. pollinator. (p) occasional pollinator. v, visitor.?, no data. Frequency: ***high, ** moderate, * low. Resources: Co, corolla- Ne, nectar. Po, pollen. Sj, stigmatic juice.

Floral Visitor	*Salvia cruik-shanksii*	*Salvia tafallae*	*Salvia tubiflora*	Visitation frequency	Resource used
Apodiformes					
Trochilidae					
Colibri coruscans			p	**	Ne
Rhodopis vesper vesper			p	***	Ne
Coleoptera	v	v		**	Co
Diptera					
Bombyliidae					
Exoprosopa sp	p			*	Ne
Syrphidae		v		**	Sj
Hymenoptera					
Apidae					
Apis mellifera	v	v		***	Ne
Anthophora tricolor	p			***	Ne
Bombus cf. coccineus	?			*	Ne
Centris neffi	v	p	v	***	Ne
Melissodes sp.		p		*	Ne
Centris sp1	p			**	Ne
Centris sp2	p			**	Ne
Chalepogenus calceolariae		p		**	Ne
Mesonychium garleppi		p		*	Ne
Paratetrapedia sp.		p		**	Ne
Thygater sp.	v	p		***	Ne
Xylocopa viridigastra	p			***	Ne
Halictidae					
Agapostemon sp.		(p)	v	**	Po, Ne
Augochlora sp.		(p)		**	Po
Megachilidae					
Megachile ecuadorius	p			***	Ne
Lepidoptera					
Sphingidae	v			*	Ne

(Fig. 6A, D-G; Table 5). Body proportions such as head size and glossa length of these bee species matched with the flower proportions (compare Tables 3 and 6) enabling contact with both pollen sacs and stigma tip. The remaining visitors either collected pollen (beetles), stole nectar by making holes on the basal side of the corolla (*Centris neffi,* Fig. 6C) or inserted their tongues into the nectar chamber without touching the reproductive organs (*Thygater* sp., and Sphingidae).

The flowers of *Salvia tafallae* were visited by 11 species, of which only bee species were regular (6 species) or occasional pollinators (2 species; Table 5). According to their body proportions, the pollinating bees fell into two groups (Fig. 7B): *Centris neffi, Melissodes* sp., *Mesonychium garleppi* and *Thygater* sp. introduced their proboscis into the corolla tube and were loaded with pollen on their heads (Fig. 6H), whereas *Chalepogenus calceolariae* and *Paratetrapedia* sp. fully introduced their heads into the corolla tube and got pollen loads on their thorax (Fig. 6I, J). Drinking time lasted 1–3 s in the first and 2–6 s in the second group. Compared to *S. cruikshanksii*, pollinator visits in *S. tafallae* were less frequent (Fig. 7A). *Apis mellifera* stole nectar from the corolla side. In contrast, *Augochlora* sp. and *Agapostemon* sp. collected pollen and might occasionally have transferred pollen to the nearby stigma (Fig. 1F).

Bees clustered in three different groups in the PCA based on morphological data. The first two main axes account for 99.00 % of the observed variance (Fig. 7B). The first axis explains 93.2 % of the variation and has a high positive correlation with distance between glossa and maxillae length. Head height was positively correlated with the second axis and the variation was 5.8 % (Fig. 5B). Bees that pollinated *Salvia cruikshanksii* flowers are grouped together (Fig. 7B; *Xylocopa* not considered). They share medium proportions of head and glossa (Table 6, Fig. 7B), while the pollinators of *S. tafallae* are classified into two groups (see Fig. 7B): a first one with medium to large heads and a long proboscis and a second one with small heads, a small proboscis length and small body size (Table 6, Fig. 7B).

Only three bee species visited both *S. cruikshanksii* and *S. tafallae* (Table 5, Fig. 7A). *Apis mellifera* was a nectar thief in both species. *Centris neffi* acted as pollinator in *S. tafallae* and as nectar thief in *S. cruikshanksii. Thygater* sp. pollinated *S. tafallae* and stole nectar in *S. cruikshanksii*: it released the staminal lever mechanism, but due to its body proportions did not contact the reproductive parts and transfer pollen.

3.7. Breeding system

The three *Salvia* species showed a high natural fruit (83.9–98.5 %) and seed set per fruit (2.16–3.65; Table 7). Accordingly, total seed set per plant was 89.9 % in *S. tafallae*, 58.7 % in *S. tubiflora* and 45.3 in *S. cruikshanksii*. The highest values were found in the annual *S. tafallae*. The three species also produced fruits under xenogamy (38.1–41.7 %) and geitonogamy (33.3–80 %) conditions.

- In *Salvia cruikshanksii*, seed set per fruit did not significantly differ from natural seed set in the geitonogamy test (Kruskal-Wallis test, $p = 0.81$); Table 7). In contrast, the xenogamy test resulted in the lowest values and showed a significant difference with natural fruit set (Kruskal-Wallis test, $p = 0.003$). A high fruit set was observed in the geitonogamy test and under natural conditions (80 % and 83.9 %, respectively) with seed set rates of 54 % and 54.2 %. In the xenogamy text both values were lower with only 41.7 % fruit set and 45 % seed set per fruit (Table 7).
- In *Salvia tafallae,* fruit set was very high under natural conditions (98.5 %) and significantly lower in the geitonogamy and xenogamy tests (33.3 % and 38.1 %, respectively; Table 7). A similar result was obtained in the seed set per fruit, with high rates under natural conditions (91.2 %), followed by the geitonogamy and xenogamy tests (75 % and 62.5 %, respectively; Table 7).
- In *Salvia tubiflora*, seed set per fruit showed the highest values in the xenogamy test (81.3 %; Table 7) significantly differing from natural fruit set (62.1 %; Kruskal-Wallis test, $p = 0.048$), but not from the geitonogamy test (61.4 %; Kruskal-Wallis test, $p = 0.53$; Table 7). The percentage of fruit set was highest under natural conditions

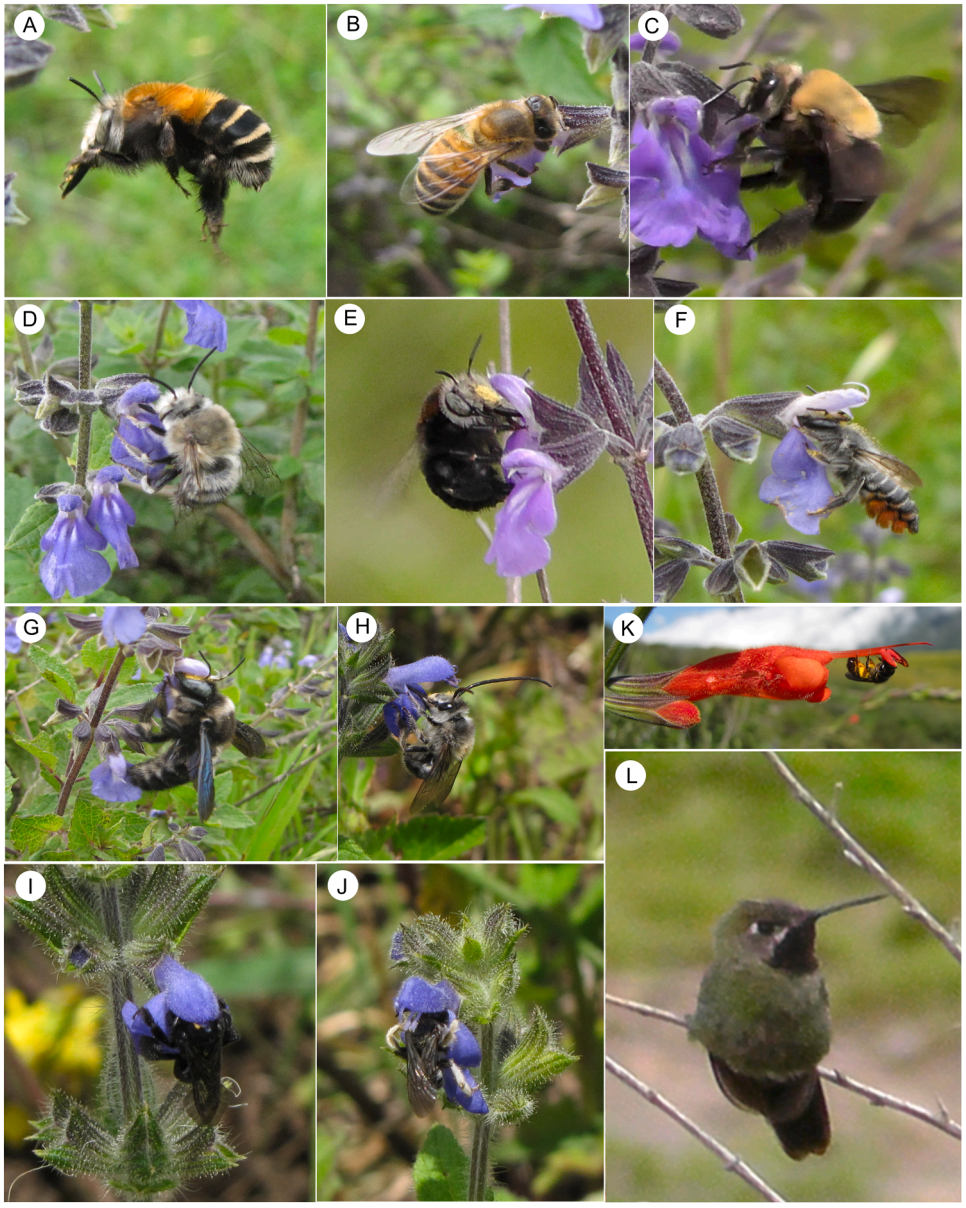

Fig. 6. Pollinators and visitors recorded on *Salvia cruikshanksii* (A-G), *S. tafallae* (H-J) and *S. tubiflora* (K, L). **A.** *Anthophora tricolor*. **B.** *Apis mellifera* (nectar thief). **C.** *Centris neffi* (nectar thief). **D.** *Centris* sp1. **E.** *Centris* sp2. **F.** *Megachile ecuadorius*. **G.** *Xylocopa viridigastra*. **H.** *Thygater* sp. **I.** *Paratetrapedia* sp. **J.** *Chalepogenus calceolariae*. K. *Agapostemon* sp., feeding on pollen. **L.** Female of *Rhodopis vesper vesper*.

(94.7 %), high in the geitonogamy test (78.6 %) and only moderate in the xenogamy test (47.1 %; Table 7).

4. Discussion

Pollinator partitioning allows species to co-exist and co-flower without affecting species integrity (Botes et al., 2008; Aguilar-Rodríguez et al., 2019). The latter is promoted by the existence of prezygotic and postzygotic barriers reproductively isolating the species from each other (Armbruster and Herzig, 1984; Grant, 1994). In our study, we found that the three *Salvia* species investigated were isolated from each other by a combination of ethological and mechanical barriers, which act in addition to potential postzygotic barriers. Colour (blue vs. red), scent, and general flower construction (e.g., landing place present vs. absent) separate bee-pollinated from bird-pollinated flowers, whereas species-specific odour bouquets and flower proportions distinguish the bee-pollinated flowers from each other.

4.1. Pollinator shift from bees to birds

Salvia tubiflora is the only bird-pollinated species among the study species. It significantly differs from the bee-pollinated species in flower size, colour, scent, and nectar characteristics excluding bees mechanically, olfactorily and visually. The corolla of *S. tubiflora* is red and, according to the bee visual modelling, inconspicuous to bees. Since the red stimulus is not very well perceived by bees due to their trichromatic visual system (Chittka and Waser, 1997), the red flower colour can be interpreted as a mechanism to avoid bees without losing attractiveness for birds (Castellanos et al., 2004; Lunau et al., 2011; Wester et al., 2020). Besides, the red corolla colour can reduce nectar and pollen theft by bees via visual exclusion (Wester et al., 2020).

The olfactory sense of birds is usually considered to be relatively unimportant though hummingbird studies showed that some species had >50 olfactory receptors (Steiger et al., 2008) and that at least one species responded to scent in respective experiments (Goldsmith and Goldsmith, 1982). Consistent with the general expectation, *S. tubiflora*

Table 6
Morphometric data of bee species studied. a-c. see Fig. 3.2B. Mean (mm) and SD = standard deviation. n = sample size (in brackets). Proboscis length (d) = $b + c$.

Bee species	Head height (a)	Maxillae length (b)	Glossa length (c)	Proboscis length (d)
Anthophora tricolor	3.94 ± 0.18 (6)	2.28 ± 0.26 (6)	3.63 ± 0.23 (6)	5.92 ± 0.24 (6)
Centris neffi	4.85 ± 0.33 (5)	2.47 ± 0.26 (5)	5.17 ± 0.35 (5)	7.64 ± 0.47 (5)
Centris sp1	3.67 ± 0.38 (5)	2.06 ± 0.52 (5)	3.40 ± 0.44 (5)	5.47 ± 0.11 (5)
Centris sp2	3.56 ± 0.55 (5)	1.97 ± 0.15 (5)	3.29 ± 0.61 (5)	5.26 ± 0.18 (5)
Chalepogenus calceolariae	2.55 ± 0.18 (6)	1.94 ± 0.21 (6)	2.28 ± 0.17 (6)	4.22 ± 0.2 (6)
Megachile ecuadorius	3.66 ± 0.11 (7)	2.2 ± 0.09 (7)	3.49 ± 0.46 (7)	5.7 ± 0.25 (7)
Mesonychium garleppi	4.73 ± 0.22 (3)	2.24 ± 0.10 (3)	5.16 ± 0.06 (3)	7.4 ± 0.05 (3)
Paratetrapedia sp	2.7 ± 0.06 (4)	1.9 ± 0.2 (4)	2.18 ± 0.39 (4)	4.08 ± 0.15 (4)
Thygater sp	3.49 ± 0.09 (6)	2.38 ± 0.11 (6)	5.36 ± 0.24 (6)	7.75 ± 0.17 (6)
Xylocopa viridigastra	5.21 ± 0.44 (10)	3.74 ± 0.28 (10)	3.37 ± 0.50 (10)	7.06 ± 0.49 (10)
Colletidae	2.96 ± 0.27 (3)	2.11 ± 0.04 (3)	2.79 ± 0.52 (3)	4.9 ± 0.39 (3)

emitted the lowest number of scent compounds. Only 3,3-dimethyl octane, β-bisabolene, 2,5-dimethyl nonane and an unidentified sesquiterpene were detected in high to moderate concentrations. These compounds are frequently emitted by leaves and other green parts of plant species like *Eucalyptus globules, Salvia rosmarinus, Solanum melongena, Verbena triphylla* and *Zygopetalum maxillare* and they are associated with a defensive function against herbivory (see Kessler and Baldwin, 2001; Rabeschini et al., 2021). Therefore, we think that *S. tubiflora* flowers do not attract birds (or bees) using olfactory signals; instead, they use visual signals, which they presumably associate with high amounts of dilute nectar.

Hummingbird pollination is common among species of *Salvia* subgen. *Calosphace* (e.g., Dieringer et al., 1991; Cuevas et al., 2018), with an estimated number of 37 species in the Peruvian Andes (Cairampoma, 2021). Based on phylogenetic data (Fragoso-Martínez et al., 2018), bee- and bird-pollinated species appear intermixed in the same clades of *Salvia* subgen. *Calosphace* resulting in two controversial interpretations. Whereas Kriebel et al. (2020, 2023) argued that birds were the primary pollinators in the subgenus showing many reversals to bee-pollinated species, other authors held the opinion that bee pollination represented the ancestral pollination system and that a pollinator shift to birds happened several times in parallel (Wester and Claßen-Bockhoff, 2007; Fragoso-Martínez et al., 2018; Benitez-Vieyra et al., 2019; Sazatornil et al., 2023). *Salvia tubiflora* belongs to section *Biflorae* which includes four bird-pollinated species in Peru (Cairampoma, 2021). The section seems to have evolved from a bee-pollinated ancestor since this condition is present in early diverging species of the section (among them *S. tafallae*). It is thus likely that the species of section *Biflorae* (or their close ancestors) adapted to bird pollination due to pollinator-driven speciation in the past.

4.2. Floral scent may contribute to pollinator constancy in bee-pollinated *Salvia* species

Salvia cruikshanksii and *S. tafallae* share the characteristics of bee-pollinated species in terms of having bilabiate flowers with a landing place, blue flower colours and relatively small amounts of concentrated nectar. Based on our study, both species were pollinated by six and eight insect, mainly bee species. Despite flowering side by side, they do not share a single pollinator indicating that the species differ in certain floral traits. In contrast to birds, both visual and olfactory signals guide bees to their food plants (Michener, 2007; Burger et al., 2008, 2010a, 2010b). Whereas colour perception by bees has been investigated in bee- and bird-pollinated *Salvia* species (Wester et al., 2020), little is known about the specificity of floral volatiles in *Salvia*. To our knowledge, the present study is the first to compare floral scent and flower colour in a field study of *Salvia* flowers.

Bees are predominantly attracted by colours ranging from yellow to blue and UV-blue (according to bee perception, e.g., Chittka and Kevan, 2005; Wester et al., 2020). They learn to discriminate similar colours in flowers, but with smaller colour distances accuracy in flower discrimination decreases (Dyer and Chittka, 2004). In our study, the distance between the colour loci was below the 0.1 unit threshold needed for good colour discrimination (Chittka and Kevan, 2005). Therefore, it is likely that the bees cannot discriminate the blue tones of the flowers from a distance. However, the nectar guides in *S. cruikshanksii* clearly differ from the flower colour and may serve a role in pollinator behaviour at short distances (Hansen et al., 2012; de Jager et al., 2017).

Floral volatiles play an important role in bee-pollinated flowers signalling foodplants (e.g., Knudsen et al., 2006; Wright and Schiestl, 2009; Burger et al., 2010a; Schiestl, 2015). In our study, the predominant compounds were mono- and sesquiterpenes in *S. cruikshanksii* and monoterpenes in *S. tafallae*. Interestingly, each of the two bee-pollinated species was characterized by a unique bouquet of volatiles, with distinct compositions and percentages of the compounds.

- The dominant compounds in *S. cruikshanksii* were β-bourbonene and γ-terpinene followed by trans-β-ocimene and methyl benzoate. These compounds are known (in low proportions of ≤ 2.5 %) as flower volatiles in other plant groups, mainly attracting *Apis* and *Xylocopa* species (Rabeschini et al., 2021 and references therein); γ-terpinene has been found in *Moringa oleifera* attracting *Xylocopa* (Rabeschini et al., 2021). Interestingly, one *Xylocopa* species (*X. viridigastra*) was also present among the pollinators of *S. cruikshanksii*. Based on this finding, we speculate that the principal volatiles found in *S. cruikshanksii may* specifically attract species of the bee genus *Xylocopa*.
- *Salvia tafallae* emitted a more complex odour with twice as many compounds than *S. cruikshanksii*. Sabinene and an unidentified monoterpene had the highest relative concentrations in the floral scent bouquet of *S. tafallae.* Sabinene is a common volatile reported in flowers and leaves of diverse plant taxa, including several *Salvia* species (see El-Sayed, 2024). Therefore, we hypothesise that this chemical may be associated with the attraction of distinct pollinators in relation to *S. cruikshanksii*.

Interspecific differences in floral scent are well-known and interpreted as adaptations to certain pollinators (Huber et al., 2005; Knudsen et al., 1993; Raguso and Pichersky, 1995; Raguso et al., 2003; Jürgens et al., 2003). Pollinators can learn and discriminate specific scents even when these differ only in a single compound (e.g., bees: Vareschi, 1971; moths: Balkenius and Kelber, 2006; butterflies: Andersson, 2003). This fits with the finding that bee-pollinated flowers usually differ only in few recognizable floral volatiles, rather than in a wide diversity of chemicals (Huber et al., 2005; Rabeschini et al., 2021). The specific attraction of pollinators might be guided by innate preferences (Schiestl and Schlüter, 2009; Dötterl and Vereecken, 2010; Burger et al., 2010a, b), or by learning (e.g., to maintain floral constancy; Waser, 1986). Since the bouquet composition is different between *Salvia cruikshanksii* and *S. tafallae*, it is possible that the bees use scent to distinguish the two *Salvia* species. However, further work is needed to shed light on the role

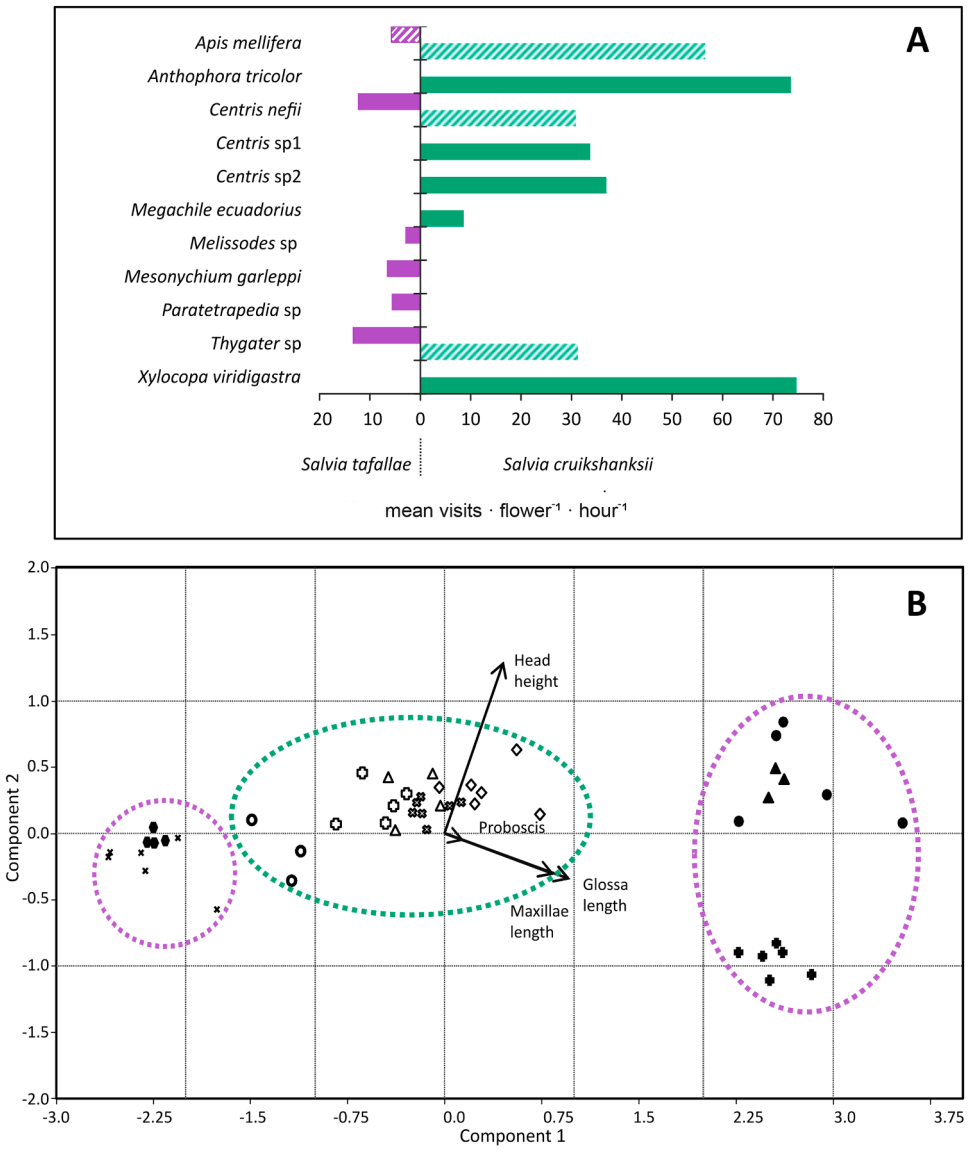

Fig. 7. Frequency of floral visits and morphometry of bee pollinators and floral visitors. **A.** Main pollinators in *Salvia cruikshanksii* (green) and *Salvia tafallae* (violet) and number of flowers visited per hour. Bars in lines indicate visitors not pollinating the flowers **B.** Principal component analysis (PCA) plot based on morphological bee datasets. Bee species group in green dotted circle are pollinators of *S. cruikshanksii*, while bee species group in violet dotted circles are pollinators of *S. tafallae*. ◇ *Anthophora tricolor*, ● *Centris neffi*, △ *Centris* sp1, ✚ *Centris* sp2, ✖ *Chalepogenus calceolariae*, ⊠ *Megachile ecuadorius*, ○ *Melissodes* sp, ▲ *Mesonychium garleppi*, ⬢ *Paratetrapedia* sp, ✚ *Thygater* sp.

of floral traits on pollinator attraction in *Salvia*, and particularly on the role of scent in pollinator partitioning.

4.3. Pollinators and pollinator partitioning in bee-pollinated Salvia species

Compared to hummingbirds, which usually hover in front of the flowers, bees need a landing platform. They physically manipulate the flowers, presumably exerting strong selection pressure on floral traits which might promote morphological convergence among species (Benitez-Vieyra et al., 2019). Against this background, it is interesting that the two bee-pollinated species *S. cruikshanksii* and *S. tafallae* significantly differed in their flower proportions.

Mechanical isolation is well-known in *Salvia* and usually associated with divergent use of the same pollinators (Grant and Grant, 1964; Claßen-Bockhoff et al., 2004; Wei et al., 2017; Celep et al., 2020; Cultid-Medina et al., 2021). However, in *S. cruikshanksii* and *S. tafallae*, specific pollen deposition on the pollinator's body mediated by the staminal lever mechanism is irrelevant because their distinct pollinator spectra prevent pollinator sharing. One might assume that it is rather the relative length of flower tube vs. proboscis and the relative size of flower entrance vs. head size that allow or reject bees access to nectar. However, even though the floral proportions are significantly different between the two *Salvia* species, they do not necessarily exclude all possible pollinators. Based on the relative proportions, the pollinators of *S. cruikshanksii* could also reach the nectar in flowers of *S. tafallae* and small bees could also crawl into the flower tube of *S. cruikshanksii*. We therefore conclude that scent is likely the most important prezygotic isolating factor among the bee-pollinated *Salvia* species investigated.

The bees found to be pollinators are solitary bees (Aguilar, 1961, 1965), which are known for their highly specialised sensory and learning capabilities (Michener, 2007). Little is known about the ecology of the bees from the Andean highlands as biological studies are rare (Michener, 2007; Gonzalez and Engel, 2004; Gonzalez et al., 2004, 2006; Gonzalez and Ospina, 2008). A specific affinity for *Salvia* flowers

Table 7
Pollination experiments data in *Salvia cruikshanksii, S. tafallae* and *S. tubiflora.* Results are expressed in "Mean ± SD (N)". SD = standard deviation, *n* = sample size.

	Natural pollination	Xenogamy	Geitonogamy
S. cruikshanksii			
n	112	12	15
Fruit set (%)	83.9	41.7	80
Mean seeds per flower	2.16 ± 1.14	1.8 ± 1.06	2.17 ± 1.33
Seed set per fruit (%)	54	45	54.2
S. tafallae			
n	132	21	18
Fruit set (%)	98.5	38.1	33.3
Mean seeds per flower	3.65 ± 0.78	2.5 ± 1.43	3 ± 1.68
Seed set per fruit (%)	91.2	62.5	75
S. tubiflora			
n	94	17	14
Fruit set (%)	94.7	47.1	78.6
Mean seeds per flower	2.48 ± 1.14	3.25 ± 1.77	2.45 ± 1.44
Seed set per fruit (%)	62.1	81.3	61.4

has been observed in unrelated Andean oligolectic bees (e.g. *Anthophora walteri* (Apidae), *Caupolicana* (Colletidae), *Megachile* (Megachilidae); see Michener et al., 2003; Gonzalez and Chavez, 2004; Michener, 2007). When they drink nectar from *Salvia* flowers, modified hairs in the face allow them to collect pollen (Gonzalez and Chavez, 2004; Michener, 2007). In our study, bee species with modified hairs (*Anthophora tricolor, Megachile ecuadorius*) visited *Salvia cruikshanksii* for nectar but may also collect pollen to provisioning their brood cells.

As for foraging behaviour, Arroyo et al. (1982) investigated bee communities in the Chilean Andes. They suggested that high elevation (2700–3100 m) favours opportunistic feeding strategies and observed that Halictidae displayed foraging constancy. We cannot confirm these findings. Although our study was conducted at high elevation (2900–3000 m), we found specialized anthophorid and megachilid bees, while Halictidae showed a generalist behaviour (e.g. during the search for nectar in *Salvia tubiflora*). One reason for the different findings might be the lower latitude in Peru influencing biotic and abiotic conditions (see Baumgartner and Roubik, 1989). These are warmer, more humid and more biodiverse in the Peruvian than in the Chilean Andes and impact on the high floral availability and the occurrence of other flowering species that might be exploited by the bees (e.g. Asteraceae species).

4.4. Pollinator behaviour influences the degree of in- vs. outbreeding

In the three studied *Salvia* species, the highest fruit set was achieved under natural conditions indicating that the activity of pollinators might increase the reproductive success. However, we acknowledge that there might be some problems with the experiments, and therefore, our results should be interpreted with caution. Nevertheless, we have demonstrated that the three *Salvia* species are self-compatible and employ a mixed mating system, which seems to be predominant among *Salvia* species (Owens and Ubera Jiménez, 1992; van Treuren et al., 1993; Ott et al., 2016; Cairampoma et al., 2020; Ajani et al., 2022; Naghiloo et al., 2022; Xiao et al., 2023; Surina et al., 2024).

The bird-pollinated *Salvia tubiflora* and the bee-pollinated *Salvia cruikshanksii* are perennial shrubs, therefore they are not as strongly dependent on sexual reproduction as the annual *S. tafallae*, which has only a single season to reproduce (Stebbins, 1950; Baker, 1959). Wiens (1984) observed higher seed set in annuals than perennials and Wyatt (1982) stated that in self-compatible species, small floral display sizes could more effectively promote xenogamy. Our results confirm these observations as the smallest plant size and floral display are associated with the highest percentage of fruit set and seed set per fruit, and the shortest duration of anthesis in the annual *S. tafallae*. However, we are not able to rule out that autogamy does not significantly contribute to the higher fruit set in *S. tafallae.* As anthesis started in the morning with a receptive stigma followed by pollen release, and only lasted a single day, the whole plant was most likely multicyclic protogynous (Lloyd and Webb, 1986). Interestingly, flowering sequence and foraging behaviour appeared to be less relevant as all flowers of an inflorescence were synchronised in terms of the pollen release and receptive phases.

In contrast, *S. cruikshanksii* displayed protandry which, at the beginning of flowering, would promote xenogamy if bees move in an acropetal direction (Harder et al., 2004). They got first into contact with the stigma depositing foreign pollen and then touched the thecae removing self pollen from the plant. However, anthesis lasted for five days and the reproductive phases of the primary and secondary flowers overlapped within the inflorescence, increasing opportunities for geitonogamy. Compared to *S. tafallae*, the species appeared less adapted to xenogamy corresponding to its perennial lifetime.

Salvia tubiflora differed from the bee-pollinated species in presenting flowers in spikes instead of thyrses. This reduced the degree of reproductive phase overlap, but, as the birds approached the inflorescence in random patterns, geitonogamy might largely happen. Additionally, the flowers bloomed for around four days. Though they were protogynous, they released pollen most of the time, again potentially contributing to geitonogamy. Self-pollination was promoted by the territorial behaviour of the main pollinator *Rhodopis vesper vesper* as the bird visited several flowers of an inflorescence during one visit (see Kalin de Arroyo, 1976; Levin et al., 1971; Feisinger, 1978). Furthermore, *R. vesper vesper* attempted to exclude other hummingbirds that might carry outcross pollen (Feisinger, 1978). On the other hand, the trapliner behaviour of *Colibri coruscans* promoted outcrossing when visiting different populations of *S. tubiflora* from far distances. Due to its short visits, however, the final impact on the seed set caused by *C. coruscans* might be small compared to the active and frequent visits of *R. vesper*.

Though the results of our experiments are insufficient to be interpreted in more detail, they indicate that the three *Salvia* species most likely share a mixed mating system. Such systems are highly flexible. Whereas the combination of self-pollination with xenogamy is an adaptation to maximize seed set, the degree of in- vs. outbreeding can be regulated by minute changes in temporal patterns (Xiao et al., 2023). This enables the plant to adapt to varying environmental conditions, particularly at disturbed areas.

5. Conclusions

The three *Salvia* species investigated evolved specific signals which allowed them to attract a differential subset of the available pollinator community. Interestingly, each *Salvia* species exhibits the classical characteristics of its respective bee- and bird floral syndrome (sensu Vogel, 1954). The lack of any signs of transition and the assumed distant relationship of the three species lead to the conclusion that the species adapted to their types of pollinators (i.e. pollinated by bees or birds) before they got into geographical contact.

The finding that the floral bouquets significantly differ between the bee-pollinated *S. cruikshanksii* and *S. tafallae* opens a new dimension in the research on *Salvia.* Our results indicate that sympatric *Salvia* species may avoid pollinator sharing by emitting species-specific floral scents, but future research must confirm this by experiment. Blue flower colour can act as a general attractant for bee species and nectar guides as signals in close range of the flower. The relative proportions between flowers and pollinators differ among partnerships but are rarely precise enough to only allow a single bee species access to nectar. This is shown in studies of sympatric *Salvia* species in which pollinators are shared and hybridisation minimized by species-specific proportions of the staminal lever arms (e.g., Celep et al., 2020). In our study species, the staminal lever mechanism plays a minor role in pollinator exclusion, whereas the specificity of floral scent together with morphometric differences and visual signals leads to a complete partitioning of pollinators.

The Andes represent one of the global biodiversity hotspots (Gentry, 1982; Myers et al., 2000; Pennington et al., 2010). Nevertheless, the patterns underlying diversification are still little understood (Mutke and Weigend, 2017). In Peru, at least 71 *Salvia* species occur, among them 37 hummingbird- and 22 bee-pollinated species (the remaining ones are not clearly assignable; Cairampoma, 2021). In many places, they co-occur (Benitez-Vieyra et al., 2019) as in our study and therefore pollinator niche partitioning is likely to occur avoiding pollen loss and potential hybridisation events. Future studies are needed to elucidate whether our findings are representative of sympatric *Salvia* species assemblages, whether scent specificity is common in the genus and how far it is a key for floral isolation in co-existing *Salvia* species.

CRediT authorship contribution statement

Lianka Cairampoma: Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Juan A. Tello:** Investigation. **Carlos Martel:** Formal analysis. **Manfred Ayasse:** Resources. **Regine Claßen-Bockhoff:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the German Academic Exchange Service (DAAD), and the equal opportunity officer of the Faculty of Biology, Johannes-Gutenberg-University Mainz. The first author gratefully acknowledges the funding given by the re-entry scholarship and the State Scholarship Foundation of the Rhineland-Palatine, Germany. We thank Dr. C. Rasmussen and M.Sc. E. Sanchez for helping with the identification of bees as well as the Entomology Laboratory and the Department of Ornithology of the Museum of Natural History of San Marcos University for allowing us to work with the bee and hummingbird collections. We thank Gabriela Ortiz for her assistance in the fieldwork, Joshua Wachlin for discussion and the editor and reviewers for their valuable comments on the manuscript.

Data availability

Data will be made available on request.

References

Adams, R.P., 2017. Identification of essential oil components by gas chromatograph/mass spectrometry, 5th ed. Allured Publishing Corporation, Carol, Stream, IL.
Ajani, Y., Jamzad, Z., Claβen-Bockhoff, R., 2022. Floral biology in the endemic Iranian *Salvia majdae* - implications for taxonomy, character evolution and conservation. Flora 287, 151986.
Aguilar, P.G., 1961. Insectos polinizadores más comunes en Lima y alrededores. Rev. Peru. Entom. Agr. 4, 81–82.
Aguilar, P.G., 1965. Algunas consideraciones sobre los insectos polinizadores en los alrededores de Lima. Rev. Peru. Entom. 8, 138–145.
Aguilar-Rodríguez, P.A., Tschapka, M., García-Franco, J.G., Krömer, T., MacSwiney, G. M.C., 2019. Bromeliads going batty: pollinator partitioning among sympatric chiropterophilous Bromeliaceae. AoB Plants 11 (2) plz014.
Andersson, S., 2003. Foraging responses in the butterflies *Inachis io, Aglais urticae* (Nymphalidae), and *Gonepteryx rhamni* (Pieridae) to floral scents. Chemoecol 13, 1–11.
Armbruster, W.S., Edwards, M.E., Debevec, E.M., 1994. Floral character displacement generates assemblage structure of Western Australian triggerplants (*Stylidium*). Ecology 75, 315–329.
Armbruster, W.S., Herzig, A.L., 1984. Partitioning and sharing of pollinators by four sympatric species of *Dalechampia* (Euphorbiaceae) in Panama. Ann. Mo. Bot. Gard. 71, 1–16.
Arroyo, M.T.K., Primack, R., Armesto, J., 1982. Community studies in pollination ecology in the high temperate Andes of central Chile. I. Pollination mechanisms and altitudinal variation. Am. J. Bot. 69, 82–97.
Baker, H.G., 1959. Reproductive methods as factors in speciation in flowering plants. Cold Spring Harbor Symp. Quant. Biol. 24, 177–191.
Balkenius, A., Kelber, A., 2006. Colour preferences influence odour learning in the hawkmoth, *Macroglossum stellatarum*. Naturwiss 93, 255–258.
Baumgartner, D.L., Roubik, D.W., 1989. Ecology of necrophilous and filth-gathering stingless bees (Apidae: meliponinae) of Peru. J. Kansas Entomol. Soc. 62, 11–22.
Benitez-Vieyra, S., Pérez-Alquicira, J., Sazatornil, F.D., Domínguez, C.A., Boege, K., Pérez-Ishiwara, R., Fornoni, J., 2019. Evolutionary transition between bee pollination and hummingbird pollination in *Salvia*: comparing means, variances and covariances of corolla traits. J. Evol. Biol. 32, 783–793.
Botes, C., Johnson, S.D., Cowling, R.M., 2008. Coexistence of succulent tree aloes: partitioning of bird pollinators by floral traits and flowering phenology. Oikos 117, 875–882.
Brako, L., Zarucchi, J., 1993. Catálogo de las angiospermas y gimnospermas del Perú. Monograph. Syst. Bot. Mo. Bot. Gard. 45, 1–1286.
Burger, H., Ayasse, M., Häberlein, C.M., Schulz, S., Dötterl, S., 2010b. *Echium* and *pontechium* specific floral cues for host–plant recognition by the oligolectic bee *Hoplitis adunca*. S. Afr. J. Bot. 76, 788–795.
Burger, H., Dötterl, S., Ayasse, M., 2008. Importance of olfactory and visual cues of *Echium* for host-plant finding of the oligolectic bee *Osmia adunca* (Megachilidae). Mitt. D.G. a.a.E. 16, 163–166.
Burger, H., Dötterl, S., Ayasse, M., 2010a. Host–plant finding and recognition by visual and olfactory floral cues in an oligolectic bee. Funct. Ecol. 24, 1234–1240.
Cairampoma, L., 2021. The Genus *Salvia* in the Andes - taxonomical, distributional and ecological perspectives. Johannes-Gutenberg-University Mainz, Germany. PhD thesis.
Cairampoma, L., Tello, J.A., Claßen-Bockhoff, R., 2020. Pollination in the desert: adaptation to bees and birds in *Salvia rhombifolia*. Int. J. Plant Sci. 181, 857–870.
Castellanos, M.C., Wilson, P., Thomson, J.D., 2004. Anti-bee and pro-bird changes during the evolution of hummingbird pollination in Penstemon flowers. J. Evol. Biol. 17, 876–885.
Celep, F., Atalay, Z., Dikmen, F., Doğan, M., Sytsma, K.S., Claßen-Bockhoff, R., 2020. Pollination ecology, specialization, and genetic isolation in sympatric bee pollinated *Salvia* (Lamiaceae). Int. J. Plant Sci. 181, 800–811.
Chittka, L., 1992. The colour hexagon: a chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. J. Comp. Physiol. A 170, 533–543.
Chittka, L., Kevan, P.G., 2005. Flower colours as advertisement. In: Dafni, A., Kevan, P. G., Husband, B.C. (Eds.), Practical Pollination Biology. Enviroquest Ltd, Cambridge, pp. 157–230.
Chittka, L., Waser, N.M., 1997. Why red flowers are not invisible to bees. Isr. J. Plant Sci. 45, 169–183.
Claßen-Bockhoff, R., Speck, T., Tweraser, E., Wester, P., Thimm, S., Reith, M., 2004. The staminal lever mechanism in *Salvia* L. (Lamiaceae): a key innovation for adaptive radiation? Org. Divers. Evol. 4, 189–205.
Cuevas, E., Espino, J., Marques, I., 2018. Reproductive isolation between *Salvia elegans* and *S. fulgens*, two hummingbird-pollinated sympatric sages. Plant Biol. 20, 1075–1082.
Cultid-Medina, C.A., González-Vanegas, P.A., Bedolla-García, B.Y., 2021. Wild bees as floral visitors to *Salvia assurgens* (Lamiaceae): a contribution to the pollination ecology of a white-flowered endemic Mexican sage. Acta Bot. Mex. 128, e1785. https://doi.org/10.21829/abm128.2021.1785.
Dafni, A., Kevan, P.G., Husband, B.C., 2005. Practical Pollination Biology. Eniroquest Lrd. Onatrio.
de Jager, M.L., Willis-Jones, E., Critchley, S., Glover, B.J., 2017. The impact of floral spot and ring markings on pollinator foraging dynamics. Evol. Ecol. 31, 193–204.
Dieringer, G., Ramamoorthy, T.P., Lezama, P.T., 1991. Floral visitors and their behavior to sympatric *Salvia* species (Lamiaceae) in Mexico. Acta Bot. Mex. 13, 75–83.
Dötterl, S., Vereecken, N.J., 2010. The chemical ecology and evolution of bee–flower interactions: a review and perspectives. Can. J. Zool. 88, 668–697.
Dyer, A.G., Chittka, L., 2004. Biological significance of distinguishing between similar colours in spectrally variable illumination: bumblebees (*Bombus terrestris*) as a case study. J. Comp. Physiol. A 190, 105–114.
El-Sayed, A.M., 2024. The pherobase: database of pheromones and semiochemicals [online]. Available from http://www.pherobase.com.
Epling, C., 1947. Natural hybridization of *salvia apiana* and *S. mellifera*. Evolution 1, 69–78.
Feisinger, P., 1978. Ecological interactions between plants and hummingbirds in a successional tropical community. Ecol. Monogr. 48, 269–287.
Fenster, C.B., Armbruster, W.S., Wilson, P., Thomson, J.D., Dudash, M.R., 2004. Pollination syndromes and floral specialization. Ann. Rev. Ecol. Evol. Syst. 35, 375–403.
Fragoso-Martínez, I., Martínez-Gordillo, M., Salazar, G.A., Sazatornil, F., Jenks, A.A., García Peña, M., Barrera-Aveleida, G., Benitez-Vieyra, S., Magallón, S., Cornejo-Tenorio, G., Granados Mendoza, C., 2018. Phylogeny of the neotropical sages (*Salvia* subg. *Calosphace*; Lamiaceae) and insights into pollinator and area shifts. Plant. Syst. Evol. 304, 43–55.
Gentry, A.H., 1982. Neotropical floristic diversity: phytogeographical connections between central and South America, pleistocene climatic fluctuations, or an accident of the Andean orogeny? Ann. Mo. Bot. Gard. 69, 557–593.
Goldsmith, K., Goldsmith, T., 1982. Sense of smell in the black-chinned hummingbird. Condor 84, 237–238.

Gonzalez, V.H., Chavez, F., 2004. Nesting biology of a new high Andean bee, *Anthophora walteri* Gonzalez (Hymenoptera: apidae: anthophorini). J. Kansas Entomol. Soc. 77, 36–44.
Gonzalez, V.H., Engel, M.S., 2004. The tropical Andean bee fauna (Insecta: hymenoptera: apoidea), with examples from Colombia. Entom. Abh. 62, 65–75.
Gonzalez, V.H., Mantilla, B., Palacios, E., 2006. Foraging activity of the solitary andean bee, *anthophora walteri* (Hymenoptera: apidae, Anthophorini). Rev. Colomb. Entomol. 32, 73–76.
Gonzalez, V.H., Mejia, A., Rasmussen, C., 2004. Ecology and nesting behavior of *Bombus atratus* Franklin in Andean Highlands (Hymenoptera: apidae). J. Hym. Res. 13, 234–242.
Gonzalez, V.H., Ospina, M., 2008. Nest structure, seasonality, and host plants of *Thygater aethiops* (Hymenoptera: apidae, Eucerini) in the Andes. J. Hym. Res. 17, 110–115.
Grant, K.A., Grant, V., 1964. Mechanical isolation of *Salvia apiana* and *salvia mellifera* (Labiatae). Evolution 18, 196–212.
Grant, V., 1994. Modes and origins of mechanical and ethological isolation in angiosperms. Proc. Natl. Acad. Sci. U.S.A. 91, 3–10.
Haque, M.S., Ghoshal, K.K., 1981. Floral biology and breeding system in the genus *Salvia* L. Proc. Indian Nat. Sci. 5, 716–724.
Hansen, D.M., van der Niet, T., Johnson, S.D., 2012. Floral signposts: testing the significance of visual 'nectar guides' for pollinator behaviour and plant fitness. Proc. Roy. Soc. B: Biol. Sci. 279, 634–639.
Harder, L.D., Jordan, C.Y., Gross, W.E., Routley, M.B., 2004. Beyond floricentrism: the pollination function of inflorescences. Plant Spec. Biol. 19, 137–148.
Huber, F.K., Kaiser, R., Sauter, W., Schiestl, F.P., 2005. Floral scent emission and pollinator attraction in two species of *Gymnadenia* (Orchidaceae). Oecologia 142, 564–575.
Jürgens, A., Witt, T., Gottsberger, G., 2003. Flower scent composition in *Dianthus* and *Saponaria* species (Caryophyllaceae) and its relevance for pollination biology and taxonomy. Biochem. Syst. Ecol. 31, 345–357.
Kalin de Arroyo, M.T., 1976. Geitonogamy in animal pollinated tropical angiosperms. A stimulus for the evolution of self-incompatibility. Taxon 25, 543–548.
Kessler, A., Baldwin, I.T., 2001. Defensive function of herbivore-induced plant volatile emissions in nature. Science 291, 2141–2144.
Knudsen, J.T., Eriksson, R., Gershenzon, J., Stahl, B., 2006. Diversity and distribution of floral scent. Bot. Rev. 72, 1–120.
Knudsen, J.T., Tollsten, L., Bergström, L.G., 1993. Floral scents - a checklist of volatile compounds isolated by head-space techniques. Phytochemistry 33, 253–280.
Kriebel, R., Drew, B.T., Drummond, C.P., González-Gallegos, J.G., Celep, F., Mahdjoub, M.M., Rose, J.P., Xiang, C.L., Hu, G.X., Walker, J.B., Lemmon, E.M., Lemmon, A.R., Sytsma, K.J., 2019. Tracking the temporal shifts in area, biomes, and pollinators in the radiation of *Salvia* (sages) across continents: leveraging Anchored Hybrid Enrichment and targeted sequence data. Am. J. Bot. 106, 573–597.
Kriebel, R., Drew, B.T., González-Gallegos, J.G., Celep, F., Heeg, L., Mahdjoub, M.M., Sytsma, K.J., 2020. Pollinator shifts, contingent evolution, and evolutionary constraint drive floral disparity in *Salvia* (Lamiaceae): evidence from morphometrics and phylogenetic comparative methods. Evolution 74, 1335–1355.
Kriebel, R., Rose, J.P., Drew, B.T., González-Gallegos, J.G., Celep, F., Heeg, L., Mahdjoub, M.M., Sytsma, K.J., 2023. Model selection, hummingbird natural history, and biological hypotheses: a response to Sazatornil et al. Evolution 77, 646–653.
Levin, D.A., Kerster, H.W., Niedzlek, M., 1971. Pollinator flight directionality and its effect on pollen flow. Evolution 25, 113–118.
Lloyd, D.G., Webb, C.J., 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms. I. Dichogamy. N. Z. J. Bot. 24, 135–162.
Lunau, K., Papiorek, S., Eltz, T., Sazima, M., 2011. Avoidance of achromatic colours by bees provides a private niche for hummingbirds. J. Exp. Biol. 214, 1607–1612.
Macbride, F., 1960. Flora of Peru. Field Museum of Natural History, Chicago.
Macior, L.W., 1983. The pollination dynamics of sympatric species of *Pedicularis* (Scrophulariaceae). Am. J. Bot. 70, 844–853.
Michener, C.D., 2007. The Bees of the World. Johns Hopkins University Press, Baltimore.
Michener, C.D., Engel, M.S., Ayala, R., 2003. The bee genus *caupolicana* in Central America (Hymenoptera: colletidae). J. Kansas Entomol. Soc. 76, 160–171.
Mutke, J., Weigend, M., 2017. Mesoscale patterns of plant diversity in Andean South America based on combined checklist and GBIF data. Ber. Reinhold-Tüxen-Ges. 29, 83–97.
Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858.
Naghiloo, S., Nikzat-Siahkolaee, S., Esmaillou, Z., Claßen-Bockhoff, R., 2022. Pollination efficiency and pollen limitation in bistaminate vs tetrastaminate Lamiaceae. Plant Spec. Biol. 37, 91–102.
Ott, D., Hühn, P., Claßen-Bockhoff, R., 2016. *Salvia apiana*: a carpenter bee flower? Flora 221, 82–91.
Owens, S.J., Ubera Jiménez, J.L., 1992. Breeding systems in Labiatae. In: Harley, R.M., Reynolds, T. (Eds.), Advances in Labiate Science. Royal Botanic Gardens, Kew, pp. 257–280.
Paulus, H.F., Gack, C., 1990. Pollinators as prepollinating isolation factors: evolution and speciation in *Ophrys* (Orchidaceae). Isr. J. Plant Sci. 39, 43–79.
Pauw, A., 2006. Floral syndromes accurately predict pollination by a specialized oilcollecting bee (*Rediviva peringueyi*, Melittidae) in a guild of South African orchids (*Coryciinae*). Am. J. Bot. 93, 917–926.
Pennington, R.T., Lavin, M., Särkinen, T., Lewis, G.P., Klitgaard, B.B., Hughes, C.E., 2010. Contrasting plant diversification histories within the Andean biodiversity hotspot. Proc. Natl. Acad. Sci. U.S.A. 107 (31), 13783–13787.
Rabeschini, G., Bergamo, P.J., Nunes, C.E.P., 2021. Meaningful words in crowd noise: searching for volatiles relevant to carpenter bees among the diverse scent blends of bee flowers. J. Chem. Ecol. 47, 444–454.
Raguso, R.A., Levin, R.A., Foose, S.E., Holmberg, M.W., Mcdade, L.A., 2003. Fragrance chemistry, nocturnal rhythms and pollination "syndromes'' in *Nicotiana*. Phytochemistry 63, 265–284.
Raguso, R.A., Pichersky, E., 1995. Floral volatiles from *Clarkia breweri* and *C. concinna* (Onagraceae): recent evolution of floral scent and moth pollination. Plant Syst. Evol. 194, 55–67.
Ramamoorthy, T.P., Elliott, M., 1998. Lamiaceae de México: diversidad, distribución, endemismo y evolución. In: Ramamoorthy, T.P., Bye, R., Lot, A., Fa, J. (Eds.), Diversidad Biológica de México: Orígenes y Distribución. Instituto de Biología, UNAM, México, pp. 501–525.
Rieseberg, L.H., Willis, J.H., 2007. Plant speciation. Science 317, 910–914.
Ruchisansakun, S., Tangtorwongsakul, P., Cozien, R.J., Smets, E.F., van der Niet, T., 2016. Floral specialization for different pollinators and divergent use of the same pollinator among co-occurring *Impatiens* species (Balsaminaceae) from Southeast Asia. Bot. J. Linnean Soc. 181, 651–666.
Sazatornil, F., Fornoni, J., Fragoso-Martínez, I., Pérez-Ishiwara, R., Benitez-Vieyra, S., 2023. Did early shifts to bird pollination impose constraints on *Salvia* flower evolution? Evolution 77, 636–645.
Schemske, D.W., Bradshaw Jr, H.D., 1999. Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). Proc. Nat. Acad. Sci. 96, 11910–11915.
Schiestl, F.P., 2015. Ecology and evolution of floral volatile-mediated information transfer in plants. New Phytol. 206, 571–577.
Schiestl, F.P., Schlüter, P.M., 2009. Floral isolation, specialized pollination, and pollinator behavior in orchids. Ann. Rev. Entomol. 54, 425–446.
Schulenberg, T.S., Stotz, D.F., Lane, D.F., O'Neill, J.P., Parker III, T.A., 2007. Birds of Peru. Princeton field guides, New Jersey.
Stebbins, G.L., 1950. Variation and Evolution in Plants. Columbia Univ. Press, New York.
Steiger, S.S., Fidler, A.E., Valcu, M., Kempenaers, B., 2008. Avian olfactory receptor gene repertoires: evidence for a well-developed sense of smell in birds? Proc. R. Soc. B Biol. Sci. 275, 2309–2318.
Surina, B., Balant, M., Glasnović, P., Gogala, A., Fišer, Ž., Satovic, Z., Liber, Z., Radosavljević, Classen-Bockhoff, R., 2024. Lack of pollinators selects for increased selfing, restricted gene flow and resource allocation in the rare Mediterranean sage *Salvia brachyodon*. Sci. Rep. 14 (1), 5017.
van Treuren, R., Bijlsma, R., Ouborg, N.J., van Delden, W., 1993. The effects of population size and plant density on outcrossing rates in locally endangered *Salvia pratensis*. Evolution 47, 1094–1104.
Vareschi, E., 1971. Duftunterscheidung bei der Honigbiene - Einzelzellableitungen und Verhaltensreaktionen. Z. Vergl. Physiol. 75, 143–173.
Vogel, S., 1954. Blütenbiologische Typen als Elemente der Sippengliederung, dargestellt anhand der Flora Südafrikas. Bot. Stud. 1, 1–388 [2012 translated to English; Floral-biological syndromes as elements of diversity within tribes in the Flora of South Africa. Shaker, Aachen.].
Waser, N.M., 1986. Flower constancy: definition, cause, and measurement. Am. Nat. 127, 593–603.
Wei, Y., Huang, Y., Li, G., 2017. Reproductive isolation in sympatric *Salvia* species sharing a sole pollinator. Biodivers. Sci. 25, 608–614.
Wester, P., Cairampoma, L., Haag, S., Schramme, J., Neumeyer, C., Claßen-Bockhoff, R., 2020. Bee exclusion in bird-pollinated *Salvia* flowers: the role of flower color versus flower construction. Int. J. Plant Sci. 181, 770–786.
Wester, P., Claßen-Bockhoff, R., 2007. Floral diversity and pollen transfer mechanisms in bird-pollinated *Salvia* species. Ann. Bot. 100, 401–421.
Wester, P., Claßen-Bockhoff, R., 2011. Pollination syndromes of new world *salvia* species with special reference to bird pollination. Ann. Mo. Bot. Gard. 98, 101–155.
Wiens, D., 1984. Ovule survivorship, brood size, life history, breeding systems, and reproductive success in plants. Oecologia 64, 47–53.
Will, M., Claßen-Bockhoff, R., 2017. Time to split *Salvia* s.l. (Lamiaceae) – new insights from Old World *Salvia* phylogeny. Mol. Phyl. Evol. 109, 33–58.
Wood, J.R.I., 2007. The Salvias (Lamiaceae) of Bolivia. Kew Bull. 62, 177–221.
Wright, G.A., Schiestl, F.P., 2009. The evolution of floral scent: the influence of olfactory learning by insect pollinators on the honest signalling of floral rewards. Funct. Ecol. 23, 841–851.
Wyatt, R., 1982. Inflorescence architecture: how flower number, arrangement, and phenology affect pollination and fruit-set. Am. J. Bot. 69, 585–594.
Xiao, H.W., Huang, Y.B., Liu, Q.S., Claßen-Bockhoff, R., Tian, R.N., Wei, Y.K., 2023. Mixed mating patterns in morphologically diverse bumblebee-pollinated *Salvia* species from China. Biol. J. Linn. Soc. 143, blad164. https://doi.org/10.1093/biolinnean/blad164.