

Outcrossing effects on the reproductive performance of *Hypericum cumulicola*, an endangered Florida scrub endemic¹

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TRAGER, M. D. (Dept. of Wildlife Ecology and Conservation, University of Florida, Gainesville, FL 32601), E. S. MENGES (Archbold Biological Station, PO Box 2057, Lake Placid, FL 33862), P. F. QUINTANA-ASCENCIO (Dept. of Biology, University of Central Florida, Orlando, FL 32816), AND C. W. WEEKLEY (Archbold Biological Station, PO Box 2057, Lake Placid, FL 33862). Outcrossing effects on the reproductive performance of *Hypericum cumulicola*, an endangered Florida scrub endemic. J. Torrey Bot. Soc. 132: 204–213. 2005.—We investigated the effects of self-fertilizing and within-population outcrossing in two populations of *Hypericum cumulicola*, a perennial herb endemic to xeric scrub habitats on central Florida's Lake Wales Ridge. To examine the relationship between pollen source and reproductive performance, we compared fruit set, seed set, percent germination and net fertility (mean number of seedlings produced per flower within a pollination treatment) of hand-pollinated selfed and outcrossed flowers. There were no significant effects of categorical pollen source (self, near outcross, far outcross) on fruit set, and logistic regression showed no effect of interplant distance. Overall and at one of the two populations, far outcrosses had higher seed set than selfs and we found a weak but significant positive correlation between outcrossing distance and seed set. Percent seed germination was unaffected by pollen source. Net fertility was significantly correlated with outcrossing distance overall and at one of the two sites. These results show that pollen source may influence reproductive performance in *Hypericum cumulicola*, but the effects are fairly weak and inconsistent among populations, suggesting that outcrossing distance is not an important factor affecting small-scale population dynamics of this species.

Key words: Florida scrub, *Hypericum cumulicola*, inbreeding, Lake Wales Ridge, mating system, outcrossing.

Plant species with restricted geographic ranges and small population sizes tend to have relatively low genetic diversity and high rates of biparental inbreeding (Karron 1987, Keller and Waller 2002). Consequently, crosses between genetically related parents may produce fewer or

less fit offspring due to prezygotic factors or poor postzygotic performance (Sobrevila 1988, Montalvo 1992, Byers 1995, Keller and Waller 2002, Souto et al. 2002). Although genetic purging may reduce the potential negative effects of inbreeding (Husband and Schemske 1996, Byers and Waller 1999), many small and genetically depauperate populations nevertheless face greater risk of extinction from environmental and demographic stochasticity (Shaffer 1987, Tanaka 2000, Dennis 2002). As such, examining the effects of inbreeding in natural populations may have important implications for protecting population health and conserving genetic diversity of many rare plant species (Hamrick et al. 1991, Ellstrand and Elam 1993, Byers 1998, Keller and Waller 2002, Paschke et al. 2002).

Even among self-compatible plant species, outcrossing within and among populations often improves reproductive performance. Outcross-

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ing has been shown to increase fertilization rate (Sobrevila 1988, Waser and Price 1991), seed set (Waser and Price 1989, Dudash 1990, Montalvo 1992, Moran-Palma and Snow 1997), seed mass (Mitchell-Olds and Waller 1985, Montalvo 1992), percent germination (Liu and Koptur 2003) and progeny fitness (Mitchell-Olds and Waller 1985, Dudash 1990, Waser and Price 1994, Byers 1998). The magnitude of outcrossing benefits is often inversely correlated with the genetic similarity of the parent plants (Waser and Price 1991). However, crossing between genetically dissimilar parents may also result in outbreeding depression manifested as low fertilization rate, reduced seed set or maladaptation of offspring for the maternal environment due to different local selective pressures (Waser and Price 1989, 1994, Parker 1992, Fenster and Galloway 2000). In such cases, the spatial scale at which decreased reproductive performance occurs due to negative outcrossing effects is determined by the interaction of habitat differences and the spatial-genetic patterns resulting from natural rates of gene flow within and among populations (Price and Waser 1979, Waddington 1983).

For species with limited pollinator movement and seed dispersal, the distance between plants is often correlated with their degree of genetic similarity, and, therefore, spatial relationships have been used as surrogate measures of the presumed relatedness of individuals (Newport 1989, Escudero et al. 2003). If inbreeding and outbreeding depression occur simultaneously in plant populations with strong spatial-genetic structures, there may be an intermediate optimal outcrossing distance that represents the levels of genetic similarity between parents most conducive to high reproductive performance (Price and Waser 1979, Waddington 1983, Schierup and Christiansen 1996, Paschke et al. 2002). The scale at which inbreeding and outbreeding depression occur varies among species and populations. Whereas some species apparently do not experience reduced performance from inbreeding (Newport 1989) or local outcrossing (Byers 1998, Fenster and Galloway 2000), in other species these phenomena occur at a scale of several meters (Price and Waser 1979, Waser and Price 1989, 1991, 1994, Moran-Palma and Snow 1997, Paschke et al. 2002). It is also important to note that although individual crosses may be affected by inbreeding or outcrossing, population dynamics and total allelic diversity may remain unchanged due to confounding factors

such as random mating, gene flow, maternal effects and habitat quality (Waser and Price 1991, Moran-Palma and Snow 1997, Keller and Waller 2002).

In this study, we examined the effects of selfing and within-population outcrossing on the reproductive performance of *Hypericum cumulicola*, a federally endangered herb endemic to central Florida's Lake Wales Ridge (USFWS 1999). Our primary objective was to compare the reproductive performance of selfed and outcrossed flowers and to examine the effects of outcrossing distance. Because outcrossing effects can manifest at different stages of reproduction and offspring life (Schemske 1983, Dudash 1990, Montalvo 1992, Aizen and Feinsinger 1994, Husband and Schemske 1996, Keller and Waller 2002), we examined performance at three important measures of reproductive output: fruit set, seed set and germination, from which we were able to calculate a fourth cumulative measure of net fertility. Given the mating system of the species and the small size, isolation and low genetic diversity of many *H. cumulicola* populations (Dolan et al. 1999), we hypothesized that fruit set, seed set, germination and net fertility would be lower in selfed flowers compared to outcrossed flowers. We also hypothesized that the small interplant distances within populations would preclude an optimal outcrossing distance at the scale we studied and, therefore, performance of outcrossed flowers would be positively correlated with the distance between the pollen donor and the maternal plant. We compared results between two natural populations of *H. cumulicola* to contrast population-level differences in outcrossing effects.

Study species. *Hypericum cumulicola* (Adams) P. Small (Hypericaceae) is a short-lived perennial herb endemic to xeric scrub habitats on the Lake Wales Ridge, a globally important center of plant endemism (Christman and Judd 1990, Dobson et al. 1997, Menges 1999). This species is primarily restricted to bare sand gaps in patches of Florida rosemary (*Ceratiola ericoides*) scrub, but also occurs in scrubby flatwoods and sand firebreaks and roads (Abrahamson et al. 1984, Quintana-Ascencio and Morales-Hernandez 1997). Within sites, occupancy of individual gaps is spatially and temporally dynamic, with the highest levels of seedling recruitment and population densities occurring shortly following fire (Quintana-Ascencio and Menges 1996, Quintana-Ascencio et al. 2003).

Individual plants produce up to several thousand flowers during the period of April to October, each of which may result in the development of one fruit containing from 10 to 25 seeds, and most germination occurs during the winter and early spring.

Flowers are usually borne on multiple stems and are receptive between approximately 0730 and 1130 hrs. *Hypericum cumulicola* is self-compatible but herkogamous (male and female reproductive organs are separated spatially) and, therefore, requires insect visitors for high rates of pollination (Evans et al. 2003). The most common pollinators are small Halictid bees (*Dialictus* spp. and *Augochloropsis sumptuosa*), but other bees (Apidae: *Bombus pennsylvanicus*) and bee flies (Bombiliidae: *Geron* spp.) also visit receptive flowers (Evans et al. 2003, M. D. Trager pers. observation). In *H. cumulicola*, insect visitation rate increases seed set (Boyle and Menges 2001) and insufficient pollinator service may limit seed set in this species (Evans et al. 2003). Evans et al. 2003 observed that flowers pollinated by donors from more than 5 m away had higher seed set than selfed flowers in one population, but outcrossing effects on germination and the effects of varying interplant distance on outcross performance have not been studied previously. Pollinator observations have shown that most pollen transfer occurs among individuals within relatively small areas and most seed dispersal also occurs within a few meters of the maternal plant (Dolan et al. 1999, Boyle and Menges 2001). Consequently, *H. cumulicola* populations have low genetic diversity and show a strong spatial-genetic pattern in which plants separated by less than 15 to 20 m are on average significantly more related than expected from baseline variation within populations (Dolan et al. 1999, Menges et al. 2001, P. F. Quintana-Ascencio unpubl. data).

Methods. **STUDY SITES.** We conducted this study during the summer of 2002 at two protected areas near the southern end of Florida's Lake Wales Ridge, Lake June in Winter State Park (LJSP) and Lake Placid Scrub Preserve (LPS). Plants used for experimental crosses were located in bare-sand gaps within patches of xeric sand pine scrub. This vegetation type is characterized by the dominance of Florida rosemary, scrub oaks (*Quercus geminata*, *Q. inopina*, *Q. chapmanii* and *Q. myrtifolia*) and repent palmettos (*Sabal etonia*, *Serenoa repens*) (Abrahamson et al. 1984, Menges 1999). The LJSP

site had been burned recently (Spring 2000) and had more open sand, fewer Florida rosemary plants and a higher density of *H. cumulicola* than the LPS site, which had not been burned for over 10 years. Although the sites differed in time since fire and were separated by approximately 8 km, both represented appropriate habitat, were relatively isolated and hosted *H. cumulicola* populations of 120 to 200 individuals.

EXPERIMENTAL DESIGN. At each site we conducted experimental crosses on 20 focal plants selected haphazardly from each site to achieve a range of interplant distances. The plants also varied in height, number of stems and the density of conspecifics within a 1 m radius. Pollinators were excluded from two to four flowering stems per focal plant with plastic mesh pollination bags (Applied Extrusion Technologies) suspended on freestanding wire frames. We also excluded pollinators from the nearest neighbors of focal plants for shorter periods to assure that donor pollen from these plants was uncontaminated by insect visitors. Open-pollinated control flowers were selected from unbagged branches.

The presence and abundance of open flowers each day was unpredictable; therefore, we conducted crosses opportunistically with the goal of achieving four self-pollinations, near outcrosses, far outcrosses and open pollinations per plant. Because focal plants varied in conspecific density and we did not have an *a priori* reason to believe that certain categories of interplant distance would differ, continuous distances rather than predetermined categories were used for the analysis of outcrossing distance (Newport 1989). However, the near outcrosses were always conducted with the nearest neighbor to a given focal plant (median 1.08 m, range 0.03 to 12.43 m), which may have important ecological implications regardless of the exact interplant distance since neighbors are the most likely source of pollen. The far outcrosses, which were conducted between focal plants within each site that were not nearest neighbors, comprised a larger range of distances (median 23.3 m, range 0.5 to 104 m). Although the interplant distances comprised in these categories overlapped as an artifact of our haphazard method of selecting focal plants, most crosses in each category were not within the range of the other and, more importantly, we believed that the separation of nearest neighbors from other individuals would be ecologically meaningful.

We conducted hand-pollinations at the peak of

flowering season from 22 July to 26 August 2002. We emasculated flowers receiving pollen and applied donor pollen to each of the three stigmas. Selfed flowers were autogamous, as opposed to geitonogamous crosses. All flowers, including the open-pollinated controls, were individually marked with colored marking tape attached to the stem. We recovered mature fruits from hand-pollinated flowers 4 to 5 weeks after pollination and determined fruit set and seed set in the lab. Apparently healthy seeds were immediately placed on filter paper in petri dishes and subjected to a February temperature and light regime in a Conviron (Controlled Environments Limited) growth chamber to approximate conditions during the period of highest natural germination. The filter paper was moistened and replaced regularly and germination was monitored daily to prevent mold growth. The seeds that did not germinate within a few weeks were usually obviously dead—either shriveled or covered with fungal hyphae—and, as such, we did not test for viability of ungerminated seeds.

DATA ANALYSIS. At each reproductive stage we compared the performance of selfed, near outcrossed, far outcrossed and open-pollinated flowers and analyzed the effects of interplant distance on cross outcome where appropriate. We analyzed each parameter cumulatively and for many tests we also present results for LPS and LJSP individually to show differences between the two sites. For the analysis of fruit set, we used χ^2 tests to assess differences among pollen source treatments and logistic regression to assess the effect of interplant distance and maternal plant characteristics on the likelihood that flowers produce a fruit. The data for seed set, germination and net fertility could not be transformed to a normal distribution and, therefore, we used Wilcoxon signed rank tests to determine pairwise differences between treatments. To control for differences in performance among maternal plants in the categorical analyses of seed set, germination and net fertility, within each plant we aggregated all flowers subjected to a given treatment and then relativized these values by setting the highest mean equal to 1.0 and the others as proportions before conducting the tests. For these analyses, we were only able to include plants that had mean values for selfed, near and far outcrossed treatments. We performed Spearman rank correlations to determine outcrossing distance effects on seed set, germination and net fertility and used the regression

curve-fitting procedure of SPSS to indicate the presence of an optimal outcrossing distance. In the tests using continuous distances as the predictor variable, we treated each flower individually regardless of maternal plant. We used SPSS 11.0 (SPSS, Inc. 2002) for all analyses.

Results. We followed 501 *H. cumulicola* flowers (139 self, 103 near outcross, 126 far outcross, 133 open-pollinated) and recovered fruits from 447 of them (89.2 percent). By every measure of reproductive performance except germination percentage, which was universally very high, the naturally pollinated open flowers performed significantly better than those that were hand-pollinated. Because the data from these flowers were so disparate and were not focal to our study, we present here only the results from hand-pollinated crosses (self, near and far outcross) to best examine the effects of pollen source and crossing distance rather than including results that may have confounded our inability to mimic natural pollination processes for this species with actual effects of pollen source.

In a small number of cases (15 across both sites and all three treatments included here), the seeds had not yet matured when we collected the fruits, which allowed measurement of fruit set and seed set but not of germination. Additionally, 17 fruits had already matured and dehisced, thus allowing measurement of fruit set but precluding measurement of seed set or germination. The 22 fruits in which the seeds were partially or fully depredated by an unidentified beetle were not included in the analyses except for fruit set. Fruits at the LPS site were depredated at a significantly higher frequency than those at LJSP ($n = 315$, $\chi^2 = 10.95$, d.f. = 1, $P = 0.001$). Additionally, the treatments at LPS differed in the frequency of predation ($n = 165$, $\chi^2 = 6.838$, d.f. = 1, $P = 0.033$, with far outcrosses being attacked the most frequently (11 of 59) followed by near outcrosses (6 of 47) and selfs (2 of 59).

FRUIT SET. Of 333 hand-pollinated flowers available for analysis from the two sites, 278 (83.5 percent) produced a fruit. Overall, there were no significant differences among pollination treatments in overall proportional fruit set ($n = 333$, $\chi^2 = 0.80$, d.f. = 2, $P = 0.67$). Additionally, there were no significant differences in fruit set among treatments within sites (LPS: $n = 179$, $\chi^2 = 0.991$, d.f. = 2, $P = 0.609$; LJSP: $n = 154$, $\chi^2 = 0.899$, d.f. = 2, $P = 0.651$) or within treatments between sites (self: $n = 126$,

Table 1. Logistic regression testing the effects of plant characteristics (conspecific density within 1 m radius, total height, number of stems) and outcrossing distance on fruit set. Only outcrossed flowers for which we had the necessary data were used in this analysis ($n = 207$). The model has fairly low explanatory power (Nagelkerke $r^2 = 0.103$), but was significant overall (Likelihood ratio $\chi^2 = 12.904$, d.f. = 4, $P = 0.012$).

Variable	d.f.	β	SE	Wald χ^2	Sig.
Density	1	-0.080	0.056	1.994	0.158
Height	1	-0.023	0.022	1.017	0.313
No. stems	1	-0.086	0.030	8.355	0.004
Outcrossing distance	1	0.0	0.0	1.595	0.207
Constant	1	3.898	1.501	6.741	0.009

$\chi^2 = 0.007$, d.f. = 1, $P = 0.935$; near outcross: $n = 93$, $\chi^2 = 1.883$, d.f. = 1, $P = 0.176$; far outcross: $n = 114$, $\chi^2 = 0.998$, d.f. = 1, $P = 0.318$). Logistic regression using only the outcrossed flowers showed no significant effect of crossing distance on fruit set, but did show a significant positive effect of number of stems of the maternal plant on the likelihood of a flower producing a fruit (Table 1). Because exploratory analysis showed no significant differences between LPS and LJSP and because including subsets of predictor variables in the model did not produce different results, here we only present the results of the model that included all variables and all flowers from both sites.

SEED SET. Overall, there was significantly lower seed set in the self-pollinated flowers than in the far outcrossed flowers (Wilcoxon signed rank test, $n = 31$, $Z = -2.107$, $P = 0.035$), but there were no significant differences in other

pairwise comparisons of the treatments (self vs near outcross: $Z = -1.092$, $P = 0.275$; near vs far outcross: $Z = -1.568$, $P = 0.117$). The median values for seed set of the treatments (selfed = 5.0, near outcross = 5.0, far outcross = 7.0) suggested that far outcrosses performed better than flowers in the other treatments. The far outcrossed flowers also had the highest median proportional seed set at both sites; however, when analyzed separately, the sites showed divergent patterns of relationships between the treatments (Fig. 1). At LPS, the self treatment had significantly lower seed set than the near outcrossed flowers ($n = 16$, $Z = -2.229$, $P = 0.026$), but there was only a marginal difference between selfed and far outcrossed flowers ($Z = -1.817$, $P = 0.069$) and no difference between near and far outcrosses ($Z = -0.175$, $P = 0.861$). At LJSP, near outcrossed flowers had marginally lower seed set than far outcrossed flowers ($n = 15$, $Z = -0.931$, $P = 0.053$) but selfed flowers did not significantly differ from either of the outcross treatments (self vs near outcross: $Z = -0.282$, $P = 0.778$; self vs far outcross: $Z = -1.193$, $P = 0.223$).

Spearman rank correlation analysis of the effect of interplant distance showed that overall seed set was weakly but significantly related to outcrossing distance ($n = 137$, $r_s = 0.224$, $P = 0.009$). However, like the results for the categorical treatments, this relationship was not consistent between sites (Fig. 2; LPS: $n = 73$, $r_s = 0.140$, $P = 0.238$; LJSP: $n = 64$, $r_s = 0.317$, $P = 0.011$). As the best fit to the data was achieved with a linear model, regression analysis showed no evidence of an intermediate optimal outcrossing distance.

GERMINATION. Germination percentage was very high across all treatments at both sites. Over half of all fruits had 100 percent seed germination and there were no differences in proportional percent germination among categorical

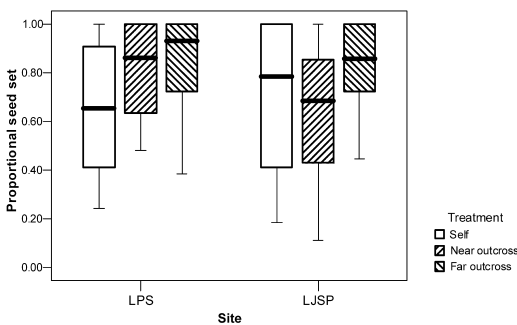


FIG. 1. Boxplots of proportional relative seed set of treatments at both sites showing minimum, 25% quartile, median, 75% quartile and maximum values. At LPS, the self treatment had significantly lower seed set than the near outcross ($P = 0.026$), and marginally insignificantly lower seed set than the far outcross ($P = 0.069$). At LJSP, the near outcrosses had marginally significantly lower seed set than the far outcrosses ($P = 0.053$). Each pollen source treatment resulted in the highest relative mean seed set in at least one maternal plant.

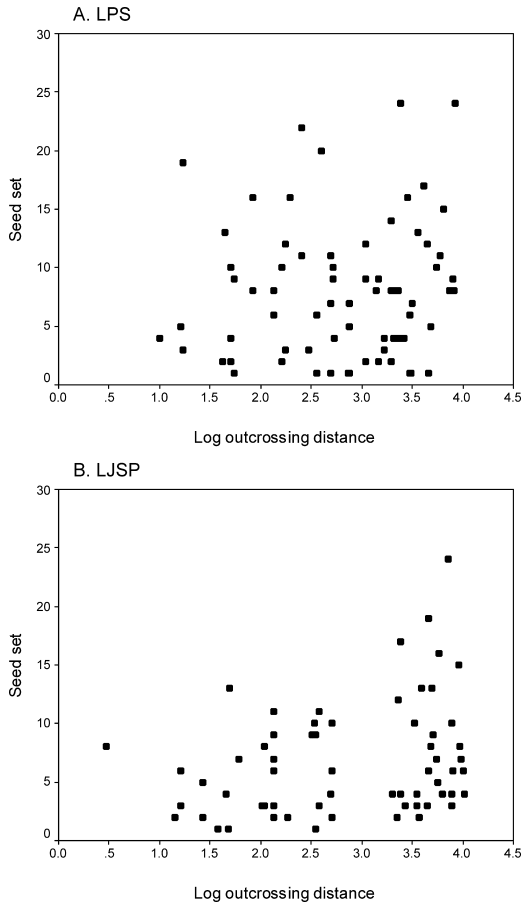


FIG. 2. Scatterplots of log outcrossing distance and seed set for LPS (A) and LJSP (B). The positive correlation was only significant at LJSP (LPS: $n = 73$, $r_s = 0.140$, $P = 0.238$; LJSP: $n = 64$, $r_s = 0.317$, $P = 0.011$).

treatments. Additionally, there was no effect of outcrossing distance on percent germination (Overall: $n = 144$, germination = 90.1%, $r_s = -0.034$, $P = 0.685$; LPS: $n = 78$, germination = 88.8%, $r_s = -0.026$, $P = 0.824$; LJSP: $n = 66$, germination = 91.6%, $r_s = 0.079$, $P = 0.527$).

NET FERTILITY. We calculated net fertility as the mean number of seedlings resulting from all flowers subjected to a given treatment on each plant. Thus, this measure reflected plant variation in fruit set, seed set and percent germination to give a cumulative measure of reproductive performance. Wilcoxon signed rank tests showed no significant pairwise differences among categorical pollination treatments at either LPS or LJSP (Fig. 3). At LPS, the self treatment had marginally significantly lower seed set

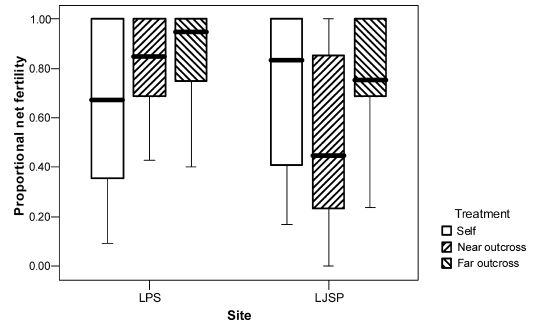


FIG. 3. Boxplots of proportional net fertility of treatments at both sites showing minimum, 25% quartile, median, 75% quartile and maximum values. The results were similar to those for seed set but all treatments were more variable due to differences in percent germination.

than far outcrosses ($n = 17$, $Z = -1.655$, $P = 0.098$) but there were no other pairwise differences (self vs near outcross: $Z = -1.412$, $P = 0.158$; near vs far outcross: $Z = -0.876$, $P = 0.381$). At LJSP, near outcrossed flowers had marginally significantly lower total net fertility than far outcrossed flowers ($n = 16$, $Z = -1.758$, $P = 0.079$) but there were no other pairwise differences (self vs near outcross: $Z = -1.345$, $P = 0.179$; self vs far outcross: $Z = -0.465$, $P = 0.642$). Accordingly, there were also no significant differences overall among categorical treatments in proportional net fertility ($n = 33$; self vs near outcross: $Z = -0.093$, $P = 0.926$; self vs far outcross: $Z = -1.300$, $P = 0.194$; near vs far outcross: $Z = -1.796$, $P = 0.073$).

As was the case for the seed set analysis, overall there was a positive and significant correlation between outcrossing distance and net fertility ($n = 168$, $r_s = 0.240$, $P = 0.002$) despite the presence of many aborted fruits. However, this relationship was inconsistent between sites, with LJSP showing a weak significant correlation ($n = 82$, $r_s = 0.290$, $P = 0.008$) and LPS having only a marginally significant and fairly weak correlation ($n = 86$, $r_s = 0.181$, $P = 0.096$; Fig. 4).

Discussion. Given the low genetic diversity, clear spatial-genetic structure and low rates of long-distance gene flow within and among *Hypericum cumulicola* populations (Dolan et al. 1999, Boyle and Menges 2001, Menges et al. 2001), we expected selfed flowers to have lower performance than outcrossed flowers, with the benefits of outcrossing increasing with distance. However, contrary to expectation, our study did

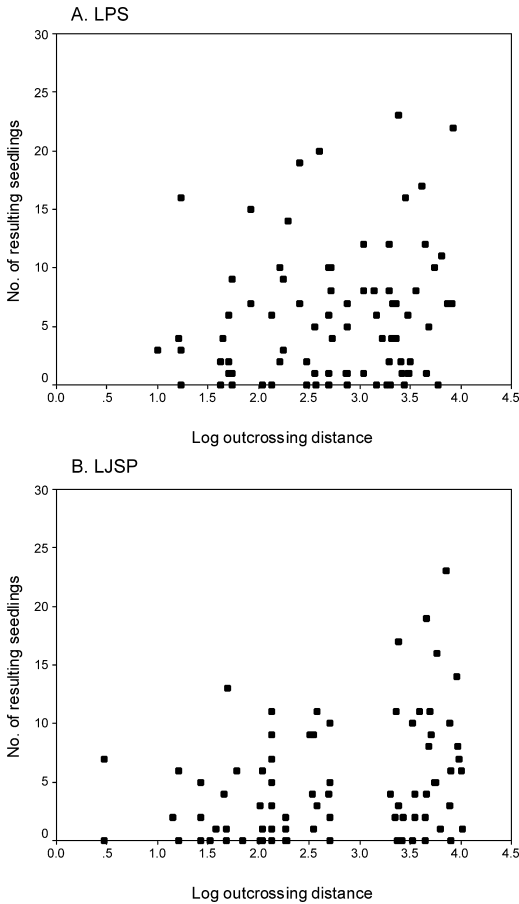


FIG. 4. Scatterplots of log outcrossing distance and net fertility for LPS (A) and LJSP (B). The positive correlation was only significant at LJSP, but was nearly so for LPS (LPS: $n = 86$, $r_s = 0.181$, $P = 0.096$; LJSP: $n = 82$, $r_s = 0.290$, $P = 0.008$).

not find convincing evidence of strong negative inbreeding effects or strong positive outcrossing effects in *H. cumulicola*. Although there were differences between the categorical pollination treatments for seed set and net fertility, and outcrossing distance affected both of these parameters overall, we did not detect significant effects of interplant distance on fruit set or germination percentage.

Evans et al. (2003) found no significant difference in *H. cumulicola* fruit set between self and outcross treatments which, in corroboration with our study, suggests that this may be a common feature across populations of this species. However, Evans et al. (2003) found higher seed set in outcrossed than in selfed flowers, whereas there were few statistically significant differences between selfed and outcrossed flowers in our

study. In our analysis of outcrossing distance, we only found significant evidence for increased seed set with interplant distance overall and at the LJSP site, but these correlations were quite weak, suggesting that other factors are also important for determining seed set. The most parsimonious explanation for these results is that inbreeding depression likely does occur to at least a limited extent in *H. cumulicola*, but it appears to be mediated by other factors and therefore the degree to which it affects reproductive performance varies among individuals and populations. This conclusion is supported by our results demonstrating that the number of stems of maternal plants, a possible indication of health, was the only factor that significantly affected fruit set and that frequency of seed predation, presumably related to environmental conditions, differed significantly between the two sites.

Several studies have found that the number of seeds produced in a cross is affected by interplant distance (Price and Waser 1979, Schemske 1983, Byers 1995, Paschke et al. 2002). Seed set can be influenced by prezygotic mechanisms that impede or prevent fertilization (Waser and Price 1991, Montalvo 1992, Richards 1997, Souto et al. 2002) and by postzygotic mechanisms such as selective abortion of seeds that are likely to have low fitness (Waser and Price 1989). Why might the beneficial effects of outcrossing in *H. cumulicola* be more apparent in seed set rather than fruit set or percent germination? Firstly, in this species fruits form regardless of the number of seeds they contain as long as there is at least one viable seed. Therefore, fruit set alone is not a reliable measure of reproductive output. Secondly, it is possible that maternal plants selectively abort non-germinable seeds and only those that are healthy develop to maturity. If so, this would explain why germination percentage of the intact recovered seeds did not vary among treatments and was universally very high.

Another potential explanation for our results is that outcrossing effects are manifested in post-germination offspring performance, and thus were undetected in our study. Research on other plant species has shown that the positive effects of outcrossing are only evident in the survivorship, growth and reproduction of the immediate progeny or future generations (Mitchell-Olds and Waller 1985, Waser and Price 1989, 1994, Parker 1992, Schemske 1983, Byers 1998). Addressing this issue with *Hypericum cumulicola*

would require planting seedlings resulting from hand-pollinations back into their populations of origin or growing them in a common garden to compare long-term fitness (Dudash 1990).

Despite performing hand pollinations that encompassed the distance of most natural pollen movement, we found no evidence for an intermediate optimal outcrossing distance in *H. cumulicola*. Indeed, the relationship between interplant distance and seed set overall and at LJSP, although fairly weak, was positive and best explained with a linear model in the SPSS curve-fitting procedure. There are two likely explanations for this result: 1) *H. cumulicola* simply does not experience outbreeding depression, or 2) our crosses were not sufficiently distant to detect it. It is possible that within the restricted range of this species' microhabitat adaptations are minimal and reproductive compatibility among populations is high (Price and Waser 1979). However, this is unlikely considering that most genetic variation in *H. cumulicola* occurs among rather than within populations (Dolan et al. 1999, Menges et al. 2001). We therefore find it more likely that outbreeding depression may occur, but was not detectable at the relatively small spatial scale at which we conducted this experiment. If this explanation is correct, then outbreeding depression is unlikely to affect natural populations of this species due to limited pollen movement over large distances (Boyle and Menges 2001), but we would expect to find evidence of outbreeding depression in crosses between disjunct populations that do not exchange genetic material under most natural conditions.

The results of many analyses differed between the Lake Placid Scrub and Lake June in Winter State Park populations. Although the two populations were similar in size, there were more reproductive plants and more suitable habitat patches at LJSP, both factors that may be correlated with higher genetic diversity at the population level (Ellstrand and Elam 1993, Paschke et al. 2002). Since fire can affect the population density and spatial-genetic structure of *H. cumulicola* populations (Quintana-Ascencio and Menges 1996, Quintana-Ascencio et al. 2003), the different fire regimes of the sites could also account for some of the differences in outcrossing effects. The history of the populations is poorly known, but their age, previous bottlenecks, founder effects or their level of isolation from other populations could also affect the current spatial-genetic structure. Regardless of the

causes for differences between these two populations, it is clear that plant breeding system studies conducted at only one or two sites may not represent the variety of effects that inbreeding and outcrossing may have in populations that differ in size, density, genetic diversity, disturbance regime and reproductive history (Aizen and Feinsinger 1994).

In this study we found limited evidence of inbreeding depression or outcrossing benefits in *H. cumulicola*, and these effects are probably not manifested at a magnitude that would directionally affect most populations of this species. Nevertheless, fire suppression and further fragmentation of *H. cumulicola* habitat could decrease population sizes and reduce genetic diversity such that inbreeding depression could significantly reduce reproductive performance, leading to a local extinction vortex (Tanaka 2000, Dennis 2002, Quintana-Ascencio et al. 2003). Fire may also indirectly affect population health through altering microhabitat conditions and interspecific interactions (Quintana-Ascencio and Morales-Hernandez 1997). In our study, for example, we suspect that the higher rate of seed predation at the LPS site may reflect an increase in the density of the beetle seed predator due to a longer interval without fire reducing its population. However, with appropriate fire management of its habitat, the potential negative effects of inbreeding on the reproductive performance of *Hypericum cumulicola* should be alleviated by the creation of more suitable bare sand patches, which result in larger population sizes and greater habitat connectivity to facilitate gene flow through pollen and seed dispersal.

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