

Incomplete partitioning of pollinators by *Linum suffruticosum* and its coflowering congeners

Rocío Pérez-Barrales¹  | W. Scott Armbruster^{2,3} 

¹Botany Department, University of Granada, Granada 18071, Spain

²School of Biological Sciences, University of Portsmouth, Portsmouth, PO1 2DY, UK

³Institute of Arctic Biology, University of Alaska, Fairbanks, AK 99775, USA

Correspondence

Rocío Pérez-Barrales, Botany Department, University of Granada, Granada 18071, Spain.
 Email: rpbarrales@ugr.es

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Abstract

Premise: *Linum suffruticosum* shows variations in pollinator fit, pollen pickup, and local pollinators that predict pollen deposition rates. The species often coflowers with other *Linum* species using the same pollinators. We investigated whether *L. suffruticosum* trait variation could be explained by local patterns of pollinator sharing and associated evolution to reduce interspecific pollen transfer.

Methods: Pollinator observations were made in different localities (single species, coflowering with other congeners). Floral traits were measured to detect differences across populations and from coflowering species. Reproductive costs were quantified using interspecific hand pollinations and measures of pollen-tube formation, combined with observations of pollen arrival on stigmas and pollen-tube formation after natural pollination in allopatric and sympatric localities.

Results: The size and identity of the most important pollinator of *L. suffruticosum* and whether there was pollinator sharing with coflowering species appeared to explain floral trait variation related to pollinator fit. The morphological overlap of the flowers of *L. suffruticosum* with those of coflowering species varied, depending on coflowering species identity. A post-pollination incompatibility system maintains reproductive isolation, but conspecific pollen-tube formation was lower after heterospecific pollination. Under natural pollination at sites of coflowering with congeners, conspecific pollen-tube formation was lower than at single-species localities.

Conclusions: Trait variation in *L. suffruticosum* appears to respond to the most important local pollinator. Locally, incomplete pollinator partitioning might cause interspecific pollination, imposing reproductive costs. These reproductive costs may generate selection on floral traits for reduced morphological overlap with coflowering congeners, leading to the evolution of pollination ecotypes.

KEYWORDS

character displacement, heterostyly, inaccuracy, interspecific pollen transfer, Linaceae, pollen tube, pollination ecotypes, pollinator importance, reproductive interference

A correlation between population differentiation in floral traits and differences in local pollinators is a necessary condition for identifying pollination ecotypes. The mechanism of ecotype formation is thought to relate commonly to the mechanical fit of flowers to locally important pollinators, thereby determining frequencies of pollen pickup and deposition. The fine-tuning between plants and their local pollinators creates selection on floral traits and may result in

evolutionary divergence and phenotypic differentiation (Armbruster, 1985; Armbruster et al., 1994; Johnson, 1997; Pérez-Barrales et al., 2007; Anderson and Johnson, 2008; Cosacov et al., 2014; Newman et al., 2014; Newman and Anderson, 2020). The formation of pollination ecotypes through the adaptation to the most important pollinator(s) represents an example of the "Grant–Stebbins" model for pollinator-driven diversification (Johnson, 2010) and how

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specialization in pollination systems may lead to reproductive isolation and speciation (Stebbins, 1974; Grant, 1994; Kay and Schemske, 2003; Kephart and Theiss, 2004; van der Niet et al., 2006; but also see Armbruster and Muchhala, 2009).

Studies of pollination-ecotype formation focus on trait variation related to changes in pollinator guilds or functional groups of pollinators by looking at shifts in pollination systems or pollination specialization (sensu Fenster et al., 2004, but see Gómez et al., 2014 for ecotype formation in a species with a generalized pollination system). However, generalization in pollination may be more the rule than the exception (Waser et al., 1996; cf. Johnson and Steiner, 2000), and species often share pollinators with other coflowering members in the community (Olesen and Jordano, 2002; Bosch et al., 2009). In recent years, there has been renewed interest in quantifying the way pollinator sharing determines pollen transfer between species, confirming that coflowering species commonly receive pollen from, and send pollen to, several other species (Morales and Traveset, 2008; Arceo-Gómez and Ashman, 2011; Ashman and Arceo-Gómez, 2013; Fang and Huang, 2013; Armbruster et al., 2014; Fang et al., 2019; Moreira-Hernández and Muchhala, 2019; Ashman et al., 2020; Zhang et al., 2021). Through pollinator sharing, coflowering species influence the pollination of their neighbors (Lázaro et al., 2009; Carvalheiro et al., 2014). Hence, trait differentiation might be context-dependent, influenced by the magnitude and sign of these pollinator-mediated plant–plant interactions and the consequences for plant fitness (Sargent and Ackerly, 2008; Pauw, 2013).

The sign of plant–plant interactions mediated by pollinators underpins how pollination-related traits evolve in communities (Ghazoul, 2006; Mitchell et al., 2009). More than 80% of angiosperms depend on animal pollinators for reproduction to transfer pollen to stigmas and form seeds (Ollerton et al., 2011), so knowledge of how coflowering species affect each other's reproduction is crucial to understanding the mechanisms that allow pollinator sharing. Therefore, it is essential to distinguish between the effects of pre- and post-pollination events of the plant–pollinator–plant interaction. Positive effects emerge when coflowering facilitates the pollination service (Gross et al., 2000; Moeller, 2004; Ghazoul, 2006). Diverse communities provide more flowers and resources for pollinators, which maintains the diversity and abundance of the local pollinator community, increasing visitation (Stone et al., 1998; Gross et al., 2000; Moeller, 2004; de Jager et al., 2011; Wei et al., 2021). In contrast, post-pollination events might weaken or cancel the positive effects, if pollinator sharing leads to interspecific pollen transfer. The positive effects of coflowering can be diminished or even offset if pollinator movements between species reduce plant fitness via pollen misplacement, arrival of heterospecific pollen to the stigmas, or if one of the coflowering species is more attractive and reduces the visitation to others (Sargent and Ackerly, 2008; Moreira-Hernández and Muchhala, 2019). For example, Bergamo et al. (2020) showed that

floral abundance and trait similarity increased pollinator attraction, but these translated into a negative correlation with pollen-tube formation.

The pervasive nature of interspecific pollination in plant populations sets the ecological context for the evolution of strategies to avoid or ameliorate the costs of pollinator sharing with neighbors (Arceo-Gómez and Ashman, 2011; Ashman and Arceo-Gómez, 2013; Arceo-Gómez et al., 2016; Fang et al., 2019; Arceo-Gómez, 2021). Plants may reduce the number of shared pollinators, fully partition the pollinator community (Armbruster and Herzig, 1984; Wolfe and Sowell, 2006; Pick and Schlindwein, 2011), or use the same pollinator in different ways. The negative effects of using the same pollinator species can be reduced or eliminated by plants reducing phenological overlap during the flowering season or having flowers open at different times of the day (Armbruster, 1985; Stone et al., 1998; Raine et al., 2007; Botes et al., 2008). However, flowering phenologies in seasonal environments are often constrained by the duration of the growing season, constraining when pollination interactions can occur (Stiles, 1977; Poole et al., 1979; Flo et al., 2018; Segrestin et al., 2018). Under these circumstances, divergence in traits involved in pollinator fit, pollen pickup, and deposition ought to facilitate the use of different pollinator body parts and reduce the likelihood of interspecific pollen transfer (Howell, 1977; Armbruster et al., 1994, 2014; Murcia and Feinsinger, 1996; Muchhala and Potts, 2007; Tong and Huang, 2018; Newman and Anderson, 2020). For closely related species, which generally share similar floral traits and pollinators due to phylogenetic conservatism, small morphological changes can reduce interspecific pollination (Armbruster et al., 1994; Aizen and Rovere, 2010; Davies et al., 2013; Mesquita-Neto et al., 2015, 2018; E-Vojtkó et al., 2020). Notwithstanding, it is important to distinguish between trait differences reflecting (1) the reduction of reproductive interference evolving after (and in response to) species experience sympatry, and (2) differences that evolve through drift or ecological sorting (Armbruster, 1986, 1994; Eaton et al., 2012; Eisen and Geber, 2018).

An avenue of research receiving increasing attention is the study of plant–plant interactions mediated through pollen–pistil interactions and pollen performance when plants receive a mix of conspecific and heterospecific pollen (Ashman et al., 2020). Conspecific pollen-tube performance might depend on the absolute or relative amounts of conspecific and heterospecific pollen reaching stigmas, the timing of the arrival of conspecific relative to heterospecific pollen, or the identity and diversity of heterospecific pollen donors (Armbruster and Herzig, 1984; Arceo-Gómez and Ashman, 2011; Bruckman and Campbell, 2016; Tong and Huang, 2016; Cavalcante et al., 2020; Coetzee et al., 2020). Heterospecific pollen-tube formation might depend on the phylogenetic distance between species or the presence of interspecific incompatibility systems that operate similarly to the self-incompatibility reaction (Grant, 1966; Broz and Bedinger, 2021; Zou et al., 2022). Because interspecific

pollination is generally costly, tolerance to receiving pollen from other species can reduce or mitigate fitness costs, allowing pollinator sharing and species coexistence (Arceo-Gómez et al., 2016; Moreira-Hernández et al., 2019; Streher et al., 2020). Comparisons of pollen–pistil interactions in sympatric and allopatric communities, combined with pollen performance using hand-pollination experiments in controlled conditions, can help to infer how species are affected by other coflowering pollen donors and the potential consequences for trait variation and differentiation (Arceo-Gómez and Ashman, 2014).

In this study, we provide new data on the pollination ecology of *Linum suffruticosum* to add to the original observations that variation in traits controlling pollinator fit evolved in response to the size and behavior of the most important pollinator(s) (Armbruster et al., 2006). *Linum suffruticosum* presents an unusual type of distyly, where stamens and styles bend and twist in three dimensions (also known as 3D reciprocity). In this way, short organs (pin anthers and thrum stigmas) appear pressed against the petals and facing toward the center of the flower, while tall organs (thrum anthers and pin stigmas) are at the center of the flower and facing outwards (Appendix S1). This arrangement causes nectar-seeking visitors to contact thrum anthers and pin stigmas dorsally (nototriby) and pin anthers and thrum stigmas ventrally (sternotriby; Armbruster et al., 2006).

During the spring bloom in Spain, it is common to observe several *Linum* species growing together and blooming simultaneously (Pérez Latorre et al., 2004). We confirmed these observations with our own, finding that *L. suffruticosum* occurs alone or with one to several other *Linum* species, including *L. tenue*, *L. narbonense*, and *L. viscosum*, which are also distylous, but belong to different subclades (Ruiz-Martín et al., 2018), and present the reproductive organs of pin and thrum flowers at the center of the flower and facing outward (Appendix S1). In preliminary observations, we found that these *Linum* species shared pollinators, with individuals moving between species. The study of distylous flowers in the context of pollinator sharing with close relatives is interesting because the polymorphism requires cross-pollination between morphs for seed production (disassortative pollination, i.e., pollen from tall and short anthers to tall and short stigmas, respectively; Darwin, 1877). Distylous species usually have a heteromorphic incompatibility system that prevents self-fertilization and mating between individuals of the same morph (Barrett, 2002). Hence, coflowering with closely related species might alter pollen transfer patterns if species display a similar arrangement of tall and short organs. For example, in *Primula*, Keller et al. (2012) found that intraspecific reciprocity was greater than interspecific reciprocity. However, hybrid formation in sympatric localities appeared associated with overlap in reproductive organs between species (Kálmán et al., 2004; Keller et al., 2016). Hence, the study of distylous flowers is particularly valuable because it allows one to investigate whether the variation in

the mechanics of pollination (pollinator fit and reciprocity) can be predicted from the floral morphology of congeneric species present in the community.

In this study, we investigated the variation of traits involved in pollinator fit, pollen pickup, and pollen deposition in *L. suffruticosum* to determine whether there was population differentiation and whether it could be explained as adaptation that minimizes reproductive interference imposed by coflowering species or instead as an adaptive response to the most important local pollinators. Data sets were generated in single-species localities and coflowering localities, comprising three parts: (1) Description of the pollinator community to determine pollinator sharing, behavior, and identification of the most important pollinator (Armbruster, 1988; Aigner, 2001; Mayfield et al., 2001) to detect geographical and flower-community variation across multiple populations of *L. suffruticosum*. (2) Descriptions of the morphological variation of floral traits to quantify the magnitude of phenotypic overlap among *Linum* species in multivariate phenotypic space. Individual comparisons of traits directly involved in pollen transfer, namely pollinator fit, length of tall organs (pin stigmas, thrum anthers), and short organs (thrum stigma, pin anther), were also used to determine the degree of phenotypic overlap and potential pollen transfer between species. Then, a linear regression model was used to assess whether the size of the most important pollinator predicted the values of traits involved in pollinator fit across multiple *L. suffruticosum* populations and across species. Adaptive inaccuracy calculations (Armbruster et al., 2004, 2009, 2017) were used to determine whether, for those traits directly involved in pollen transfer, deviations from their phenotypic target were smaller in coflowering localities of *L. suffruticosum*. (3) Measurements to determine whether receiving pollen from other *Linum* species imposes reproductive costs on *L. suffruticosum*. The reproductive costs were assessed in two ways: first, by hand-pollinating *L. suffruticosum* stigmas with different mixes of con- and heterospecific *Linum* pollen and comparing pollen-tube formation; second, by comparing the amount and rate of *Linum* pollen arrival and pollen-tube formation between single-species and coflowering localities.

MATERIALS AND METHODS

Study species

Linum suffruticosum L. (Linaceae) is a perennial, woody shrub species complex with white to cream-colored flowers, distributed in the southwestern Mediterranean Region (Afonso et al., 2021). *Linum suffruticosum* populations can co-occur with other *Linum* species, although co-occurring *Linum* species do not necessarily flower at the same time (R. Pérez-Barrales, personal observation). In this study, localities were selected according to whether *L. suffruticosum* populations bloomed on their own (hereafter single-species

locality, see justification below) or coflowered with other *Linum* species (hereafter coflowering localities). Coflowering localities included the distylous species *L. narbonense* and *L. viscosum* (both perennials with a woody base and herbaceous stems). Other localities had *L. tenue*, an annual, distylous herb. In two localities, flowering of *L. tenue* started as flowering in *L. suffruticosum* ended (i.e., the two species co-occurred but did not coflower, an observation confirmed in three consecutive years). In the other two localities, both species coflowered. Appendix S2 includes the list of *L. suffruticosum* localities surveyed, indicating the presence of other coflowering *Linum* species, the data sets generated, and the experiments conducted.

Description of pollinator community, insect behavior, and pollinator importance

Pollinators were observed in a single-species locality (San Juan de la Peña) and four coflowering localities (Aisa, Borau, Oroel and Triste) in the Central Pyrenees in Spain (CP hereafter) in June between 2008 and 2010 (Appendix S2). The pollination ecology of *L. suffruticosum* at localities in southwestern Spain (SW hereafter) was observed in 2005 and has been reported previously (Armbruster et al., 2006). The closest study localities were ca. 2 km apart. At each locality, 2–4 people observed floral visitors during 2–3 consecutive days between 10:30–11:00 and 17:30–18:00 in plots of ca. 1–1.5 m². Plots contained one, two, or three *Linum* species, and observations were made at intervals of 10–20 min (depending on insect activity), after which observers moved to a different, randomly selected plot. During the observations, it observers noted the insect type or species to the lowest possible taxonomic level, reward collected (pollen or nectar), whether insects contacted anthers and stigmas, the body parts of contact, and whether insects moved between *Linum* species. At the end of the observations, samples of most floral visitors were collected for species identification (most species were identified to the genus level). In the lab, body length was measured for those insects that contacted anthers and stigmas of both pin and thrum flowers (only these insects can potentially transfer pollen between morphs). Then, floral visitors were classified into functional type according to their feeding behavior (pollen or nectar) and body size. For each floral visitor and locality, we calculated the following: (1) the individual probability of contact with anthers and stigmas (number of visits with a contact/total number of visits) for pin and thrum flowers to then estimate the mean probability of organ contact (mean value of the probability of contact with tall and short organs of pin and thrum flowers), (2) visitation rate (number of visits/hour), (3) the relative frequency of visits, and (iv) pollinator importance (the product of visitation rate and mean probability of contact; Armbruster, 1988; Aigner 2001; Mayfield et al., 2001). The floral-visitor data across sites were used to compare pollinator communities according to the taxonomic identity

and functional types. These data allowed comparisons of five pollinator communities from CP and two from SW (see Armbruster et al., 2006 for more details on the pollinator observations in SW). The insect community was analyzed with permutational analysis of the variance (PERMANOVA; $N = 9999$ permutations) using distance matrices based on the Bray–Curtis dissimilarity index to test whether the frequency (%) of visits per insect type in *Linum* differed across the locality type. The same approach was used in coflowering localities to test for differences in the pollinator communities of coflowering *Linum* species. The analysis was repeated using only the data from *L. suffruticosum* populations to detect differences in the pollinator community composition across all localities for that species.

Floral measurements and morphological overlap among *Linum* species

Each spring from 2008 to 2010, measurements of floral traits were taken in five single-species localities and four coflowering localities (two sites with *L. narbonense*, one with *L. viscosum*, and another with both species; Appendix S2). These localities were re-visited between 2010 and 2016 to confirm that *L. suffruticosum* flowered alone or coflowered with other *Linum* species. In two of the single-species localities, the flowering of *L. tenue* started as the flowering of *L. suffruticosum* ended (see justification above). Hence, these two localities were considered single-species localities in terms of the likelihood of sharing pollinators with other coflowering *Linum* species. Flower samples were collected along 10–15 longitudinal transects of 25–50 m long each, collecting one flower/plant every 1.5–2 from ≥ 30 plants (Appendix S3). Digital images of flowers were taken from the top and lateral view to measure the following traits in pin and thrum flowers: (1) flower diameter, (2) flower tube width, (3) gap, as the minimum distance between the petal and the upper sexual organs, which corresponds to the distance a pollinator must span (i.e., pollinator “height”, tarsus to notum) to receive pollen from thrum anthers and deposit it on pin stigmas (Armbruster et al., 2006), determining a major component of pollinator fit, (4) sepal length, (5) calyx width, (6) anther height (measured as the length of the stamen), (7) stigma height (measured as the length of the pistil) and (8) herkogamy (Appendix S1). The lateral view images were taken by removing two petals. All measurements were made with 0.01 mm precision using Image J 1.40 (<http://rsb.info.nih.gov/ij/>). The corresponding traits were also measured in *L. narbonense* and *L. viscosum* flowers collected from coflowering localities (Appendix S2 and S3). *Linum tenue* samples were collected from the two localities where it co-occurs with *L. suffruticosum* (Appendices S2, S3). Only reproductive organs were measured for this species since petals dropped soon after sample collection (it was not possible to obtain the top and lateral pictures).

Principal component analysis (PCA) was used to summarize quantitative trait variation and covariation of

all floral traits in and among *L. suffruticosum*, *L. narbonense*, and *L. viscosum*. Data from each morph were analyzed separately. The analyses included all samples measured with no locality distinction to determine whether the three species are morphologically well differentiated. The first and second principal component (PC) scores were retrieved to apply a one-way ANOVA, using the PC scores as the dependent variable and species as the factor. Welch's ANOVA and post hoc Games–Howell tests were used when heteroscedasticity was detected (Keller et al., 2012). Generalized linear mixed effect models (GLMM) were implemented to determine the level of floral phenotypic overlap between *L. suffruticosum* and other *Linum* species in coflowering localities, using species pairs separately, with a focus on the traits involved in pollinator fit (gap), and in pollen pickup and deposition as dependent variables, modeled with a normal distribution.

The analyses of traits involved in pollen pickup and deposition were conducted under the assumption that pollination in distylous flowers depends on pollen flow between reciprocal anthers and stigmas of similar height (i.e., tall reproductive organs: pin stigmas and thrum anthers, short reproductive organs: thrum stigmas and pin anthers). For the “gap” trait, the GLMM included the fixed factor species and the random factor, locality. For the analysis of the height of reciprocal reproductive organs, the GLMM included the fixed factors, species and organ height (tall or short), and their interaction term. The random factors were locality and flower sample. Each species pair was analyzed separately using the data from the localities in which species coflowered. For *L. tenue*, the data used in the analysis for organ height came from the localities where it co-occurs (it was not possible to obtain morphological data from the coflowering localities used in the study of natural pollination). Post hoc analyses were conducted to identify differences between species for each trait and organ height. Failure to identify statistically significant differences (sample size of the post hoc comparisons was similar between the groups compared) was interpreted as the two species having substantial overlap for the trait analyzed (which could lead to interspecific pollination when pollinators are shared).

Character divergence and ecotypic differentiation in *L. suffruticosum*

Population differentiation for the gap trait in *L. suffruticosum* was analyzed using a generalized linear model to test for differences among populations, differences between morphs, and the importance of interactions. A linear regression model was used to evaluate the association between the average population gap and the body size of the most important pollinator observed on *L. suffruticosum* flowers, across localities (Table 1; data for the SW localities were retrieved from Armbruster et al., 2006). The analysis was repeated, after adding the data from coflowering

TABLE 1 Mean body length (mm), standard deviations (SD) and sample sizes (*N*) for *Usia* (Bombyliidae) flies and bees collected (see Appendices S2–S11) in Triste, Borau, Oroel, Aisa, and San Juan de la Peña localities in Spain during the observations of pollinators on *Linum suffruticosum*, *L. narbonense*, and *L. viscosum*.

Insect functional group	Mean body length (mm)	SD	<i>N</i>
<i>Usia</i> species			
Extra-large (EL) (<i>U. pubera</i>)	7	1.77	9
Large (L) (<i>U. pusilla</i> , <i>U. pubera</i> , and <i>Usia</i> sp. nov.)	3.95	0.69	19
Medium (M) (<i>U. pusilla</i> and <i>U. martini</i>)	2.75	0.35	10
Small (s) (<i>U. pucilla</i>)	1.92	0.11	5
Very small (ss) (<i>U. pusilla</i>)	1	0	2
Small bees	4.53	0.55	4
Large bees	8.3	1.1	10

L. narbonense and *L. viscosum*. The insect-body size was calculated as the average of the localities and of the pollinator species, when the most important pollinators included two pollinator species.

The methodology of adaptive inaccuracy (Armbruster et al., 2004, 2009, 2017) was used to investigate whether traits directly involved in pollen transfer were closer to their phenotypic adaptive target and more differentiated from other species in coflowering localities than in single-species localities, assuming that costly interspecific pollen transfer creates selection to avoid similar trait morphology among coflowering species. Adaptive inaccuracy is a concept that stems from the notion that traits evolve toward their optimal values to maximize fitness (Armbruster et al., 2004; Hansen et al., 2006) so that natural selection operates to decrease deviations between the trait and its phenotypic optimum. Adaptive inaccuracy is estimated by partitioning deviations from the phenotypic optimum into mean and variance components (Armbruster et al., 2009). In the present study, the phenotypic optimum for the fit with pollinators entails that both pin and thrum flowers match in mean gap values. Similarly, in distylous species, reciprocity means that the phenotypic optimum for tall anthers is the population mean of tall stigmas, and the phenotypic optimum for short anthers is the population mean of short stigmas. Hence, we expect that coflowering localities of *L. suffruticosum* will have lower inaccuracy values for gap and reciprocity than do populations in single-species localities. Inaccuracy for gap was estimated as follows:

$$\text{Gap inaccuracy} = (\overline{\text{Gap}}_{\text{Pin}} - \overline{\text{Gap}}_{\text{Thrum}})^2 + V_{\text{gapPin}} + V_{\text{gapThrum}}, \quad (1)$$

where $\overline{\text{Gap}}_{\text{Pin}}$ and $\overline{\text{Gap}}_{\text{Thrum}}$ is the gap locality mean for Pin and thrum, respectively, and $V_{\text{gap}_{\text{Pin}}}$ and $V_{\text{gap}_{\text{Thrum}}}$ stand for the variance in gap for pin and thrum, respectively.

Inaccuracy for reciprocity was estimated as described by Armbruster et al. (2017):

$$\text{Tall organ inaccuracy} = (\bar{A} - \bar{S})^2 + V_A + V_S \quad (2)$$

$$\text{Short organ inaccuracy} = (\bar{a} - \bar{s})^2 + V_a + V_s \quad (3)$$

where \bar{A} , \bar{S} , \bar{a} , and \bar{s} are the locality mean for thrum anther height, pin stigma height, pin anther height, and thrum stigma height, respectively, and V_A , V_S , V_a , and V_s are the variances of the mentioned organs, respectively. The inaccuracy values were divided by the squared mean of the traits under study to obtain standardized values (Armbruster et al., 2009). Comparisons between single-species and coflowering localities of *L. suffruticosum* for the average inaccuracy values of gap, tall and short organs were made using an unpaired *t*-test, implementing a bootstrap procedure ($N = 20,000$ permutations with replacement; Manly, 1998).

Experimental assessment of pollen-tube formation after interspecific pollination

Before the study of pollen-tube formation after interspecific pollination, it was necessary to determine the location of the incompatibility reaction in *L. suffruticosum* (see Appendix S4). All pollen tubes were arrested in the stigmatic tissue after both self-pollination and intramorph pollination (hand pollinations between flowers of the same morph). After intermorph pollination (pollination between flowers of different morphs), pollen tubes grew along the stylar tissue toward the ovary. Hence, interspecific hand-pollination experiments were conducted using intermorph pollinations to compare pollen-tube formation following pollination with different mixes of conspecific and heterospecific pollen.

Pollen-tube formation was studied in two localities in the SW (Puerto de las Palomas) and CP (Oroel). The interspecific pollen donor in Puerto de las Palomas were *L. tenue* plants collected from a locality 2 km away. The pollen donors in Oroel were *L. narbonense* and *L. viscosum*, which coflower with *L. suffruticosum* (Appendix S2). In each locality, all hand pollinations were done within 1 week. Daily, 10–15 cuttings of *L. suffruticosum* pin and thrum plants and cuttings from the pollen donors were collected and stored in vials with water (plant cuttings had one to three mature flower buds). In the lab, upon flower opening in the morning, *L. suffruticosum* recipient flowers were emasculated using a stereomicroscope to eliminate pollen contamination. The hand-pollination treatments included (1) interspecific pollination or (2) pollination with a mixture of conspecific and heterospecific pollen in newly open flowers (“Pollen Mix”), (3) interspecific pollination in newly

open flowers, then intraspecific pollination 4 h later [“Inter + intra (4h)”]. The control treatment included (4) intraspecific (intermorph) pollination in newly open flowers (“Intraspecific”) to compare it against “Pollen Mix”, and (5) intraspecific pollination in flowers 4 h after opening [“Intraspecific (4h)”] to compare it against “Inter + intra (4h)”. Pollinations were done using a stereomicroscope by rubbing stigmas with two anthers, using a single donor for treatments (1), (4), and (5). For treatment (3), interspecific pollination was completed using a single anther, and a second anther from *L. suffruticosum* 4 h later. Flowers were left in vials with water for 24 h to allow the formation of pollen tubes and then fixed and stored in 3:1 v/v ethanol–acetic acid solution. All treatments were completed for at least 15 flowers of each morph and for each interspecific pollen donor. In the lab, pollen-tube growth was observed using aniline blue staining (Martin, 1959), modified for *Linum* (Murray, 1986). Fixed flowers were softened for 3 h in 5 M NaOH. Then, three styles per flower were stained in decolorized aniline blue (0.1% v/v in 0.1 M K_3PO_4) for 2.5 h and mounted on slides in 50% v/v glycerol. Using a UV fluorescence microscope, the measurements recorded included: the number of pollen grains on the stigma (it was not possible to obtain accurate counts with the stereomicroscope) and the number of pollen tubes that reached stylar tissues.

Generalized linear mixed effect models (GLMMs) were used to determine differences in the probability of observing pollen tubes (Number of pollen tubes in the style/Number pollen grains on the stigma; hereafter, “probability of pollen-tube formation”) and the number of pollen tubes observed in the style (hereafter, “pollen-tube number”) in the different pollination treatments. The model included the fixed factors pollination treatment and morph, their interaction term, and the random factor *L. suffruticosum* plant branch as pollen recipient. The probability of pollen-tube formation was modeled using the binomial distribution with the probit link function. Pollen-tube number was analyzed using the negative binomial distribution. Post hoc comparisons were conducted to detect response-variable differences between morphs within each treatment. Within a morph, to test the effect of interspecific pollination on pollen-tube formation, the treatment comparisons included: Pollen Mix and Intraspecific to determine the effect of pollen composition, and Inter + intra (4h) and Intraspecific (4h) to test for the effect of the sequence of arrival of heterospecific pollen. Comparisons between the treatments Intraspecific and Intraspecific (4h) were made to investigate whether stigma aging affects pollen-tube formation. The analyses were done independently for each heterospecific pollen donor. Most samples receiving only heterospecific pollen showed pollen germination, but pollen-tube growth stopped in the stigmatic tissue soon after its initiation (*L. tenue*: 96 of 100 stigmas, *L. narbonense*: 45 of 55 stigmas; *L. viscosum*: all 100 stigmas); hence, this treatment was excluded from the statistical analyses.

Comparison of pollen deposition and pollen-tube formation in single-species and coflowering localities

In June 2014, flowers exposed to 1 day of natural pollination were collected from the SW and CP regions in single-species and coflowering localities (Appendix S2). The coflowering localities from SW included *L. tenue* (different localities from those in the morphological data analyses). In CP, one *L. suffruticosum* locality (Oroel) had *L. narbonense* and *L. viscosum*, while the other had only *L. viscosum* (Aisa) (both included in the morphological data analyses). Within each region, all localities were sampled on the same day, collecting flowers in the evening (the distance between localities ranged between 6 and 15 km). A single flower from each of 30 to 50 individual plants was collected in the two morphs and left in water vials for 24 h to allow for pollen-tube growth, then stored in the fixative solution. In the lab, the same protocol as above was used to quantify pollen on the stigma and observe pollen-tube formation under the microscope. Pollen of *Linum* is distinguishable from pollen from other species, but the pollen of the other various *Linum* species were not, because pollen-grain size overlaps among species (R. Pérez-Barrales, personal observation). In addition, after germination, pollen size changes slightly, making it impossible to obtain precise size estimates to distinguish all species. Generalized linear mixed effect models were used to investigate differences between single-species and coflowering localities of *L. suffruticosum* with respect to the number of *Linum* pollen grains observed on the stigmas, percentage of *Linum* pollen observed on the stigma (Number of *Linum* pollen grains/Total number pollen grains on the stigma), the probability of pollen-tube formation (Number of pollen tubes formed/Number pollen grains on the stigma), and pollen-tube number (as described above). The models included the factors, morph, locality type (single-species vs. coflowering localities), region (SW vs. CP), and all interaction terms. The variable plant (from which flowers had been collected) was included as a random factor. The variables representing probabilities (percentage of *Linum* pollen observed on the stigmas, probability of pollen-tube formation) and variables representing counts (number of *Linum* pollen grains observed on the stigmas, pollen-tube number) were modeled with binomial distribution (using the probit link function) and negative binomial distributions, respectively. Pairwise post hoc comparisons were conducted upon detection of statistical significance of interaction terms.

Pollinator-community data were analyzed using *adonis*, implemented in the R package *vegan* (Oksanen et al., 2013). Principal component analyses and one-way ANOVA comparisons of PC1 and PC2 were made in SPSS version 20.0 (IBM, Armonk, NY, USA). Generalized mixed-effects models were made with the R package *lme4* (Bates et al., 2015). The package *car* (Fox and Weisberg, 2019) was used to conduct the likelihood ratio test (ANOVA type III) and linear models. The significance of factors was tested

with the R package *lmerTest* (Kuznetsova et al., 2017), and the contrast test was made using the R packages *emmeans* (Lenth et al., 2020) and *multcomp* (Hothorn et al., 2008). The R package *ggplot2* (Ginestet, 2011) was used to create figures. R version 3.6.2 was used for all analyses (R Core Team, 2019).

RESULTS

Description of pollinator community, insect behavior, and pollinator importance

Appendices S5 to S15 include the lists of floral visitors, the number of pin and thrum flowers visited, the probability of contact with anthers and stigmas, the visitation rate (number of visits/hour), the relative frequency of visits (%), the mean probability of contact, and pollinator importance (the product of the visitation rate and the mean probability of contact with anthers and stigmas). The average number (minimum-maximum) of hours of observation accumulated per species and locality was 18.2 h (11.1–33.3). In all localities, and for *L. suffruticosum* (except for the Aisa locality) and *L. narbonense*, bee flies (*Usia* spp.; Bombyliidae) of various body sizes were responsible for more than 50% of visits. The exception was *L. viscosum*, where bees represented over 40% of the floral visitors in the Oroel locality (Appendix S11), and most of the visits in the Aisa locality (Appendix S13). Coflowering *Linum* species appeared to share pollinators in all localities. Based on feeding behavior and body size (Table 1), the functional groups included: (1) very small (<1 mm) dipterans, including *Usia pusilla*, *Delia platura* (Anthomiidae), and *Empis pennipes* (Empididae); (2) small (~1.92 mm) *U. pusilla*; (3) medium (~2.75 mm) *U. pusilla* and *U. martini*; (4) large (~3.95 mm) *U. pusilla*, *U. pubera*, and *Usia* sp. nov.; (5) extra-large (≥7 mm) *U. pubera*, *Systoechus ctenopterus* (Bombyliidae), and *Bombylius major* (Bombyliidae). Except for *Systoechus ctenopterus* and *Bombylius major* (both of which hover while visiting flowers to drink nectar), these groups crawled down the petals to the bottom of the flower, fed on nectar, and contacted tall organs (pin stigmas and thrum anther) dorsally (thorax and abdomen) in all *Linum* species. The contact of *L. suffruticosum* short organs (pin anthers and thrum stigmas) occurred ventrally. In the other *Linum* species, these functional groups usually contacted short organs dorsally and less often ventrally. (6) Other dipterans (Syrphidae, Muscidae, and Sarcophaginae) fed only on pollen, landed on the flower, and ventrally touched stigmas and anthers of pin and thrum flowers, respectively (no contact with pin stamens or thrum stigmas). (7) Large bees (≥8 mm), including species of *Andrena*, *Anthidium*, *Anthophora*, *Ceratina*, *Halictus*, *Lasioglossum*, *Megachille*, *Apis mellifera*, *Bombus* cf. *terrestris*, and *Osmia* cf. *rufa* collected pollen most of the time, and nectar occasionally. Bees of this group usually landed on flowers and touched anthers and stigmas from pin and thrum flowers ventrally

and laterally, but not pin stamens or thrum stigmas. (8) Small bees (~4.55 mm, genera *Chelostoma*, *Halictus*, *Lasioglossum*, *Megachille*, and *Ceratina* cf. *cucurbitina*) usually fed on nectar, rarely collected pollen, and visited flowers much like *Usia* species, making contact with *L. suffruticosum* tall and short organs dorsally and ventrally, respectively. (9) Several lepidopterans (Hesperiidae, Lycaenidae, Pieridae, Zygenidae) and *Macroglossum stellatarum* collected nectar and only rarely touched anthers or stigmas with their tongues. (10) Coleopterans (Cerambycidae and Tenebrionidae) consumed pollen, styles, and stigmas.

The analysis across all sites showed that the taxonomic and functional-group compositions of the pollinator community varied significantly across localities (PERMANOVA, $F_{1,40} = 1.983$, $P = 0.02$; $F_{10,40} = 1.224$, $P = 0.04$; respectively). The comparisons of the pollinator community across *L. suffruticosum* localities (e.g., using the observations obtained only for that species) revealed significant differences in the pollinator community according to the taxonomic classification ($F_{1,28} = 1.725$, $P = 0.04$) and marginal differences for functional classification ($F_{10,28} = 1.684$, $P = 0.09$). Comparisons within coflowering localities revealed different patterns. In Triste, *L. suffruticosum* and *L. narbonense* showed differences (although marginal) in the taxonomic classification ($F_{1,15} = 1.934$, $P = 0.09$), stronger than in the functional classification, which had no significant differences ($F_{1,16} = 1.061$, $P = 0.19$). Pollinator importance differed slightly between species: medium *Usia* flies were most important on *L. suffruticosum* (Appendix S5), whereas large *Usia* followed by medium *Usia*, were most important on *L. narbonense* (Appendix S6), with *Usia* flies moving between the two *Linum* species.

PERMANOVA analysis using data from Borau showed that the taxonomic and functional pollinator communities in *L. suffruticosum* and *L. narbonense* were similar ($F_{1,11} = 1.439$, $P = 0.27$ and $F_{6,11} = 1.327$, $P = 0.25$ respectively). Extra-large *U. pubera* was the important pollinator for both *Linum* species, and it moved between them, but this bee fly species' importance value was larger for *L. suffruticosum* than for *L. narbonense* (Appendices S7, S8).

In Oroel, the taxonomic and functional pollinator communities on *L. suffruticosum* differed from those on *L. narbonense* and *L. viscosum* ($F_{1,24} = 2.149$, $P = 0.06$ and $F_{9,24} = 1.635$, $P = 0.01$, respectively). Medium and large *Usia* flies contributed to more than 60% of the visits to *L. suffruticosum* and were the most important pollinators (Appendix S9). In *L. narbonense*, extra-large *U. pubera* flies were the most important pollinators (Appendix S10). Several insects visited *L. viscosum*, but small *Lasioglossum* bees had the largest pollinator-importance value, followed by extra-large *U. pubera* (Appendix S11). In this site, large *Usia* flies moved among the three *Linum* species.

For the Aisa locality, the PERMANOVA analyses revealed that the taxonomic and functional pollinator communities both differed between *L. suffruticosum* and *L. viscosum* ($F_{1,13} = 2.351$, $P = 0.06$ and $F_{5,13} = 3.001$, $P = 0.002$). For *L. suffruticosum*, the pollinator-importance

value was larger for small *Lasioglossum* bees, whose body size is similar to a large *Usia* (Table 1; Appendix S12). Large *Halictus* bees, followed by *Ceratina* cf. *cucurbitina*, were the most important pollinators of *L. viscosum* (Appendix S13). The two *Linum* species shared pollinators species, although pollinator movement between species was not seen. Finally, the observations made at the San Juan de la Peña site showed *Usia* flies making more than 90% of the visits to allopatric *L. suffruticosum*, with medium *Usia* being the most important pollinators (Appendix S14).

Floral measurements and morphological overlap among *Linum* species

Trait mean \pm SD and sample size for each species, morph, and locality are included in Appendix S3. The PCA for pin flowers showed that the first and second axes accounted for 43.38% and 30.03% of the variance, respectively. PC1 captured the heights of sexual organs and sepal length, and they all loaded with positive values (Table 2). PC2 captured herkogamy, flower tube width, and calyx width, which also loaded with positive values. The one-way ANOVA on PC1 and PC2 showed that the three species were significantly differentiated (PC1: Welch's $F_{2,122.73} = 450.35$, $P < 0.001$, all post hoc pairwise comparisons $P < 0.001$; PC2: $F_{2,128.35} = 128.345$, $P < 0.001$, all post hoc pairwise comparisons $P < 0.001$). The results of the PCA for thrum flowers showed that the first and second axes accounted for 51.05% and 23.26% of the variance, respectively. PC1 captured sepal length, anther, and stigma height, all loading with positive values (Table 2). PC2 captured flower tube width, calyx width, and gap, which loaded with positive values. The one-way ANOVA on PC1 and PC2 axis showed that the three species were significantly differentiated (PC1: Welch's $F_{2,114.45} = 536.621$, $P < 0.001$, all post hoc pairwise

TABLE 2 Factor loadings, eigenvalues, and percentage of variance explained by PC1 and PC2 in pin (P) and thrum (T) flowers for *Linum suffruticosum*, *L. narbonense*, and *L. viscosum*.

Floral trait	P flowers		T flowers	
	PC1	PC2	PC1	PC2
Flower diameter	0.69	0.48	0.72	0.45
Flower tube width	-0.36	0.73	-0.42	0.72
Gap	0.46	0.60	0.51	0.62
Sepal length	0.93	-0.23	0.93	-0.20
Calyx width	0.18	0.72	0.28	0.79
Anther height	0.91	-0.28	0.96	-0.23
Style length	0.97	-0.04	0.77	-0.20
Herkogamy	0.04	0.80	0.83	0.02
Eigenvalue	3.47	2.40	4.08	1.86
% of Variance	43.38	30.03	51.05	23.26

comparisons $P < 0.001$; PC2: Welch's $F_{2,140.69} = 195.87$, $P < 0.001$, all post hoc pairwise comparisons $P < 0.001$). The scatterplot of PC1 and PC2 for both pin and thrum flowers shows some overlap between *L. suffruticosum* and *L. viscosum*, while *L. narbonense* was the most differentiated (Figure 1).

The phenotypic-overlap analysis of the gap trait showed statistically significant differences between *L. suffruticosum* and the coflowering *Linum* species (*L. narbonense*: $\chi^2 = 25.299$, $df = 1$, $p < 0.0001$; *L. viscosum*: $\chi^2 = 296.96$, $df = 1$, $p < 0.0001$). Coflowering localities of *L. suffruticosum* with *L. narbonense* had smaller gap values (mean [95% CI]; *L. suffruticosum*: 1.42 [1.06, 1.79], *L. narbonense*: 1.70 [1.32, 2.05]). Similarly, *L. suffruticosum* gap in coflowering localities with *L. viscosum* was also smaller (*L. suffruticosum*: 1.42 [1.19, 1.64], *L. viscosum*: 2.51 [2.27, 2.73]). However, the average difference with *L. narbonense* was ca. 0.2 mm and 1.1 mm with *L. viscosum*. The analysis to estimate differences in phenotypic overlap for tall and short reproductive organs between species in coflowering localities had statistically significant differences for the term species (except for *L. viscosum*), organ height, and the interaction term (Table 3). In general, values of tall and short organs were smaller in *L. suffruticosum* (Appendix S15). The difference with respect to the tall organs of *L. narbonense* was greater (4 mm) than for short organs (ca. 2 mm). The same patterns occurred for the comparison between *L. suffruticosum* and *L. tenue* (average difference for tall organs: 3.5 mm; average difference for short organs: 0.5 mm, all $P < 0.0001$). In contrast, the height of *L. suffruticosum* and *L. viscosum* short organs were similar, and differences occurred only for tall organs (average difference of 1.6 mm, $P < 0.0001$; Appendix S15). Altogether, these results show that the short organs of *L. suffruticosum* are more similar in height to the short organs of the other *Linum* species, while tall organs are more divergent.

Character divergence and ecotypic differentiation in *L. suffruticosum*

The GLM analysis of gaps revealed significant differences among populations ($F_{8,521} = 38.19$, $P < 0.0001$) and between morphs ($F_{2,520} = 26.79$, $P < 0.0001$), but the interaction term population \times morph was not significant ($F_{8,512} = 1.57$, $P = 0.1$). There was a positive association between gap and the body length of the most important pollinator for *L. suffruticosum*, although the relationship was not significant ($F_{1,5} = 1.57$, $P = 0.3$). In contrast, the association between variables was positive and statistically significant when *L. narbonense* and *L. viscosum* data were included in the analysis ($F_{1,10} = 8.69$, $P = 0.01$; Figure 2), with ca. 41% of the variation in gap explained by the variation in the body size of the most-important pollinator on each species, in each locality.

Inaccuracy values of gap and tall and short organs are included in Appendix S16. The comparison of inaccuracy

TABLE 3 Results of the GLMM to determine differences in morphological overlap in organ height for tall (pin stigmas, thrum anthers) and small organs (thrum stigmas, pin anthers) in coflowering localities of *Linum suffruticosum* with *L. narbonense* and *L. viscosum*, and co-occurring localities with *L. tenue*. (See Appendix S2 for the list of localities, Appendix S3 for mean heights of anthers and stigmas of pin and thrum flowers of each species per locality, and Appendix S15 for the estimated average height of tall and small organ per species pair.) For all cases, $df = 1$.

Factor	<i>L. narbonense</i>		<i>L. viscosum</i>		<i>L. tenue</i>	
	χ^2	p	χ^2	p	χ^2	p
(Intercept)	390.92	<0.001	799.99	<0.001	603.511	<0.001
Species (S)	405.1	<0.001	0.72	0.40	21.307	<0.001
Organ height (OH)	2581.96	<0.001	893.65	<0.001	563.613	<0.001
S \times OH	580.93	<0.001	367.47	<0.001	507.461	<0.001

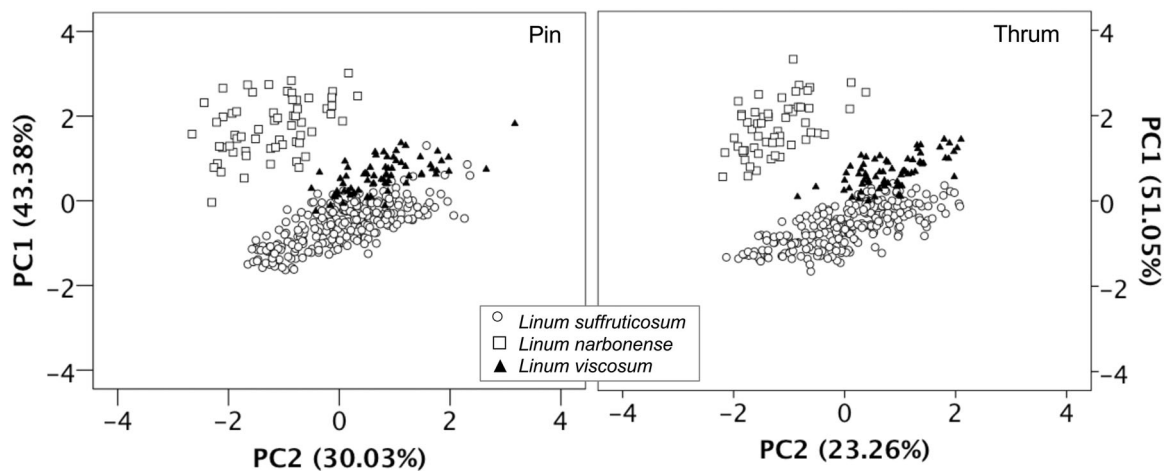


FIGURE 1 Scatterplots of principal component analyses of flower diameter, flower tube width, gap (distance between petal “floor” and stigma or anther), sepal length, calyx width, anther height, stigma height, and herkogamy in pin and thrums flowers, showing the first two principal components (PC1, PC2). The two PCs together explain 73.41% of the total variance in pin and 74.31% in thrums (see Table 3 for factor loadings per floral trait and eigenvalues).

values failed to detect significant differences in gap between locality types ($t = -0.79$, $df = 3.29$, $P = 0.7$ after bootstrapping estimation, mean \pm 95% CI of single-species locality: 11.13 ± 2.92 , coflowering localities: 15.01 ± 15.24) or in tall organs ($t = 0.99$, $df = 5.88$, $P = 0.2$ after bootstrapping estimation, single-species locality: 5.11 ± 3.54 , coflowering localities: 3.68 ± 2.11). In contrast, for short organs, there was a tendency for smaller inaccuracy values in coflowering

localities (2.03 ± 0.93) than in single-species localities (2.82 ± 1.14). However, the differences were only marginally significant ($t = 1.5785$, $df = 6.78$, $P = 0.09$ after bootstrapping estimation).

Experimental assessment of pollen-tube formation after interspecific pollination

More than 1500 stigmas were observed under the microscope (564, 524, and 480 for the assays using *L. tenue*, *L. narbonense*, and *L. viscosum* as heterospecific pollen donors, respectively). Table 4 contains the results of the GLMMs. The results of the three assays revealed similar patterns for the *Linum* species used as pollen donors (Figure 3). For all pollen donors, the probability of pollen-tube formation showed significant effects for the cross-pollination treatment, and for the interaction term cross-pollination treatment \times morph (Figure 3A, C, E). In contrast, differences between morphs only emerged when *L. tenue* acted as the heterospecific pollen donor (Table 4). The analyses using pollen-tube number revealed differences for all the terms of the analysis when *L. tenue* was the heterospecific pollen donor (Figure 3B). In contrast, when the pollen donor was *L. narbonense*, differences appeared for the cross-pollination treatment and the interaction term (Table 4, Figure 3D). When *L. viscosum* acted as a pollen donor, none of the terms was significant (Figure 3F). The post hoc pairwise comparisons revealed that pin and thrum flowers showed similar patterns within treatment for both dependent variables (all P -values > 0.2), with few exceptions (*L. tenue*: lower pollen-tube number in thrum flowers for the treatment Intraspecific (4h), $P = 0.004$, Figure 3B;

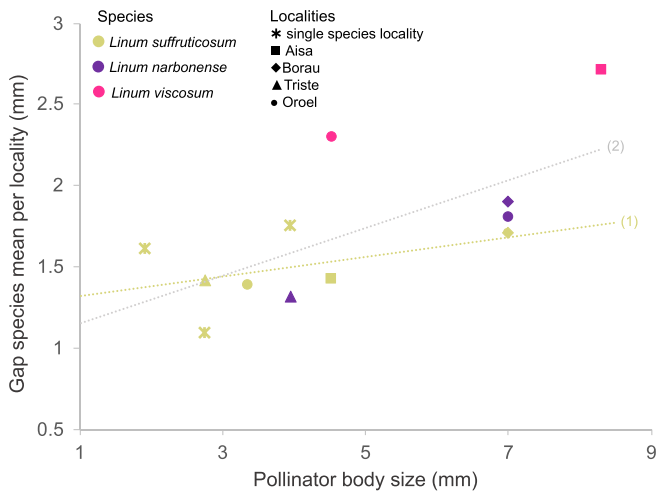


FIGURE 2 Linear relationship between population variation in gap (the minimum distances between the petals and stigmas of the pin morph and between the petals and anthers of the thrum morph; representing the mean gap value per locality and species) and the length of the most important pollinator (pollinator body size) in mm using (1) only *Linum suffruticosum* localities, and (2) adding data for *L. narbonense* and *L. viscosum*.

TABLE 4 Results of the GLMM to test the effect of the hand-pollination treatments using different interspecific pollen donors and determine differences in the probability of observing pollen tubes (Number of pollen tubes in the style/Number pollen grains on the stigma, probability of pollen tube formation) and the number of pollen tubes observed in the style (pollen tube number) under the different pollination treatments (see Material and Methods) in *Linum suffruticosum* stigmas.

Interspecific pollen donor	Probability of pollen tube formation	χ^2	df	p	Pollen tube number	χ^2	df	p
<i>L. tenue</i>	(Intercept)	39.74	1	<0.0001	(Intercept)	178.75	1	<0.0001
	Cross pollination (CT)	49.45	3	<0.0001	Cross pollination (CT)	18.82	3	0.0003
	Morph (M)	4.12	1	0.04	Morph (M)	14.22	1	0.0002
	CT \times M	12.19	3	0.006	CT \times M	11.68	3	0.009
<i>L. narbonense</i>	(Intercept)	125.92	1	<0.0001	(Intercept)	0.47	1	0.49
	Cross pollination (CT)	25.46	3	<0.0001	Cross pollination (CT)	25.90	3	<0.0001
	Morph (M)	0.88	1	0.35	Morph (M)	0.06	1	0.81
	CT \times M	17.61	3	0.0005	CT \times M	11.88	3	0.007
<i>L. viscosum</i>	(Intercept)	246.65	1	<0.0001	(Intercept)	2.09	1	0.15
	Cross pollination (CT)	12.10	3	0.007	Cross pollination (CT)	6.07	3	0.12
	Morph (M)	2.71	1	0.1	Morph (M)	0.58	1	0.45
	CT \times M	8.51	3	0.04	CT \times M	3.76	3	0.29

L. narbonense: lower probability of pollen-tube formation and pollen-tube number in pin for Pollen Mix, $P < 0.001$; Figure 3C, D). The comparisons to test for the effect of receiving a mix of pollen onto stigmas of newly open flowers (comparison Intraspecific against Pollen Mix) showed a decline in the probability of pollen-tube formation in the pin morphs when the pollen donor in Pollen Mix was *L. tenue* ($P < 0.05$, Figure 3A) and in the thrum morph when the pollen donor was *L. viscosum* ($P < 0.05$, Figure 3E). The effect of the sequence of arrival of heterospecific pollen [comparison between the pollination treatments Intraspecific (4h) and Inter + intra (4h)] showed differences for the probability of pollen-tube formation in both morphs and for pollen-tube number in Pin stigmas only when *L. tenue* was the pollen donor ($P < 0.001$, Figure 3A, B). Finally, stigma aging did not affect pollen-tube formation [comparison Intraspecific and Intraspecific (4h)], except for *L. suffruticosum* Thrum flowers from the locality Puerto de las Palomas (decline of pollen-tube formation when pollen was applied 4 h after flower opening, $P = 0.003$, Figure 3B). All these results suggest that the pollen-load identity and the arrival sequence of heterospecific pollen influenced the probability and number of

pollen tubes formed in *L. suffruticosum* stigmas. Nonetheless, the magnitude of the responses depended on the pollen donor and the intrinsic fertility of the *L. suffruticosum* localities under study.

Comparison of pollen deposition and pollen-tube formation in single species and coflowering localities

Appendix S17 includes the sample size, locality mean value and SD of the total number of pollen grains and number of *Linum* pollen grains on the stigmas, % *Linum* pollen grains on the stigma, the probability of pollen-tube formation, and pollen-tube number observed in the styles in the *L. suffruticosum* single-species and localities with coflowering *Linum* species. Stigmas with no pollen grains were excluded from the analyses. Table 5 includes the results of the GLMMs, and Figure 4 represents the estimates for all dependent variables. The two morphs were significantly different only for % *Linum* pollen grains on the stigma, with thrum stigmas having higher percentages than Pin stigmas (Figure 4C, D; Appendix S17). The term locality type

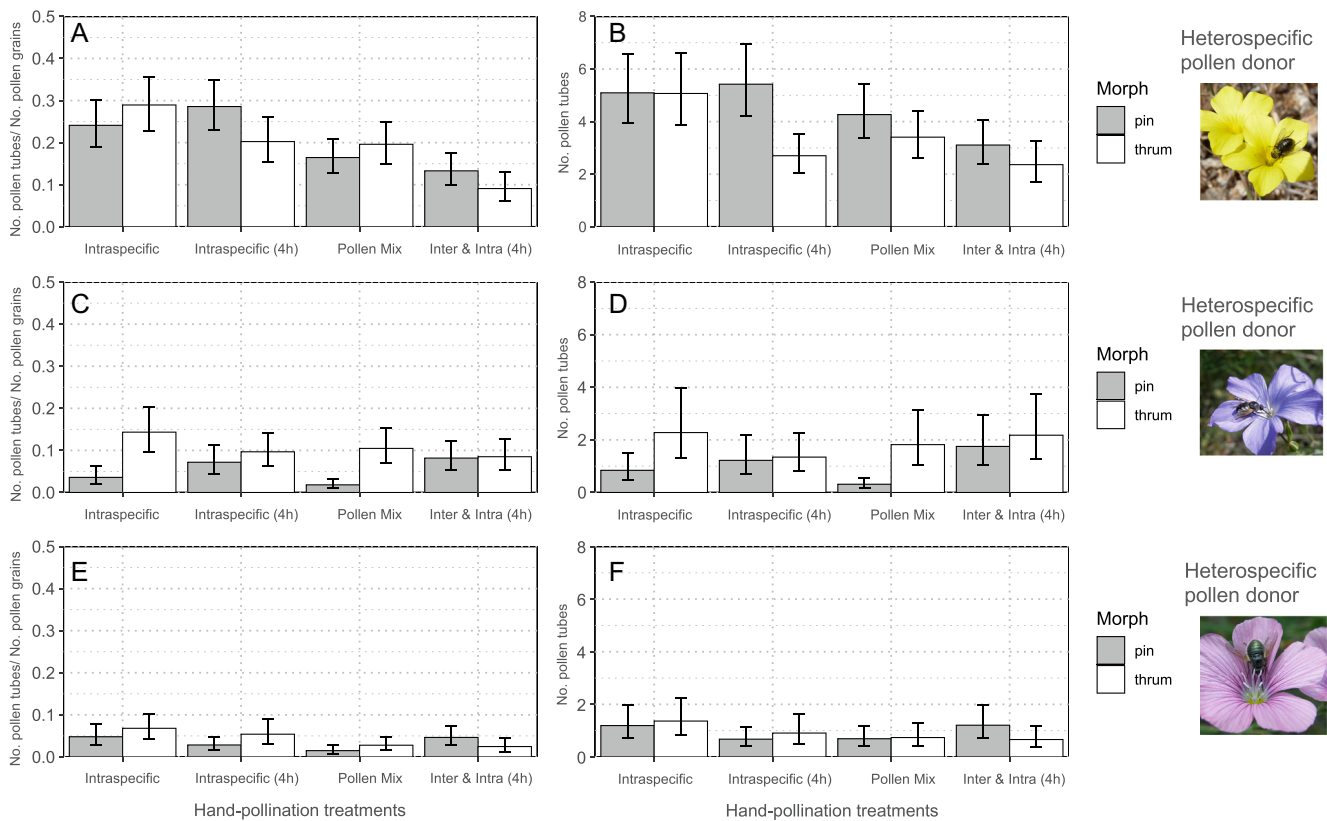


FIGURE 3 Mean and 95% CI of probability of tube formation (No. pollen tubes/No. pollen grains) and pollen-tube number (No. pollen tubes) observed in the styler tissue of *Linum suffruticosum* after experimental hand pollinations using pollen from different species, including intraspecific pollinations in newly open flowers (Intraspecific), 4 h after flower opening [Intraspecific (4)], pollination with a mix of conspecific (*L. suffruticosum*) and heterospecific pollen (Pollen mix), and combining a sequence of interspecific pollination in newly open flowers followed by intraspecific pollination 4 h after flower opening [Inter and intra (4h)]. Left panels: results for the probability of pollen-tube formation; right panels: pollen-tube number. The interspecific pollen donors were (A, B) *L. tenue*, (C, D) *L. narbonense*, and (E, F) *L. viscosum*.

TABLE 5 Results of the GLMM to investigate differences in stigmas of *Linum suffruticosum* morphs (pin and thrum) from different localities (single species vs. coflowering locality) in two regions (SW Spain and Central Pyrenees) in the number of *Linum* pollen grains on the stigmas, the % *Linum* pollen grains, the probability of pollen tube formation, and pollen tube number under natural conditions of open pollination (in all cases, the degrees of freedom were 1).

Factor	No. of <i>Linum</i> pollen grains		% <i>Linum</i> pollen grains		Probability of pollen tube formation		Pollen tube number	
	χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>
(Intercept)	354.54	<0.001	32.41	<0.001	158.00	<0.001	8.69	0.003
Morph (M)	2.09	0.15	16.48	<0.001	0.50	0.40	0.58	0.45
Locality type (L)	3.87	0.05	2.10	0.15	5.86	0.02	5.82	0.01
Region (R)	3.04	0.08	34.87	<0.001	13.04	0.003	8.03	0.005
M × L	4.35	0.03	0.61	0.43	0.02	0.89	1.23	0.27
M × R	0.01	0.91	7.58	0.006	0.31	0.58	0.15	0.70
L × R	0.72	0.40	0.01	0.92	2.47	0.12	1.95	0.16
M × L × R	1.55	0.21	2.35	0.13	1.20	0.27	2.26	0.13

showed significant differences for number of *Linum* pollen grains on the stigmas (Figure 4A, B), probability of pollen-tube formation (Figure 4E, F), and pollen-tube number (Figure 4G, H). In all cases, single-species localities displayed larger values for these three variables than did coflowering localities. The term region showed significant differences for all dependent variables, all pollination variables being consistently larger in SW than in CP. The interaction term morph × locality type was significant only for the number of *Linum* pollen grains on the stigma (Figure 4A, B). The post hoc pairwise comparisons showed that both pin and thrum stigmas received similar quantities of *Linum* pollen in single-species and coflowering localities (for both, $P > 0.1$), but the differences between morphs were larger in coflowering localities ($P < 0.0001$) than in single-species localities ($P = 0.06$). The significant interaction term morph × region, for the variable % *Linum* pollen on the stigma, was the result of both morphs exhibiting larger % of *Linum* pollen in SW than CP, but the difference between morphs was larger in CP than SW (all $P < 0.0001$, see Appendix S17). Within the region, differences between morphs for % *Linum* pollen on the stigma were significant in CP (thrum stigmas had larger values than pin stigmas, $P < 0.0001$) but not in SW ($P = 0.12$).

DISCUSSION

Variation in pollinator communities, pollinator importance, and pollinator partitioning

Our study confirmed previous observations of pollinators on *L. suffruticosum* (Armbruster et al., 2006). Several species of *Usia* bee flies were the most important pollinators in most localities, and the body sizes of the flies varied substantially intra- and interspecifically. The same *Usia* species also visited

sympatric *L. narbonense* and *L. viscosum*, although bee visitation was relatively more important for *L. viscosum*. The comparison of the pollinator communities across localities revealed different patterns from pollinator functional-group and taxonomic perspectives. Similar differences have been found in other Mediterranean species in the Iberian Peninsula (Herrera, 1988; Gómez et al., 2007, 2014). The diversity of visitors agrees with reports on other *Linum* species (Kearns, 1992; Simbaña and Tye, 2009; Seçmen et al., 2010; Harris and Koptur, 2022; Foroozani et al., 2023), although *L. pubescens* was visited almost entirely by *Usia* flies (Johnson and Dafni, 1998; Lebel et al., 2018). In our study, comparing floral visitors between *Linum* species from coflowering localities provided useful information about pollinator sharing and the potential for pollen transfer between species. In localities with coflowering *Linum* species, *Usia* flies, and other shared pollinators regularly moved between *Linum* species. Localities with coflowering *L. narbonense* revealed weak pollinator-community differences between the two *Linum* species, as measured by both the frequency of visitation and the pollinator importance (Armbruster, 1988; Aigner, 2001; Mayfield et al., 2001). In contrast, localities with coflowering *L. viscosum* showed substantial differences between *Linum* species at both taxonomic and functional-group levels. We were unable to obtain systematic observations on *L. tenue*. Foroozani et al. (2023) recently reported visits of *Usia cf. pusilla* and small *Lasioglossum* visiting *L. tenue* flowers in populations near the ones used in the present study in SW Spain. These authors observed similar insect behaviors as seen in *L. suffruticosum* (landing on the petal and crawling down the tube to obtain nectar). Our casual observations in *L. tenue* are consistent with these reports. Altogether, the pollinator observations revealed that, although pollinator importance differed among *Linum* species, pollinator partitioning was only partial, with substantial pollinator sharing and visitor movement between *Linum* species.

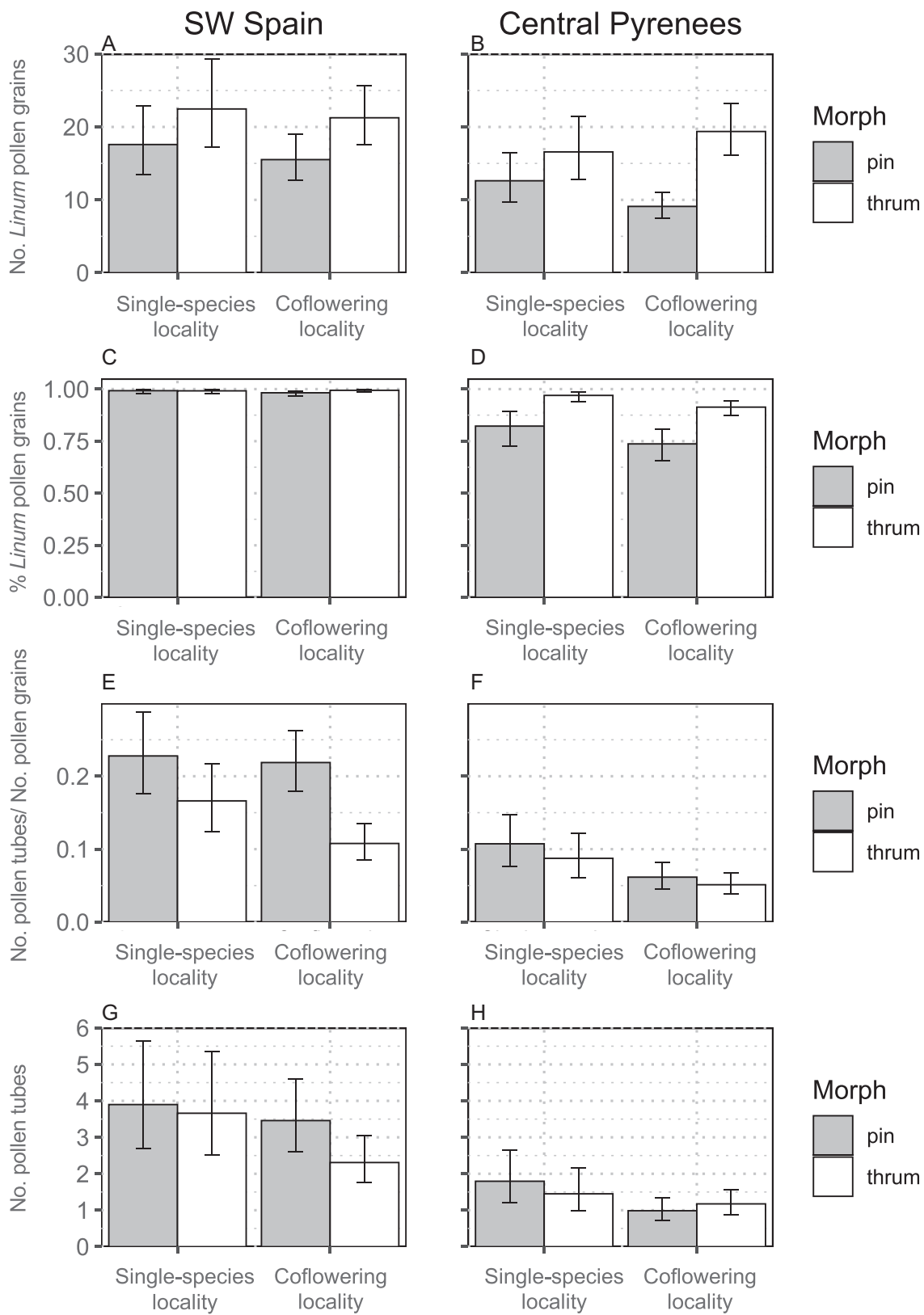


FIGURE 4 Mean and 95% CI of the number of *Linum* pollen grains (No. *Linum* grains) and proportion of *Linum* pollen grains (% *Linum* pollen grains) observed on the stigmas, probability of tube formation (No. pollen tubes/No. pollen grains) and pollen-tube number (No. pollen tubes) of flowers collected after one day of natural pollination, in single-species localities (localities with only *Linum suffruticosum*) and coflowering localities (localities where other *Linum* species were found flowering with *L. suffruticosum*) in southwestern (SW) Spain (panels A, C, E, G) and in the Central Pyrenees in Aragón (Spain) (panels B, D, F, H).

Patterns of phenotypic variation of floral traits and implications for pollen transfer between species

The PCA revealed that *Linum* species differ substantially from one another in floral morphology. For both morphs, anther height, stigma height, and sepal length explained the main differences among species, followed by variation in gap and calyx width. Univariate comparisons in gap (a proxy for pollinator fit) and the height of tall and short reproductive organs using the data from coflowering localities (or co-occurring for *L. tenue*) revealed interesting patterns. *Linum suffruticosum* had the lowest gap value, but the difference from other species was small (0.2 mm difference from *L. narbonense* and 1.1 mm difference from *L. viscosum*). The difference in height between *L. suffruticosum* tall organs with coflowering *Linum* was important (differences ranging between 4 mm and 1.6 mm). In contrast, the differences in height for short organs were negligible, particularly for the comparison with *L. tenue* (0.5 mm) and *L. viscosum* (no difference). If coflowering species have similar gap values, they could use the same pollinator size with respect to flower fit, which was observed in the coflowering localities with *L. narbonense*. Likewise, small differences in the height of reproductive organs could lead to pollen flow between species if they share individual pollinators. The heights of short organs (pin anthers and thrum stigmas) of *L. suffruticosum* were similar to those of *L. viscosum*, and, to a lesser degree, *L. tenue* and *L. narbonense*. The low pollinator sharing between *L. suffruticosum* and *L. viscosum* should limit interspecific pollination. In contrast, in the other two species, pollinator sharing was substantial, so interspecific pollen transfer could occur if differences in organ height do not translate into different pollen placements on the body of the pollinator (Murcia and Feinsinger, 1996; Massinga et al., 2005; Pérez-Barrales et al., 2006; Raupp et al., 2020; Furtado et al., 2023 [in this issue]). Unfortunately, these data are not yet available for *Linum*. But the observations of anther contact with the body of pollinators, particularly *Usia*, suggests that the pollen of *L. narbonense* and *L. viscosum* (and possibly *L. tenue*, Foorozani et al., 2023) is placed dorsally for pin and thrum flowers, and less likely ventrally. This nototribic contact probably occurs because the tall and short reproductive organs of these species are at the center of the flower (Appendix S1). In contrast, the morphology of *L. suffruticosum* reproductive organs (short organs pressed against the petals facing toward the center of the flower, tall organs at the center of the flower and facing outward, Appendix S1) allows *Usia* flies to contact *L. suffruticosum* tall organs dorsally and short organs ventrally. Therefore, *L. suffruticosum* pin stigmas probably pick up more heterospecific pollen than do thrum stigmas (Tong and Huang, 2016), while thrum stigmas receive more conspecific pollen from pin anthers (i.e., disassortative pollen transfer). The different pollen placement on the body of pollinators (nototribic for the tall organs of all species and for the short organs of *L. narbonense*, *L. viscosum*, and *L. tenue*; sternotribic for the

short organs of *L. suffruticosum*) should contribute to the segregation of pollen flow by species. Testing this hypothesis requires future research.

Analyses of phenotypic variation in tall and short organs of other distylous species have revealed that intraspecific overlap in positions of compatible organs (reciprocity) is generally higher than the corresponding positional overlap between species, presumably reducing pollen flow between sympatric species (Keller et al., 2012; Cardoso et al., 2022). Positional overlap between compatible reproductive organs of different *Primula* species has been shown to result in more interspecific pollen movement, contributing to the formation of hybrids (Kálmán et al., 2004; Keller et al., 2016). Observations of pollen placement on the bodies of pollinators and contact with reproductive organs suggest that morphological differences in floral traits can substantially reduce (but not completely prevent) interspecific pollen transfer (Armbruster et al., 1994, 2014; Muchhala and Thomson, 2012; Tong and Huang, 2018; Newman and Anderson, 2020; Moreira-Hernández et al., 2023 [in this issue]). Thus, species differences in positions of “compatible” organs probably do not, by themselves, generate reproductive isolation. Instead, they can substantially increase reproductive fitness when related species occur sympatrically and share individual pollinators (Armbruster and Muchhala, 2009; Armbruster et al., 2014). The study of pollen placement on pollinators, in combination with assessing variation in organ height and gap, will help to understand the consequences of trait differences and similarities for plant fitness in *Linum* species regularly occurring in sympatry.

Character divergence and ecotypic differentiation in *L. suffruticosum*

Gap differences between *L. suffruticosum* populations in SW Spain have been interpreted as reflecting local adaptation to the most effective pollinator, in terms of flower fit with pollinators and their contact with anthers and stigmas (Armbruster et al., 2006, 2009). The present study allowed us to assess this hypothesis using a larger number of study populations. In doing so, we found that, in coflowering localities, *L. suffruticosum* can experience reproductive interference by sharing individual pollinators with other *Linum* species. The relationship between gap size in *L. suffruticosum* populations and the size of the most important local pollinator was positive, but not statistically significant, probably because of the small sample size. The addition of *L. narbonense* and *L. viscosum* data strengthened the relationship, by increasing the sample size and allowing us to explore a wider range of trait values and pollinator sizes. These results need to be interpreted cautiously, but the positive association between a trait involved in the fit with pollinators (gap) and the size of the most important local pollinator suggests that the variation in gap (intra- and interspecifically) is consistent with the formation of pollination ecotypes, as described in other species (Armbruster, 1985; Armbruster

et al., 1994; Johnson, 1997; Pérez-Barrales et al., 2007; Anderson and Johnson, 2008; Cosacov et al., 2014; Newman et al., 2014; Newman and Anderson, 2020). What remains to be tested is whether reproductive interference from sympatric congeners determines ecotype formation (Armbruster, 1985) or whether apparent ecotypic variation emerges through other mechanisms (Armbruster et al., 1994; Eaton et al., 2012; Eisen and Geber, 2018).

At present, we lack experimental data to test the hypothesis of ecotype formation in response to reproductive interference in *Linum*. However, the morphological analyses using adaptive-inaccuracy estimates may help us to address this hypothesis. Adaptive inaccuracy estimates the phenotypic load (maladaptation) that results from the morphological departure from the phenotypic optimum of a population (Armbruster et al., 2004, 2009). Therefore, we can assume that natural selection will favor phenotypes with a morphology different from that of coflowering species because a similar morphology would lead to pollen misplacement and loss from the pollen pool (decreasing the male component of fitness). We would also determine the arrival of heterospecific pollen on stigmas potentially reducing seed set (decreasing the female component of fitness; Armbruster et al., 1994, 2014; Muchhala and Thomson, 2012; Tong and Huang, 2018; Moreira-Hernández et al., 2023 [in this issue]). Hence, lower inaccuracy values for pollinator fit or reciprocity of tall and short organs in coflowering localities would represent indirect evidence of character displacement. The inaccuracy values for gap and tall organs were similar between single-species and coflowering localities of *L. suffruticosum*. In contrast, inaccuracy values were lower for short-organ reciprocity in coflowering localities. These results must also be interpreted cautiously, but the fact that the height of the short reproductive organs of *L. suffruticosum* was close to the short organs of other coflowering *Linum* species suggests that thrum stigmas and pin anthers could experience interspecific pollen transfer and consequent reproductive interference. Reproductive interference could lead to lower inaccuracy and higher reciprocity, as observed in the short reproductive organs in coflowering localities. The current reports for heterostylous species show that there is a tendency of lower inaccuracy values for short organs, a pattern attributed to developmental variation, which is often smaller in short organs (Faivre, 2000; Faivre and Mcdade, 2001; Armbruster et al., 2017; Jacquemyn et al., 2018; Matias et al., 2020; Furtado et al., 2021; Foroozani et al., 2023). In the future, the combination of flower morphology data and pollen transfer rates from more populations will help in testing these hypotheses.

Quantifying the costs of interspecific pollen transfer and implications for pollen-tube formation in controlled and natural conditions in *L. suffruticosum* localities

Experimental hand pollinations are a practical approach to measuring the female reproductive costs of receiving pollen

from other species. When the pollen grains of different species cannot be distinguished by their features, experimental pollination can help in interpreting data obtained under natural conditions. In the present study, hand-pollination experiments revealed the presence of post-pollination reproductive isolation between species, because heterospecific pollen-tube growth was halted soon after its initiation and pollen did not form pollen tubes in the stylar tissue. These observations were similar to the incompatibility reaction observed after self-pollination, so the incompatibility system in *L. suffruticosum* may adaptively preclude pollen-tube formation after interspecific pollinations (Grant, 1969; Broz and Bedinger, 2021). The lack of pollen-tube formation could also be a non-adaptive consequence of species divergence in that none of the interacting species are closely related or belong to the same subclade (Ruiz-Martin et al., 2018, but see Zou et al., 2022). Our surveys did not reveal obvious hybrids, although hybrids between *Linum* species have been obtained experimentally (Ockendon, 1968; Seetharam, 1972). However, reproductive isolation gained through the action of the incompatibility system does not alleviate the fitness costs of pollen misplacement or interspecific pollen transfer to stigmas (Muchhala et al., 2010; Moreira-Hernández et al., 2019). Our experiments revealed that, for all heterospecific pollen donors, the probability of pollen-tube formation decreased when stigmas received a mix of intra- and interspecific pollen, as opposed to purely conspecific pollen (Arceo-Gómez and Ashman, 2011; Celaya et al., 2015). In contrast, the sequential pollen arrival (interspecific followed by intraspecific) reduced the probability of pollen-tube formation only when *L. tenue* was the interspecific pollen donor (see also Armbruster and Herzig, 1984; Bruckman and Campbell, 2016; Coetzee et al., 2020). The proportion of intraspecific pollen that stigmas receive might determine the magnitude of effects (assuming that the mixed treatment included 50% pollen of each species; see Moreira-Hernández et al., 2019). However, the different responses regarding the sequence of arrival observed in the localities used for the experiment point to differences in the tolerance of heterospecific pollen on stigmas. Interestingly, the *L. suffruticosum* locality where *L. tenue* was used as pollen donor is a single-species locality; these plants experienced a stronger reduction of conspecific-pollen performance than did plants from coflowering localities, which were pollinated with *L. narbonense* and *L. viscosum* pollen (see Material and Methods, Appendix S2). Variation in the tolerance to interspecific pollen based on a previous history of pollen exposure has been identified as an adaptive mechanism to reduce reproductive costs (Grant, 1966; Arceo-Gómez et al., 2016; Fang et al., 2019; Moreira-Hernández et al., 2019; Streher et al., 2020; Hao et al., 2023 [in this issue]).

The hand-pollination experiment provided a baseline to interpret pollen–pistil interactions in natural conditions in single-species vs. coflowering localities. Pollen-tube growth in the styles of *L. suffruticosum* occurred after intraspecific, disassortative (between-morph) pollination

and not from pollination with other pollen sources (but see Faivre, 2000). This result is critical for interpreting pollination in natural conditions, because it was not possible to distinguish the pollen from different *Linum* species (or morphs) in natural conditions. Notably, fertility differed between the *L. suffruticosum* localities used in the hand-pollination experiments (more pollen tubes formed after intraspecific pollination in the locality in SW Spain than in the Central Pyrenees; Figure 3). These differences might reflect regional differences in fertility since the average pollen-tube numbers in natural and experimental conditions were similar (an average of four and two pollen tubes per style in SW Spain and Central Pyrenees localities, respectively; Figures 3B, 3D, 3F, 4G, 4H). Despite the different pollen tube number between regions, there were significant regional differences in the pollen arrival on the stigmas and pollen-tube formation in natural conditions. The SW Spain localities received more *Linum* pollen and at higher rates than those in the Central Pyrenees. These regional differences ought to reflect variation in the composition and activity levels of the pollinator community, e.g., more diverse and generalized in the Central Pyrenees and more specialized in SW Spain (Armbruster et al., 2006). Comparisons of pollination between locality types revealed that single-species localities received more *Linum* pollen and had a higher probability of pollen-tube formation than coflowering localities. More diverse pollinator communities and pollinator sharing in coflowering localities may lead to higher rates of interspecific pollen (from coflowering *Linum* and other species), compromising pollen-tube formation by conspecific pollen (Bergamo et al., 2017; Streher et al., 2020). Altogether, our data appear to show a correlation between the variation between pollinator communities and the rates of intraspecific (intermorph) pollen arrival and pollen-tube formation at multiple scales (regional differences and between locality types). It remains unknown whether this scenario creates opportunities for microgametophytic competition and selection of traits affecting fit with pollinators and pollen pickup and deposition (Herrera, 2004; Bolstad et al., 2010; Pérez-Barrales et al., 2013; Castilla et al., 2016; Harder et al., 2016; Opedal et al., 2016; Mazer et al., 2018; Christopher et al., 2020; Albertsen et al., 2021; Furtado et al., 2021).

CONCLUSIONS

The present work confirmed the previous description of the tight relationship between *Usia* bee flies and *L. suffruticosum* (Armbruster et al., 2006), but also in other coflowering *Linum* in sympatric localities, with different levels of pollinator sharing. Importantly, the variation in *L. suffruticosum* traits involved in fit and pollen pickup and deposition had different magnitudes of phenotypic overlap with other coflowering *Linum*. The pollinator observations and flower-trait variation suggest that interspecific pollen

transfer could create reproductive interference, which might drive trait evolution and divergence. While *L. suffruticosum* incompatibility system appeared to maintain reproductive isolation between species, the combination of hand pollinations and observation of pollen tubes in conditions points to costs associated with interspecific pollination. Altogether, results using the different data sets suggest that *L. suffruticosum* population differentiation in traits directly affecting pollen transfer evolved to reduce interspecific pollination, giving rise to pollination ecotypes. However, other mechanisms might also, or instead, generate the patterns observed. For example, it remains unknown whether flower color can determine pollinator preference and visitation (Villalvazo-Hernández et al., 2022) or whether species display differences in flowering phenology that could reduce the costs associated with pollinator sharing (Waser and Real, 1979; Armbruster, 1985; Stone et al., 1998). Also, historical effects and ecological sorting could explain contemporary trait variation (Armbruster et al., 1994; Eaton et al., 2012; Eisen and Geber, 2018). Future work should incorporate these sources of variation along with measurements of the efficiency of different pollinators and reproductive costs to tease apart the ultimate causes of trait differentiation and ecotype formation in *L. suffruticosum*.

AUTHOR CONTRIBUTIONS

Both authors conceptualized the study and collected data in the field. R.P.B. performed the lab work, analyzed the data, and wrote the original manuscript. W.S.A. advised on adaptive inaccuracy analyses and reviewed and edited the manuscript.

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DATA AVAILABILITY STATEMENT

Raw data related to this work are available at Zenodo: <https://doi.org/10.5281/zenodo.7897527>.

ORCID

Rocío Pérez-Barrales  <http://orcid.org/0000-0002-2472-2214>

W. Scott Armbruster  <http://orcid.org/0000-0001-8057-4116>

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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. *Linum* species included in the study (thrum, left frontal image and pin, right frontal image) and floral traits measured.

Appendix S2. *Linum suffruticosum* localities used in the study.

Appendix S3. Sample size, mean, and standard deviation of floral traits from pin (P) and thrum (T) flowers of *Linum suffruticosum*, *L. narbonense*, and *L. viscosum* localities.

Appendix S4. Description of the incompatibility system and the location of the incompatibility reaction in *Linum suffruticosum*.

Appendix S5. Pollinators of *Linum suffruticosum* at the Triste site and number of visits, probability of anther and stigma contact for pin (P) and thrum (T) flowers, visitation rate, relative frequency of visits, mean probability of contact, and most important pollinator.

Appendix S6. Pollinator assemblage in *Linum narbonense* at Triste site, including number of visits, probability of

anther and stigma contact for pin (P) and thrum (T) flowers, visitation rate, relative frequency of visits, mean probability of contact, and most important pollinator.

Appendix S7. Pollinator assemblage in *Linum suffruticosum* at Borau site, including number of visits, probability of anther and stigma contact for pin (P) and thrum (T) flowers, visitation rate, relative frequency of visits, mean probability of contact, and most important pollinator.

Appendix S8. Pollinator assemblage in *Linum narbonense* at Borau site, including number of visits, probability of anther and stigma contact for P and T flowers, visitation rate, relative frequency of visits, mean probability of contact, and most important pollinator.

Appendix S9. Pollinator assemblage in *Linum suffruticosum* at Oroel site, including number of visits, probability of anther and stigma contact for pin (P) and thrum (T) flowers, visitation rate, relative frequency of visits, mean probability of contact, and most important pollinator.

Appendix S10. Pollinator assemblage in *Linum narbonense* at Oroel site, including number of visits, probability of anther and stigma contact for pin (P) and thrum (T) flowers, visitation rate, relative frequency of visits, mean probability of contact, and most important pollinator.

Appendix S11. Pollinator assemblage in *Linum viscosum* at Oroel site, including number of visits, probability of anther and stigma contact for pin (P) and thrum (T) flowers, visitation rate, relative frequency of visits, mean probability of contact, and most important pollinator.

Appendix S12. Pollinator assemblage in *Linum suffruticosum* at Aisa site, including number of visits, probability of anther and stigma contact for pin (P) and thrum (T) flowers, visitation rate, relative frequency of visits, mean probability of contact, and most important pollinator.

Appendix S13. Pollinator assemblage in *Linum viscosum* at Aisa site, including number of visits, probability of anther and stigma contact for pin (P) and thrum (T) flowers, visitation rate, relative frequency of visits, mean probability of contact, and most important pollinator.

Appendix S14. Pollinator assemblage in *Linum suffruticosum* at San Juan de la Peña site, including number of visits, probability of anther and stigma contact for pin (P) and thrum (T) flowers, visitation rate, relative frequency of visits, mean probability of contact, and most important pollinator.

Appendix S15. Mean value and 95% CI of tall organs (pin stigmas, thrum anthers) and short organs (pin anthers and thrum stigmas) in coflowering localities of *Linum suffruticosum* with *L. narbonense* and *L. viscosum*, and co-occurring localities with *L. tenue*.

Appendix S16. Mean² standardized inaccuracy values for gap, tall organs (pin stigmas and thrum anthers) and small organs (thrum stigmas and pin anthers).

Appendix S17. Sample size (N), mean values and standard deviation (SD) of the number of pollen grains, number of *Linum* grains on the stigmas, % *Linum* pollen grains on the stigmas, probability of pollen-tube formation and pollen tube number observed in the style of *L. suffruticosum*, in single species localities and coflowering localities after 1 day of exposure to natural pollination conditions in the SW Spain and the Central Pyrenees.

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