



Facilitated fecundity in sand flax: Pollination in an endangered herb of pine rocklands

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ABSTRACT

Reproductive success in flowering plants depends on floral biology, breeding system, and availability of compatible mates and pollinators. Here, we examine the pollination biology of *Linum arenicola* (Small) H.J.P Winkler (Linaceae), sand flax, a federally endangered herb of pine rocklands in the Lower Florida Keys (USA). We observed the timing of floral anthesis, fruiting, florivory, and frequency of flower visits by insects. We compared fruiting in flowers that were open-pollinated, bagged to exclude floral visitors, and self-pollinated by hand to determine whether pollinator visits enhance or are necessary for reproduction. Fruit set was equally high between flowers open to pollinators and those that were hand-pollinated, but both were significantly higher compared to flowers from which pollinators were excluded. We observed that the wilting of petals late in the day in unvisited flowers often initiates self-pollination, but flowers are frequently visited by small bees and flies earlier in the day, suggesting that early day visits by those insects increase pollen deposition and potential for outcrossing. Despite high fruit set from autonomous self-pollination and occasional flower visitation, sexual reproduction is limited by herbivores: nearly half of all flowers produced were consumed by Key deer and galling midges. Optimum fruit set is not achieved from autonomous selfing alone, and pollinators play an important role in fruit production of the rare sand flax. Conservation efforts that enhance populations of suitable pollinators could increase the likelihood of fruit set from a diversity of pollen parents in rare plants like the endangered sand flax.

1. Introduction

Pollination and breeding systems of flowering plants determine the quantity and quality of offspring. Yet, how reproduction occurs is unknown for many species of conservation concern. Most flowering plants benefit from animal-mediated pollination to produce seed (Mitchell et al., 2009; Ollerton et al., 2011) but have varying degrees of dependency on this relationship. In the genus *Linum* (Linaceae), reproductive modes, including floral traits associated with pollination, are diverse (Murray, 1986). Many *Linum* species are self-incompatible and heterostylous (Kearns and Inouye, 1994; Lewis, 1943). Other *Linum* species are homostylous and self-compatible, and some rely on autonomous self-pollination and fertilization (Jahnke and Etterson, 2019; Uno, 1984). New types of heteromorphism are still being discovered in the genus (Armbruster et al. 2006).

Floral traits and breeding systems are diverse but appear to be linked in *Linum*. The most well-known and well-studied species,

L. usitatissimum, is a homostylous and self-pollinating (Williams et al., 1990) annual herb that is cultivated for its fibers and oil-bearing seeds since Neolithic times (Heywood and Zohary, 1995). Similarly, the homostylous and autonomous annual herb, *L. sulcatum*, produces fruits only from self-pollen (Jahnke and Etterson, 2019). However, heterostyly and heteromorphic incompatibility are also commonly linked, especially in the Mediterranean basin and South Africa (Ruiz-Martín et al., 2018; McDill et al., 2009). In the European *Linum perenne* group, most species are heterostylous and self-sterile, whereas a single homostylous species in this group, *L. leonii*, is self-fertile (Ockendon, 1968).

Despite extensive experimental research on breeding systems within *Linum*, contributions of pollinators to reproduction are rarely examined. One exception is the apparent pollination by flies in the self-incompatible and heterostylous *Linum lewisii* (Kearns and Inouye, 1994). Autonomously self-pollinating plants may also benefit from pollinators if visits lead to higher conspecific pollen loads (Kalisz and Vogler, 2003). Indeed, autonomously selfing plants are frequently

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pollen-limited (Martén-Rodríguez and Fenster, 2010). Mixed mating systems, in which flower visitors are not required, may provide reproductive assurance when pollinators are scarce (Kalisz et al., 2004). In such systems, pollinator visits can provide opportunities for either cross-pollination, facilitated within-flower selfing, or geitonogamous selfing (Goodwillie et al., 2005).

Allee-effects (low abundance of mates or effective pollinators) are thought to be strong selective forces in transitioning from self-incompatible to selfing lineages (Kalisz and Vogler, 2003). As flowering plant species become increasingly rare, reproduction by selfing may prevail (Eckert et al., 2010). Autonomous selfing is particularly common among early colonizing plants that typically occur in low abundance and outside the range of their pollinators (Baker, 1955; Grossenbacher et al., 2017). Selfing has also been consistently observed in island species (Barrett, 1985; Martén-Rodríguez et al., 2015). Comparative studies of breeding systems including island and mainland plants indeed suggest that self-compatibility occurs at higher frequencies on islands, 66%, compared to 41% of mainland species (Grossenbacher et al., 2017).

We examined the pollination system of the two extant island populations of the endangered sand flax, *Linum arenicola* (Small) H.J.P Winkler. With the exception of seed germination studies (Pérez and Chuman, 2020) and taxonomic research (Rogers, 1963), its reproductive biology has not been studied. *Linum arenicola* is homostylous and exhibits floral traits that suggest self-compatibility in the genus, although open flowers are occasionally visited by pollinators (B. Harris personal observation). We previously observed that corollas, stamens, and styles of open flowers appeared to lose turgor pressure late in the day and intertwine. Some flower petals neatly abscised earlier in the day, but petals were persistent in many plants with mature fruits (B. Harris, personal observation). Persistent petals (Uno, 1984), and ‘petal scraping’ (Jahnke and Etterson, 2019) are known to facilitate autonomous self-pollination in some species of *Linum*; we therefore expected to find a similar situation in *L. arenicola*.

Here, we conducted field observations to discover salient details of the floral biology and reproductive success with and without visitors. Our aim was to understand what proportion of fruit set results from open-pollination by insects, autonomous self-pollination without insects, and self-pollination by hand. Further, herbivory damage to flowers (florivory) can impede pre-dispersal seed production, negating benefits conferred by pollinators. Studying the floral biology and breeding system concurrently with pollinator interactions and florivory allows for a better understanding of how and when reproduction likely occurs. Considering the pervasiveness of selfing in island taxa (Grossenbacher et al., 2017) and the consistent links between homostyly and self-pollination across the genus (Murray, 1986), we predict that the island populations of *L. arenicola* are self-compatible but may benefit from pollinator visitation.

2. Methods

2.1. Study sites

Our study was conducted from 2015 to 2016 in the Lower Florida Keys, USA (Fig. 1). Study sites were chosen where islands contained patches with at least 30 individuals within a 25 m radius. Because this species is rare on the islands, we were somewhat limited in our study design. We used published field surveys (Hodges and Bradley, 2006), expert knowledge from US Fish and Wildlife Service staff, and our own searches to locate *L. arenicola* populations. Five sites qualified for inclusion in our study: four on Big Pine Key within National Key Deer Refuge, and one on Lower Sugar Loaf Key adjacent to Florida Fish and Wildlife Conservation Commission land (Fig. 1).

On Big Pine Key, all sample sites were within the fire-dependent pine rockland ecosystem. The highest abundance of *L. arenicola* plants occurred on a fire-break trail, an area cleared of trees and shrubs. The second and third sites were inland and far from any roads, and the fourth

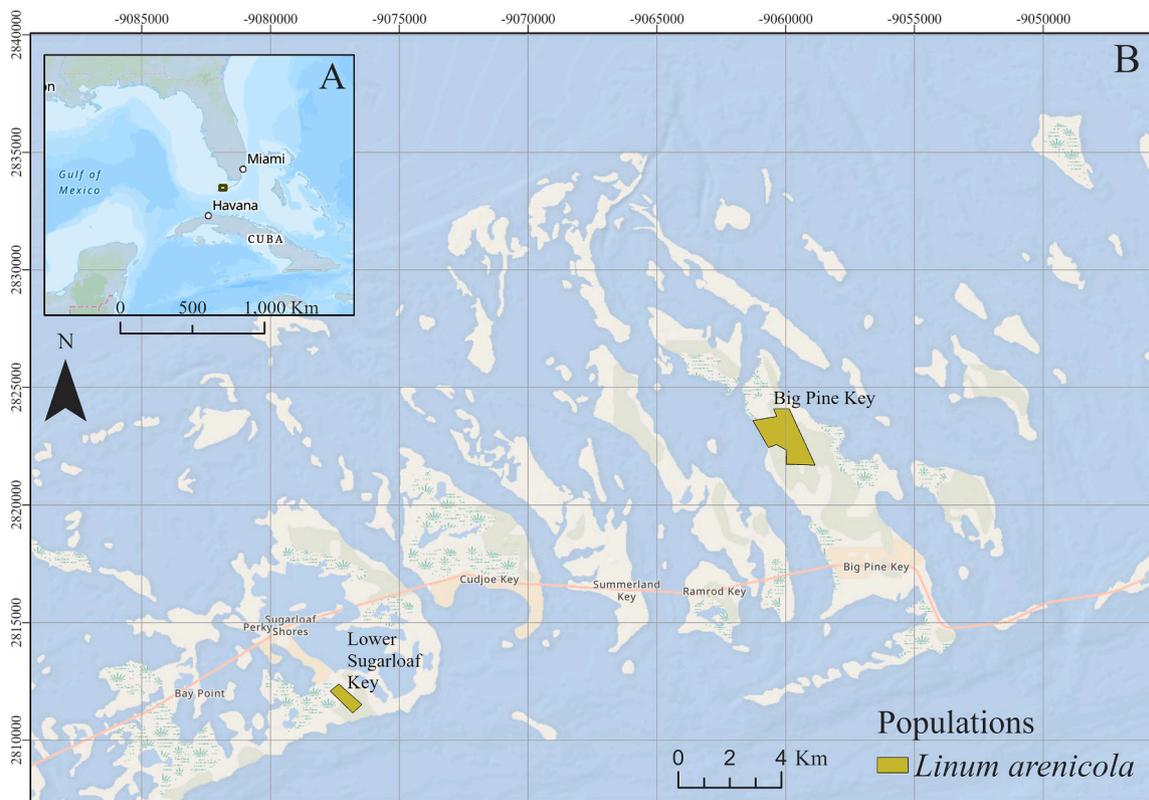


Fig. 1. Map of study sites of *Linum arenicola*. (A) Location of Lower Florida Keys. (B) General locations of sites on Big Pine Key and Lower Sugarloaf Key (Exact locations obscured to protect illegal collecting of endangered species).

was at the entrance of a residential subdivision that is sporadically mowed. On Big Pine Key, the substrate in all sites is naturally exposed oolitic limestone rock characteristic of the pine rocklands in the Lower Keys (Snyder et al., 1990). The fifth site was on an adjacent island, Lower Sugarloaf Key; though forested lands on this island are not managed as they are on Big Pine Key, this sand flax population occurred on limestone gravel along a roadside that was very sparsely vegetated and mowed once annually.

2.2. Study species

The genus *Linum* (Linaceae) has approximately 200–230 species (Bailey et al., 1976); 11 species are recognized in the state of Florida (Wunderlin et al., 2022). *Linum arenicola*, sand flax, is a thin grass-like perennial herb between 35 and 53 cm tall with one or several stems arising from the base, some of which are branching (Bradley and Gann, 1999; FNAI, 2000). Each stem has one to several flowers, each borne on pedicels 2 mm long on branches or terminal stems, with petals 4.5–5.5 mm long (Bradley and Gann, 1999; Bradley and van der Heiden, 2013). This species is in the yellow-flowered clade of *Linum*, and based on morphological similarities, its closest relative is thought to be *L. bahamensis*, a species endemic to islands of the Bahamas (Rogers, 1963).

L. arenicola has been of federal conservation concern since 1975 and was officially listed federally endangered in 2016 (USFWS, 2016). It is endemic to south Florida and the lower Florida Keys, USA (USFWS, 2016) and has been declining in abundance across its narrow range (Hodges and Bradley, 2006). Unlike many widespread species of *Linum* (McDill et al., 2009), *L. arenicola* occurs only in disturbed uplands, dry marl prairie, and pine rocklands of peninsular south Florida and the lower Florida Keys (Bradley and Gann 1999; Bradley and van der Heiden 2013; FNAI, 2000). Pine rocklands are a globally rare, fire-adapted ecosystem (Jones and Koptur, 2017; Snyder et al., 1990), and *L. arenicola* thrives under conditions of high disturbance and open canopy areas.

2.3. Floral biology

At each site, we chose 15 or more plants on which we measured whole plant and floral traits. Individual plants were tagged in 2015, and pedicels of flower buds were marked, so that we could observe patterns of bud-, flower-, and fruit production over time. Individual flowers were followed from bud through fruiting. Observations of flower anthesis, senescence, and fruiting were made daily over a period of 12 days. We continued to use these tagged plants for two years to observe flower visitors, florivory, and to measure fruit set in open-pollinated plants. We added new plants to be observed as individuals terminated flowering or perished.

2.4. Pollination experiments

2.4.1. Pollinator exclusion and open-pollination experiments

The contribution of pollinator visitation to fruit set and the probability of autonomous self-pollination was assessed by bagging flowers of some plants to exclude pollinators and comparing fruit set to unmanipulated flowers with access to pollinators. Very fine, small mesh nylon netting was used to make light-weight bags that were propped up so that the bags would not bend or break the fragile plant stems. One to three flower buds were randomly selected on tagged plants and their pedicels marked before bagging plants. Pedicels were inconspicuously labeled with indelible markers (Sharpie®), as tags were too heavy for plants. Marked buds were followed until fruit set or flower abortion, and bags were removed after flower senescence. Fruit set was distinguished from aborted fruits by a 1–2 mm diameter swelling of the calyx-covered ovaries, or a near tripling in width. Reproductive success was assessed by the presence of mature fruits containing seeds. The pollinator

exclusion experiment was conducted on Big Pine Key along the fire break trail, as this site had the largest population away from roadsides. Less than ten percent of plants that were flowering on a given day were bagged to reduce negative impacts on the population. The bagging experiment was repeated five times between June and July of 2015.

Fruit and seed set in *L. arenicola* flowers were measured under natural field conditions for comparison with flowers excluded from visitors. Individual plants were tagged, and pedicels of open flowers were marked (as described above) with indelible markers. Pollinators readily visited marked flowers and unmarked alike. An open-pollination treatment was conducted at all sites on both islands between April and August for two years. Sites were visited approximately twice monthly, at which time up to twenty percent of open flowers were marked and followed. When examining plants for fruit set, herbivore damage to flowers and fruits was recorded. Damaged flowers and fruits were excluded from fruit set analyses, as herbivory generally occurred before fruit set could be observed to occur. Any other visible animal associations were also noted, such as presence of herbivores or herbivore enemies that could illuminate our interpretation of pollination and fruit maturation. Mature fruits were collected from plants to calculate seeds per fruit under natural conditions.

2.4.2. Self-pollination experiments

We performed self-pollinations on greenhouse-grown plants, not in the field. Eleven *L. arenicola* plants were grown from seeds collected on Big Pine Key. Seeds were germinated on wet cloth in high humidity and seedlings were transplanted to individual trays filled with a potting medium of 70% mixed perlite/rock and 30% potting soil in a greenhouse. Twelve of the 20 seeds germinated, all germinated seeds survived transplanting, and all but one transplant grew to maturity. Once plants matured, they were watered from the bottom to prevent potential pollen movement by water. Pollen was transferred within individual flowers to determine fruit set rates from self-pollen. Upon flower opening, we moved pollen from the anthers to the stigmas of the same flower using a dull dissecting pin, which was wiped clean with ethanol and tissue between flowers. Subsequent fruit and seed set were recorded for each hand-pollinated flower. This self-pollination experiment was performed over two days on every flower that opened during that time. We did not perform any cross-pollinations on those greenhouse plants.

2.5. Pollinator observations

Patches of open flowers (2 m x 2 m) were observed in timed segments to document the structure and composition of the community of flower visitors. Observations were conducted from shortly after sunrise, when *L. arenicola* flowers were at full anthesis, until 14:00 when most flower petals had abscised or wilted, and pollinators were no longer active at flowers. In each observation period, we counted open flowers in the 2 m × 2 m patches to standardize visitation rates to patches of different sizes. We also used the counts of open flowers in each 2 m × 2 m observation patch to assess if pollinator visitation rates varied by flower density. We counted visitors over four observation periods of 15 min each, distinguishing pollinators from mere visitors by observing whether they contacted anthers or stigmas of one or more flowers in the patch. Flower visitors were identified visually, collected for lab identification, or from photos taken during timed observations. From these data visitation frequency was estimated across the pollinator assemblage. Pollinators were grouped according to morphological and functional similarities. Interaction frequency per group is reported as visits per flower per hour ($\text{vis} \cdot \text{flwr}^{-1} \cdot \text{h}^{-1}$) to *L. arenicola* flowers.

Pollinator observations were carried out at all sites and occurred concurrently with the open-pollination experiment. Observations were approximately bi-weekly, between April and July in 2015 and 2016. Pollinator observations were recorded on sunny, low cloud-cover days, with low wind-speed. Dewy and cloudy mornings sometimes delayed flower opening or pollinator activity, and on these days, timed

observations were postponed until insects were active and flowers fully opened.

2.6. Data analyses

Analyses were performed in R version 4.0.2 (R Core Team, 2018). We used a binary logistic regression (logit link) to test for the likelihood that any of the three treatments (bagged, open-pollinated, self-pollinated) would produce fruit. Each flower was considered an individual unit in the model. We calculated robust standard errors clustered by individual plants to adjust for sampling dependence (Harrell, 2015). Island (Big Pine Key and Lower Sugarloaf Key) was included as a fixed effect in the model. We used Tukey post-hoc testing in the multcomp package (Hothorn et al., 2008) to determine if and where differences occurred among treatments. We used a Poisson regression (log link) to analyze differences in seed counts per fruit of open-pollinated flowers between the two islands.

To test whether functional groups of visitors differed in their visitation rate, a Poisson regression was implemented. Counts of visits for each morpho-taxon during an observation period were offset by the log of the product of flowers and hours ($\log[\text{flowers} \cdot \text{min}/60]$) in each observation to standardize for different observation lengths and flower patch sizes. Both island and counts of open flowers in the patch were added as covariates. Terms were added sequentially, and Likelihood Ratio Tests compared deviances of terms to a null model of random interactions without explanatory variable groupings. For all generalized linear models, we assessed model fit and model assumptions using likelihood ratio tests and goodness-of-fit χ^2 tests of residual deviances and degrees of freedom. For over-dispersed count data, we used negative binomial regressions. Results of Poisson regressions are reported as back-transformed (i.e., exponentiated) estimates and 95% confidence intervals (CI). Plant trait measurements are reported as means and standard errors. To visualize differences in the composition of the pollinator assemblages on each island, we constructed a weighted bipartite graph with aid of the bipartite package (Dormann et al., 2008). Pollinator visitation frequencies to individual flowers ($\text{vis} \cdot \text{flwr}^{-1} \text{h}^{-1}$) were calculated before averaging visitation rates for each island.

Data will be archived in the FIU Digital Portal at the following URL: <https://doi.org/10.34703/gzx1-9v95/OKZJYH>

3. Results

3.1. Plant and floral biology

Plants flowered mainly between March and October, although some individuals could be found in flower year-round. Reproductively mature plants ($N = 187$) were on average 33.5 cm tall (± 0.76 cm), with an average of 1.76 ± 0.08 stems from the base and 3.17 ± 0.18 flowering branches. Most individuals sampled across all experiments had at least one open flower per plant on any given day (1.55 ± 0.05 , $N = 642$), although one plant at the firebreak site had 18 open flowers in early July.

Flower anthesis began at first exposure to sunlight. The five petals were convolute in bud, and at complete opening petals became flat to revolute. Corollas were approximately 1 cm in diameter at full opening. In all *L. arenicola* flowers observed, the five styles were equal in height to the five stamens (i.e., homostylous) and both anthers dehiscence and stigmas receptivity occurred seemingly simultaneously, and shortly after full flower opening. Our hand-pollination experiments on newly opened flowers in the greenhouse produced fruit, demonstrating stigma receptivity at flower opening. Flowers were open for one day only, and generally senesced at peak temperatures mid-day (between noon and 16:00). The capsular fruits matured within two weeks and produced a maximum of 10 seeds from five carpels, each separated by a false septum. The seeds produced were shiny and tan in color.

Flower petal abscission or persistence appears to be influenced by

pollen deposition before flower senescence is initiated. On 12 separate pollinator observation periods occurring after mid-day, we noted that petals abscised neatly following a pollinator visit and that pollen was visible on stigmas with aid of a $10 \times$ magnification hand lens (Fig. 2E, left side). In bagged flowers, petals did not abscise by late afternoon, and we generally observed wilting and intertwining of the five stamens with the five unfused styles causing stigmas and anthers to contact; this was followed by a wilting inward of the petals. When bags were removed, the stigmas of untagged flowers were covered in pollen. Petals of bagged flowers were wilted inward and faded to white after a few days (Fig. 2F). Persistent petals were common in nearly all flowers excluded from pollinators, including bagged plants in the field. Flowers in the pollinator-free greenhouse that were not hand-pollinated also exhibited persistent petals and most appeared to set fruit.

3.2. Breeding system

Bagged flowers with visitors excluded ($N = 57$) produced a considerable amount of fruit ($68 \pm 6\%$), but substantially less than flowers open to visitors ($89 \pm 1\%$, $N = 542$) or those self-pollinated by hand ($96 \pm 4\%$, $N = 26$; Fig. 3). Although open flowers had higher fruit set than bagged flowers (Table 1; Fig. 3), open-pollinated flowers were equally as likely to set fruit as those that we self-pollinated in the greenhouse ($z = 1.066$, $P = 0.51$, Tukey Contrasts). Fruit set in open-pollinated flowers was consistently high for both island populations and did not differ significantly between them ($z_{(621)} = 0.68$, $P = 0.50$), but seeds per fruit collected were 1.4 times higher for open-pollinated flowers on Big Pine Key than Lower Sugarloaf Key ($z = -4.0$, $P < 0.001$; Table 1).

Of all 969 flowers on 561 plants tagged in the field, more than one-third were consumed or destroyed by herbivores (Table 1). Flowers and immature fruits were destroyed by stem herbivores or flower bud herbivores. Herbivory of stems and flowering inflorescences was highest on Big Pine (46.8% of all plants), but stem herbivory was almost absent from the Lower Sugarloaf Key population (3.5%) and damage to flowers nonexistent. On several occasions, we observed the federally endangered Key Deer (*Odocoileus virginianus* subsp. *clavium*) consuming whole branching stems of flowers and fruits in the Big Pine Key populations.

Bud herbivory was also common on Big Pine Key and was mostly evident in the bagged experiment, where young flower buds were tagged instead of open flowers, as in the open-pollination experiments. Most bud herbivory consisted of galled flower buds (Fig. 2G). Galling became noticeable in late-stage buds as they swelled and emerging petal tips became sunburst orange to red in color. Galled flowers never opened, eventually aborting and dropping to the ground. We successfully reared a single midge (Cecidomyiidae) to maturity from one galled flower bud. On a few occasions, while observing flowers, we noticed small parasitoid wasps seemingly ovipositing in galled flower buds (Fig. 2G) and pupal cases protruding from a few galled flower buds before they aborted.

3.3. Pollinator assemblage

Thirteen arthropod species were collected and categorized within nine groups as flower visitors (Table 2). Two groups were removed from analysis, as they visited flowers less than 3 times over the two-year sampling period. Most visits to *L. arenicola* flowers were made by small bees (Halictidae, Apidae, & Colletidae; Fig. 2A, C, & D), although medium sized bees (Megachilidae) and flies (Syrphidae; Fig. 2B) were also frequent visitors (Fig. 4). During the 53 timed observations (12.7 h) to 337 flowers between April and July, 37 small bees made 57 visits to flowers at a frequency of 0.70 visits per flower per hour ($\text{vis} \cdot \text{flwr}^{-1} \text{h}^{-1}$). Leaf-cutter bees (Megachilidae) and flower flies (Syrphidae) were also frequent flower visitors (0.30 and 0.14 $\text{vis} \cdot \text{flwr}^{-1} \text{h}^{-1}$, respectively), but both with significantly weaker interactions with *L. arenicola* than small bees (Table 3; Fig. 4B). The remaining 17% of visits were nearly evenly distributed among the six other groups: large bees (Apidae), metallic sweat bees (Augochlorinae), sulfur butterflies (Pieridae), skippers

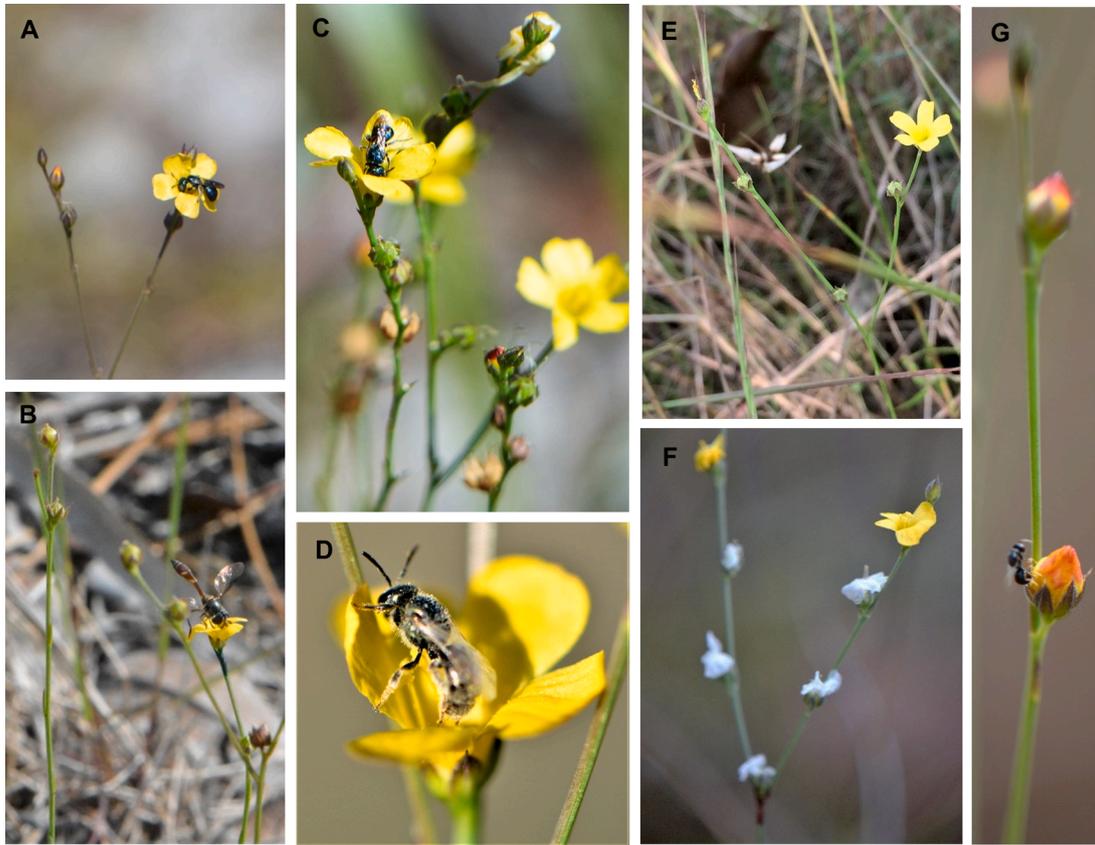


Fig. 2. *Linum arenicola* (Linaceae) floral visitors, flower behavior, and florivory. Flower Visitors: (A) *Ceratina dupla* (small Bee) visiting a flower. (B) *Pseudodorus clavatus*. (C) *Lassioglossum* sp. (D) *Lassioglossum* sp. (E) Typical specimen shown growing among grasses and displaying an open flower, mature fruits, and a visited flower with newly abscised petals at the far left. (F) Persistent petals of unvisited flowers turning white and wilting inward. (G) A likely parasitoid oviposits into flower bud galls formed by midges (Cecidomyiidae).

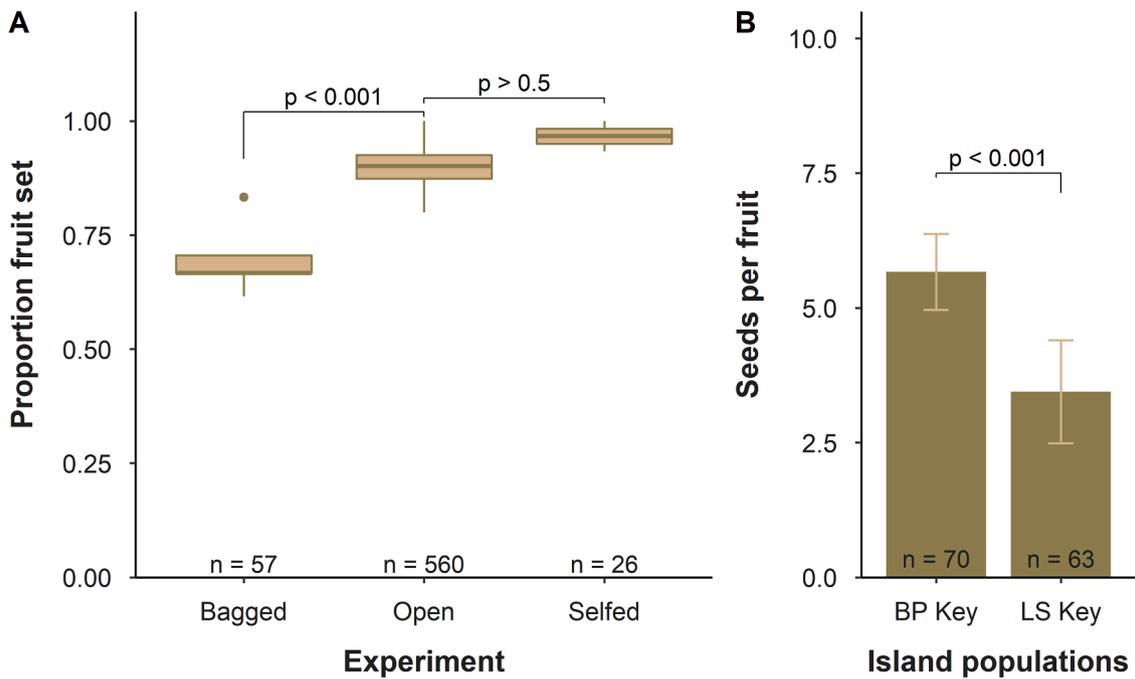


Fig. 3. Reproductive output of tagged *Linum arenicola* flowers (n) among treatments and island populations. A) Boxplots showing the daily proportion of fruit set for each experimental treatment. An outlier is indicated with a dot. B) Seed counts per fruit in open pollination treatment of tagged flowers on Big Pine Key (BP Key) and Lower Sugarloaf Key (LS Key). Maximum seed set = 10.

Table 1

Pre-seed dispersal herbivory of tagged *Linum arenicola* (Linaceae) flowers on Big Pine Key (BP Key) and Lower Sugarloaf Key (LS Key) in the lower Florida Keys. Counts and percent of all tagged flowers damaged by the likely herbivores as the result of whole stem removal, flower bud galling, or pre-seed dispersal fruit predation are given.

Herbivory	BP Key	LS Key	Herbivore
Stem	271 (35%)	7 (3.5%)	Key deer
Bud	48 (6.8%)	0	Flower gall midge
Fruit	22 (2.8%)	0	Lepidoptera larvae
None	367 (46%)	193 (96.5%)	

Table 2

Descriptions of flower visitor groups to *Linum arenicola* flowers in the Lower Florida Keys. Scientific names and families of species comprised within groups based on size and ability to identify on the wing. Species identity was determined from published works (Michener, 2007; Pascarella, 2000) and species distributions (discoverlife.org); ^asingle visits by morpho-taxa excluded from analysis.

Groups	Species	Family	Common Name
Hymenoptera, Apoidea (Bees)			
Small Bees	<i>Ceratina dupla</i> (Say)	Apidae	Small Carpenter bees
	<i>Lassioglossum</i> spp.	Halictidae	Sweat bees
	<i>Hylaeus formosus</i> (Krombein)	Colletidae	Yellow-faced bees
Augochlorinae	<i>Augochloropsis anonyma</i> (Cockerell)	Halictidae	Metallic sweat bee
Megachilinae	<i>Megachile georgica</i> (Cresson)	Megachilidae	Leafcutter
	<i>Megachile petulans</i> (Cresson)	Megachilidae	Leafcutter
	<i>Anthidiellum notatum</i> subsp. <i>rufimaculatum</i> (Latreille)	Megachilidae	Florida rotund-resin bee
Large Bees	<i>Centris errans</i> (Fox)	Apidae	Florida locust-berry bee
Other Hymenoptera			
Formicidae ^a		Formicidae	Ant
Vespidae ^a	<i>Pachodynerus erynnis</i> (Lepeletier)	Vespidae	Black and red potter wasp
Diptera (Flies)			
Syrphidae	<i>Pseudodorus clavatus</i> (Fabricius)	Syrphidae	Flower Fly
Lepidoptera (Butterflies and Skippers)			
Pieridae	<i>Phoebis sennae</i> (Linnaeus)	Pieridae	Cloudless sulfur
Hesperiidae	<i>Hylephila phyleus</i> (Drury)	Hesperiidae	Fiery skipper

(Hesperiidae), wasps, and ants (Formicidae). Exponentiated coefficients (Table 3) suggest that mean visitation frequency by megachilid bees is 59.7% lower than that of small bees, the most frequent visitor, and 77.2% lower for syrphid flower flies after controlling for flower patch size and island location. Average visitation frequency among all morpho-taxa, collectively, was 1.37 vis·flwr⁻¹h⁻¹ or approximately seven visits to a single flower per day.

In the same model, while controlling for visitor group and flower patch size, visitation rates among groups were different between the two islands. Flowers on Big Pine Key received more visits than those on Lower Sugarloaf Key (Table 3), and the composition of visitors was different (Fig. 4A). All pollinator groups were observed visiting flowers on Big Pine Key, but only small bees visited flowers of the Lower Sugarloaf Key population.

4. Discussion

Comparable to other species of *Linum*, homostyly is linked with autonomous self-pollination and some degree of self-compatibility in *Linum arenicola*. More than half of all bagged flowers reproduced autonomously and nearly all of flowers self-pollinated by hand produced fruit with seeds. Nevertheless, several species of pollinators were relatively frequent flower visitors, and flowers with access to pollinators had higher reproductive success.

The small, open flowers and the lack of style polymorphism in this species (Ruiz-Martín et al., 2018) suggested a generalist pollination system (Hetherington-Rauth and Johnson, 2020), and we observed this pattern in the composition of flower visitors. Various functional pollinator groups visited the flowers, including several species of distantly related small bees, medium-sized bees, flies, and, on rare occasions, ants, wasps, and small butterflies. Although taxonomically distinct, members sharing functional traits may provide similar selective pressures on flowers, and for most plants, certain functional groups provide superior pollination services compared with other groups of flower visitors (Fenster et al., 2004). For instance, functional traits like body size (Muchhala et al., 2009), tongue length (Arbulo et al., 2011), and width of mouthparts (Barrios et al., 2016; Koptur et al., 2020) could influence pollen dispersal distances and efficiency of pollination. The small-medium sized bees and hovering flower flies that visited sand flux flowers are well-suited to the small flower openings, easily available pollen and nectar, and thin fragile stems incapable of supporting larger pollinators. Although a variety of pollinators visit flowers, small bees are likely the most important visitors to *L. arenicola* flowers. Leaf-cutter bees and flower flies supplemented visits on Big Pine Key, but only small bees were present at flowers on Lower Sugarloaf Key (Fig. 4). Although fruit set was similar between populations, seeds per fruit was higher on Big Pine Key where the pollinator assemblage was more diverse at flowers. Contrary to expectation, flowers in larger flower patch sizes received fewer visits than smaller patches (Table 3), though this difference was modest and perhaps inconsequential. For instance, our model suggests a 0.20% increase in visitation frequency for every one flower less in a flower patch.

Despite high fruit and seed set in natural populations, flower and fruit herbivory were high. Florivory is often overlooked in reproductive studies but can significantly affect plant fitness. Consumption of flowers, fruits, or seeds results in lost reproductive effort (Cardel and Koptur, 2010), energy, and lower offspring potential (Mothershead and Marquis, 2000). Considering that we mostly tagged only open flowers in our experiments, herbivory is likely even more pervasive than we reported due to overlooked bud herbivory. Most herbivory, however, was by removal of whole stems and flowering inflorescences. Key Deer (*Odocoileus virginianus clavium*) are the only abundant grazers on Big Pine Key, and likely the primary stem herbivores. Further, Key Deer are rarely present on Lower Sugarloaf Key, and plants on that island were not browsed. Although flowers and immature fruits were lost to herbivory, many fruits with ripe seeds were also consumed. The potential for endozoochorous seed dispersal by Key deer of *L. arenicola* seeds exists (Janzen, 1984) and merits investigation. Considering such high rates of herbivory, large population sizes of *L. arenicola* may be necessary to counter herbivore pressure to the Big Pine Key population of this endangered species.

Details of the floral biology combined with pollination experiments and flower visitation suggest that *L. arenicola* has a mixed mating system. Our observations suggest that late-day intertwining of stamens and styles in flowers, further pressed together by upturned and persistent petals, likely facilitates self-pollination. Delayed self-fertilization via persistent flower petals or sepals occurs in other homostylous *Linum* species (Jahnke and Etterson, 2019; Uno, 1984) as well as other self-compatible flowering plants (Geiger et al., 2010). Although we cannot be certain that selfing is delayed without controlled timed pollination experiments, selfing did not occur before flower opening.

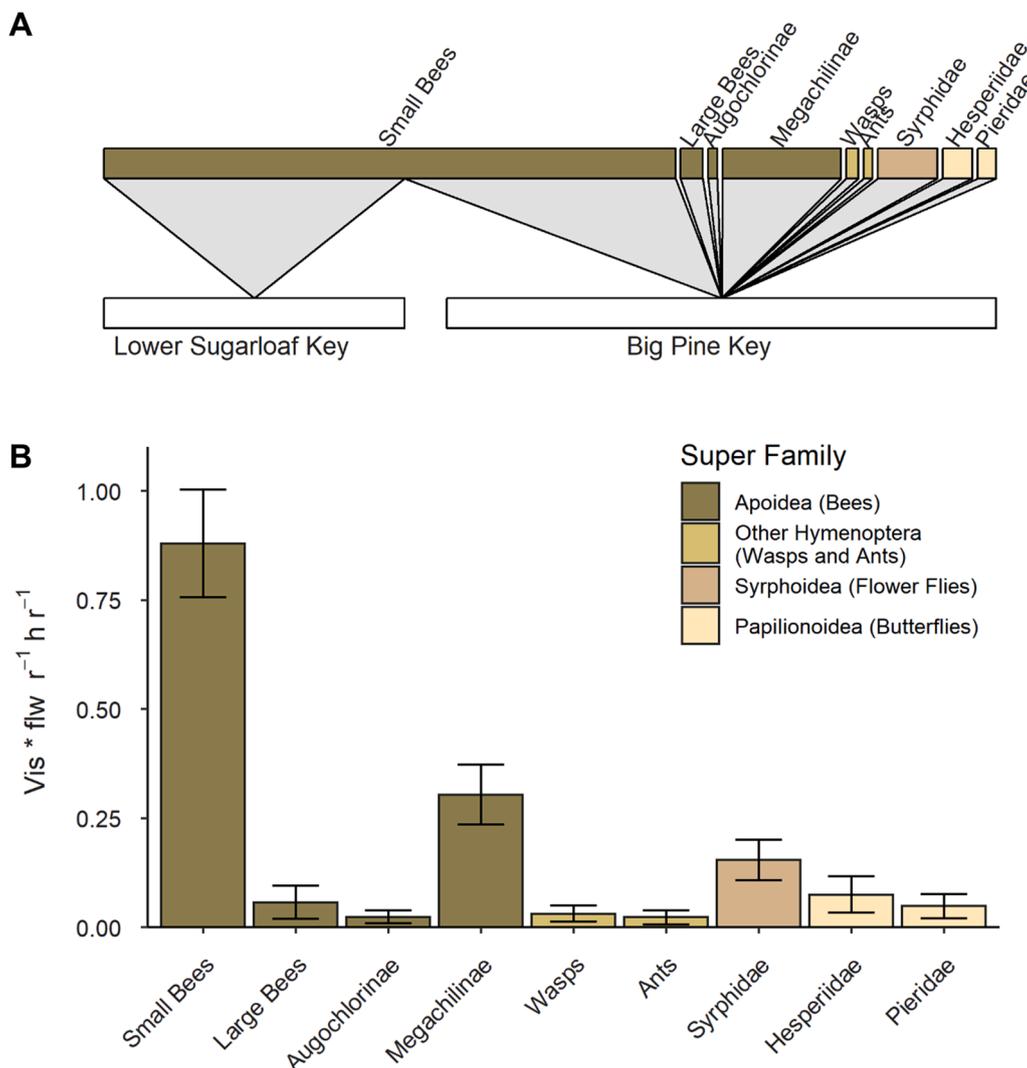


Fig. 4. Flower visiting assemblage of *Linum arenicola* in the lower Florida Keys (A) Interaction network of the two island populations. Width of bars and links represent the relative proportions of flower visits among pollinator groups and the relative proportions visiting flowers on each island. (B) Means and standard error of visitation frequency across the pollinator assemblage.

Table 3

Results of poisson regression of flower visitation with island and flower patch size as covariates. Log odds ratios, standard errors, exponentiated coefficients, and significance values of our flower visitor observations to *Linum arenicola* in the Lower Florida Keys. Z-values were obtained from Wald tests and significance codes indicate level of significance of P-values. ^a Small bees observed on Big Pine Key is the model intercept and reference for the pollinator assemblage and population.

Variables	Coefficients	Std. Errors	Odds Ratios	Z value	Pr(> z)	significance
Small bees ^a	0.106	0.180	1.112	0.591	0.554271	NS
Large bees	-2.944	0.592	0.053	-4.971	6.67E-07	***
Augochlorinae	-2.944	0.592	0.053	-4.971	6.67E-07	***
Megachilinae	-0.908	0.247	0.403	-3.674	0.000239	***
Syrphidae	-1.478	0.307	0.228	-4.809	1.52E-06	***
Hesperidae	-2.944	0.592	0.053	-4.971	6.67E-07	***
Pieridae	-2.944	0.592	0.053	-4.971	6.67E-07	***
Island: LS Key	-0.971	0.353	0.379	-2.748	0.005996	**
Patch size	-0.002	0.001	0.997	-3.265	0.001096	**

Significance codes: *** = 0.001; ** = 0.01; * = 0.05.

Stigmas observed at flower opening were free of pollen. Upon close observation, the unfused stigmas and styles only appeared to touch because of heavy winds, insect movement, and when stigmas and styles appeared to lose turgor pressure and intertwine late in the day. We speculate that selfing is delayed in this species; this warrants further experimental research. Delayed selfing and attractive, chasmogamous flowers may reduce reliance on complete self-pollination by providing

opportunities for outcrossing (Goodwillie and Weber, 2018). If delayed selfing is acting in *L. arenicola*, then pollinator visits early in anthesis may be beneficial for increasing the potential for cross-fertilization.

Baker’s Law suggests that autonomous self-pollination is a beneficial breeding system strategy for island species (Baker, 1967, 1955), facilitating colonization and persistence of colonizing species. Not only is this trend globally evident among island and mainland species

(Grossenbacher et al., 2017), but self-incompatibility appears to have broken down in island populations of several species with larger ranges (Barrett, 1985; Schueller, 2004; Martén-Rodríguez et al., 2015). In *L. arenicola*, autonomous self-pollination was not always successful in bagged flowers. Reproductive output was higher in flowers with access to pollinators, perhaps because of increased conspecific pollen deposition. As such, combined pollinator visitation and autonomous self-pollination (Kalisz and Vogler, 2003) may have resulted in enough pollen deposition for fruit and some seed production in most *L. arenicola* flowers. Although some outcrossing was likely when mates were nearby and pollinator visits were frequent, the parental source of pollen needs to be quantified, as *Linum* plants often have several stems and multiple flowers open on the same day. Controlled emasculations could further elucidate the contribution of pollinators to cross-pollination in these populations, while controlled cross-pollinations and measurements of seed production could elucidate the degree of self-compatibility in this species and if mixed mating is acting. For example, *L. sulcatum* plants are not receptive to outcrossed pollen from mates and only produce fruit when self-pollinated (Jahnke and Etterson, 2019).

From our limited breeding system experiments, *L. arenicola* appears to be highly self-compatible. Flowers self-pollinated by hand nearly all produced fruit, which was significantly higher than those relying on autonomous self-pollination alone. However, the environmental conditions of the greenhouse under which the selfing experiment was conducted could have influenced fruit set. As such, direct comparisons between our greenhouse and field experiments are less clear. Soil nutrient and water conditions are known to influence floral traits and indirectly affect pollination and reproduction (Burkle and Irwin, 2009). However, in *Linum lewisii*, a perennial herb, reproductive responses to water and nutrient supplementations were delayed by a year (Burkle and Irwin, 2009). Clearly, a more detailed investigation of fruit and seed set in controlled hand-pollination experiments is needed.

Despite the importance of island taxa to global biodiversity (Kier et al., 2009), island species have the highest extinction toll of all “protected” species, yet efforts for their conservation are consistently underfunded (Restani and Marzluff, 2002). Islands across the Lower Florida Keys archipelago, particularly within the pine rockland ecosystem, are frequently exposed to disruptive events such as hurricanes, periodic storm surge, and fire (Jones and Koptur, 2017; Saha et al., 2011). Anthropogenic factors add further complexity to managing endangered species. For instance, seasonal insecticide spray for mosquito control reduces the frequency of pollinators visiting *L. arenicola* flowers (Harris, 2016). Considering such concerns, autonomous selfing may be advantageous and is thought to enhance reproductive fitness in variable environments (Kalisz and Vogler, 2003), as are the pine rocklands for the sand flax. With adequate pollinators present, occasional outcrossing by flower visitors could rescue populations from inbreeding depression, while selfing may alleviate complete dependency on pollinators in uncertain times (Goodwillie and Weber, 2018). While perpetual selfing may confer a short-term advantage when mates and pollinators are scarce (Spigler and Kalisz, 2017), it can lead to inbreeding depression from reduced heterozygosity and an inability to adapt to change (Charlesworth and Charlesworth, 1987; Cheptou, 2019).

5. Conclusions

Breeding system, pollinator interactions, and florivory are all factors that influence reproduction. Knowledge of these factors is critical for conservation of endangered plants. Quality and quantity of offspring determine a species' ability to avoid extinction, and for flowering plants, this is often dependent on interactions with mutualist pollinators and seed dispersers (Bond, 1994). Pollinators may deposit self-pollen or xenogamous pollen to outcross some flowers, but unvisited flowers only receive their own pollen via autonomous self-pollination. Regardless of pollen source, *L. arenicola* appears to benefit from a mixed mating

system, such that reproductive output is higher when flowers have access to pollinators than in a pollinator-free environment. Examining seed viability resulting from different pollination treatments could elucidate the potential for inbreeding depression and inform how extant populations can best be conserved. Future experiments could reveal if persistent petals are clear indicators of autonomous selfing, thus enabling conservation managers to indirectly monitor pollinator activity on *Linum arenicola* flowers.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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