

## RESEARCH ARTICLE



# A test of the reproductive assurance hypothesis in *Ipomoea hederacea*: does inbreeding depression counteract the benefits of self-pollination?

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## Abstract

**Premise:** Darwin proposed that self-pollination in allegedly outcrossing species might act as a reproductive assurance mechanism when pollinators or mates are scarce; however, in natural populations, the benefits of selfing may be opposed by seed discounting and inbreeding depression. While empirical studies show variation among species and populations in the magnitude of reproductive assurance, little is known about the counterbalancing effects of inbreeding depression.

**Methods:** By comparing the female reproductive success of emasculated and open-pollinated flowers, we assessed the reproductive assurance hypothesis in two Mexican populations of *Ipomoea hederacea*. In one population, we assessed temporal variation in reproductive assurance for three years. We evaluated inbreeding depression on seed production, seedling germination, and dry plant mass by contrasting self- and cross-hand pollination treatments in one population for two years.

**Result:** The contribution of self-pollination to female reproductive success was high and consistent between populations, but there was variation in reproductive assurance across years. Inbreeding depression was absent in the early stages of progeny development, but there was a small negative effect of inbreeding in the probability of germination and the mass of adult progeny.

**Conclusions:** Self-pollination provided significant reproductive assurance in *I. hederacea* but this contribution was variable across time. The contribution of reproductive assurance is probably reduced by inbreeding depression in later stages of progeny development, but this counter effect was small in the study populations. This study supports the hypothesis that reproductive assurance with limited inbreeding depression is likely an important selective force in the evolution of self-pollination in the genus *Ipomoea*.

## KEYWORDS

autonomous self-pollination, Convolvulaceae, floral traits, inbreeding depression, *Ipomoea hederacea*, pollination, reproductive assurance

Seminal work conducted by Darwin (1876) demonstrated that in many plant species, the offspring resulting from outcrossing is generally more vigorous than the offspring derived from self-pollination. This phenomenon, known as inbreeding depression (ID), is currently recognized as one of the most important selective forces in the evolution of plant reproductive systems (Lloyd, 1979; Charlesworth and Willis, 2009). The favorable consequences of outcrossing on progeny performance and genetic diversity have been well established (e.g., González-Varo et al., 2010;

Aguilar et al., 2019); however, the recurrent loss of self-incompatibility, the repeated evolution of autonomous self-pollination mechanisms, and the widespread occurrence of highly selfing and mixed-mating systems in plants indicate that, under certain conditions, natural selection has favored the evolution and maintenance of self-fertilization (Stebbins, 1957; Goodwillie et al., 2005). The main factors proposed as drivers of these common evolutionary transitions include the genetic transmission advantage of selfing (Fisher, 1941), the necessity of short-lived plant species to

reproduce rapidly (Aarssen, 2000), selection for traits that promote reproductive isolation in sympatric congeners (e.g., Smith and Rausher, 2006), selection to increase reproductive success in the absence of mates or pollinators (Darwin, 1876; Lloyd and Schoen, 1992), and selection for reproductive traits that allow the colonization of ephemeral environments or insular habitats after long-distance dispersal (Baker, 1955; Stebbins, 1957). The latter two explanations are encompassed within the reproductive assurance (RA) hypothesis because they imply that self-fertilization may be adaptive if it ensures the production of offspring in pollinator- or mate-depauperate environments.

Empirical assessments of the RA hypothesis have found that evidence is not consistent among species, nor across time or space. A few studies have directly demonstrated a direct association between low pollinator visitation and the reproductive benefit conferred by autonomous self-pollination (e.g., Kalisz and Vogler, 2003; Teixido and Aizen, 2019), while other studies have not detected a significant contribution of self-pollination to plant fitness (Eckert and Schaefer, 1998). Some studies have reported that the benefits of autonomous self-pollination are restricted to certain sites or years (Goodwillie, 2001; Zhao et al., 2006; Martén-Rodríguez and Fenster, 2010; Schoupe et al., 2017), and a limited number of studies have found that the benefits of RA are dependent on the counteracting effects of ID (*Decodon verticillatus*, Eckert and Barrett, 1994; *Aquilegia canadensis*, Herlihy and Eckert, 2002; *Collinsia verna*, Kalisz et al., 2004; *Rhododendron ferrugineum*, Delmas et al., 2014). Thus, despite great advances in our understanding of the evolution of self-fertilization (Karron et al., 2012), surprisingly little empirical work has been conducted to quantify the overall benefit of autonomous self-pollination mechanisms taking into account the effects of ID (Brys et al., 2013; Busch and Delph, 2012).

Like most reproductive strategies, autonomous self-pollination has costs and benefits that depend on factors such as the timing of pollination, pollinator availability, life-history strategy, and the genetic consequences of this pollination mode (Eckert et al., 2006). For example, a greater benefit of self-pollination to plant fitness is expected in species with short life cycles than in their long-lived relatives. For instance, the production of self-fertilized seeds in perennial plants may compromise the resources required to develop outcrossed seeds in future seasons (Eckert and Herlihy, 2004), but this is not the case for annual species. The advantages of self-fertilization for annual species may be particularly significant when these plants occur in early successional or ephemeral habitats, where mates and/or pollinators may be in short supply. Finally, the benefits and costs of self-pollination both in annuals and perennials are strongly influenced by mating patterns and levels of ID (Duminil et al., 2009).

The role of ID in the evolution of mixed-mating systems has been widely discussed; however, it is not yet clear to what extent and how ID reduces the benefits of autonomous self-pollination in plants with different life-history traits.

In general terms, the reduced fitness of self-fertilized progeny is explained by two effects of homozygosity: the increased expression of partially deleterious alleles at multiple homozygous loci, and a reduction in the number of loci with heterozygote advantage; the former one is thought to be the most important mechanism underlying ID (Charlesworth and Willis, 2009). Theoretically, in large outcrossing populations, highly deleterious mutations should be purged by self-fertilization even if they occur at low frequency; in contrast, purging of additive mutations of mild effect should be much slower (Lande et al., 1994). However, the level of ID is also dependent on plant life histories and mating systems. For instance, frequent selfers and annuals tend to have lower ID than outcrossers and perennials, two traits that tend to be correlated (Angeloni et al., 2011; Friedman, 2020).

Variation in ID is also expressed differently across the life-cycle stages of the progeny. Species that reproduce primarily by selfing tend to have lower ID than outcrossers for early fitness traits (e.g., seed production and germination), suggesting that lethal mutations, which are more common in early stages of development, are effectively purged by inbreeding (Husband and Schemske, 1996; but see Zhang et al., 2014). Nonetheless, selfing species tend to express ID in late life-cycle traits similar to outcrossing species (Husband and Schemske, 1996). Given this background, one expectation is that plants with mixed-mating systems maintain a genetic load that causes ID mostly in the late stages of progeny development. In some plant species, ID along with seed discounting may offset the RA advantage obtained through self-pollination (Herlihy and Eckert, 2002; Delmas et al., 2014). Therefore, assessments of ID are highly relevant in studies that evaluate the RA hypothesis in natural populations.

The genus *Ipomoea* (Convolvulaceae) is characterized by substantial variation in life history and reproductive traits, both within and among species (Brown and Clegg, 1984; Chemás-Jaramillo and Bullock, 2002; Carranza, 2008). Variation is evident in traits such as color, size, herkogamy (i.e., stigma-anther distance), and breeding system. Furthermore, the loss of self-incompatibility has occurred in several clades, indicating multiple origins of self-fertility (Rosas-Guerrero et al., 2011; McDonald et al., 2011). In addition, many *Ipomoea* species are annual and they often occupy early successional, disturbed, and ruderal habitats. Despite this variation, most self-compatible species have relatively large attractive flowers, suggesting that they possibly maintain mixed mating systems. However, little is known about the mechanisms that determine the mating system in morning glories, except for a few well-studied species, e.g., *I. purpurea* and *I. hederacea*, which show low but varying levels of ID (Chang and Rausher, 1999; Hull-Sanders et al., 2005; Mason et al., 2015; Van Etten et al., 2021). Other factors also appear to contribute to the maintenance of mixed mating in *I. purpurea* (e.g., pollen discounting, Chang and Rausher, 1998; selection for selfing in rare herbicide-resistant *I. purpurea* plants, Kuester

et al., 2017). More studies are required to understand the relationship between RA and ID in the evolution of self-pollination in morning glories.

The main goal of this study was to assess the contribution of self-pollination to female fitness in *I. hederacea*, considering the potential reproductive losses associated with ID. Specifically, we conducted a field experiment to quantify RA in two natural populations of *I. hederacea* in the state of Veracruz, Mexico. In one population, we conducted RA experiments for three consecutive years, and estimated ID for fruit set, seed production, and progeny performance over two years. To determine the relationship between floral traits, pollinator visitation, and RA we also compared a one-year sampling of flower size, herkogamy, and pollinator visitation rates between populations. Given that self-compatible *Ipomoea* species have annual life histories and establish new populations in disturbed habitats each year, we hypothesized that RA would vary among populations and years. Based on what is known for ID in annual self-compatible *Ipomoea* species, we predicted low ID in *I. hederacea*.

## MATERIALS AND METHODS

### Description of species and study sites

*Ipomoea hederacea* Jacq., is an annual, self-compatible herb (Ennos, 1981; Smith and Rausher, 2006), native to the Americas but is currently widespread in most continents (Austin and Huáman, 1996; Felger et al., 2012). The reproductive biology and genetics of *I. hederacea* have been studied in southeastern U.S. populations, where bumblebees are reported as the main pollinators (e.g., Stucky, 1984; Wolfe and Sowell, 2006; Smith and Rausher, 2006, 2008), and in eastern Mexican populations, where flowers are mainly visited by native bees (e.g., Euglossini) and less frequently by honeybees and butterflies (Delgado-Dávila et al., 2016). Mating system estimates range from highly selfing to mixed mating across various U.S. populations (Ennos, 1981; Hull-Sanders et al., 2005). In Mexico, eastern populations of *I. hederacea* are fully self-compatible (Delgado-Dávila et al., 2016). The flowering of Mexican populations is reported to occur during the months of August–November (Felger et al., 2012). However, in the region of Los Tuxtlas, Veracruz, *I. hederacea* blooms from January to April, while in Coatepec, Veracruz, flowering occurs from September to November (Delgado-Dávila and Martín-Rodríguez, unpublished data). Flowers are bell-shaped with purple corolla limbs and white inner tubes. They have five stamens that differ in length, resulting in a gradient of stigma-anther distances (Delgado-Dávila et al., 2016). Anthesis occurs between 4:30–11:00 h and anther dehiscence starts approximately 10 h before anthesis. Fruits are dry capsules that generally contain 1–6 seeds.

We conducted fieldwork in Veracruz, Mexico, during the flowering seasons of 2012–2014. *Ipomoea hederacea*

grows on roadsides and fallow fields often surrounded by secondary tropical forest and cultivated lands. The Catemaco population (18°24'N, 95°06'W; 340 m a.s.l.) was located in southeastern Veracruz along the edge of the Catemaco lake. The Coatepec population (19°27'N, 96°57'W, 1200 m a.s.l.) was in Central Veracruz within a shade-grown coffee plantation.

### Spatial variation in floral morphology

To quantify variation in floral morphology between populations, 32 plants of *I. hederacea* were selected at each population, and the following variables were measured in one flower per plant: corolla length, corolla diameter, pistil length, and the length of the two longest stamens (S4 and S5). Measurements were performed with a caliper (0.1 mm accuracy). In addition, herkogamy, or stigma-anther distance, was determined as the difference between pistil height and the height of each stamen. We assessed differences in flower size and the absolute value of the stigma-anther distance between populations with a multivariate analysis of variance (MANOVA), using the MANOVA function from the stats package in R (R Core Team, 2017). To determine differences in floral traits among populations, after MANOVA we performed a univariate analysis of variance (ANOVAs) using the 'summary.aov' function on each response variable.

### Pollinator visitation

Direct observations and recordings of floral visitors were conducted from 7:00–11:00 h for periods of 15 min (a total of 96 periods in Catemaco in 2013, and 38 in Coatepec in 2014) throughout the flowering season, in three flowering patches at each site. For every pollinator visit, we recorded insect morphospecies, the number of flowers visited, time of visitation, and visitor contact with stigmas and anthers. We calculated overall and total visitation rates by each functional group of pollinators as the mean number of visits per flower per 15 min. Total visitation rates between populations were compared using generalized linear models (GLMs) in R, following a Poisson distribution with a square-link function. We tested for overdispersion in the model through the dispersion test function from the 'AER' package in R (Kleiber and Zeileis, 2008).

### Reproductive assurance: spatial variation

To determine if different populations varied in the contribution of autonomous self-pollination to fruit and seed production, we sampled 50 plants at two locations: Catemaco,  $N=27$ ; and Coatepec,  $N=23$ ; and conducted the following treatments on two flowers per plant: (1) Natural pollination of emasculated flowers ( $F_E$ ) and (2) Natural

pollination of intact flowers ( $F_I$ ). In the first treatment, flower buds were emasculated and bagged in the late afternoon to be exposed to natural pollination the following morning at 05:00 h; these plants potentially received outcross pollen and geitonogamous self-pollen (i.e., pollen from flowers on the same plant). In the second treatment, flowers were tagged and subsequently exposed to natural pollination (unmanipulated); these flowers potentially received outcross and geitonogamous pollen, as well as self-pollen from the same flower. Each flower was followed until fruit maturation.

We collected mature capsules 45–50 days after flowering, counted the seeds produced by each capsule, and estimated the mean fruit set per plant (fruits/flowers) and the mean seed set per flower per plant (seeds/mean number of ovules). The number of ovules per plant was fixed at a mean of six per flower according to Stucky (1985), and to our own ovule counts. We calculated total reproductive output as mean fruit set  $\times$  mean seed set for emasculated ( $F_E$ ) and control flowers ( $F_I$ ). The contribution of autonomous selfing to total female reproductive success was calculated as  $RA = 1 - (F_E/F_I)$ , following Eckert et al. (2006). RA index values range from 0 to 1, where zero indicates no contribution of autonomous self-pollination to the natural seed set, and one indicates that all seed production under natural pollination conditions results from autonomous self-pollination.

Spatial variation in RA was analyzed for fruit set and seed production. For fruit set, we conducted an analysis of deviance (difference of deviances, twice the log-likelihood from nested models), after generalized linear mixed models (GLMM), with the 'glmer' function from the 'lme4' package in R (Bates et al., 2015). Pollination treatment, population, and their interaction were specified as independent variables, fruit set as the response variable, and plant nested within treatment as a random factor; we used a binomial distribution with a logit link function (McCullagh and Nelder, 1989; Fox et al., 2015). For seed production, we conducted an analysis of deviance after a GLM with the 'glm' function from the 'stats' package in R (McCullagh and Nelder, 1989; Fox et al., 2015; R Core Team, 2017). The number of seeds per fruit was specified as the response variable, following a Poisson distribution with a square-link function.

## Reproductive assurance: temporal variation

We conducted the experiment described above for three years in the Catemaco population only: 2012 ( $N = 12$ ), 2013 ( $N = 27$ ) and 2014 ( $N = 12$ ). We tested for differences in fruit set between treatments and years with an analysis of deviance after GLMM; plant nested within treatment was included as a random factor. For seed production, we used analysis of deviance after GLM, with a quasi-Poisson distribution and square-link function to deal with overdispersion. Model specifications and tests for overdispersion

were set as specified above. For all GLMM and GLM models, comparisons between pollination treatments, populations, and years were calculated with contrast weight vectors, using the 'glht' function from 'multcomp' package in R (Hothorn et al., 2008).

## Inbreeding depression

We quantified the effects of inbreeding on fruit set, seed production, and progeny performance over two consecutive years in the Catemaco population. A total of 38 plants were subjected to the following two treatments: (1) hand self-pollination: flowers were manually pollinated with pollen from the two longest anthers of the flower; and (2) hand cross pollination: emasculated flowers were manually pollinated with pollen obtained from the two longest anthers of two–three donor plants. We avoided crossings between closely related individuals by collecting pollen for outcross hand-pollinations from patches that were separated from the focal plants by at least 20 m. We followed plants bi-weekly and recorded fruit and seed production approximately one month later.

All seeds produced under different pollination treatments were counted and weighed independently on an analytical balance to a precision of 0.001 mg. Seeds that weighed more than 1 g were scarified with 98%  $H_2SO_4$ , then washed with distilled water and randomly planted in germination pots. Seeds weighing less than 1 g were previously determined as unviable; therefore, they were not planted. Potted seedlings were placed in an open field exposed to natural conditions until they were harvested. Germination was monitored daily for four months in 2012 and six months in 2013; germination monitoring was longer in 2013 because the seeds took longer to germinate. The proportion of germinated seeds and the number of days to germinate were recorded for each treatment. Some seeds remained ungerminated at the end of each follow-up period; therefore, time to germination data correspond to right-censored observations (McNair et al., 2013). To determine the effects of inbreeding on progeny quality, seedlings were transplanted to potting bags and grown for four months the first year and six months the second year. Plants were harvested to measure dry mass including aerial stems and roots.

The effect of pollination treatment and year on fruit set, seed viability, and progeny performance were analyzed with linear models. For fruit set and seed production, we tested models with the same specifications as described in the RA section. Seed mass and whole plant mass were analyzed through ANOVA after linear models, using 'lm' functions from the 'stats' package in R (R Core Team 2017). Pollination treatment and year were specified as independent variables, while the mass of viable seeds, aborted seeds, and whole plant mass were indicated as response variables. Because seed mass is often negatively correlated with seed number, the number of viable and aborted seeds were used



as covariates in their respective analyses. We also calculated an index of ID that estimates the relative performance of selfed and outcross individuals for each of the four reproductive traits following Ågren and Schemske (1993), as  $RP = 1 - w_s/w_o$  if  $w_s \leq w_o$ ; or  $RP = w_o/w_s - 1$  if  $w_s > w_o$ ; where  $w_s$  corresponds to the trait fitness after self-pollination and  $w_o$  is the fitness after outcross-pollination. The index varies from  $-1$  to  $1$ , where negative values indicate that self-pollination outperforms cross pollination, and positive values indicate the opposite trend.

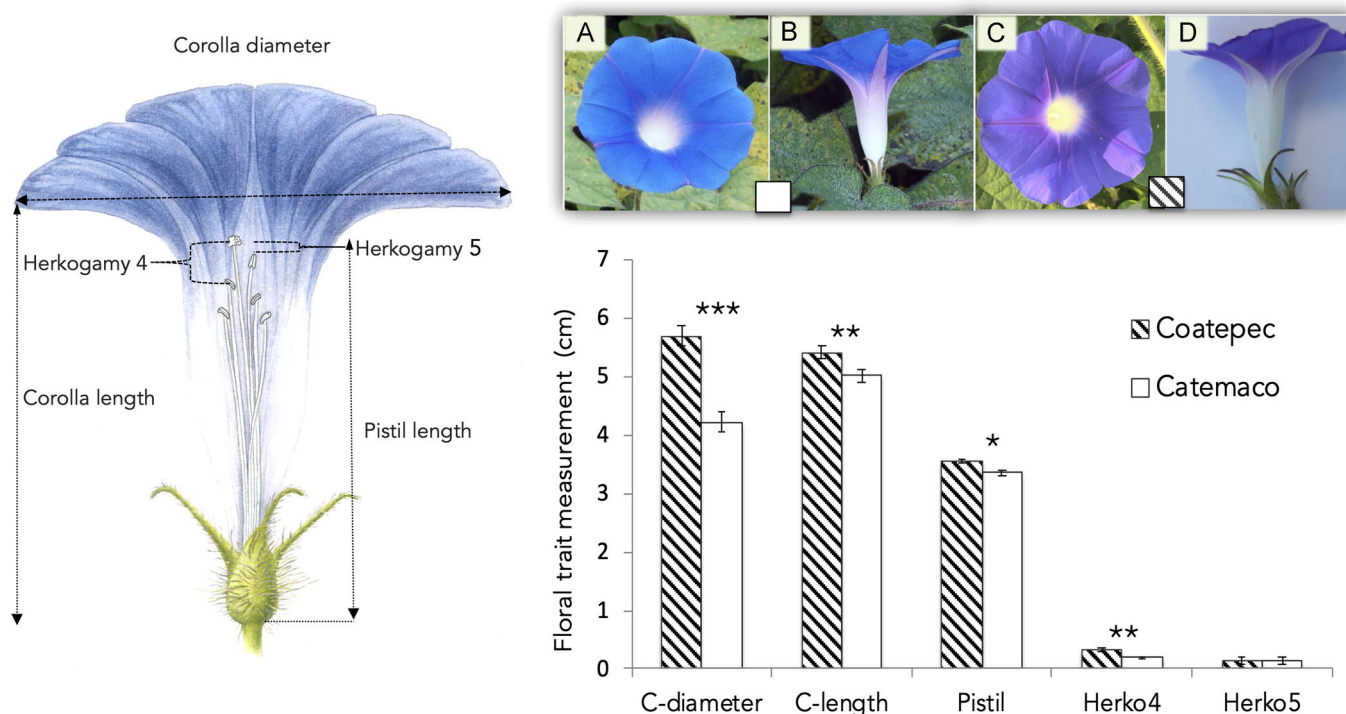
To evaluate the number of days seeds took to germinate, a survival analysis (time-to-event analysis) was used (Clark et al., 2003; Onofri et al., 2010). To test for differences in germination times between pollination treatments, cure-mixtures models were used for each year (Onofri et al., 2011). Pollination treatment was specified as the independent variable, while the response variable was defined as a survival object that includes the two variables of day of germination and germination success in the 'survival' package (Therneau and Grambsch, 2000; Therneau, 2021). The model was fitted to the observed data by using the 'flexsurvcure' function from the 'flexsurvcure' package in R (Amdahl, 2017). Using the akaike information criterion (AIC) value, a log-normal cumulative distribution was chosen for the fraction of germinated seeds and a logistic distribution for the fraction of ungerminated seeds. The fit of the cure model is shown in a graphical comparison between observed and predicted data in Onofri et al. (2011).

## RESULTS

### Spatial variation in floral morphology and pollinator visitation frequency

Floral traits differed between populations (Wilk's  $\lambda = 0.44$ ;  $F_{7,56} = 10.10$ ;  $P < 0.001$ ); specifically, flowers from Coatepec were larger than flowers from Catemaco (Appendix S1). Pistil length and stigma-anther distance (herkogamy) of the fourth stamen (HER4) were greater in flowers from the Coatepec population ( $F_{1,62} = 5.58$ ,  $P = 0.02$ ;  $F_{1,62} = 9.15$ ,  $P = 0.004$  respectively; Figure 1). The stigma-anther distance of the fifth stamen was  $0.2$  cm on average in both populations; however, the position of the stamens relative to the stigma was not always consistent. Stamen four had anthers above the stigma in 16% of flowers in Catemaco and none of the flowers in Coatepec. In contrast, stamen five had anthers that were above the stigma in 53% of the flowers in Catemaco, and above or at the same level of the stigma in 25% of the flowers in Coatepec.

There was no difference in total pollinator visitation between *I. hederacea* populations ( $\chi^2 = 0.90$ ,  $df = 1$ ,  $P = 0.34$ ), and mean pollinator visitation rates were  $0.10 (\pm 0.03 \text{ SE})$  visits per flower  $0.25 \text{ h}^{-1}$  for the Coatepec population, and  $0.17 (\pm 0.05 \text{ SE})$  visits per flower  $0.25 \text{ h}^{-1}$  for the Catemaco population. The main pollinators in both populations were native bees in the genus *Euglossa*, although in the Coatepec population there were also butterflies and *Apis mellifera*.

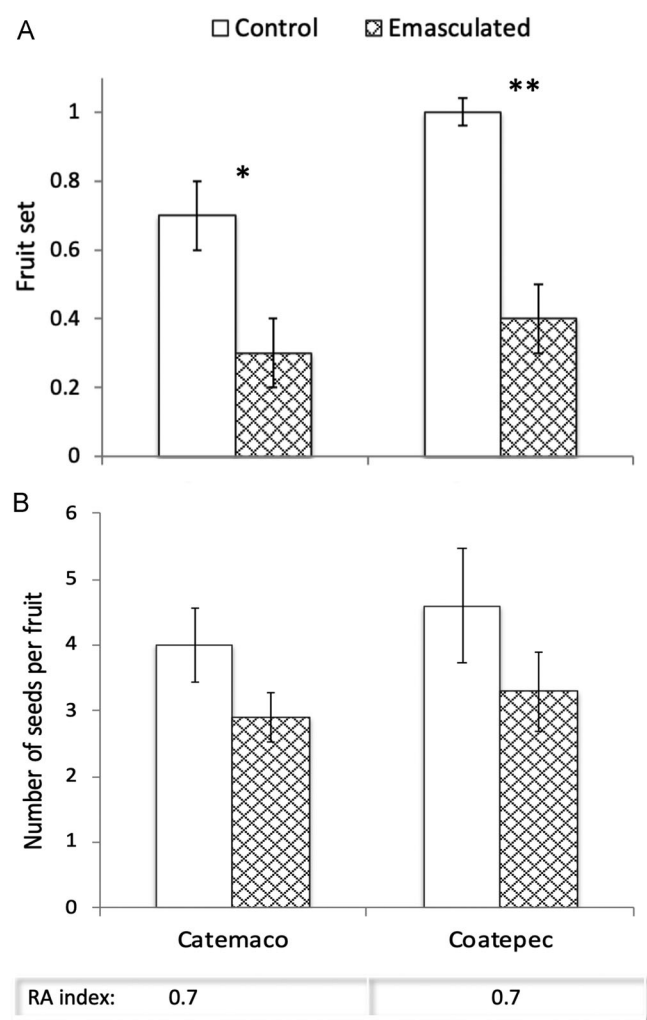


**FIGURE 1** (A, B) Flowers of *Ipomoea hederacea* from Coatepec and (C, D) Catemaco, Veracruz, Mexico, and floral traits variation between populations. Floral traits (mean  $\pm$  SE) are: C-diameter = corolla diameter, C-length = corolla length, HER4 = herkogamy for stamen four, HER5 = herkogamy for stamen five. Asterisks indicate significant differences between populations. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ . Drawing by Pedro Emiliano Cortéz Rodríguez

## Reproductive assurance: Spatio-temporal variation

Fruit set was higher in open-pollinated than in emasculated flowers in both Catemaco and Coatepec populations ( $\chi^2 = 10.58$ ,  $df = 1$ ,  $P = 0.001$ ; Figure 2A), and there was no significant interaction between population and treatment ( $\chi^2 = 1.83$ ,  $df = 1$ ,  $P = 0.18$ ). Seed production was marginally different between treatments ( $\chi^2 = 4.83$ ,  $df = 1$ ,  $P = 0.03$ ; Figure 2B), and no significant interaction was found either ( $\chi^2 = 0.006$ ,  $df = 1$ ,  $P = 0.94$ ). The contribution of autonomous self-pollination to overall reproductive output—measured as the RA index—was high in both Coatepec and Catemaco populations (RA = 0.7).

For the analysis of temporal variation in RA, there were significant treatment effects for fruit set and seed

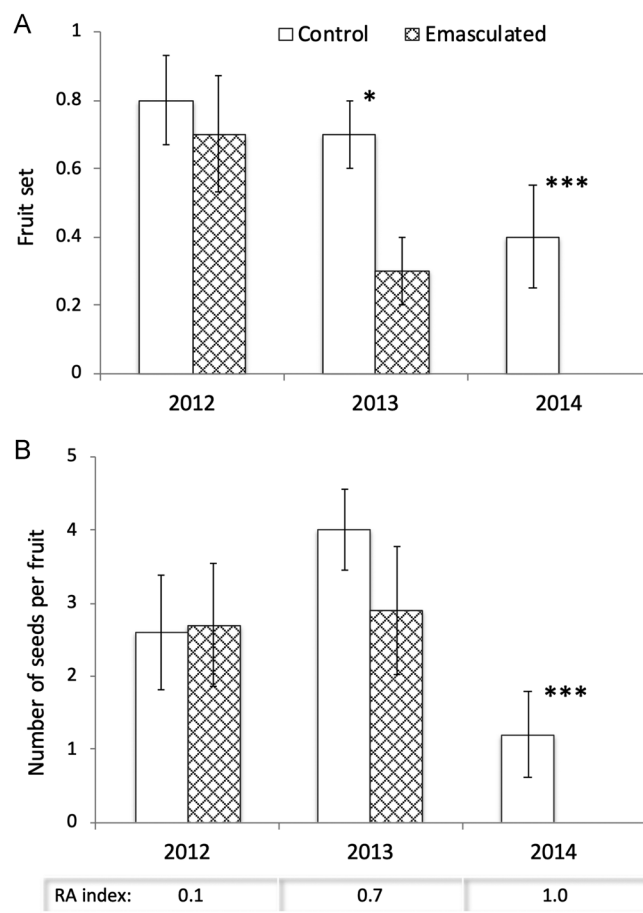


**FIGURE 2** (A) Fruit set and (B) seed set of paired pollination treatments (open-pollination control, emasculated open-pollination, mean  $\pm$  SE) in two populations of *I. hederacea* in the state of Veracruz, Mexico in 2013. Asterisks indicate significant differences between treatments at: \* $p < 0.05$ ; \*\* $p < 0.01$ . The reproductive assurance index was calculated as  $RA = 1 - (F_E/F_I)$ , where  $F$  corresponds to the natural pollination of emasculated (E) and intact (I) flowers

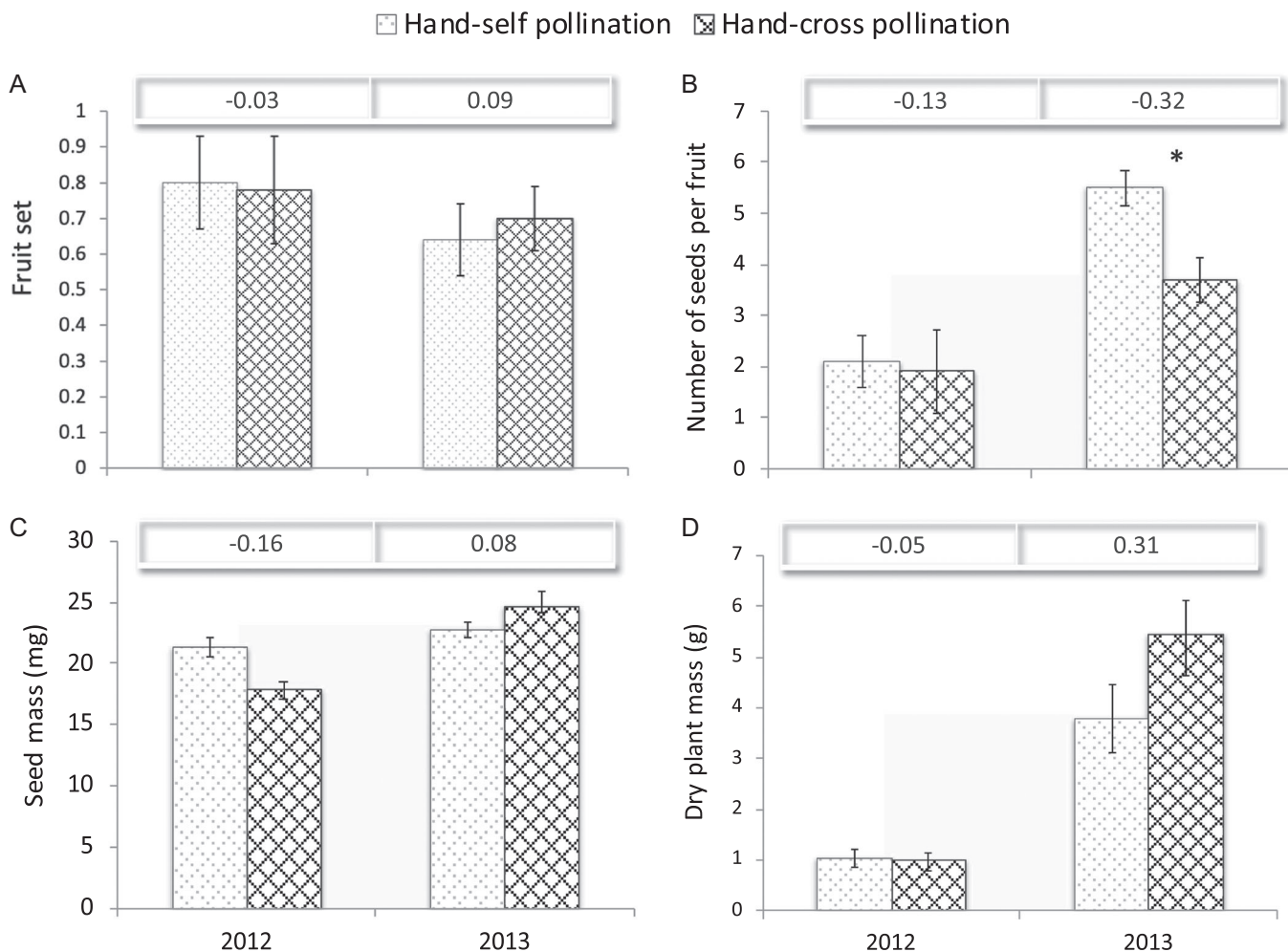
production ( $\chi^2 = 6.79$ ,  $df = 1$ ,  $P = 0.01$ ;  $\chi^2 = 7.39$ ,  $df = 1$ ,  $P = 0.01$  respectively; Figure 3), a significant effect of year on fruit and seed production ( $\chi^2 = 6.34$ ,  $df = 2$ ,  $P = 0.04$ ;  $\chi^2 = 35.82$ ,  $df = 2$ ,  $P < 0.001$ , respectively), and a marginally significant interaction between treatment and year for seed production ( $\chi^2 = 6.57$ ,  $df = 2$ ,  $P = 0.04$ ). There was a general trend for greater fruit set and seed production in open-pollinated than emasculated flowers, with significant contrast effects for fruit set both in 2013 and 2014 (Figure 3A), and for seed production in 2014 (Figure 3B), but no effects on 2012. The contribution of autonomous pollination to overall female reproductive success was low in 2012 (RA = 0.1), but higher in 2013 (RA = 0.7) and 2014 (RA = 1.0).

## Inbreeding depression

Fruit set did not significantly vary between treatments ( $\chi^2 = 0.14$ ,  $df = 1$ ,  $P = 0.71$ ; Figure 4A) and there was no



**FIGURE 3** Temporal assessment of reproductive assurance in a population of *I. hederacea* from Catemaco, Veracruz, Mexico during 2012, 2013, and 2014 comparing (A) mean  $\pm$  SE fruit set and (B) seed production, between pollination treatments (unmanipulated open-pollination, emasculated open-pollination). Asterisks indicate significant differences between treatments at: \* $p < 0.05$ ; \*\*\* $p < 0.001$ . The reproductive assurance index was calculated as  $RA = 1 - (F_E/F_I)$ , where  $F$  corresponds to the natural pollination of emasculated (E) and intact (I) flowers



**FIGURE 4** Assessment of inbreeding depression in a population of *I. hederacea* from Catemaco, Veracruz, Mexico conducted in 2012 and 2013. Letters indicate (A) mean  $\pm$  SE fruit set, (B) seed production, (C) seed mass, and (D) dry mass of F1 progeny, comparing hand self-pollination control and hand cross-pollination treatments. Asterisks indicate significant differences between treatments at  $*p < 0.05$ . The numbers above bars are estimates of inbreeding depression based on Ågren and Schmske (1993); index values go from -1 (selfed progeny advantage) to 1 (outcross progeny advantage), see the ID section in the Methods section for details

interaction effect between treatment and year ( $\chi^2 = 0.11$ ,  $df = 1$ ,  $P = 0.74$ ). Seed production was significantly higher for the self-pollination than the outcross treatment ( $\chi^2 = 5.05$ ,  $df = 1$ ,  $P = 0.02$ ; Figure 4B) and there was no significant interaction effect ( $\chi^2 = 0.95$ ,  $df = 1$ ,  $P = 0.33$ ). For seed mass, there was no treatment effect ( $F_{1,183} = 3.57$ ,  $P = 0.06$ ; Figure 4C), but there was a significant interaction showing opposite trends for selfed and outcrossed seed mass in 2012 and 2013 ( $F_{1,183} = 11.43$ ,  $P = 0.001$ ). For aborted seeds, there were no significant effects of pollination treatment or interaction between treatment and year for either seed production or seed mass (Appendix S2).

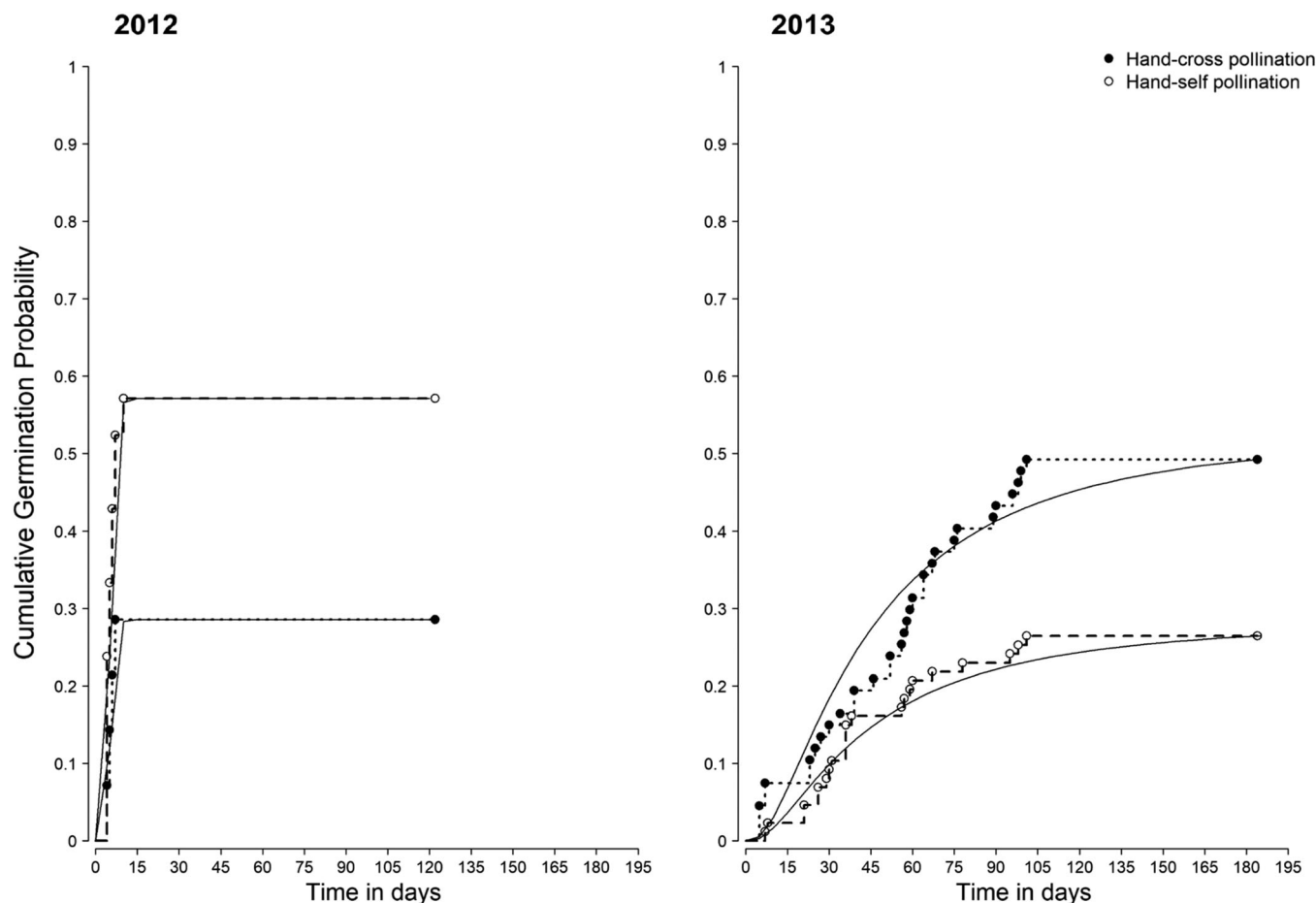
The percentage of germination was 38% of the viable seeds (total seeds sown = 188; 28 in 2012 and 160 in 2013). Germination time did not differ between treatments for year 2012 ( $\chi^2 = 2.83$ ,  $df = 1$ ,  $P = 0.09$ ; Figure 5); however, for year 2013 outcrossed seeds had a significantly greater probability of germinating than selfed seeds ( $\chi^2 = 8.52$ ,  $df = 1$ ,  $P = 0.003$ ; Figure 5). When the plant mass of F1 progeny was

compared, there was a significant main effect of treatment ( $F_{1,61} = 4.66$ ,  $P = 0.03$ ; Figure 4D), but a posteriori comparisons indicated no significant differences between pollination treatments each year. There was no interaction effect between treatment and year ( $F_{1,61} = 1.86$ ,  $P = 0.18$ ).

## DISCUSSION

### Reproductive assurance

The repeated evolution of self-compatibility in large plant genera such as *Ipomoea*, is a strong indicator that self-pollination is beneficial for plant fitness, at least under certain circumstances. Empirical evidence indicates that self-pollination provides RA in some species at certain times or locations (e.g., Kalisz and Vogler, 2003; Moeller, 2006; Martén-Rodríguez and Fenster, 2010; Schoupe et al., 2017; Teixido and Aizen, 2019), but not in other species



**FIGURE 5** Probability of seed germination in *Ipomoea hederacea* for hand self-pollination (open circles) and hand cross-pollination (solid circles) in 2012 and 2013. Dotted lines show the Kaplan-Meier estimates of germination for each pollination treatment. Solid lines show the parametric cure models. Differences were only significant in 2013

(e.g., Eckert and Schaefer, 1998; Eckert, 2000). Given that ID is the strongest selective force against self-fertilization, this study evaluated the contribution of self-pollination to female reproductive success in eastern Mexican populations of *I. hederacea*, considering the potential counteracting effect of ID. We found that autonomous self-pollination conferred considerable RA in natural populations of *I. hederacea*, but the magnitude of RA varied over the three study years; meanwhile, ID was negligible in the early stages of progeny development and low in later stages.

Assessments of geographic variation in RA have found that the contribution of self-pollination varies in association with different floral or environmental variables. For example, in *Collinsia verna* and *Tuberaria guttata*, RA was negatively associated with pollinator availability (Kalisz and Vogler, 2003; Teixido and Aizen, 2019), and corolla diameter (Teixido and Aizen, 2019), while across populations of *Centaureum* spp., RA was negatively correlated with the level of herkogamy, but not with other floral traits (Schoupe et al., 2017). In *I. hederacea*, we found differences in corolla size between the Catemaco and Coatepec populations, but this variation did not lead to spatial differences

in the magnitude of RA. Other studies have also found that the contribution of autonomous self-pollination to reproduction varies according to flower size categories (Elle and Carney, 2003) and herkogamy levels (Eckert and Herlihy, 2004; Chen et al., 2009). These studies show that the traits associated with RA vary among plant species; however, herkogamy is considered the most important floral trait associated with the capacity to self-fertilize (Moeller, 2006; Brys and Jacquemyn, 2011; Roldán and Ashworth, 2018).

In morning glories, the distance between the stigma and the two tallest anthers is variable but frequently small (Chemás-Jaramillo and Bullock, 2002), and it is considered an important determinant of the mating system (e.g., Duncan and Rausher, 2013). Moreover, herkogamy tends to be smaller or absent in self-compatible *Ipomoea* species in contrast to their self-incompatible relatives (Delgado-Dávila et al., 2016). In *I. hederacea*, the fourth and fifth anthers were at the stigma level or above more frequently in Catemaco than in Coatepec. However, this situation was not reflected in a greater magnitude of RA in Catemaco, possibly because absolute values of herkogamy remain very



small in both populations (Figure 1). Such herkogamy values suggest a high potential for competing and delayed self-pollination (sensu Lloyd and Schoen, 1992); thus, the overall high values of RA recorded in this study are possibly the result of reduced herkogamy combined with low pollinator visitation in both populations—on average, less than one visit per flower per hour, where anthesis lasts 5–6 h. Nevertheless, RA was not consistently high across years, suggesting factors other than herkogamy influence the contribution of autonomous self-pollination to plant reproduction in *I. hederacea*.

Temporal variation in RA has been documented in various studies (e.g., Goodwillie, 2001; Martén-Rodríguez and Fenster, 2010). In *I. hederacea*, there was variation in RA values for the Catemaco population with RA being negligible in 2012, but relatively high in 2013 and 2014 (Figure 3). Temporal variation in RA is expected when there is variation in the abundance of mates or pollinators (Lloyd and Schoen, 1992), but empirical evidence for this is still limited. In a seminal study, Kalisz and Vogler (2003) found that the contribution of self-pollination to fruit production in bee-pollinated *Collinsia verna* varied along with changes in pollinator visitation, both across populations and years. Likewise, in hawkmoth-pollinated *Lilium formosanum*, RA was significant across years but greater the year of higher pollination failure (Rodger et al., 2013). Meanwhile, in vertebrate-pollinated plants, RA was significant and consistent across years in hummingbird-pollinated Gesneriads and *Aquilegia canadensis*, while it was consistently absent in bat-pollinated species (Herlihy and Eckert, 2002; Martén-Rodríguez and Fenster, 2010). A possible explanation is that insect-pollinated species may experience larger variation in pollinator visitation and effectiveness than vertebrate-pollinated species, potentially causing greater temporal fluctuations in RA. Being an insect-pollinated species, the interannual variation in RA observed in *I. hederacea* is likely to reflect temporal variation in pollinator visitation in the human-disturbed habitats this species currently occupies, although this has yet to be confirmed by empirical work. Because particular floral visitors differ in pollination effectiveness in other *Ipomoea* species (Santiago-Hernández et al., 2019), it will be necessary to assess pollinator visitation frequency and effectiveness to elucidate the factors underlying temporal variation in RA in *I. hederacea*.

The results of this study support the idea that RA, through autonomous self-pollination, reduces pollen limitation in *I. hederacea*, as it may also occur in other self-compatible *Ipomoea* species with low pollinator visitation rates (Rosas-Guerrero et al., 2011; Delgado-Dávila et al., 2016; Hassa et al., 2020). A previous study showed that fruit and seed production in *I. hederacea* and *I. purpurea* were not limited by pollen in contrast to two self-incompatible congeners, despite the lower pollinator visitation rates (Delgado-Dávila et al., 2016). Furthermore, in the present study, fruit and seed set values of outcrossed plants in the ID experiment mirror those of open-pollinated flowers in the RA experiment, both in 2012 and 2013

(Figures 3A, 3B and 4A, 4B). Overall, these results show that RA is contributing to the lack of pollen limitation in *I. hederacea*, as observed in Catemaco during 2014, where emasculated flowers did not set any fruit or seed (Figure 3).

One potential limitation of studies that use emasculation treatments to test for RA is that emasculation itself may negatively affect floral longevity, plant reproductive success, or pollinator attraction (Dart and Eckert, 2013). However, the emasculation of hand-pollinated flowers in a previous study did not reduce fruit or seed production in *I. hederacea* or any other *Ipomoea* species (Delgado-Dávila et al., 2016). Moreover, there were no differences in fruit set and seed set between emasculated and control flowers in 2012 in Catemaco, when fruit set values were the highest. Furthermore, although most pollinators of *I. hederacea* are bees, none of the bee visitors were observed collecting pollen. Finally, anthesis lasts only a few hours and shortening of floral longevity upon emasculation was not observed (Delgado-Dávila and Martén-Rodríguez, personal observation). Therefore, we are confident that the reported results on RA are not related to the emasculation of flowers.

## Inbreeding depression

Inbreeding depression is considered an important evolutionary force counteracting the advantages of selfing; however, few studies have evaluated ID in assessments of RA (e.g., Eckert and Barrett, 1994; Eckert, 2000; Herlihy and Eckert, 2002; Kalisz and Vogler, 2003; Kalisz et al., 2004; Weber and Goodwillie, 2009; Delmas et al., 2014). This study showed that RA provided by self-pollination is not countered by ID in the early stages of progeny development in *I. hederacea*. However, inbreeding affected later stages of plant development, such as time to germination, and plant biomass of the F1 progeny, although this counterbalancing effect was small and observed only in 2013. Results for other annual morning glory species have shown similar patterns; specifically, ID effects on germination and later stages of development were significant in U.S. populations of *I. hederacea* in greenhouse experiments, but not under field conditions (Hull-Sanders et al., 2005), while in *I. purpurea*, ID was low but varied with herkogamy levels (Chang and Rausher, 1999). Meanwhile, a recent study has shown that in U.S. populations of *I. purpurea*, highly selfing plants resistant to glyphosate have lower ID than susceptible plants with mixed mating systems (Van Etten et al., 2021). Overall, these results support the general pattern that short-lived frequently selfing species generally express low levels of ID; however, in *Ipomoea*, genetic loads are large enough to generate some ID for certain life-cycle stages or under particular environmental conditions.

The magnitude of ID reported in this study for *I. hederacea* was low across life-cycle stages and years, although outcrossed progeny outperformed selfed progeny in the probability of germination and adult plant mass (Figures 4 and 5). Other studies have also reported ID in

late but not in early life-cycle stages in natural populations (e.g., *Silene vulgaris*, Glaetli and Goudet, 2006). This may be explained by the fact that deleterious mutations tend to have more severe effects in the early stages of progeny development (e.g., seed formation, germination), and that selection against these mutations should be most effective during early life-cycle stages, particularly in short-lived plants (Husband and Schemske, 1996; Melser et al., 1999). Furthermore, if purging of lethal alleles occurs in early development, ID in later life-cycle stages would be primarily determined by mildly deleterious mutations (Husband and Schemske 1996). It is important to indicate that differences in the magnitude of ID among life-cycle stages were found in a global meta-analysis, showing a trend for lower ID in early fitness traits in self-compatible plants, particularly for germination (Angeloni et al., 2011). In our study, there was no ID for the earliest fitness traits (fruit and seed production and seed mass); however, there was a small but significant ID effect on germination time (Figure 5). To understand the importance of RA and ID in the evolution of self-compatibility in the genus *Ipomoea*, future studies should explore ID across all life-cycle stages in different native species and different environments.

Most studies of RA that consider the effects of ID have found high levels of ID (Herlihy and Eckert, 2002; Eckert and Barrett, 1994), even for early life-cycle traits (Delmas et al., 2014). However, these cases include mainly perennial species, which are thought to be under strong selection against selfing, both via ID and gamete discounting. In annual *Ipomoea* species, however, the advantages of self-fertilization may outweigh the disadvantages of ID when selfing provides RA under the variable mate and pollinator availability to which these species are commonly exposed. Annual species tend to have lower genetic loads and smaller levels of ID than perennial species (Angeloni et al., 2011; Friedman, 2020). The low values of ID found in this study, as well as in U.S. populations of *I. hederacea*, also support this idea (Hull-Sanders et al., 2005). Annual morning glory species that occur in human-disturbed habitats possibly occur as metapopulations, with population dynamics involving frequent colonization and extinction events and distinct genetic structuring, as was demonstrated for eastern U.S. populations of *I. hederacea* (Campitelli and Stinchcombe, 2014). In such a context, both RA and ID should be relevant to the maintenance of morning glory populations.

## CONCLUSIONS

The assessment of RA conducted in our study demonstrated that self-pollination makes an important contribution to female reproductive success in Mexican populations of *I. hederacea* that occur in ephemeral human-disturbed environments. The temporal variation in RA found in our study highlights the importance of including temporal assessments of RA in natural populations. Variation in RA

may be related to the low and possibly variable visitation rates in *I. hederacea*, also reported in other *Ipomoea* species (Rosas-Guerrero et al., 2011; Delgado-Dávila et al., 2016). Finally, the reproductive advantage provided by self-pollination is not countered by ID in early life-cycle stages, which has also been shown in other *Ipomoea* species (e.g., *I. purpurea*, Chang and Rausher, 1999); however, results indicate that ID in later stages of development is potentially important and should be further studied. Overall, these findings support the notion that RA is an important selective force in the evolution of self-pollination in the genus *Ipomoea*.

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## AUTHOR CONTRIBUTIONS

R.D.D. and S.M.R. designed the study and wrote the manuscript. R.D.D. processed and analyzed the data.

## DATA AVAILABILITY STATEMENT

Data used in all the analyses are available through Zenodo: <https://doi.org/10.5281/zenodo.5091713> (Delgado-Dávila and Martín-Rodríguez, 2021).

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

**Appendix S1.** Means  $\pm$  SE of floral traits for two Mexican populations of *Ipomoea hederacea*.

**Appendix S2.** Analysis of deviance for number and mass of aborted seeds in *Ipomoea hederacea*.

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