Reproductive biology of three sympatric endangered plants endemic to Florida scrub

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Abstract
We investigated the reproductive biology of three plants endemic to rosemary scrub habitats on the Lake Wales Ridge of Florida, USA. We used hand-pollination experiments and observations of flowers and their insect visitors to determine their mating systems and pollination. Fruit or seed set after self pollination was 94, 97, and 8% of fruit or seed set after cross pollination in *Eryngium cuneifolium* (Apiaceae), *Hypericum cumulicola* (Hypericaceae), and *Liatris ohlingerae* (Asteraceae) respectively, indicating that the first two are self-compatible and the last is obligately outcrossing. All three depend on insects for seed production (4–7% fruit or seed set without insects). Diverse insects visit flowers of *E. cuneifolium* (101 species recorded), whereas *L. ohlingerae* is visited predominantly by butterflies and *H. cumulicola* by one genus of bees (Dialictus, Halictidae). Our data indicate pollinator visitation does not currently limit seed production in *E. cuneifolium* or *H. cumulicola*, but does in *L. ohlingerae*. Despite the features they share (habit, habitat, disturbance regime), we found unique aspects of these species’ reproductive biology yielding unique risks to population viability. We suggest that multispecies recovery plans must consider several aspects of the biology of species with superficial similarities to be successful.

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Keywords: *Eryngium cuneifolium*; Florida scrub; *Hypericum cumulicola*; *Liatris ohlingerae*; Mating system

1. Introduction

Through its effects on demography and population genetics, reproductive biology has important consequences for the viability of rare plant populations. Seed production of plants that rely on pollinators may be limited by pollinator abundance or behavior (pollinator limitation; Bierzychudek, 1981). Pollinator limitation or, at its extreme, pollinator failure could cause seed production to fall below the level necessary for population viability (Lamont et al., 1993; Bond, 1994; Steiner and Whitehead, 1996; Mawdsley et al., 1998; Spira, 2001). Mating patterns affect the amount of genetic variation found in plant populations (Hamrick et al., 1991; Ellstrand and Elam, 1993; Hamrick and Godt, 1996). Genetic variation is important for genetic viability, since evolutionary responses to environmental change hinge upon such variation (Fisher, 1958; Huenneke, 1991). The loss of self-incompatibility alleles in self-incompatible species poses a special threat to genetic viability (Les et al., 1991; DeMauro, 1993; Schemske et al., 1994). Genetic viability may also be affected negatively by inbreeding in the presence of inbreeding depression (Charlesworth and Charlesworth, 1987; Lande, 1995; Lynch et al., 1995) or positively by inbreeding to the extent that deleterious alleles can be purged (Husband and Schemske, 1996; Byers and Waller, 1999).

The Lake Wales Ridge (LWR) of central Florida, USA is an ancient dune system covering ~150 × 15 km, with more than 20 species of federally-listed, narrowly-endemic higher plants (Christman and Judd, 1990; Dobson et al., 1997; USFWS, 1999). Declines of these plants have been caused primarily by habitat loss and fire suppression. More than 15 years ago, Peroni and Abrahamson (1985) estimated that just 16% of the historic extent of scrub habitat on the LWR remained. In
this study we examine the reproductive biology of three of these species, *Eryngium cuneifolium* Small (Apiaceae), *Hypericum cumulicola* (Small) W. P. Adams (Hypericaceae), and *Liatris ohlingerae* B. L. Rob. (Asteraceae). All three are herbaceous perennials found in rosemary scrub habitats and are federally listed as endangered species. Rosemary scrub occurs on elevated patches (“islands”) of white sands throughout the Lake Wales Ridge and other ridges in Florida (Menges and Hawkes, 1998; Menges, 1999). Fire is an important ecological force in rosemary scrub, which is dominated by the shrub, *Florida rosemary* (*Ceratiola ericoides*). Gaps among individuals of *C. ericoides* are large immediately after fire and close with time-since-fire (Menges and Hawkes, 1998). At least 10 years are required before a sufficiently continuous mass of fuel accumulates for fire to return. Historical fire return intervals in rosemary scrub probably ranged from 20 to 100 years, but fire has been suppressed over large parts of the LWR for the last 60 years (Menges, 1999).

The species studied here have common and unique characteristics. If their similarities (habit, habitat, and disturbance regime) lead to similar reproductive biology, then detailed knowledge of the reproductive biology of each species would not be necessary. Alternatively, differences in their life span, reliance on seeds to recover from fire, specialization to post-fire gaps, and hence population dynamics could generate different degrees of selection for reproductive assurance. Differences in reproductive biology resulting from these different selective pressures could then require species-specific conservation approaches. To address the hypothesis that these species are reproductively uniform and evaluate risks to population viability arising from reproductive biology, we investigated the floral biology, flowering phenology, pollination, and mating systems of *E. cuneifolium*, *H. cumulicola*, and *L. ohlingerae*.

1.1. The species

Although all three species grow in rosemary scrub, they vary in a number of ecological traits (Table 1). Only *L. ohlingerae* is commonly found in both rosemary scrub and a more mesic, oak-dominated community, scrubby flatwoods. Scrubby flatwoods have few gaps between shrubs (Young and Menges, 1999), a shorter average fire return interval (5–20 years; Menges, 1999), and are more abundant and spatially continuous on the Lake Wales Ridge. The species also differ in postfire recovery and how they respond to community-level changes with time-since-fire. Individuals of *E. cuneifolium* and *H. cumulicola* are killed by fire, but populations recover from dormant seeds in the soil (Quintana-Ascencio and Morales-Hernandez, 1997; Quintana-Ascencio and Menges, 2000). In contrast, *L. ohlingerae* resprouts after fire from a belowground corm (Menges and Kohfeldt, 1995), though resprouting is low after some fires (Weekley, C. W., Menges, E. S., unpublished data). Recruitment of *L. ohlingerae* seedlings appears episodic (Herndon, 1997). *E. cuneifolium* is most sensitive to shrub encroachment into the open microsites in which it does best (Menges and Kimmich, 1996; Menges and Hawkes, 1998). *H. cumulicola* is somewhat less sensitive to competition from shrubs (Quintana-Ascencio and Morales-Hernandez, 1997; Quintana-Ascencio and Menges, 2000), and *L. ohlingerae* often grows among shrubs. From *E. cuneifolium* to *H. cumulicola* to *L. ohlingerae*, these three species form a series from shorter to longer-lived and greater to less demographic fluctuation (Menges et al., 2001; Menges, E. S., unpublished data). In addition, the first two species, which depend on seeds to recover from fire, appear to have metapopulation dynamics (Quintana-Ascencio and Menges, 1996; Quintana-Ascencio et al., 1998), which is not evident in *L. ohlingerae*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Habit (and max lifespan)</th>
<th>Range, no. extant populations</th>
<th>Habitat</th>
<th>Specialization to open space</th>
<th>Post-fire regeneration</th>
<th>Median population size (and size fluctuation)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Eryngium cuneifolium</em></td>
<td>Perennial herb (10 year)</td>
<td>43 km</td>
<td>Rosemary scrub</td>
<td>High</td>
<td>Seeds, limited resprouting</td>
<td>4280 (high)</td>
</tr>
<tr>
<td><em>Hypericum cumulicola</em></td>
<td>Perennial herb (10 year)</td>
<td>84 km</td>
<td>Rosemary scrub</td>
<td>Moderate</td>
<td>Seeds</td>
<td>539 (high)</td>
</tr>
<tr>
<td><em>Liatris ohlingerae</em></td>
<td>Perennial herb (&gt; 10 year)</td>
<td>109 km</td>
<td>Rosemary scrub, scrubby flatwoods</td>
<td>none</td>
<td>Resprouting</td>
<td>179 (low)</td>
</tr>
</tbody>
</table>

Maximum lifespan was estimated from demographic monitoring of *E. cuneifolium* and *H. cumulicola*, and from observations of *L. ohlingerae*. The range of each species is expressed in km north–south along the long axis of the ~160 × 20 km Lake Wales Ridge, in Florida, USA.

a From Menges et al. (2001)
b From Dolan et al. (1999)
c From (Menges and Hawkes, 1998, Quintana-Ascencio and Menges, 2000).
The three species also differ in genetic variation, as detected by isozymes. L. ohlingerae has the most variation (percent of loci polymorphic, number of alleles per polymorphic loci, and observed heterozygosity; Dolan et al., 1999). Allelic diversity is also distributed most evenly in L. ohlingerae, indicated by a greater proportion of genetic variation found within than among populations (Ficient 0.120; Dolan et al., 1999). In contrast, H. cumulicola has little variation, which is strikingly structured, both at regional (Ficient 0.730; Dolan et al., 1999) and local scales (Quintana-Ascencio et al., 1998), whereas E. cuneifolium is intermediate in these respects (Ficient 0.445; Dolan et al., 1999). The isozyme data further suggest that inbreeding is low in E. cuneifolium (FIs 0.078) and L. ohlingerae (FIs 0.144), and high in H. cumulicola (FIs 0.729).

2. Materials and methods

2.1. Floral biology and flowering phenology

The flowers of E. cuneifolium, H. cumulicola and L. ohlingerae are perfect; thus we examined the spatial and temporal arrangement of mature male and female sexual parts within flowers. We define male sexual maturity as the period from when anthers dehisce to when pollen is no longer present or fresh, and female sexual maturity as the period of stigmatic receptivity, during which stigmas evolve bubbles of oxygen when submerged in hydrogen peroxide (Kearns and Inouye, 1993). Peak stigmatic receptivity was determined by examining the surface area and texture of stigmas, and the rate of oxygen evolution from samples of flowers of known age. We observed the phenology of marked flowers of each species in early-flowering individuals in natural populations at Archbold Biological Station, Highlands County, Florida (ABS). These observations, and observations of plant- and population-level reproductive phenology, contribute to our understanding of the potential for pollen transfer within vs. among plants.

2.2. Pollination

We considered any insects observed on flowers potential pollinators and recorded several aspects of their behavior: their contact with male and female parts of flowers; foraging and grooming behavior; the length of their visit; the number of flowers or inflorescences visited; and the species of the next plant they visited. Rarely were observers able to record all of this information for a single visitor. Observers also described insect visitors to the best of their ability or collected voucher specimens. The insects were identified and curated by Dr. Mark Deyrup (Archbold Biological Station) and are housed in the insect collection at ABS.

Our goal was to categorize each plant’s pollination in two respects: as generalist (many pollinating insect species) vs. specialist (few pollinating insect species), and in terms of the type of insects visiting the flowers (bees, bee-flies, flies, butterflies, moths, beetles, etc.). These categorizations are a first step in understanding both the plants’ pollination biology and the potential extent of gene flow through pollen movement.

All observations of insect visitors were made in natural populations at ABS. Each observation period lasted from 15 to 30 minutes and included a single focal plant. Insect behavior on flowers was recorded between 0720 h and 1005 h in H. cumulicola, and between 1035 h and 1415 h in E. cuneifolium, sampling the period of peak visitation in each species. Most observations of E. cuneifolium and H. cumulicola were made in 1995 at a site where the density of both species was high. Additional observations of insect visitors to flowers of H. cumulicola were made in 1996 at two populations with moderate and low densities of H. cumulicola. The observations of H. cumulicola were made over 12 days spanning May, June and July in 1995, and 3 days in August in 1996, totaling 765 min. Observations of E. cuneifolium were made over 3 days in September, 1995 totaling 130 min. Visits to flowers of L. ohlingerae were so infrequent that timed watches were not practical; instead, observations were made while pollinating flowers in the mating system experiment. The site at which these observations were made supports a relatively large, dense population of L. ohlingerae.

2.3. Mating system

We tested the mating systems of these plants using controlled hand pollination experiments with five (E. cuneifolium and H. cumulicola) or four (L. ohlingerae) pollination treatments during the 1995 flowering season. The treatments tested for spontaneous (autonomous) self-pollination, asexual reproduction in flowers (agamospermy), and self-compatibility, while cross and open pollinations served for comparisons. These treatments were achieved using various combinations of insect exclusion, emasculation, and hand-pollination (Table 2). It was not possible to emasculate flowers of L. ohlingerae because of their floral structure (see Section 3), thus we did not test for agamospermy in this species. We relied on the spatial and temporal separation of sexes within flowers of L. ohlingerae to prevent pollen contamination within flowers. We used plastic mesh bags (Applied Extrusion Technologies, maximum mesh size 0.9 mm) to exclude insect visitors. Plants were chosen for the experiment from natural populations of each species at ABS (20 plants of H. cumulicola, 25 of L. ohlingerae and E. cuneifolium).

Inflorescences or flowering stalks were selected for the experiment according to their phenological state and
polination treatments are described in the text and correspond to labels in Table 2 and Figs. 1–3. The average number of flowers per plant and pollination treatment is followed in parentheses by the range of flower numbers and the number of

<table>
<thead>
<tr>
<th>Pollination treatment</th>
<th>Eryngium cuneifolium</th>
<th>Hypericum cumulicola</th>
<th>Liatris ohlingerae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auto</td>
<td>22 (14–27, N = 21)</td>
<td>45 (3–84, N = 19)</td>
<td>23 (19–32, N = 19)</td>
</tr>
<tr>
<td>Agam</td>
<td>23 (12–28, N = 20)</td>
<td>18 (5–34, N = 20)</td>
<td></td>
</tr>
<tr>
<td>Self</td>
<td>20 (8–33, N = 20)</td>
<td>19 (8–34, N = 20)</td>
<td>21 (10–49, N = 18)</td>
</tr>
<tr>
<td>Cross</td>
<td>19 (5–28, N = 21)</td>
<td>16 (4–32, N = 20)</td>
<td>22 (13–40, N = 14)</td>
</tr>
</tbody>
</table>

The average number of flowers per plant and pollination treatment is followed in parentheses by the range of flower numbers and the number of plants. Pollination treatments are described in the text and correspond to labels in Table 2 and Figs. 1–3.
Flowers of each gender are generally present within a plant. Flowering occurs for about a month in September. Flowers of *Hypericum cumulicola* are also perfect, with small, bright yellow petals and numerous stamens (mean = 27, S.D. = 2.3, n = 13). The flowers are borne in panicles of spikes. Pollen is the only reward for insect visitors. Each flower opens with the morning light and withers by late morning (around 11:00 h). During this interval, both male and female sexual parts are mature. Thus, flowers of *H. cumulicola* lack the temporal separation of genders seen in flowers of *E. cuneifolium* (Table 4). However, the genders are separated spatially (herkogamy). The styles (3–4) in flowers of *H. cumulicola* are typically bent at a 90° angle near the ovary, positioning the stigmas away from the upright-standing cluster of stamens. The degree of spatial separation is variable among flowers within and among individuals. Flower production per plant can be quite large (up to 4218 flowers; Quintana-Ascencio, P. F., unpublished data), but occurs over an extremely long flowering season lasting from April to November. On all but the largest plants, from zero to a few flowers open per day. Peak flowering occurs in August.

*L. ohlingeriae* has from 20 to 25 tubular, bright purple, perfect disk flowers per head, and several heads per plant. The flowers produce nectar and are typical of the Asteraceae. Five fused anthers surround the two-lobed style. The anthers dehisce on the first day the flower opens, and the pollen is carried on the stylar lobes as they grow up and out of the corolla, emerging by late morning on the first day of flowering. The stigmatic surface is located on both margins of each stylar lobe below the region where the pollen is deposited. This part of the styal lobe emerges from the corolla mid-day on the second day of flowering, and is receptive both that day and the third day of flowering. Thus, flowers of *L. ohlingeriae* are both protandrous and herkogamous. Flowers in male and female phase are present within heads, and among heads within a plant. Like *E. cuneifolium*, flowering lasts for about a month, but in August.

### 3.2. Pollination

The diversity of insects visiting flowers of *E. cuneifolium* was the striking feature of this plant’s pollination. This was obvious after observing flowers for a limited time; thereafter, we focused our efforts on documenting this diversity. We collected insects from five orders, 39 families, 80 genera, and 101 species from *E. cuneifolium* flowers (Appendix). Flowers of *E. cuneifolium* were visited frequently in the 130 min of timed observations: an
average of 0.31 visits per min per plant. Most foraging visitors focused on nectar, but halictid and megachilid bees were among the few observed to collect pollen. These bees, as well as the syrphid flies, were the most common visitors (88% of visits). In the dense population where visitation was observed, insects often moved among flowers on a plant, or traveled short distances (<5 m) when they moved among plants.

Flowers of *H. cumulicola* were visited by native solitary bees (*Dialictus* and *Augochloropsis*, Halictidae) bumblebees (*Bombus*, Apidae), and a small bee-fly (*Geron* sp., Bombyliidae). The average rate of visitation was 0.11 visits per min per plant in 765 minutes of observations. Visits by *Dialictus* constituted 81% of all visits. Foraging by *Dialictus*, and the second most frequent visitor, *Augochloropsis*, left most flowers cleaned of pollen within the first hour after opening. Individual flowers lost most of their pollen after a single visit. *Dialictus* are small enough that they often did not contact the stigmas while standing on the anthers gathering pollen. When *Dialictus* were observed to contact stigmas it was often as they first arrived on the flower. *Augochloropsis* and *Bombus* are large enough that they contacted anthers and stigmas simultaneously. The bombyliid may have foraged for pollen using its proboscis, but did not appear to carry pollen on its body. It visited flowers throughout the day, including times that flowers had neither pollen nor receptive stigmas.

Flowers of *L. ohlingerae* produce nectar; this, in addition to their color (purple) and morphology (tubular) give the expectation that they are butterfly pollinated (Faegri and van der Pijl, 1979). Butterflies were observed visiting the flowers, especially skippers, sulfurs, and swallowtails (Hesperidae, Pieridae, and Papilionidae, respectively). Their visits were infrequent, mostly occurring between 1100 h and 1500 h. Pollen was visible on the butterflies’ hairy bodies, and even on their proboscides. The butterflies were often observed to move among plants of *L. ohlingerae*, rather than to flowers of other species, at this relatively high-density site.

### Table 4
Traits affecting the likelihood of self-pollination and predicted mating systems of three Florida scrub endemics

<table>
<thead>
<tr>
<th>Trait</th>
<th><em>Eryngium cuneifolium</em></th>
<th><em>Hypericum cumulicola</em></th>
<th><em>Liatris ohlingerae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichogamous (protandrous)</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Herkogamous</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Flowering flowers/plant</td>
<td>Many</td>
<td>Few</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Autonomous pollination</td>
<td>No</td>
<td>Some (7% fruit set)</td>
<td>No</td>
</tr>
<tr>
<td>Self-compatible</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Predicted mating system</td>
<td>Mixed</td>
<td>Mixed</td>
<td>Outcrossing</td>
</tr>
</tbody>
</table>

Pollination treatment had a highly significant effect on fecundity in all three species (Figs. 1–3; except Fig. 2b). Seed set in *E. cuneifolium* (4%, Fig. 1) and fruit set in *H. cumulicola* (2%, Fig. 2a) were very low when we tested for agamospermy by emasculating and isolating flowers. Seed set in *E. cuneifolium* (4%, Fig. 1) and fruit set in *H. cumulicola* (7%, Fig. 2a) were also very low when we tested for spontaneous self-pollination. Seed (*E. cuneifolium*) and fruit set (*H. cumulicola*) in this second treatment was a small fraction of that when flowers were hand-pollinated with self pollen (6 and 16%, respectively). Flowers given self pollen produced 94% of the seeds (*E. cuneifolium*, Fig. 1) and 98% of the fruits (*H. cumulicola*, Fig. 2a) produced by flowers given cross pollen. Seed and fruit set, respectively, was not significantly different between the self and cross treatments in either of these species (*F*1, 164 = 0.76, *P* = 0.38, *E. cuneifolium* and *F*1, 73 = 0.19, *P* = 0.66, *H. cumulicola*). While fruit set was not affected by pollen type in *H. cumulicola*, seed set from outcross pollinations was greater than seed set from self pollinations (*F*1, 51 = 6.05, *P* = 0.01) or open pollinations (*F*1, 51 = 3.43, *P* = 0.06; Fig. 2b). Uncontrolled insect visitation resulted in high seed set (80%, *E. cuneifolium*) and moderate fruit set (44%, *H. cumulicola*) in these two species, either higher than the cross treatment (*E. cuneifolium*, *F*1, 164 = 7.22, *P* = 0.01) or no different (*H. cumulicola*, *F*1, 73 = 0.27, *P* = 0.61).
Flowers of *L. ohlingerae* isolated from insects were also unsuccessful (4% seed set, Fig. 3). In contrast to *E. cuneifolium* and *H. cumulicola*, seed production from hand-pollinations using self pollen was low in *L. ohlingerae* (4%), 8% of what flowers given cross pollen produced. The difference between self and cross treatments was highly significant (*F*<sub>1, 54</sub> = 43.39, *P* < 0.0001). In addition, seed set was significantly lower in open pollinated flowers than flowers given cross pollen (*F*<sub>1, 54</sub> = 5.46, *P* = 0.02).

4. Discussion

4.1. Patterns of mating

Patterns of reproduction, i.e. asexual vs. sexual and selfing vs. crossing, have important consequences for the amount and distribution of genetic variation (Hamrick et al., 1991; Karron, 1991). These three hercaceous species showed little evidence of asexual reproduction or spontaneous pollination within flowers (“agami” and “auto” treatments, Figs. 1–3). Reproduction without insect visitors occurred at its highest rate in *H. cumulicola*, in which male and female parts mature synchronously within flowers, but was still minimal (7% fruit set, Fig. 2a).

Limits to pollen transfer within plants increase from *E. cuneifolium* to *H. cumulicola* to *L. ohlingerae*. The first two are self-compatible: seed set of *E. cuneifolium* and fruit set of *H. cumulicola* in flowers given self pollen was indistinguishable from that in flowers given cross pollen. Inflorescences of *E. cuneifolium* are sequentially male and then female. This sort of pattern of synchronized protandry is known in other members of the Apiaceae, such as *Aralia hispida* (Thompson & Barrett, 1981; Barrett, 1984). But because plants of *E. cuneifolium* typically have many inflorescences, blooming within a short flowering season, there is ample opportunity for selfing. In contrast, on all but the largest plants of *H. cumulicola*, few flowers are open per plant, limiting opportunities for pollen transfer within plants. Our data indicate that *L. ohlingerae* is self-incompatible: seed set from hand-pollination with self pollen was a small fraction of seed set from hand-pollination with cross pollen. Genetically based mate incompatibility systems are known from many other species in the Asteraceae (Richards, 1997). We conclude that *E. cuneifolium* and *H. cumulicola* are likely to have mixed mating systems, whereas *L. ohlingerae* is obligately outcrossing (Table 4).

Our results regarding patterns of mating and gene flow via pollination are generally consistent with the isozyme data for these species (Dolan et al., 1999). The obligate outcrosser *L. ohlingerae* has higher allelic diversity, more heterozygosity, and less spatial structuring of allelic diversity than the other two species. Low spatial structuring of allelic diversity is consistent with our observation of butterfly visitation to *L. ohlingerae* flowers, since butterflies can carry pollen substantial distances. The two species with mixed mating systems, *E. cuneifolium* and *H. cumulicola*, have less allelic diversity and heterozygosity and more patchily distributed allelic diversity. Allelic diversity is distributed most patchily in *H. cumulicola* (*F*<sub>ST</sub> = 0.730), which we observed to be visited predominantly by small-bodied solitary bees that are unlikely to transfer pollen over large distances. Boyle and Menges (2001) also reported that visitation of *H. cumulicola* was dominated by small solitary bees. It is also possible that high differentiation among populations of *H. cumulicola* is generated by metapopulation dynamics (Quintana-Ascencio and Menges, 1996; Quintana-Ascencio et al., 1998).

Superficially, the isozyme data conflict with our suggestion that *E. cuneifolium* should show the highest degree of selfing. Instead, the isozyme data suggest that selfing is most prevalent in *H. cumulicola* (*F*<sub>IS</sub> = 0.729), followed by *L. ohlingerae* (*F*<sub>IS</sub> = 0.144), and last by *E. cuneifolium* (*F*<sub>IS</sub> = 0.078). However, this species-level statistic obscures heterogeneity found among loci and populations of *E. cuneifolium* that is not found in the other two species. In all cases where genotype frequencies of *H. cumulicola* and *L. ohlingerae* deviated significantly from random expectation, the deviation was positive, indicating inbreeding. But in *E. cuneifolium*, 75% of the cases of significant deviation from random expectation were positive and 25% were negative (Dolan et al., 1999). Thus the low species-level value of *F*<sub>IS</sub> in *E. cuneifolium* does not indicate a consistent pattern of outbreeding. The heterogeneity of inbreeding vs. outbreeding in *E. cuneifolium* and the evidence of strong selfing in *H. cumulicola* are surprising and worth further investigation.

4.2. Limits on seed production: pollen quantity and quality

Seed production may be limited by the number of pollen grains that reach stigmas (pollinator or pollen limitation; Bierzychudek, 1981). Much evidence indicates that pollinator limitation is a widespread phenomenon, though often variable (see the reviews of Burd, 1994, and Larson and Barrett, 2000). All of the plants we studied rely on insects for pollen transfer and are subject to pollinator limitation. We did not detect pollinator limitation in *E. cuneifolium* or *H. cumulicola*. Uncontrolled insect visitation led to high (80% seed set) or moderate (44% fruit set) fecundity in these two species, respectively, which was not improved upon by hand pollination (Figs. 1 and 2). Both were observed to have high insect visitation rates. In contrast, seed set in *L. ohlingerae* flowers given open access to insect visitors was significantly improved upon by hand-pollination with cross pollen, and insect visitation rates were low.
Pollinator limitation or failure is thought to be more likely in plants that are pollinated by one or few insect species (Bond, 1994; Kearns and Inouye, 1997; Johnson and Steiner 2000; Spira, 2001). The diversity of floral visitors we observed decreased from *E. cuneifolium* to *L. ohlingerae*. *E. cuneifolium* seems to have a generalist pollination system, whereas *L. ohlingerae* may rely upon one guild of insects: butterflies. We caution that our data for these two species are preliminary. Visitation of *Hypericum cumulicola* flowers is dominated by *Dialictus* spp., among a handful of other native solitary bees (this study and Boyle and Menges, 2001). A study of the effectiveness of *Dialictus* bees vs. other, larger-bodied bees in causing fruit and seed set would resolve whether the pollination of *H. cumulicola* is as specialized as it appears. But the halictid bees that visit *H. cumulicola* are not specialized with respect to the plants they visit: they are known to visit flowers of other species (Deyrup, M., unpublished data). Generalization from the insect’s point of view should reduce the risk of reciprocal declines between pollinators and plants, as with generalization from the plant’s point of view (Deyrup and Menges, 1997).

Pollinator limitation may also arise via low pollinator visitation rates in small, isolated, or low-density populations. Evidence has accumulated for these patterns in plant populations (Schaal, 1978; Jennersten, 1988; Kunin, 1993, 1997; Aizen and Feinsinger, 1994; Agren, 1996; Groom, 1998; Oostermeijer et al., 1998; Kearns et al., 1998; Spira, 2001). In fact, there is evidence that visitation to flowers of *H. cumulicola* is positively density-dependent, and that seed set increases with visitation rate (Boyle and Menges, 2001). Plant densities of *E. cuneifolium* and *H. cumulicola* decline with time-since-fire (Menges, E. M., Quintana-Ascencio, P. F., unpublished data), suggesting that pollinator visitation could cycle with fire and plant population dynamics. Plant density is chronically low in *L. ohlingerae* (Dolan et al., 1999; Menges et al., 2001), which is particularly problematic since seed set with self pollen or without insects is so poor.

Seed set can be limited by pollen quality, via a genetically based self-incompatibility system (Byers and Meagher, 1992; Byers, 1995; Ramsey and Vaughton, 2000; Wolf and Harrison, 2001) or inbreeding depression (Brown and Kephart, 1999; Fischer and Matthies, 1997; Bosch and Waser, 1999). Self-incompatibility in *L. ohlingerae* could limitits seed productivity. We found no evidence of inbreeding depression in *E. cuneifolium*, but seed set of *H. cumulicola* is higher given pure outcross pollen compared to either self or open pollinated fruits (Fig. 2b). Inbreeding depression reduced seed production in *H. cumulicola* by 13% [1−\(w_s/w_c\); Eq. (1) in Johnston and Schoen, 1994]. Potential limits on seed production increase from *E. cuneifolium* to *H. cumulicola* to *L. ohlingerae* (Table 5).

The differences in reproductive biology that we discovered among these three rare species are consistent with selection for reproductive assurance, and illustrate a pattern of “compensation” described by Bond (1994). *L. ohlingerae* has a belowground organ (corm) that resists fire-induced mortality and confers longer lifespan. This, combined with its lack of specialization to post-fire open microhabitats, leads to less turnover of individuals per unit time and hence the need for fewer seeds to maintain a stable population size. This species has the most traits that can limit seed production (self-incompatibility, pollination by a limited guild of insects). The species with minimal belowground storage organs, *E. cuneifolium* and *H. cumulicola*, are shorter-lived, are specialized to post-fire open microhabitats, rely on seeds to recover from fire, and fluctuate considerably with fire. These species have high demographic dependence on seeds (*E. cuneifolium* and *H. cumulicola*), and “compensate” for this risk with low risk of pollinator failure (generalist pollination in *E. cuneifolium*) or moderate dependence on pollination for seed production (self-compatibility, though not autonomously pollinating).

### 4.3. Implications for conservation

Our study of reproductive biology and the survey of isozymes by Dolan et al. (1999) identify three genetic effects that could affect viability in the study species. Evolutionary responses to environmental change could be constrained in *H. cumulicola* by its lack of genetic diversity, and inbreeding depression in *H. cumulicola* reduces its seed productivity. Successful matings in *L. ohlingerae* require allelic diversity at the self-incompat-

<table>
<thead>
<tr>
<th>Trait</th>
<th><em>Eryngium cuneifolium</em></th>
<th><em>Hypericum cumulicola</em></th>
<th><em>Liatris ohlingerae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecundity in open pollinated flowers</td>
<td>80% (seed)</td>
<td>44% (fruit) 65% (seed)</td>
<td>33% (seed)</td>
</tr>
<tr>
<td>Insect visitation rates</td>
<td>High</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Pollination</td>
<td>Generalist</td>
<td>Specialist (<em>Dialictus</em> spp.)</td>
<td>Moderate specialist (butterflies)</td>
</tr>
<tr>
<td>Plant density (plants/m²)</td>
<td>1.84</td>
<td>0.46</td>
<td>0.19</td>
</tr>
<tr>
<td>Ratio of self and cross pollinated treatments</td>
<td>0.94 (seed)</td>
<td>0.97 (fruit) 0.89* (seed)</td>
<td>0.08* (seed)</td>
</tr>
</tbody>
</table>

Both *E. cuneifolium* and *L. ohlingerae* have single-seeded fruits. Statistically significant differences between fecundity in the self vs. cross treatments are indicated with an asterisk.
ibility locus, as in other rare, self-incompatible plants (Les et al., 1991; DeMauro, 1993; Byers, 1995; Weekley and Race, 2001; Wolf, 2001). The latter two problems are most likely to threaten viability in small populations. In such populations, plant numbers and hence overall seed production is low, at the same time that inbreeding increases. Small populations are expected to lose self-incompatibility (SI) alleles. Populations of *H. cumulicola* and *L. ohlingeræ* are currently most threatened by habitat loss and fire suppression, which can be reversed by habitat preservation and fire management.

Our study of reproductive biology also reveals certain risks for demographic viability. Seeds are critical for persistence of *E. cuneifolium* and *H. cumulicola* populations, since fire kills all or most plants. Seed set in these two species may be increasingly limited by pollinator abundance or behavior with time-since-fire, as discussed above. *H. cumulicola* may be particularly vulnerable to pollinator limitation, since its flowers are visited by a few species of native solitary bees. The threats to *L. ohlingeræ* are different. Individuals of *L. ohlingeræ* often survive fire, and population declines between fires are less rapid, but pollinator limitation may be chronic. Though less dramatic, these declines are also serious, since plant density and seed production are chronically low and seedling recruitment is episodic. Factors influencing recruitment in *L. ohlingeræ* should be a focus of further investigation. Prescribed fire programs tailored to the population dynamics of these plants and reserve systems designed to accommodate extinction-recolonization dynamics should address both the positive and negative effects of fire on these plants.

Although the species we studied are all herbaceous perennials subject to natural disturbance via fire in rosemary scrubs, our study reveals species-specific risks to population viability related to reproductive biology. Based on this work, we suggest that reproductive biology and concomitant risks to population viability cannot be predicted from coarse-grained similarities among species, in this case, from habit, shared habitat, and shared disturbance regime. Single conservation strategies for multiple sympatric species that are grouped based on a few such coarse-grained characteristics are unlikely to be successful. Instead, knowledge of several aspects of population biology is necessary to predict risks to population viability of rare species. The sheer number and distribution of rare species requires that common conservation strategies be sought, hence further investigation should focus on whether strategies based on multiple aspects of population biology can be successful.

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**Appendix**

Insect visitors to flowers of three plants endemic to Florida scrub: *Eryngium cuneifolium, Hypericum cumulicola*, and *Liatris ohlingerae*.

In parentheses are the initials of the collector, either MD (Dr. M. Deyrup, Archbold Biological Station) or ME (M. E. K. Evans, University of Arizona).

**Eryngium cuneifolium**

**COLEOPTERA**

*CANTHARIDAE*

*Chauliognathus marginatus* (MD)

*CERAMBYCIDAE*

*Strangalia sexnotata* (MD)

**COCCINELLIDAE**

*Exochima marginipennis* (ME)

*CUCURLIONIDAE*

*Notolomus basalis* (ME)

*Odontocorynus pulverulentus* (ME)

**MELOIDAE**

*MELOIDAE*  
*Pyrota* sp. (MD)

*P. lineata* (ME)

**MELOIDAE**

*SCARABAEIDAE*

*Trigonopeltastes delta* (MD, ME)

**DIPTERA**

*BIBIONIDAE*

*Plecia nearctica* (MD, ME)

**BOMBILIIDAE**

*Chrysanthrax dispar* (MD)

*C. mira* (MD)

*Exprosopa fasciata* (MD, ME)

*E. fascipennis* (MD)

*Geron* sp. (MD, ME)

*G. vitripennis* (MD)

*Poecilognathus* sp. (MD)

*Systoechus solitus* (MD)

*Villa* sp. (MD)

**CALLIPHORIDAE**

*Cochliomyia macellaria* (MD, ME)

*Chrysomya* sp. (ME)

**CHLOROPIDAE**

*Liohippelates pusio* (MD)
Ocella sp. (MD)
Thaumatomyia sp. (ME)
CONOPIDAE
Pysoconops brachyrhynchus (MD)
P. fronto (MD)
Zodion americanum (ME)
EUPHYDRIDAE
Hydrochasma incisum (MD)
MILICHIIDAE
Milichiella sp. (MD)
Paramyia nitens (MD)
MUSCIDAE
Atherigona orientalis (MD)
OTITIDAE
Euxesta sp. (MD)
SARCOPHAGIDAE
Gynnaprosopa sp. (MD)
Oxysarcodexia sp. (MD)
Sarcodexia sternodontis (MD)
Senotania ruhriventris (ME)
S. trilineata (MD)
SEPSIDAE
Palaeosepsis pusio (MD)
STRATIOMYIDAE
Copestylum pusillum (MD, ME)
P. vinetorum (MD, ME)
P. pusilla (ME)
Pseudodoros clavatus (ME)
Toxomerus floralis (MD)
SYRPHIDAE
Augochlora pura (ME)
P. vinetorum (MD, ME)
P. pusilla (ME)
Pseudodoros clavatus (ME)
Toxomerus floralis (MD)
TACHINIDAE
Archtas sp. (MD)
Crocinosoma cernuale (MD)
Gnadochaeta sp. (MD)
Gonia sp. (MD)
Masiphya sp. (MD)
Paradidyma melania (MD)
Phytomyntera sp. (MD)
Ptilodexia sp. (MD)
Trichopoda pennipes (MD)
Vanderwulpia sequens (MD)
HETEROPTERA
Corimelaenidae
Corimelaena lateralis (ME)
PHYMATIDAE
Phymata sp. (ME)
HYMENOPTERA
APIDAE
Bombus pennsylvanicus (ME)
COLLETIDAE
Colletes sp. (MD)
COLLETIDAE
C. mandibularis (ME)
CRABRONIDAE
Ectemnius decemmaculatus (ME)
Oxybelus emarginatus (ME)
EUCARITIDAE
Orasema sp. (ME)
EUMENIDAE
Euodynerus castigatus (ME)
E. boscii (MD)
Pachydynerus erynnis (ME)
HALICTIDAE
Augochlora pura (ME)
Augochlorrella aurata (ME)
Augochloropsis sumptuosa (ME)
Dialictus miniatus (MD)
D. nympha lis (ME)
D. placidensis (MD)
Sphecodes heraclei (MD)
LEUCOSPIDIDAE
Anacrabro ocellatus (MD)
Leucospis affinis (MD)
L. robertsoni (MD)
L. slossonae (ME)
MEGACHILIDAE
Anthidiellum perplexum (ME)
Coelioxys mexicana (MD)
C. octodentata (ME)
C. sayi (MD)
Megaluchile albitarsis (MD)
M. brevis (ME)
M. mendsa (MD)
M. texana (ME)
NYSSONIDAE
Bembix sayi (MD)
Bicyrtes capnoptera (MD)
SCOLIIDAE
Campomeris plumipes (MD)
SPHECIDAE
Isodontia exornata (MD)
TIPHIIDAE
Myzinum carolinianum (MD)
M. macculatum (MD)
M. sp. (MD)
LEPIDOPTERA
ARCTIIDAE
Cisseps fulvicollis (MD)
Cisthene subjecta (ME)
Utetheisa bella (MD)
HESPERIDAE
Euphyes arpa (ME)
LYCAENIDAE
Hemiargus ceraunus (MD)
Strymon melinus (MD)
NYMPHALIDAE
Junonia coenia (ME)
PAPILIONIDAE
Eurytides marcellus (MD)
PIERIDAE
Eurema daira (MD)
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HALICTIDAE


HYMENOPTERA

Bond, W.J., 1994. Do mutualisms matter? assessing the impact of pol-

Bierzychudek, P., 1981. Pollinator limitation of plant reproductive


Aizen, M.A., Feinsinger, P., 1994. Forest fragmentation, pollination,

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Phoebis sennae

PIERIDAE

Liatris ohlingerae

COLEOPTERA

MELYRIDAE

Collops nigriceps (MD)

DIPTERA

BOMBILIIDAE

Geron sp. (ME)

G. vitripennis (MD)

HYMENOPTERA

APIDAE

Bombus pennsylvanicus (ME)

HALICTIDAE

Augochloropsis sumptuosa (ME)

Dialictus miniatulus (MD)

D. nymphalis (MD)

D. placidensis (ME)

Liatris ohlingerae

MELYRIDAE

Collops nigriceps (MD)

DIPTERA

BOMBILIIDAE

Poecilognathus sulphuea (MD)

HYMENOPTERA

HALICTIDAE

Dialictus nymphalis (MD)

LEPIDOPTERA

HESPERIIDAE

Atrytone delavare (MD)

PIERIDAE

Phoebis sennae (MD)

Byers, D.L., Meagher, T.R., 1992. Mate availability in small popula-


