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Reproductive success through high pollinator visitation rates despite self incompatibility in an endangered wallflower[1]

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PREMISE OF THE STUDY: Self incompatibility (SI) in rare plants presents a unique challenge—SI protects plants from inbreeding depression, but requires a sufficient number of mates and xenogamous pollination. Does SI persist in an endangered polyploid? Is pollinator visitation sufficient to ensure reproductive success? Is there evidence of inbreeding/outbreeding depression? We characterized the mating system, primary pollinators, pollen limitation, and inbreeding/outbreeding depression in *Erysimum teretifolium* to guide conservation efforts.

METHODS: We compared seed production following self pollination and within- and between-population crosses. Pollen tubes were visualized after self pollinations and between-population pollinations. Pollen limitation was tested in the field. Pollinator observations were quantified using digital video. Inbreeding/outbreeding depression was assessed in progeny from self and outcross pollinations at early and later developmental stages.

KEY RESULTS: Self-pollination reduced seed set by 6.5× and quadrupled reproductive failure compared with outcross pollination. Pollen tubes of some self pollinations were arrested at the stigmatic surface. Seed-set data indicated strong SI, and fruit-set data suggested partial SI. Pollinator diversity and visitation rates were high, and there was no evidence of pollen limitation. Inbreeding depression (δ) was weak for early developmental stages and strong for later developmental stages, with no evidence of outbreeding depression.

CONCLUSIONS: The rare hexaploid *E. teretifolium* is largely self incompatible and suffers from late-acting inbreeding depression. Reproductive success in natural populations was accomplished through high pollinator visitation rates consistent with a lack of pollen limitation. Future reproductive health for this species will require large population sizes with sufficient mates and a robust pollinator community.

KEY WORDS Brassicaceae; *Erysimum teretifolium*; inbreeding depression; pollen limitation; self incompatibility

In plants, mating systems are strong predictors of the distribution of genetic diversity (Glémin et al., 2006; Duminil et al., 2007, 2009), which is especially important in managing endangered species. Mating systems range from outcrossing to selfing. Outcrossing increases heterozygosity, allelic richness, and the amount of genetic diversity within-populations compared with selfing (Hamrick and Godt, 1989, 1996; Charlesworth, 2003). Outcrossing mating systems range from self incompatible (SI) to self compatible (SC). Although SI makes individuals completely reliant on extrinsic pollination agents for sexual reproduction (de Nettancourt, 1977; Pavlik et al., 1993; Ortigosa and Gómez, 2010), these individuals benefit from higher levels of genetic diversity and are less likely to express inbreeding depression than if they were SC (Charlesworth and Charlesworth, 1987; Saunders et al., 1991; Barrett, 2002; Aguilar et al., 2006; Edmands, 2007; Honnay and Jacquemyn, 2007; Richter et al., 2009). SI in rare plants presents a unique challenge—SI species are largely protected from the expression of inbreeding depression, yet require xenogamous pollination and sufficient population sizes to provide viable mates to ensure reproductive success.

Although an SI mating system has many advantages, SC individuals may benefit in certain circumstances, some of which are particularly apposite to endangered plant populations. SC can increase reproductive success in fragmented or degraded habitats where mates and/or pollinators may be limited (Pannell and

Barrett, 1998). SC individuals benefit from greater reproductive assurance than SI individuals (Eckert and Schaefer, 1998; Eckert et al., 2010), and they suffer less pollen limitation (Eckert and Schaefer, 1998; Busch, 2005; Campbell and Husband, 2007; but see reviews by Burd, 1994 and Knight et al., 2005). Furthermore, SC is a precondition for an autogamous mating system, which automatically confers a 3:2 transmission advantage for the self-compatible allele when compared with a self-incompatible allele (Fisher, 1941; Schoen and Busch, 2008; Charlesworth and Charlesworth, 2012; Igić and Busch, 2013). These short-term benefits of SC, however, are often outweighed by the long-term disadvantages of decreased heterozygosity, lower genetic diversity, and less adaptive potential compared with SI mating systems (Igić and Busch, 2013). Managing current populations of endangered species to maintain sufficient genetic diversity for future adaptability is a persistent challenge in conservation biology and relies heavily on the type of mating system (Stockwell et al., 2003).

SI prevents self fertilization through a lock-and-key system where the pistil only accepts pollen from individuals that do not share the same S-locus alleles (Charlesworth and Charlesworth, 1987; Kao and McCubbin, 1996; Mena-Ali et al., 2008; Guo et al., 2009). Following self pollination in a sporophytic SI species, cell–cell recognition between male and female components at the S-locus initiates a complex signal transduction pathway that leads to arrested growth of self-pollen tubes (Mable, 2008). Sporophytic SI, a system wherein the pollen phenotype is diploid and thus both pollen and pistil express two S alleles, is widespread in Brassicaceae, where it has been extensively characterized in several genera, including *Leavenworthia*, *Brassica*, *Capsella*, and *Arabidopsis* (Charlesworth et al., 2005; Anderson and Busch, 2006; Vekemans et al., 2014). Sporophytic SI can break down due to genome duplication events, mutations at the S-locus, or mutations downstream in the SI signaling pathway (Mable et al., 2005; Mable, 2008; Tsuchimatsu et al., 2012; Vekemans et al., 2014). Overall, previous research suggests that polyploidy events may correlate with SI breakdown in general (Barringer, 2007; Robertson et al., 2011; but see Mable, 2004). In the Brassicaceae, polyploidy has only sporadically been associated with breakdown in SI, such as in *Arabidopsis kamchatica*, *Capsella bursa-pastoris*, and numerous *Draba* spp. (Brochmann, 1993; Miller and Venable, 2000; Hintz et al., 2006; Tsuchimatsu et al., 2012). Breakdown of SI is a frequent mating system transition and leads to partial SI and eventually to SC, which can generate obligately selfing species under favorable evolutionary conditions (Grossenbacher and Whittall, 2011). The transition from SI to SC can be quantified using the index of self incompatibility (ISI) that compares the proportion of successful reproductive events (e.g., seeds or fruits) from self pollinations to that from outcross pollinations (Raduski et al., 2012). In a literature review of 1238 angiosperms, ISI appears strongly bimodal—three quarters of these species fall below 0.2 or above 0.8 (Raduski et al., 2012). The latter value is often used as a cutoff for SI (Bawa, 1974).

Translocations are increasingly used in the conservation of threatened and endangered species as a way to mitigate the loss of genetic diversity as well as ecological Allee effects (Weeks et al., 2011; International Union for Conservation of Nature/Species Survival Commission, 2013). These challenges for small populations are even more pronounced for SI taxa because of their reliance on outcrossing and need for a large number of potential mates for successful reproduction (DeMauro, 1993; Elam et al., 2007). However, promoting gene flow by translocating individuals, or mixing genetic material from multiple source populations during reintroductions, may lead to outbreeding depression if locally adapted traits are compromised or coadapted gene complexes are disrupted (Ellstrand, 1992; Frankham et al., 2011; Mijangos et al., 2015). Even in cases where outbreeding depression is not expected, such as SI taxa, conservation practitioners may still be concerned about the irreversible effects of mixing genes from different populations (Weeks et al., 2011). One important component of risk assessment for translocations is to compare the survival and reproductive success of offspring from within-population crosses vs. between-population crosses.

The wallflower genus *Erysimum* exhibits a range of mating systems and ploidy levels (Price, 1987) with a history of SI in the family (Mable, 2008; Vekemans et al., 2014). *Erysimum* is the third most diverse genus in the Brassicaceae, containing species that vary from outcrossing biennials (e.g., *E. capitatum*) to autogamous annuals (e.g., *E. cheiranthoides*), with ploidy levels ranging from diploid to hexaploid (Rossbach, 1958; Nieto Feliner, 1991; Warwick et al., 2010). The *E. capitatum* alliance, concentrated in western North America consists of approximately 25 closely related, large-flowered, biennial, hexaploid taxa including several rare or endangered edaphic endemics (Rossbach, 1958; Price, 1987; Moazzeni et al., 2014). One member of the *E. capitatum* alliance, *E. teretifolium*, is an endangered, monocarpic herb that produces a large, showy inflorescence of bright yellow, protandrous flowers that mature into siliques containing 1–54 seeds (U. S. Fish and Wildlife Service, 1998). *Erysimum teretifolium* is restricted to the Zayante sandhills of Santa Cruz County, California, United States, a habitat composed of naturally fragmented patches of uplifted, Miocene beach sand surrounded by mesic redwood forests and mixed evergreen forests.

In 1998, there were 20 known populations of *E. teretifolium* (U. S. Fish and Wildlife Service, 1998). By 2007, only 13 of these populations remained, most of which were in varying states of decline (U. S. Fish and Wildlife Service, 2007). Bonny Doon Ecological Preserve was one of the largest populations (McGraw, 2004a, b), yet has declined precipitously since 2011 from several thousand reproductively mature individuals to fewer than 10, reportedly due to reproductive failure (T. Kasteen, U. S. Fish and Wildlife Service, personal communication). Reproductive failure could have multiple causes including pollen limitation due to reduced mate availability in a SI-mating system, pollen limitation due to lack of pollinator visitation, and/or inbreeding depression caused by a recent increase in inbreeding in a historically large, outcrossing population following the breakdown of the SI system (Waites and Ågren, 2004; Campbell and Husband, 2007; Wagenius and Lyon, 2010). Potential conservation actions include reintroducing *E. teretifolium* into extirpated sites and supplementing the Bonny Doon site to augment the dwindling population. Both measures involve introducing individuals from other populations.

Appropriate management for *E. teretifolium* depends on the mating system, pollinators and pollen limitation, and the potential for inbreeding/outbreeding depression. Here, we tested for SI in *E. teretifolium* through controlled crosses and visualization of pollen tube growth. We conducted field studies to determine whether *E. teretifolium* is pollen-limited and used digital video to capture the identity and frequency of its pollinators. We also assessed whether selfed offspring show inbreeding depression and whether the offspring from crosses made between populations exhibit outbreeding depression to guide future management actions for this endangered species.

MATERIALS AND METHODS

Seed sources and growing conditions—Seeds were collected from Quail Hollow Ranch County Park (QH), San Lorenzo Valley Water District/Olympia Wellfields (SLV), Geyer Quarry/Randall Morgan Sandhills Preserve (GEY), and Bonny Doon Ecological Reserve (BD) in Santa Cruz County, California, in the summer of 2010. Seeds were sampled from three geographically distinct patches at each location, except at BD where only two patches remained (Appendix S1, see Supplemental Data with the online version of this article). The distance between patches ranged from 10 to 162 m (mean = 30.82 m). At least 15 seeds total were collected from five individuals per patch.

In August 2010, seeds were germinated and grown in a randomized design in 4-inch pots at the University of California Santa Cruz greenhouse. In March of 2012, plants were transplanted into 1-gallon pots and transported to the Santa Clara University (SCU) experimental garden (QH N = 76; SLV N = 75; GEY N = 76; BD N = 46). Plants were maintained at this outdoor facility for 6 weeks to experience late winter dormancy. In May 2012, plants began bolting and were moved to the SCU greenhouse, where temperatures were kept below 30°C, and natural sunlight was supplemented with 12 h of 400-W lighting. Fourteen individuals flowered over the next 3 months (QH N = 1; SLV N = 4; GEY N = 8; BD N = 1). Following this first year of the pollination experiment (see *Greenhouse crossing experiment* section), all plants were returned to the SCU experimental garden in December 2012 to establish winter dormancy. In March 2013, all surviving plants were moved back into the SCU greenhouse where about 80% of plants flowered, and 36 individuals were randomly selected for the greenhouse crossing experiment (QH N = 11; SLV N = 8; GEY N = 8; BD N = 9). Whenever possible, within-population crosses were conducted with plants from separate patches to reduce the probability of shared S alleles and biparental inbreeding.

Greenhouse crossing experiment—In tests for SI and assessing levels of inbreeding and outbreeding depression, each maternal plant received four treatments within the first 10 flowers of the same inflorescence. Following emasculation, the three experimental treatments were self pollination and two forms of outcross pollination: within-population and between-population crosses. All pollination types were attempted on each plant to control for maternal effects. Over 2 years, 161 crosses were made (self pollinations N = 43; within-population crosses N = 50; between-population crosses N = 68) on 50 maternal plants (QH N = 12, SLV N = 12, GEY N = 16, BD N = 10). Negative controls were emasculated, but no pollen was added.

Pollinations were conducted using standard hybridization techniques (Kearns and Inouye, 1993). The order of pollination treatments was randomized for each maternal plant. For ensuring that pollen was deposited on receptive stigmas, a dry stigma receptivity test using Peroxtesmo KO (Macherey-Nagel, Bethlehem, Pennsylvania, USA) was conducted on 27 buds (QH N = 6; SLV N = 6; GEY N = 8; BD N = 7). Stigmas became receptive on the first day through 9 d after opening. We applied pollen from a single, arbitrarily assigned donor using a toothpick within the treatment type 3, 4, and 5 d after emasculation.

Pollinated flowers took approximately 2 months to mature their seeds. Dry fruits were harvested and stored in coin envelopes until the seeds were counted and weighed. Some seeds were aborted and

removed from subsequent analyses. All viable seeds were counted and weighed as a group to estimate average seed mass.

To test for SI, we first compared the average seed count for the self-pollination treatment with the two outcross treatments combined. To determine overall effects of self pollination vs. outcross pollination, we used a Welch's t test to compare mean seed set after a Levene's test had indicated that there was unequal variance ($W_{1, 159}$ = 44.7, P < 0.0005). To compare self-pollination to within-population and between-population outcross treatments, we used the nonparametric Kruskal–Wallis statistic due to the excess of self pollinations producing zero seeds. To determine which pairwise comparisons among these three cross types were significant, we used the Dwass–Steel–Critchlow–Fligner test (Critchlow and Fligner, 1991), which provides a nonparametric, post hoc procedure that separately ranks the observations from each pair of treatments, then computes the Wilcoxon rank sum statistic for each pair (Spurrier, 2006).

The index of self incompatibility (ISI) compares reproductive success following self and outcross pollinations to assess the degree of SI (Raduski et al., 2012). Values range from zero to one, where zero signifies completely SC and one indicates SI. We calculated ISI based on the proportion of fertilized ovules following self and outcross pollinations: ISI_{seed} = 1 − [(number of fertilized self ovules / total number of self ovules) / (number of fertilized outcross ovules / total number of outcross ovules)]. We did not need to measure the number of ovules since we assume there is the same number of ovules per flower for self pollinations as outcross pollinations and therefore these values cancel in the ISI calculation. For comparison, we also calculated ISI_{fruit} based on the proportion of fruits matured following self and outcross pollinations: ISI_{fruit} = 1 − [(number of self fruits matured / total number of self pollinations) / (number of outcross fruits matured / total number of outcross pollinations)] (Raduski et al., 2012). All fruits containing ≥1 seed were considered successful.

Pollen-tube growth assay—We conducted a pollen-tube growth experiment to assess the possibility of sporophytic SI in *E. teretifolium*. Four individuals from three populations from the greenhouse crossing experiment that did not flower previously were emasculated and given one application of either self or outcross pollen in 2014. After pollination, plants were maintained under greenhouse conditions identical to that described previously (see *Greenhouse crossing experiment* section). Stigmas were harvested 24 and 48 h after pollination and stored in 10% acetic acid–90% ethanol solution (v/v) for up to 7 days. Stigmas were cleared with 8 N sodium hydroxide, dyed with a 0.1% w/v aqueous aniline blue and then washed in 0.1 N tripotassium phosphate as described by Martin (1959). Pollen-tube growth was visualized under UV light with a 4× objective using a DAPI filter cube on a Zeiss axioscope. Opened flowers of *Arabidopsis thaliana* provided a positive control for the visualization of pollen tubes, and emasculated *E. teretifolium* without pollen added was the negative control.

Pollen limitation experiment—We selected 20–23 plants at each of three field sites (GEY, QH, SLV), with two pairs of flowers at the same stage of corolla development per plant. One flower of each pair was hand-pollinated with pollen mixed from ≥2 pollen donors located a minimum of 5 m away. All experimental plants were protected from deer by topless wire cages with 4.5 cm holes to allow access by pollinators. Plants with visible effects of other herbivory were dropped from the study. We checked the resulting fruits

repeatedly. Before fruit dehiscence, we collected the matured fruits and counted both viable and aborted seeds to produce both final seed number and seed viability as a proportion of seeds initiated. We used a mixed effects logistic model to test for an effect of supplemental pollination on the probability of fruit set, and general linear mixed models to test for an effect of supplemental pollination on the (arcsine-transformed) proportion of seeds that were viable and the number of seeds per fruit. Site and treatment were fixed effects, and maternal plant was a random effect nested within site.

Pollinator observations—Digital video cameras were used to document the common diurnal pollinators for *E. teretifolium* and the frequency of visitation for distinct insect pollinator guilds. Three populations were surveyed at peak flowering over 2 years (Appendix S2, see online Supplemental Data). Three to six Canon FS22s camcorders were deployed at a site and positioned on tripods approximately 50 cm from an inflorescence between 0° (horizontal) and 30° (below horizontal). Camcorders were manually focused on staked inflorescences with 5–14 open flowers in frame (mean = 7.8 flowers/camcorder). Camcorders recorded diurnal pollinators from 07:00 to 19:00 hours. Several field surveys at dusk revealed no crepuscular pollinators (J. Whittall, unpublished observations). Data were imported from each camera using iMovie v.11 9.0.9, then exported as *.mov files and reviewed using QuickTime Player 10.2 at 1–4× recording speed.

Any insect that contacted either the anthers or the stigma was considered a pollinator. The start and stop times for each visit were extracted from the video clock. Pollinators were identified into broad functional guilds using a reference collection (M. K. Melen, unpublished), screen shots, and the advice of specialists. The number of flower-hours for each camcorder was determined by multiplying the number of video-hours by the number of visible flowers in the camcorder's view. To determine the average number of visits per flower per hour, the total number of insect visits was summed and divided by the total number of flower-hours for all camcorders. Using the average visits per flower per hour, we estimated the average number of visits to a flower by multiplying visits per flower per hour by the number of hours each flower was potentially available to pollinators: each flower lasts at least 3 days according to Parker et al. (2011), and we estimate that at a minimum, approximately half of the daylight hours (six) were conducive to pollinator activity because of temperature, wind, fog, etc. Pollinators were identified to functional guild (Table 1).

F1 fitness and inbreeding depression—To determine the effects of cross type on F1 fitness and specifically test for inbreeding and outbreeding depression, we examined germination, growth, and survival to 14 wk of the F1 generation seeds from the greenhouse crossing study in sandhill soil in fall of 2013. For 61 crosses, 10 seeds per cross were planted on each of two soil types (QH and BD) to account for the possibility of local edaphic adaptation. For 47 additional crosses that produced fewer than 20 seeds, we split the seeds evenly between the two soil types favoring BD soil when we had an odd number of seeds. In total, we planted F1 offspring from 16 self pollinations, 33 within-population crosses, and 59 between-population crosses. Seeds from each cross were germinated on approximately 30 g sandhill soil in 15 × 100 mm petri dishes with 5 mL double-distilled H_2O added to saturate the substrate. Petri dishes were sealed with Parafilm and kept at 4°C. Upon germination (17–27 d), seeds were scored as either germinated, ungerminated,

TABLE 1. *Erysimum teretifolium* pollinator visitation by guild.

Pollinator guild	Count (*N*)	Total visits per flower per hour	Mean visitation duration (min:s)
Small solitary bee	213	0.1921	01:24
Large butterfly	99	0.0893	00:08
Small beetle	59	0.0532	03:04
Large bumblebee	48	0.0433	00:35
Large beetle	32	0.0289	00:26
Large solitary bee	25	0.0226	00:10
Large fly	7	0.0063	00:16
Small hymenoptera (ant)	4	0.0036	00:17
Medium beetle	3	0.0027	01:19
Hemiptera (aphid)	3	0.0027	05:48
Hemiptera (leaf hopper)	3	0.0027	00:17
Medium social bee	3	0.0027	00:09
Spider	3	0.0027	00:04
Unknown [a]	3	0.0027	09:52
Small fly	1	0.0009	00:01
Total	506	0.4566	

[a] Unidentified insect contacting reproductive parts, yet too small to identify to guild.

or moldy; the last represented <1% of all seeds planted and was removed from all subsequent analyses. A χ^2 test was used to test for effects of year the cross was done (2012 or 2013) and cross type on germination.

To assess growth and survival, we selected up to eight seedlings per dish for transplantation into the same soil type in which they were germinated. A maximum of four seedlings were transplanted into the corners of 6.35 cm pots. Sandhill soil was immediately saturated after transplanting and misted three times per day until the seedlings were established. Plants were then watered as needed to prevent wilting. Greenhouse conditions were identical to those described for the greenhouse crossing experiments. Thirty-six pots were arranged in a checkerboard pattern within each rack. Racks were individually rotated 180° and then moved to a new location twice per week to control for position effects of the rack within the greenhouse.

Plant survival was assessed weekly, and plants lacking green leaf tissue were considered dead. For all plants surviving to 14 wk from germination, we recorded the number of leaves and the length of the longest leaf. Five hundred and eighty-five plants were harvested by clipping at the soil surface level. Aboveground plant material was dried at 40°C in 50 mL ultracentrifuge tubes for 7 days and weighed on an analytical balance for aboveground dry biomass. Belowground biomass could not be separated from the sandhill substrate.

To test for an effect of cross type on survival and growth, we used the program JMP Pro 11 (SAS, Cary, North Carolina, USA) to construct general linear mixed models with cross type, maternal source population, soil type, and a variable controlling for potential competitive effects (since each pot contained 1–4 seedlings) as predictors, and survival and biomass production as responses. All effects were treated as random except for cross type, which was treated as fixed. The response variable with the smallest effect was removed, and the likelihood was recalculated until all response variables were removed from the model. We selected among models using a biased-corrected Akaike information criterion (AICc) as a preliminary rule, then Bayesian information criterion (BIC) to break ties in which AICc values were not different by more than two AICc units. The cumulative early-stage fitness of offspring produced through self pollination (w_s) vs. outcross pollinations (w_o) can be used to

estimate overall early stage inbreeding depression (δ) = $1 - w_s/w_o$ (Charlesworth and Willis, 2009). Note that in the case of outbreeding depression, $\delta < 0$. To avoid confounding SI with inbreeding depression at the stage of seed set, we only used germination, survival to 14 wk, and dry aboveground biomass at 14 wk in our cumulative estimates of relative fitness (seed number and seed mass were not included).

In a test for inbreeding depression at later developmental stages, a subset of plants was not harvested at 14 wk, but instead planted in a common garden at Santa Clara University (progeny from self pollinations, $N = 13$; progeny from within-population crosses, $N = 34$; progeny from between-population crosses, $N = 52$). Box beds 30 cm deep, lined with weed blocker were filled with the same sandhill soil from two sandhill populations used for germination (BD, QH). Soil types were represented twice in a 2×2 block design. Plants were watered as necessary (1–2× per week). After 4 months additional growth, the longest leaf was measured on all surviving plants as a proxy for growth rate. The proportion of plants flowering was recorded after the first year of growth. Cumulative inbreeding depression for this later developmental stage was the product of the relative fitness values from the early developmental stages × the growth rate × the proportion of flowering individuals.

RESULTS

Greenhouse crossing experiment—Of 43 self pollinations, 27 produced zero seeds compared with only 17 of 118 outcross pollinations. In all cases, emasculation controls produced zero seeds. The three measures of crossing success were strongly correlated (the number of seeds per silique vs. silique length; $R^2 = 0.654$, $P < 0.0001$; the number of seeds per silique vs. average seed mass; $R^2 = 0.373$, $P < 0.0001$), so we used the most direct measure of crossing success, number of seeds per silique, for all further analyses.

Self-pollinations produced 6.5× fewer seeds than the two outcross-pollination treatments combined (mean$_{self}$ = 3.28 seeds; mean$_{outcross}$ = 21.5 seeds; Welch's *t* test, $W_{1,1450} = 101.5$, $P < 0.0005$; Fig. 1), and self pollinations were much more likely to produce zero seeds (63%) compared with the two outcross-pollination treatments combined (14%). ISI$_{seed}$ was 0.827 and ISI$_{fruit}$ was 0.565, suggesting strong SI and partial SI, respectively. Among the self pollinations that produced one or more seeds, seed set did not differ substantially among maternal source populations (QH = 40 crosses, mean seed set = 7; SLV = 33 crosses, mean seed set = 1; GEY = 61 crosses, mean seed set = 4; BD = 27 crosses, mean seed set = 1). Within-population crosses in general produced approximately 13% fewer seeds than between-population crosses (within-population crosses $N = 50$, mean = 19.96 seeds/cross; between-population crosses $N = 68$, mean = 22.69 seeds/cross; Kruskal–Wallis test statistic = 47.999, df = 4, $P < 0.001$; Dwass–Steel–Critchlow–Fligner $P < 0.0001$).

After cross type was accounted for, maternal source population significantly affected the number of seeds produced per cross ($N = 118$; Kruskal–Wallis test statistic = 15.357, df = 3, $P = 0.002$; Dwass–Steel–Critchlow–Fligner $P \leq 0.001$ for all comparisons). The BD maternal stock produced about six times fewer seeds per cross when compared with the mothers from the other three populations (Fig. 2). Furthermore, maternal plants from BD produced significantly more zero-seed outcomes than did the mothers from the other three populations combined, including all crosses (48% vs. 23%; χ^2 test, $\chi^2 = 7.08$, df = 1, $P < 0.01$).
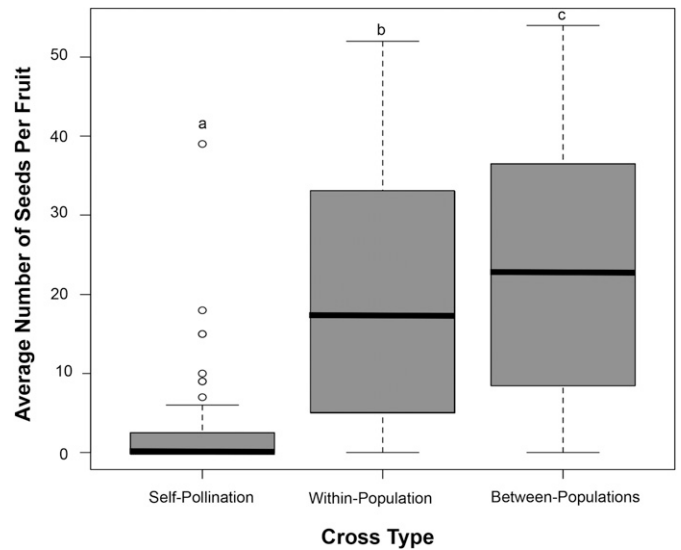
**FIGURE 1** A comparison of seed set following three pollination treatments of *Erysium teretifolium*: self pollination ($N = 43$), within-population outcross ($N = 50$), and between-population ($N = 68$) outcross (Kruskal–Wallis test statistic = 47.999, df = 4, $P < 0.001$). This Tukey boxplot shows the 1st and 3rd quartiles (gray box), median (dark horizontal bar), and whiskers of 1.5 the distance of the interquartile range. Outliers are represented as open circles. Lower case letters indicate significant differences in a pairwise post hoc test ($P < 0.05$).

Pollen-tube growth assay—Pollen tubes were arrested at the stigmatic surface in two of four self pollinations, but pollen tubes grew in all four outcross pollinations on the same maternal plants (Fig. 3). Pollen tubes that were not arrested grew all the way to the ovules.

Pollen limitation experiment—In open-pollinated (unmanipulated) flowers, fruit set was relatively high (75% ± 36% SD overall, Fig. 4A), and the proportion of viable seeds was also high (72% ± 19% SD, Fig. 4B). The probability of fruit set did not significantly increase with pollen supplementation and did not vary significantly across sites (Fig. 4A, model $R^2 = 0.0012$, $N = 228$, $P = 0.96$). The proportion of viable seeds did not show a significant effect of either pollen addition ($F_{1,115} = 1.78$, $P = 0.18$) or site ($F_{2,43} = 1.06$, $P = 0.35$, Fig. 4B). Similarly, seed number per fruit showed no significant effect of either pollen addition ($F_{1,123} = 2.79$, $P = 0.10$) or site ($F_{2,47} = 1.10$, $P = 0.34$, Fig. 4C).

Pollinator observations—We recorded 506 visits over 1108.8 flower-hours from a diversity of pollinators (0.456 visits^{-1} flower^{-1} h). If an individual flower remains open for a minimum of 3 d (Parker et al., 2011) and is available to pollinators for a minimum of half of the daylight hours (6 h/d), then each flower is predicted to be visited at least eight times. The most common pollinators were from the orders Hymenoptera ($N = 293$), Lepidoptera ($N = 99$), and Coleoptera ($N = 94$) (Fig. 5). The pollinator guild with the most visits was small solitary bee among all sites and in both years (Table 1). Visit durations ranged from <1 s to >20 min. On average, the small solitary bee pollinator guild had the longest average visit durations and small fly pollinator guild had the shortest average visit durations (Table 1).
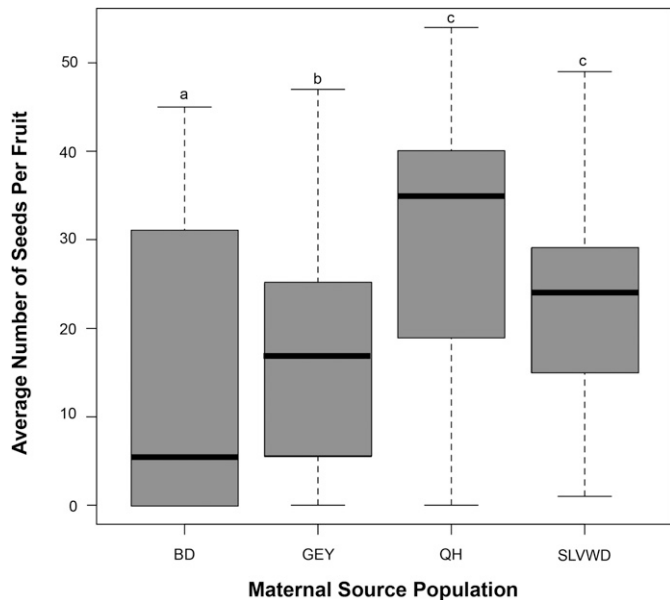
FIGURE 2 Maternal source population of *Erysium teretifolium* affects the average number of seeds per outcross fruit ($N = 118$, Kruskal–Wallis test statistic = 15.357, df = 3, $P = 0.002$). Lower case letters indicate significant differences in a pairwise post hoc test ($P < 0.001$). Sample sizes are as follows: QH = 30; SLV = 23; GEY = 47; BD = 18.

F1 fitness and inbreeding depression—Germination was very high for all F1 offspring, and it did not differ between years that the seeds were produced (χ^2 test, $\chi^2 = 0.533$, df = 1, $P > 0.1$), nor by cross type (overall average 96%; $N = 108$), self pollination (95%; $N = 16$), within-population crosses (93%; $N = 33$) and between-population crosses (97%; $N = 59$).

Overall, 51.6% of transplants survived to 14 wk. For survival, the model containing only cross type (fixed effect) and competitive effects was preferred (AICc = 90.74, BIC = 107.21, adjusted R^2 = 0.65). Cross type was a significant factor in survival ($P = 0.03$). Among the three cross types, survival was lowest for self-pollinated plants (42.2%), intermediate for within-population crosses (47.2%), and highest for between-population crosses (55.2%). Competitive effects accounted for 24.0% of the variance.

Growth analyses ultimately focused on the cumulative measure of growth, dry aboveground biomass, because it strongly correlated with leaf length and leaf number ($R^2 > 0.6$). The model for biomass production containing cross type, maternal identity, soil type, and competitive effects was preferred (AICc = 225.64, BIC = 246.57, adjusted R^2 = 0.43; online Appendix S3). Dry aboveground biomass differed significantly among the three crossing treatments, after accounting for maternal identity, soil type, and competitive effects ($P = 0.01$). Within-population outcrosses had the lowest aboveground biomass (37 mg), but self pollinations and between-population crosses produced F1 offspring with higher aboveground biomass (45 mg and 39 mg, respectively). Soil type accounted for 9.3% of the variation in biomass production, maternal identity accounted for 16.6%, and competitive effects accounted for 2.9%. Cumulative inbreeding depression (δ) comparing selfed crosses to between-population outcrosses was 0.11, and δ comparing selfed crosses to outcrosses within-populations was −0.14 (because selfed crosses outperformed those outcrosses). Using the analogous fitness ratio of $[\delta = 1 - (w_{within}/w_{between})]$ to compare within-population outcrosses with between-population outcrosses yielded a δ value of +0.22.

At later developmental stages, there was a reduction in vegetative growth of selfed progeny estimated from the length of the longest leaf (9.2 cm ± 0.72) compared with progeny from within-population crosses (10.3 cm ± 0.47) and between-population crosses (10.9 cm ± 0.55) (outcross treatments grouped in a *t* test, $t = 1.7148$, df = 92, $P = 0.0897$). In the first flowering season, only 9% of progeny from self pollinations flowered compared with 24% and 34% of individuals from within- and between-population crosses, respectively (outcross treatments grouped in a Fisher's exact test, $P = 0.17$). Using cumulative fitness from germination through the first year of flowering, δ comparing selfed crosses with outcrosses between populations was 0.80, and δ comparing selfed crosses with outcrosses within populations was 0.62. Using the analogous fitness ratio of $\delta = 1 - (w_{within}/w_{between})$ to compare within-population outcrosses with between-population outcrosses yielded a δ value of 0.48 (i.e., no outbreeding depression).

DISCUSSION

Erysimum teretifolium is a hexaploid with complete or nearly complete SI based on ISI_{seed}. Approximately 43.9% of angiosperms studied by Raduski et al. (2012) had ISI values > 0.8, a historical cutoff for SI (Bawa, 1974). In contrast, ISI_{fruit} (where all fruits producing ≥1 seed were considered successful) was only 0.565, suggesting partial SI. The ISI_{fruit} was nearly midway between the range of values considered partial SI (0.2 and 0.8) (Raduski et al., 2012).

ISI values must be interpreted in light of several biological caveats and statistical issues. Biologically, if *E. teretifolium* has transient SI—when the first flowers of an inflorescence are completely SI, but later flowers become partially or completely SC (Good-Avila and Stephenson, 2002; Goodwillie et al., 2004)—then we may have overestimated the degree of SI by using the first flowers in each inflorescence. Although we did not directly test for transient SI (after pollination treatments were applied, all subsequent flowers were removed), anecdotally, when plants not used in this experiment were left to flower in a pollinator-free greenhouse, no fruits formed, making transient SI unlikely (although herkogamy could also explain this result). Assuming transient SI applies to all ovules in a flower, this biological complication would affect both ISI calculations equally.

Alternatively, early-acting inbreeding depression can artificially elevate ISI_{seed} (Kenrick and Knox, 1989), but may be less likely to affect ISI_{fruit} (Raduski et al., 2012, but see Stone et al., 2014 for an exception). Inbreeding depression should be strongest following the initial breakdown of SI because of the substantial genetic load accumulated in SI populations, a plausible scenario in *E. teretifolium*. We saw no evidence of inbreeding depression for our earliest developmental stage (seed germination), but we did detect significant inbreeding depression at later developmental stages (survival to 14 wk). If early-acting inbreeding depression artificially elevated our ISI_{seed}, we would expect more aborted seeds following self pollination vs. outcross pollination (whereas the SI reaction occurs before fertilization on the sigma and in the style and should not lead to aborted seeds). In *E. teretifolium*, there were more than 4× more aborted seeds per pollination in the outcross treatments (7.06 ± 1.05) than in the self pollinations (1.70 ± 0.65), opposite the
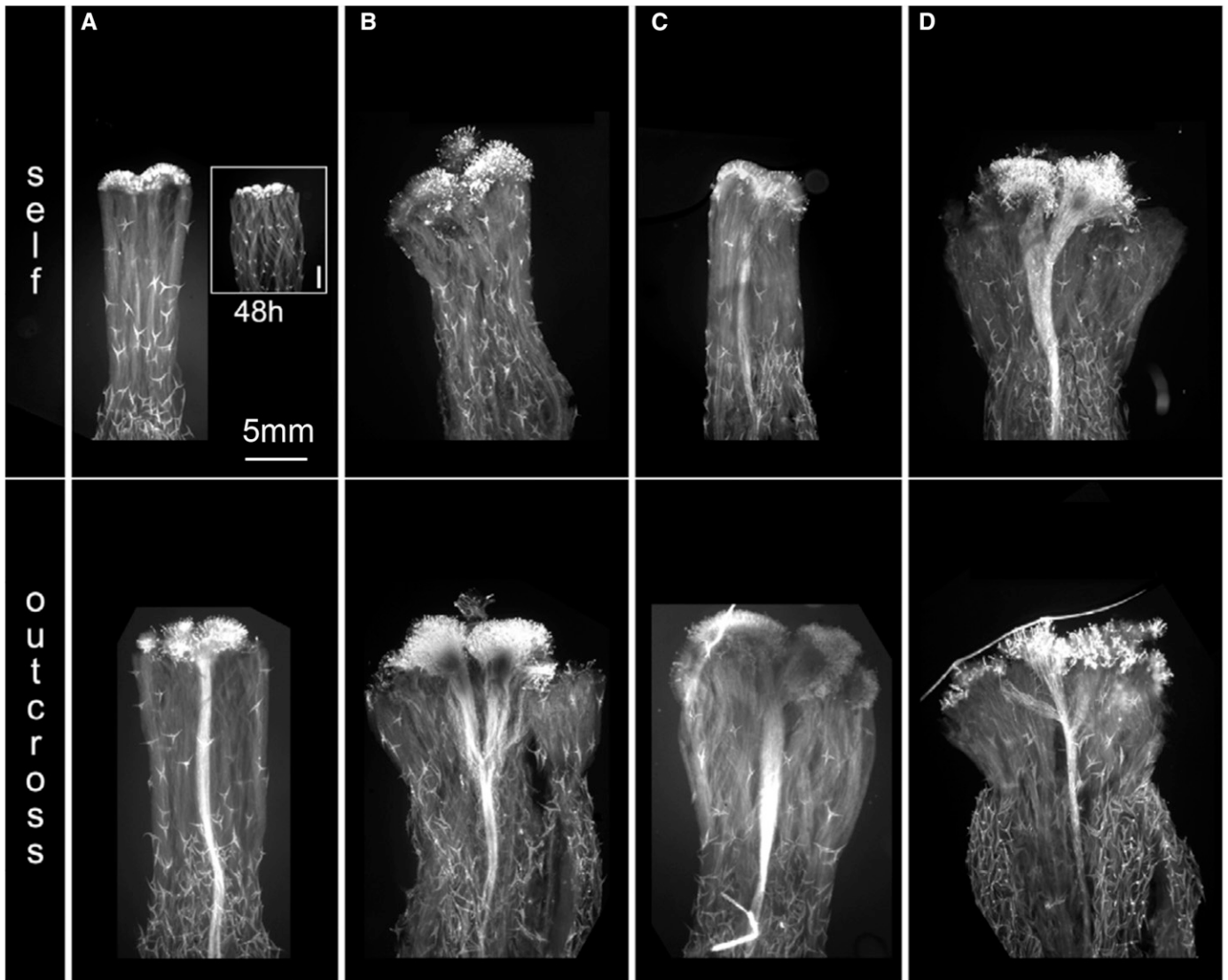
FIGURE 3 Pollen tube growth of *Erysium teretifolium* following different pollination treatments. Pistils were harvested 24 h after either self pollination or outcross pollination from the same maternal plant (A–D). Pollen and pollen tubes fluoresced under UV light; viewed at 40×. Pistils were collected and imaged at 24 and 48 h. Pollen tube growth between 24 h and 48 h did not differ substantially. The inset in A confirms no pollen tube growth after 48 h.

prediction of early-acting inbreeding depression. Therefore, it is unlikely that our ISI$_{seed}$ estimates are substantially affected by early-acting inbreeding depression.

Instead, we believe the difference in the two ISI values likely arises from the technical aspect of treating all fruits with ≥1 seed as successful, thus underestimating the SI reaction (reduces ISI$_{fruit}$) because 25% of "successful" self pollinations (4/16) produced fruits with only one seed and 43.75% produced fruits with less than five seeds (7/16). In contrast, among the 101 "successful" outcross fruits, only one had a single seed (~1%), and another five crosses had less than five seeds (6/101 = ~6%). Clearly, self fruits were much more likely to have very few seeds, thereby artificially reducing ISI$_{fruit}$ to make it appear as partial SI instead of strongly SI like we concluded from the preferred ISI$_{seed}$.

It is important to note two additional technical reasons why ISI values could be misleading. First, outcross pollen contamination would increase reproductive success of self pollinations in a truly SI species. This case is unlikely since we conducted emasculation controls on every plant and they consistently produced no fruit. Second, our ISI values are calculated based on the sum of reproductive success among all self and outcross pollinations, effectively averaging the ISI values among individuals from different populations and different types of outcross pollinations (within- vs. between-population crosses). This averaging effect is likely to produce intermediate ISI values if there is variation in the degree of the SI reaction among individuals, populations, and crosses (Raduski et al., 2012). Our ISI$_{seed}$ (0.82) is approaching the historical cutoff for SI (0.80), suggesting that the SI system may be in the early stages of breakdown. Incomplete SI is supported by our in-vitro visualization of self vs. outcross pollen-tube growth, providing independent evidence that the SI reaction is not absolute. Since the ancestral mating system for *Erysimum* is likely SI (Vekemans et al., 2014), we
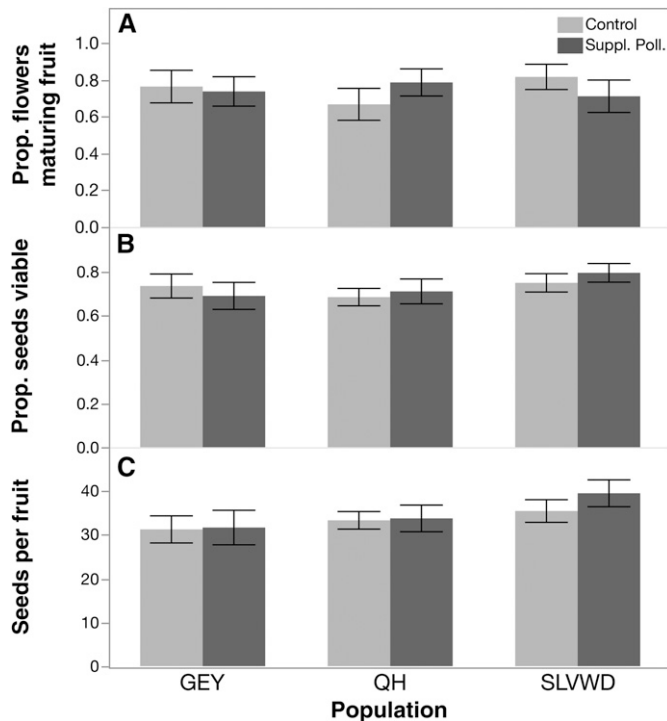
FIGURE 4 Measures of reproductive success of *Erysium teretifolium* in the field comparing open pollination (Control) and open pollination plus manual pollen supplementation (Suppl. Poll.) across three sites. Reproductive success was estimated as (A) the proportion of flowers producing a mature fruit, (B) number of seeds per mature fruit, and (C) proportion of ovules that produced viable (filled) seeds.

interpret *E. teretifolium* as potentially showing early signs of SI breakdown.

Although the causes of SI breakdown can be numerous (Mable, 2004; Anderson and Busch, 2006), polyploidy is likely a contributing factor. It is unknown whether the hexaploidy of *Erysimum teretifolium* is a result of an allopolyploid or autopolyploid event (Price, 1987), but with six alleles per pollen grain and stigma, the range of possible *S*-allele interactions is substantially larger than in a diploid system significantly complicating the SI reaction (Igić and Busch, 2013). For example, in the allopolyploid *Arabidopsis kamchatica*, the enzyme partially responsible for the self-incompatibility reaction is dysfunctional, leading to breakdown of SI (Tsuchimatsu et al., 2012).

There is modest evidence for inbreeding depression in *E. teretifolium* at early developmental stages under benign conditions. Self-progeny had a 24% decrease in fitness in survival to 14 wk compared with offspring of between-population crosses, while within-population crosses had intermediate survival. A contrasting pattern was found in the aboveground dry biomass results, in which self progeny had a higher biomass than between-population and within-population crosses. This unexpected result may have arisen if inbreeding depression at the earlier survival stage culled all but the most vigorous self progeny, leading to an overestimate of the aboveground dry biomass in self progeny. Additionally, our model shows that competition among plants sharing a pot influenced biomass production, and since more self progeny died before our measurement cutoff, the individuals remaining in those pots may have been larger as a result.

A comparison of the cumulative relative fitness of self progeny vs. outcross progeny can illuminate the overall magnitude of inbreeding depression. At early developmental stages (germination, survival to 14 wk, and biomass at 14 wk), our estimate of inbreeding depression based on between-population outcrosses is low (0.11), largely driven by the increased dry aboveground biomass of self progeny (potentially due to release from competition with other plants in the pot). After including later developmental stages (growth rate and proportion flowering in the first year), the cumulative amount of inbreeding depression is more dramatic (0.80). In comparison, Husband and Schemske (1996) revealed in a review of inbreeding depression in 54 plant species an average δ of 0.53 for predominantly outcrossing species.

Our finding that progeny from within-population crosses are 22% less fit at early developmental stages and 48% less fit from germination to the first year of flowering compared with between-population crosses suggests that the dynamics within these small populations and isolation among them may reflect the negative consequences of biparental inbreeding or the positive effects of heterosis. The latter is still theoretically possible even with low levels of migration under certain circumstances (Whitlock et al., 2000). Without controlled crosses among pedigreed parents (Heywood, 1993), biparental inbreeding depression cannot be distinguished from heterosis, but the management conclusions are the same—mixing populations increases fitness. The crossing results comparing seed set in outcrosses from different maternal source populations, where BD has a significantly lower average (Fig. 2), could partly explain the reported reproductive failure at that site. Possible causes of reduced seed set in BD maternal parents includes biparental inbreeding and/or a stronger SI reaction. Regardless, the lack of outbreeding depression suggests that future reintroduction efforts at this site *would not be harmed* by introducing seeds or plants from nearby populations.

Manifestation of inbreeding depression can be environment-dependent and will be more apparent in harsher conditions and at later life stages (Husband and Schemske, 1996; Armbruster et al., 2002; Geber and Eckhart, 2005; Charlesworth and Willis, 2009). Although we attempted to provide edaphically relevant conditions for our estimate of inbreeding depression, plants in our experiment experienced relatively benign environmental conditions in the greenhouse and experimental garden in terms of water availability and temperature extremes. Lack of strong inbreeding depression at early developmental stages was likely influenced by the reduced competitive effects within pots following increased mortality in the progeny of self pollinations. If *E. teretifolium* has been capable of some self pollination, it may have purged early-acting deleterious recessive alleles, and therefore stronger inbreeding depression would be seen at later life history stages (even beyond the proportion flowering in the 1st year) as suggested by the findings of Husband and Schemske (1996). The cumulative effects of inbreeding depression and apparent increase in its effects at later life stages in *E. teretifolium* suggest that the current study may underestimate inbreeding depression at early developmental stages, a limitation common to many inbreeding depression studies (Armbruster and Reed, 2005).

In a rare species, SI or even partial SI seems highly disadvantageous. Yet *E. teretifolium* demonstrated a high proportion of fruit set and seed viability by wild, open pollination in our pollen supplementation experiment (Fig. 4), which indicates that reproduction is not pollen limited. This seems to be the rule of most wild populations (J. Whittall, personal observation). Our pollinator

FIGURE 5 Representatives of the most common pollinators of *Erysium teretifolium*. (A) Hymenoptera, Halictidae, sweat bee; (B) Hymenoptera, Apidae, *Bombus vosnesenskii*, yellow-faced bumblebee; (C) Hymenoptera, Halictidae, sweat bee; (D) Hymenoptera, Melecta, cuckoo bee; (E) Lepidoptera, Nymphalidae, *Euphydryas chalcedona*, Chalcedon Checkerspot; (F) Lepidoptera, Hesperiidae, skipper butterfly; (G) Coleoptera, Elateridae, click beetle; (H) Coleoptera, Mordellidae, tumbling flower beetle; (I) Coleoptera, Chrysomelidae, leaf beetle.

observations indicate a high rate of visitation by a diverse assemblage of insect pollinators, and experimental supplementations show no evidence of pollen limitation in three separate populations. We estimate each flower is visited at least eight times, which should provide sufficient outcross pollen to maintain high seed set even in light of the largely SI mating system. The most common visitor to every population in both years was in the small solitary bee guild (Table 1), which may prevent pollen limitation. We also found no fitness reductions in open-pollinated offspring compared with pollen-supplemented offspring (I. M. Parker, personal observation), supporting the conclusion that abundant pollinators ensure the reproductive success of *E. teretifolium* even as it remains at least partially SI.

Restoration and reintroduction activities for *E. teretifolium* must accommodate the SI mating system while recognizing the negative fitness effects of inbreeding. The geographic isolation among populations of this endangered sandhill endemic has raised concerns about mixing seeds and plants from different populations, which might disrupt coadapted gene complexes or local adaptations. However, we failed to detect any sign of outbreeding depression in F_1 progeny emerging from crosses among populations, but hybrid breakdown could potentially manifest itself in later generations (Edmands, 2007). We cannot rule out the possibility that some genotypes may be locally adapted to their sites; our experiments show that soil type can be a significant factor in survival and growth at some life stages. However, our results suggest that the risk of insufficient genetic variation within small populations outweighs the risk of any unmeasured outbreeding depression for this species. The SI or largely SI mating system requires population sizes large

enough to ensure sufficient mates containing distinct *S* alleles. For *E. teretifolium*, the ability to maintain high seed set and offspring fitness in most of the natural populations studied here is likely due to the diversity of pollinators and high visitation rates. At the same time, the rapid decline of some *E. teretifolium* populations due to reproductive failure may be attributed to lack of unrelated mates (e.g., BD). The results of this study will aid in the development of reintroduction plans for this species and potentially other SI endemics facing similar threats.

ACKNOWLEDGEMENTS

LITERATURE CITED

Aguilar, R., L. Ashworth, L. Galetto, and M. A. Aizen. 2006. Plant reproductive susceptibility to habitat fragmentation: Review and synthesis through a meta-analysis. *Ecology Letters* 9: 968–980.

Anderson, I. A., and J. W. Busch. 2006. Relaxed pollinator-mediated selection weakens floral integration in self-compatible taxa of *Leavenworthia* (Brassicaceae). *American Journal of Botany* 93: 860–867.

Armbruster, P., and D. H. Reed. 2005. Inbreeding depression in benign and stressful environments. *Heredity* 95: 235–242.

Armbruster, W. S., C. P. H. Mulder, B. G. Baldwin, S. Kalisz, B. Wessa, and H. Nute. 2002. Comparative analysis of late floral development and mating-system evolution in tribe *Collinsieae* (Scrophulariaceae s.l.). *American Journal of Botany* 89: 37–49.

Barrett, S. C. H. 2002. The evolution of plant sexual diversity. *Nature Reviews. Genetics* 3: 274–284.

Barringer, B. C. 2007. Polyploidy and self-fertilization in flowering plants. *American Journal of Botany* 94: 1527–1533.

Bawa, K. S. 1974. Breeding systems of tree species of a lowland tropical community. *Evolution* 28: 85–92.

Brochmann, C. 1993. Reproductive strategies of diploid and polyploid populations of arctic *Draba* (Brassicaceae). *Plant Systematics and Evolution* 185: 55–83.

Burd, M. 1994. Bateman's principle and plant reproduction: The role of pollen limitation in fruit and seed set. *Botanical Review* 60: 83–139.

Busch, J. 2005. The evolution of self-compatibility in geographically peripheral populations of *Leavenworthia alabamica* (Brassicaceae). *American Journal of Botany* 92: 1503–1512.

Campbell, L. G., and B. C. Husband. 2007. Small populations are mate-poor but pollinator-rich in a rare, self-incompatible plant, *Hymenoxys herbacea* (Asteraceae). *New Phytologist* 174: 915–925.

Charlesworth, B., and D. Charlesworth. 2012. Elements of evolutionary genetics, 1st ed. Roberts and Company, Greenwood Village, Colorado, USA.

Charlesworth, D. 2003. Effects of inbreeding on the genetic diversity of populations. *Philosophical Transactions of the Royal Society of London, B, Biological Sciences* 358: 1051–1070.

Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* 18: 237–268.

Charlesworth, D., and J. H. Willis. 2009. Fundamental concepts in genetics: The genetics of inbreeding depression. *Nature Reviews. Genetics* 10: 783–796.

Charlesworth, D., X. Vekemans, V. Castric, and S. Glemin. 2005. Plant self-incompatibility systems: A molecular evolutionary perspective. *New Phytologist* 168: 61–69.

Critchlow, D. E., and M. A. Fligner. 1991. On distribution-free multiple comparisons in the one-way analysis of variance. *Communications in Statistics. Theory and Methods* 20: 127–139.

DeMauro, M. 1993. Relationship of breeding system to rarity in the lakeside daisy (*Hymenoxys acaulis* var. *glabra*). *Conservation Biology* 7: 542–550.

de Nettancourt, D. D. 1977. Incompatibility systems in angiosperms. Springer, New York, New York, USA.

Duminil, J., S. Fineschi, A. Hampe, P. Jordano, D. Salvini, G. G. Vendramin, and R. J. Petit. 2007. Can population genetic structure be predicted from life-history traits? *American Naturalist* 169: 662–672.

Duminil, J., O. J. Hardy, and R. J. Petit. 2009. Plant traits correlated with generation time directly affect inbreeding depression and mating system and indirectly genetic structure. *BMC Evolutionary Biology* 9: 177.

Eckert, C. G., S. Kalisz, M. A. Geber, R. Sargent, E. Elle, P. O. Cheptou, C. Goodwillie, et al. 2010. Plant mating systems in a changing world. *Trends in Ecology & Evolution* 25: 35–43.

Eckert, C. G., and A. Schaefer. 1998. Does self-pollination provide reproductive assurance in *Aquilegia canadensis* (Ranunculaceae)? *American Journal of Botany* 85: 919–924.

Edmands, S. 2007. Between a rock and a hard place: Evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Molecular Ecology* 16: 463–475.

Elam, D., C. Ridley, K. Goodell, and N. Ellstrand. 2007. Population size and relatedness affect fitness of a self-incompatible invasive plant. *Proceedings of the National Academy of Sciences, USA* 104: 549–552.

Ellstrand, N. C. 1992. Gene flow by pollen: Implications for plant conservation genetics. *Oikos* 63: 77–86.

Fisher, R. A. 1941. Average excess and average effect of a gene substitution. *Annals of Eugenics* 11: 53–63.

Frankham, R., J. D. Ballou, M. D. Eldridge, R. C. Lacy, K. Ralls, M. R. Dudash, and C. B. Fenster. 2011. Predicting the probability of outbreeding depression. *Conservation Biology* 25: 465–475.

Geber, M. A., and V. M. Eckhart. 2005. Experimental studies of adaptation in *Clarkia xantiana*. II. Fitness variation across a subspecies border. *Evolution* 59: 521–531.

Glémin, S., E. Bazin, and D. Charlesworth. 2006. Impact of mating systems on patterns of sequence polymorphism in flowering plants. *Proceedings. Biological Sciences* 273: 3011–3019.

Good-Avila, S. V., and A. G. Stephenson. 2002. The inheritance of modifiers conferring self-fertility in the partially self-incompatible perennial, *Campanula rapunculoides* L. (Campanulaceae). *Evolution* 56: 263–272.

Goodwillie, C., K. L. Partis, and J. W. West. 2004. Transient self-incompatibility confers delayed selfing in *Leptosiphon jepsonii* (Polemoniaceae). *International Journal of Plant Sciences* 165: 387–394.

Grossenbacher, D. L., and J. B. Whittall. 2011. Increased floral divergence in sympatric monkeyflowers. *Evolution* 65: 2712–2718.

Guo, Y. L., J. S. Bechsgaard, T. Slotte, B. Neuffer, M. Lascoux, D. Weigel, and M. H. Schierup. 2009. Recent speciation of *Capsella rubella* from *Capsella grandiflora*, associated with loss of self-incompatibility and an extreme bottleneck. *Proceedings of the National Academy of Sciences, USA* 106: 5246–5251.

Hamrick, J., and M. Godt. 1989. Allozyme diversity in plant species. *In* A. Brown, M. T. Clegg, A. L. Kahler, and B. S. Weir [eds.], Plant population genetics, breeding and genetic resources, 43–63. Sinauer, Sunderland, Massachusetts, USA.

Hamrick, J. L., and M. J. W. Godt. 1996. Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society of London, B, Biological Sciences* 351: 1291–1298.

Heywood, J. 1993. Biparental inbreeding depression in the self-incompatible annual plant *Gaillardia pulchella* (Asteraceae). *American Journal of Botany* 80: 545–550.

Hintz, M., C. Bartholmes, P. Nutt, J. Ziermann, S. Hameister, B. Neuffer, and G. Theissen. 2006. Catching a 'hopeful monster': Shepherd's purse (*Capsella bursa-pastoris*) as a model system to study the evolution of flower development. *Journal of Experimental Botany* 57: 3531–3542.

Honnay, O., and H. Jacquemyn. 2007. Susceptibility of common and rare plant species to the genetic consequences of habitat fragmentation. *Conservation Biology* 21: 823–831.

Husband, B. C., and D. W. Schemske. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* 50: 54–70.

Igić, B., and J. W. Busch. 2013. Is self-fertilization an evolutionary dead end? *New Phytologist* 198: 386–397.

International Union for Conservation of Nature/Species Survival Commission. 2013. Guidelines for reintroductions and other conservation translocations, version 1.0. IUCN Species Survival Commission, Gland, Switzerland.

Kao, T., and A. G. McCubbin. 1996. How flowering plants discriminate between self and non-self pollen to prevent inbreeding. *Proceedings of the National Academy of Sciences, USA* 93: 12059–12065.

Kearns, C. A., and D. W. Inouye. 1993. Techniques for pollination biologists. University Press of Colorado, Boulder, Colorado, USA.

Kenrick, J., and R. B. Knox. 1989. Quantitative analysis of self-incompatibility in trees of seven species of *Acacia*. *Journal of Heredity* 80: 240–245.

Knight, T. M., J. A. Steets, J. C. Vamosi, S. J. Mazer, M. Burd, D. R. Capbell, M. R. Dudash, et al. 2005. Pollen limitation of plant reproduction: Pattern and process. *Annual Review of Ecology, Evolution and Systematics* 36: 467–497.

Mable, B. K. 2004. Polyploidy and self-compatibility: Is there an association? *New Phytologist* 162: 803–811.

Mable, B. K. 2008. Genetic causes and consequences of the breakdown of self-incompatibility: Case studies in the Brassicaceae. *Genetical Research* 90: 47–60.

Mable, B. K., A. V. R. Dart, C. D. Berardo, and L. Witham. 2005. Breakdown of self-incompatibility in the perennial *Arabidopsis lyrata* (Brassicaceae) and its genetic consequences. *Evolution* 59: 1437–1448.

Martin, F. W. 1959. Staining and observing pollen tubes in the style by means of fluorescence. *Stain Technology* 34: 125–128.

McGraw, J. 2004a. Sandhills conservation and management plan: A strategy for preserving native biodiversity in the Santa Cruz sandhills. The Land Trust of Santa Cruz County, Santa Cruz, California, USA.

McGraw, J. 2004b. Interactive effect of disturbance and exotic species on the structure and dynamics of an endemic sandhills plant community. Ph.D. dissertation, University of California, Berkeley, Berkeley, California, USA.

Mena-Ali, J. I., L. H. Keser, and A. G. Stephenson. 2008. Inbreeding depression in *Solanum carolinense* (Solanaceae), a species with a plastic self-incompatibility response. *BMC Evolutionary Biology* 8: 10.

Mijangos, J. L., C. Pacioni, P. B. Spencer, and M. D. Craig. 2015. Contribution of genetics to ecological restoration. *Molecular Ecology* 24: 22–37.

Miller, J., and D. Venable. 2000. Polyploidy and the evolution of gender dimorphism in plants. *Science* 289: 2335–2338.

Moazzeni, H., S. Zarre, B. E. Pfeil, Y. J. Bertrand, D. A. German, I. A. Al-Shehbaz, K. Mummenhoff, and B. Oxelman. 2014. Phylogenetic perspectives on diversification and character evolution in the species-rich genus *Erysimum* (Erysimeae; Brassicaceae) based on a densely sampled ITS approach. *Botanical Journal of the Linnean Society* 175: 497–522.

Nieto Feliner, G. 1991. Breeding systems and related floral traits in several *Erysimum* (Cruciferae). *Canadian Journal of Botany* 69: 2515–2521.

Ortigosa, A. L., and J. M. Gómez. 2010. Differences in the diversity and composition of the pollinator assemblage of two co-flowering congeneric alpine wallflowers, *Erysimum nevadense* and *E. baeticum*. *Flora—Morphology, Distribution. Functional Ecology of Plants* 205: 266–275.

Pannell, J. R., and S. C. H. Barrett. 1998. Baker's Law revisited: Reproductive assurance in a metapopulation. *Evolution* 52: 657–668.

Parker, I. M., A. Weitz, and K. Webster. 2011. Reproductive bottlenecks in Ben Lomond wallflower (*Erysimum teretifolium*). B.S. thesis, University of California, Santa Cruz, California, USA.

Pavlik, B. M., N. Ferguson, and M. Nelson. 1993. Assessing limitations on the growth of endangered plant populations, II. Seed production and seed bank dynamics of *Erysimum capitatum* ssp. *angustatum* and *Oenothera deltoides* ssp. *howellii*. *Biological Conservation* 65: 267–278.

Price, R. A. 1987. Systematics of the *Erysimum capitatum* alliance (Brassicaceae). Ph.D. dissertation, University of California, Berkeley, California, USA.

Raduski, A. R., E. B. Haney, and B. Igić. 2012. The expression of self-incompatibility in angiosperms is bimodal. *Evolution* 66: 1275–1283.

Richter, S. C., B. I. Crother, and R. E. Broughton. 2009. Genetic consequences of population reduction and geographic isolation in the critically endangered frog, *Rana sevosa*. *Copeia* 2009: 799–806.

Robertson, K., E. E. Goldberg, and B. Igić. 2011. Comparative evidence for the correlated evolution of polyploidy and self-compatibility in Solanaceae. *Evolution* 65: 139–155.

Rossbach, G. B. 1958. The genus *Erysimum* (Cruciferae) in North America north of Mexico—A key to the species and varieties. *Madrono* 14: 261–267.

Saunders, D. A., R. J. Hobbs, and C. R. Margules. 1991. Biological consequences of ecosystem fragmentation: A review. *Conservation Biology* 5: 18–32.

Schoen, D. J., and J. W. Busch. 2008. On the evolution of self-fertilization in a metapopulation. *International Journal of Plant Sciences* 169: 119–127.

Spurrier, J. D. 2006. Additional tables for Steel–Dwass–Critchlow–Fligner distribution-free multiple comparisons of three treatments. *Communications in Statistics – Simulation and Computation* 35: 441–446.

Stockwell, C., A. Hendry, and M. Kinnison. 2003. Contemporary evolution meets conservation biology. *Trends in Ecology & Evolution* 18: 94–101.

Stone, J., E. VanWyk, and J. Hale. 2014. Transmission advantage favors selfing allele in experimental populations of self-incompatible *Witheringia solanacea* (Solanaceae). *Evolution* 68: 1845–1855.

Tsuchimatsu, T., P. Kaiser, C.-L. Yew, J. B. Bachelier, and K. K. Shimizu. 2012. Recent loss of self-incompatibility by degradation of the male component in allotetraploid *Arabidopsis kamchatica*. *PLOS Genetics* 8: e1002838.

U. S. Fish and Wildlife Service. 1998. Recovery plan for insect and plant taxa from the Santa Cruz Mountains of California. U. S. Fish and Wildlife Service Pacific Region, Portland, Oregon, USA.

U. S. Fish and Wildlife Service. 2007. Ben Lomond Wallflower (*Erysimum teretifolium*): 50-year review, summary and evaluation. U. S. Fish and Wildlife Service, Ventura, California, USA.

Vekemans, X., C. Poux, P. Goubet, and V. Castric. 2014. The evolution of selfing from outcrossing ancestors in Brassicaceae: What have we learned from variation at the S-locus? *Journal of Evolutionary Biology* 27: 1372–1385.

Wagenius, S., and S. Lyon. 2010. Reproduction of *Echinacea angustifolia* in fragmented prairie is pollen-limited but not pollinator-limited. *Ecology* 91: 733–742.

Waites, A. R., and J. Ågren. 2004. Pollinator visitation, stigmatic pollen loads and among-population variation in seed set in *Lythrum salicaria*. *Journal of Ecology* 92: 512–526.

Warwick, S. I., K. Mummenhoff, C. A. Sauder, M. A. Koch, and I. A. Al-Shehbaz. 2010. Closing the gaps: Phylogenetic relationships in the Brassicaceae based on DNA sequence data of nuclear ribosomal ITS region. *Plant Systematics and Evolution* 285: 209–232.

Weeks, A. R., C. M. Sgro, A. G. Young, R. Frankham, N. J. Mitchell, K. A. Miller, M. Byrne, et al. 2011. Assessing the benefits and risks of translocations in changing environments: A genetic perspective. *Evolutionary Applications* 4: 709–725.

Whitlock, M. C., P. K. Ingvarsson, and T. Hatfield. 2000. Local drift load and the heterosis of interconnected populations. *Heredity* 84: 452–457.