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# Self-Compatibility and Reproductive Success of *Oenothera drummondii* subsp. *drummondii*: Is It Similar between Native and Non-Native Populations?

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**Abstract:** The mating system of plants widely distributed can change in native range but also in non-native habitats. *Oenothera drummondii,* native to the coastal dunes of the Gulf of Mexico, has been introduced to Europe, Africa, Asia and Oceania. Hand self- and cross-pollination were performed to determine compatibility and to compare fruit set, fruit weight, seed set and germination characteristics from natives and non-natives populations and a comprehensive integral reproductive success index (IRSI) was built. *Oenothera drummondii* exhibited high self-compatibility and mixed reproductive systems in all populations. Characteristics of fruits and seeds from self- and cross-pollination varied within and between native and non-native populations and some had a positive clinal variation in the native range. The IRSI was sensitive to changes of fruit set, seed set and final germination of both self- and cross-pollination, showing differences between native populations. Differences in characteristics of fruits and seeds in the native and non-native ranges suggest the occurrence of distinct selection factors. The mixed reproductive system of *O. drummondii* suggests it can take advantage of local visitors in the native range, but also can provide advantages for the establishment at non-native sites giving the opportunity to interact with local flower visitors.

**Keywords:** costal dunes; cross-pollination; reproductive success index; seed germination; incompatibility index; self-pollination

## 1. Introduction

Plant reproduction is central to the studies of population ecology, since it allows an understanding of the evolutionary processes of species throughout their distribution range [1,2]. Flowering plants possess exceptionally diverse reproductive strategies [3] and mating system type determines reproductive success [4]. Sexual reproduction in hermaphroditic plants generally includes self-pollination and cross-pollination, or a combination of both strategies [5,6]. Selfing resolves competition among individual plants for pollination services when there is a limitation of pollinators or mates [7], while outcrossing reduces inbreeding and increases genetic diversity. However, outcrossing is less efficient because it requires the presence of pollen vectors and neighbouring mates [4].

Studies in natural populations have indicated that mixed mating systems are frequent [5,8]. With this mating strategy, reproduction occurs by both self-fertilization (selfing) and mating with other individuals (outcrossing). One possible explanation is that autonomous pollination provides reproductive assurance for outcrossing species when pollinators are limited [5]. However, the rate of selfing can vary widely among populations throughout their natural range as a result of both biotic and environmental factors [2]. Some plant species show selfing ability, such as those with short lifetimes or that inhabit sites with frequent disturbances [9] and selfing species are more likely to have wider geographical ranges than those without this ability [10,11].

Human activities have not only caused major disturbances of natural habitats but have also led to species dispersal beyond their native ranges [12,13]. Several studies have shown that success in the establishment and spread of these non-native species depends on particular physiological, ecological and reproductive characteristics [14,15]. However, reproductive ability is important in the naturalization process because seed production and seed germination are necessaries to maintain populations [16]. Baker [17] stated that selfing plant species that arrive at new habitats after long-distance dispersal should have an adaptive advantage, since one or more individuals will have the opportunity to start a sexually reproductive population through the selfing reproductive mechanism. Known as Baker's law, this hypothesis has been widely discussed because of contradictory findings; however, recent studies indicate that self-fertilization is a common mating mechanism, mainly in species that colonize, both naturally following long-distance dispersal and with human assistance, as in the case of invasive species [11,18].

Phenotypic traits of native populations can evolve rapidly under the environmental conditions presented by novel habitats, leading to local adaptation of the individuals [19]. Differences between native and non-native populations can be observed by growing plants of populations of both origins in controlled uniform environments such as common gardens [15]. To date, few studies have compared the reproductive success of self- and cross-pollination from native and non-native populations [20–23] and none have included almost all of the parameters that define reproductive success (v.gr., fruit set, seed set, seed germination) and the range of the species distribution.

*Oenothera drummondii* subsp. *drummondii* Hook. naturally inhabits the back beaches and the first dune ridges of the coasts of the Gulf of Mexico and southeastern USA; although, during the last century it was introduced into parts of Europe, Asia, Africa, Australia and New Zealand [24–27]. This habitat is frequently disturbed by wind, burial by sand and impacted by waves during tropical storms. The species presents self-compatible herkogamous yellow flowers with anthesis at dusk, living for one night or for a few hours into the following morning in the native range [24,28,29]. The flowers are visited and pollinated by sphingid moths (e.g., *Hyles lineata*, *Manduca* sp.), although some hymenopterans (e.g., *Lasioglossum texaum*, *Apis mellifera*) also visit the flowers [24,29]. The seeds are very small with high germination capacity and some can germinate after floating in seawater for a period of time [30]. The genus *Oenothera* L. has been widely used for studies related to variation in pollination and breeding systems; some taxa are self-compatible and outcrossing, whereas others just self-compatible and autogamous [7,28,31]. Raven [32] considers that both cross- and self-pollination can occur in Onagraceae species that present positive herkogamy.

The current distribution of *O. drummondii*, with native and non-native populations distributed over a wide geographic range, with different ecological histories and environmental conditions, presents an opportunity to explore possible variation in mating systems and reproductive success as a result of both the evolutionary history in the native populations and the possible adaptation of non-native populations of *O. drummondii* to their novel environments. Faced with these scenarios, it is possible that native populations present a predominantly outcrossed mating system adapted to their native pollinators, while non-native populations present a predominantly self-mating system, since in their new habitats, flower visitors and pollinators can be not suitable for the flowers. On the other hand, since the native range of *O. drummondii* corresponds to a latitudinal gradient, we expected that the mating systems and fruit and seed characteristics will differ among these populations, especially among the most geographical distant populations, because change in pollinator presence (more abundant in the tropic climate than subtropical).

In this paper, we addressed the following specific questions: (1) What is the mating system (outcrossing, selfing or mixed) of *Oenothera drummondii*. (2) Does the mating system vary between native populations? (3) Do fruit and seed characteristics and seed germination vary between hand-pollination treatments in the native population range? (4) Does the success of self- or cross-pollination change according to the native or non-native

status of populations? (5) Do the fruit and seed characteristics and seed germination of self- and cross-pollination vary among native and non-native populations? (6) Do the reproductive success and the self-compatibility index of non-native populations fall within the range of these parameters of native populations?

## 2. Materials and Methods

### 2.1. Taxon Studied

*Oenothera drummondii* subsp. *drummondii* is a short-lived shrub (up 4 years) that blooms from spring to autumn. In native populations, the flowers live very briefly (12–15 h) while in non-native populations, they can last for two or more days [29,33]. The flowers produce both nectar and pollen as floral rewards. Floral nectar is exuded by nectaries located at the base of tubular hypanthium. Pollen grains are connected to each other by viscin threads. The fruits are capsules with tetragonal dehiscence, which contain a large number of small seeds (ca. 200 seeds, each of 1.2 mm in length and 0.6 mm in width) that germinate readily under laboratory conditions [34].

### 2.2. Seed Collection and Plant Material

The study includes six native populations that together cover almost all of the geographic range of the natural distribution of the species in the Gulf of Mexico, four from Mexico and two from USA and four non-native populations corresponding to locations across its non-native distribution range (Spain, Israel, South Africa, Australia, Figure 1 and Supplementary Material Table S1 show the full list of the populations with their origin, countries, acronyms and geographic locations). Ripe fruits were collected during 2015 and 2016 from ca. 30 randomly selected mature-similar size plants in each population (1–5 fruits from each plant). Seeds of each population were obtained manually from fruits, pooled and stored in marked container. In December of 2016 and 2017, groups of seeds taken at random from each population were germinated in pots (15 × 25 cm) filled with a substrate composed of perlite (30%) and dune sand (70%) and regularly irrigated to field capacity. When the seedlings emerged, we removed all but the most vigorous individual in each pot, keeping a total of 18 plants from each population (180 plants in total). The plants were maintained under greenhouse conditions (temperature 25 °C, humidity 40% and natural daylight), with weekly irrigation of the substrate to field capacity, at the Centre of Technological Research and Innovation of the University of Seville (CITUS-US). In addition, the plants were fertilized every month with 200 mL of Hoagland solution (20%) throughout the study time in order not to limit their growth.

### 2.3. Pollination Treatments and Fruit and Seed Data

Flowering started nearly at the same time in all populations. Once the plants were flowering regularly, we performed the following hand-pollination treatments on the flowers of each population: (1) spontaneous selfing: the flower remained unmanipulated, (2) self-pollination: the flower was emasculated and pollinated with its own pollen. (3) cross-pollination: one flower was emasculated and pollinated using pollen from another emasculated flower of a different individual; we subsequently pollinated the latter flower with pollen from the former. All treatments were applied using fresh newly opened flowers with virgin styles that were pollen-saturated according to the pollination treatment. The plants produced different numbers of flowers, but enough to make all hand-pollination treatments on each one. Following application of the manipulation treatments, the flowers were carefully labelled and left unbagged, since the strict insect control practiced in the greenhouses ensured that no alien pollen could subsequently be deposited on the flowers.

The production and development of fruits were recorded regularly. The fruits were harvested when they were ripe, at ca. 6 weeks after pollination (fruit set) and pooled in paper bags per pollination treatment and population. With the number of fruits produced on each pollination treatment and population, we obtained the fruit set by means of the ratio of the number of flowers in the treatment and the number of fruits produced. Each

fruit was weighed on an analytical balance (Ohaus Adventurer resolution 0.1 mg). The seeds of each fruit were then extracted manually and counted. With the number of seeds of each fruit developed, we calculate the seed set for each treatment and population, as the average number of seeds per fruit. Seeds of each pollination treatment and population were pooled and stored in label plastic containers in dry conditions until germination experiments.
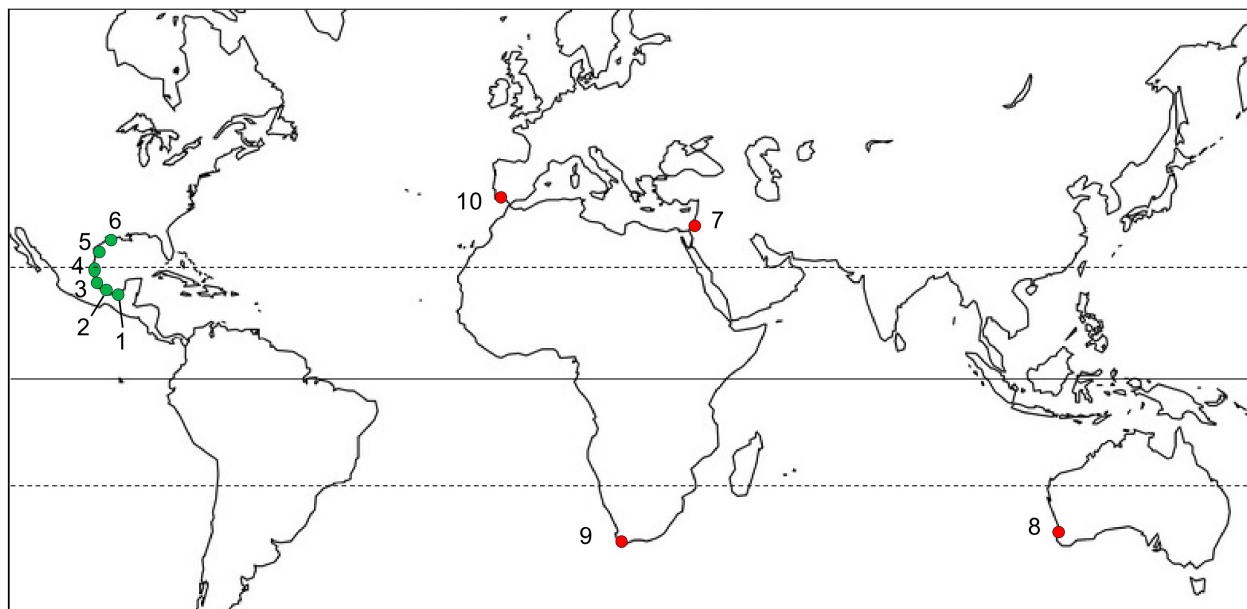


**Figure 1.** Geographical locations of the populations of *Oenothera drummondii* studied. Mexico: Native populations (green dots). Mexico: 1. Ojoshal (OJO); 2. Sontecomapan (SON); 3. La Mancha (MAN); 4. Tecolutla (TEC). US: 5. South Padre (SPA); 6. Bolivar (BOL). Non-native populations (red dots). Israel: 7. Rishon-Lezion (LEZ); Australia: 8. Mandurah (MAH). South Africa: 9. Muizenberg (MUI). Spain: 10. El Dique (DIQ). Line of Ecuador (solid black line) and intertropical region are shown (dashed black lines).

### 2.4. Germination Experiment

To evaluate the success of the hand-pollination methods, we conducted germination experiments with the seeds obtained. We only carry out germination experiments with the seeds produced by self-pollination and cross-pollination, because the seeds from the spontaneous pollination treatment were not well developed. Four sets of 100 randomly selected seeds were obtained from the seed mixture of each treatment and population (800 seeds from the two treatments per population; 8000 total experimental seeds). These seeds were placed in labelled Petri dishes with three layers of wet filter paper as a substrate and kept in germination chambers under controlled conditions (12 h light/darkness, temperature 25/20 °C). Previous studies indicate that *O. drummondii* seeds maintain high viability ($\approx$90% after ca. 2 years) and do not require any pre-germinative treatment [29]. Daily, for a 90-day period, germinated seeds were quantified and removed and distilled water added as necessary to keep the papers moist. We considered a seed to have germinated when growth of the radicle was visible. For each treatment and population studied, we recorded the time to first germination in days ($t_o$), the percentage of total germination (final germination) and the germination rate. Germination rate was estimated using a modification of the Timson index [35] as follows,

$$\text{Germination rate} = \sum G/t, \tag{1}$$

where G is the percentage of germination accumulated at one-day intervals and t is the total number of days of the experiment. Once the germination period was completed, the

crush test [36] was performed on the remaining seeds to determine if they were empty or if the embryo was still viable.

*2.5. Data Analysis*

Due to the low number of fruits and immature seeds in the spontaneous selfing treatment, all comparisons were made only between the manual self- and cross-pollination data; however, the data from spontaneous pollination are shown in Table A2. Prior to analysis, the normality of all data was tested (Shapiro-Wilk test) and germination data transformed (Arcsine). Since transformations did not produce normality of the data, non-parametric analyses were carried out. All analyses were performed using JMP (v9.0.1, SAS Institute Inc., Cary, NC, USA) and SPSS (IBM SPSS Statistics for Windows, version 25; North Castle, NY, USA).

The mating system of each population was evaluated with the self-compatibility index (SCI) [37], where:

$$SCI = \text{seed set from self-pollination/seed set from cross-pollination,} \qquad (2)$$

in which values > 1 indicate full self-compatibility. Petanidou [22] note that the SCI allows comparison of data produced under different environmental growth conditions (i.e., different habitats, greenhouse). In our study, plants were obtained from seeds from populations of different geographic locations, but which were grown under the same greenhouse conditions.

We used a Wilcoxon t-test to determine whether the fruit sizes (weight, length and width), seed characteristics (seed set, seed set mass, individual seed weight), final germination and germination rate from each hand pollination treatment differ within each population (native and non-native). Subsequently, since the native populations studied are located along a latitudinal gradient, we explored whether fruit and seed characteristics obtained in each hand pollination treatment differed between the native populations by mean Wilcoxon/Kruskall—Wallis nonparametric analyses of variance and multiple Steel—Dwass comparisons. On another hand, linear regression analysis was performed to know any relation between the reproductive traits and the latitudinal distribution of the populations. Likewise, the relationships between latitude and final germination percentage and germination rate were compared by nonparametric ANOVA and explored through regression analysis. Since each native population represents a latitudinal position along the gradient of distribution of the study species (Figure 1), the charts presented in the results section include the population acronyms instead of latitudinal data. Population acronyms follow the order of latitudinal increase (see Table A1).

Latter we then explored whether reproductive success differed between the sets of native populations and non-native populations. Each data point pertaining to fruit and seed characteristics, as well as the final germination percentage and germination rate of all populations, was labelled and pooled according to origin (native and non-native) and pollination treatment (self- and cross-pollination) for inclusion in the analysis. The data were compared through Wilcoxon non-parametric analysis of variance and multiple Steel–Dwass comparisons, where the pollination treatment (self- and cross-) in each origin (native and non-native) was considered as a factor.

Plant reproductive success has been usually determined by the fruit set, the seed set, or the final germination, because each parameter shows the individual success of different reproductive phases in plants. As far as we know, however, there is no parameter that indicates the total reproductive success in plants. In order to represent the total reproductive success in a single value, we constructed the integral reproductive success index (IRSI), which was obtained as follows:

$$IRSI = \text{fruit set} \times \text{relative seed set} \times \text{final germination,} \qquad (3)$$

where a value of 1 indicates very high success in all reproductive phases (e.g., 1.0 [fruit set] × 1.0 [relative seed set] × 1.0 [final germination] = 1); while values < 1 indicate that success could have been high in some of the reproductive phases, but low in others (e.g., 1.0 [fruit set] × 0.8 [relative seed set] × 0.5 [final germination] = 0.4). The relative seed set data for each hand pollination treatment (self- and cross-) of each population was obtained by dividing every seed set value by the highest seed set value recorded in all populations, regardless of the pollination treatment from which it was derived. We made the latter, based on the hypothesis that the highest seed set value represents that which could potentially achieved in any treatment and population. Finally, we performed a linear regression analysis, to explore whether the IRSI of each pollination treatment and the value of the SCI are related to the latitude at which each population is located.

## 3. Results

### 3.1. Compatibility, Fruit and Seed Characteristics among Native Populations

The fruit set of the two pollination treatments was high in all native populations (92–100%) and they therefore also presented a high level of compatibility (SCI 0.92–1.04) (Table A2). In general, fruit and seed characteristics did not differ between pollination treatments within the populations, but there was a tendency towards higher values in the cross-pollination treatment. The weight of fruits from cross-pollination were significantly greater in the MAN and BOL populations (Table A2).

The seeds of the two hand-pollination treatments presented a similar pattern to that of the fruits. Seed set did not differ significantly among the two hand-pollination treatment within almost all native populations; only in BOL seed set were significantly higher in the cross-pollination (Table A2). Time to first germination was similar between treatments in most native populations (4–5 d), in SON and TEC the time of first germination were greater in seeds from cross pollination (Table A3). Meanwhile, the germination rate and final germination was higher in seeds from cross pollination, although significant differing in SPA, MAN and TEC (Table A3).

On the other hand, the characteristics of fruits and seeds of each pollination treatment presented some differences between populations. The weight of the fruits derived from self-pollination did not differ significantly and showed less variability in the OJO and SON populations, but greater variability in SPA and BOL (Figure 2A), while the fruits of cross-pollination were significantly heavier in BOL and lighter in SON, but similar among the rest of the populations (Figure 2B).

The seeds of the two pollination treatments presented a similar pattern to that of the fruits. Seed set in the self-pollination treatment did not differ significantly among the populations (Figure 2C); however, seed set in the cross-pollination treatment did differ significantly, being higher in BOL and lower in SON and SPA (Figure 2D).

In general, seed germination varied among the hand-pollination treatments and populations. The populations BOL and SPA had the highest final germination percentages in both hand-pollination treatments, while OJO and SON had the lowest values in the two hand-pollination treatments (Figure 3), but only the final germination of seeds derived from the self-pollination treatment was positively related to latitude (Figure 3A,B). On the other hand, the germination rate (Timson Index) also differed among populations, showing the same pattern as that of final germination: low values in OJO and SON and higher values in SPA and BOL and again the germination rates obtained from seeds from the self-pollination treatment were significant positively related to latitude (Figure 3C).
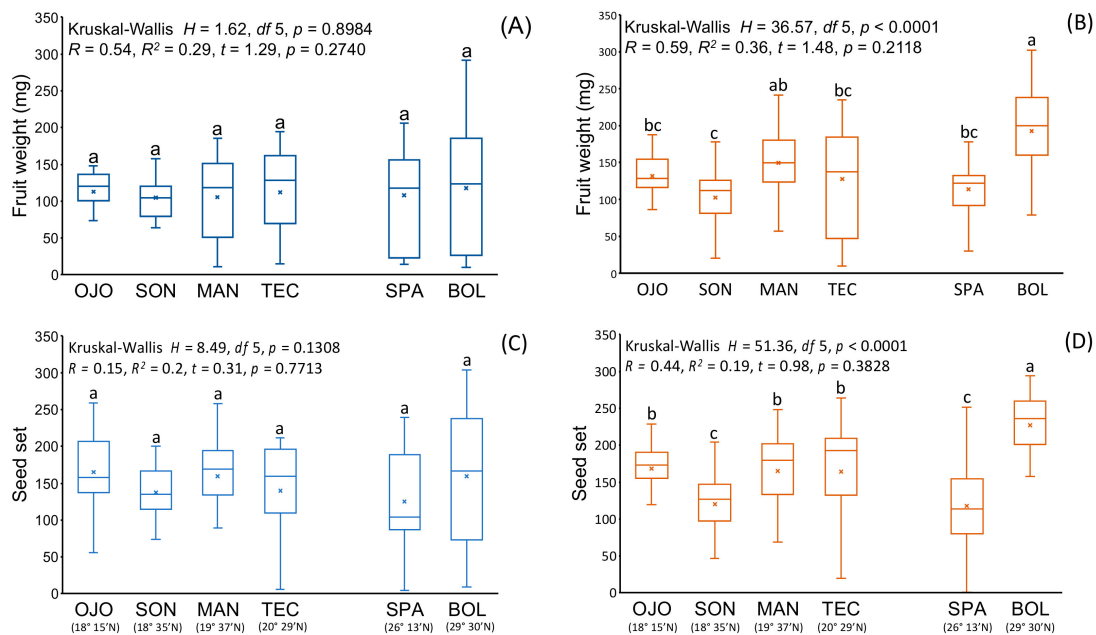
**Figure 2.** Fruit weight (**A**,**B**) and seed set (**C**,**D**) obtained by self- (blue boxes) and cross-pollination (orange boxes) in native populations of *Oenothera drummondii*. Comparison among populations (Kruskal–Wallis nonparametric ANOVA) and the relationship between the reproductive characteristic and latitude (linear regression analysis) are shown in each graph. Population acronyms are explained in Table A1. Latitudinal ubication is indicated below each acronymous. Different letters above the boxes indicate significant differences among populations (Steel–Dwass all pairs multiple comparisons *p* < 0.001). Boxes show the average (x), median (-) and quartiles.
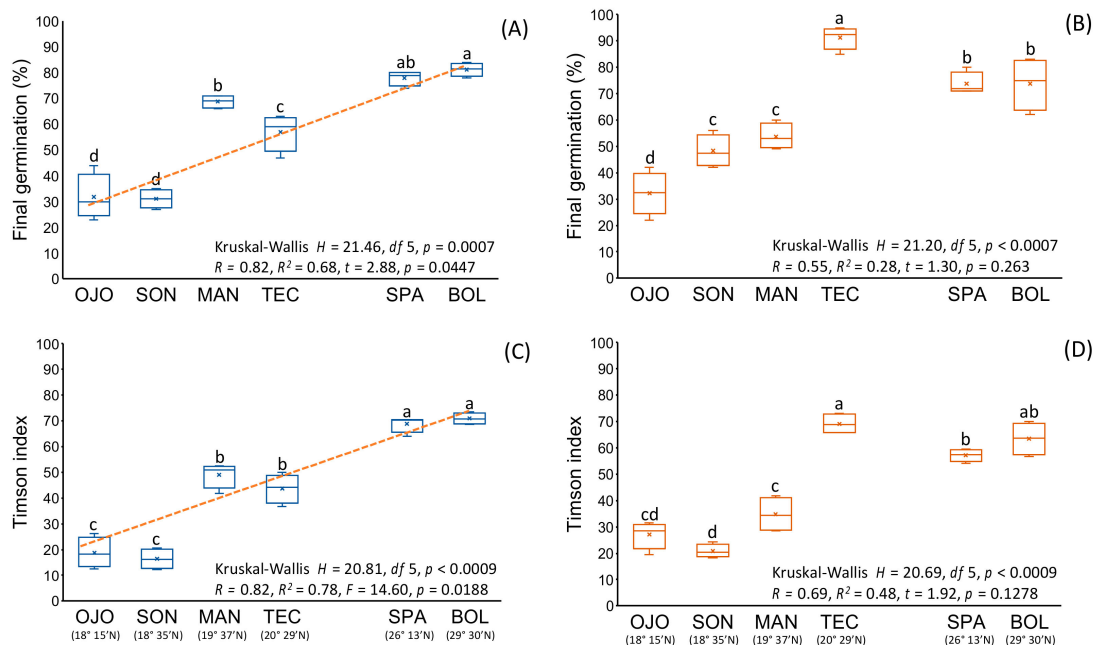


**Figure 3.** Final germination and Timson index of the seeds obtained by self- (**A**,**C**) and cross- (**B**,**D**) pollination in native populations of *Oenothera drummondii*. Comparison among populations (Kruskal–Wallis nonparametric ANOVA) and the relationship between the reproductive characteristic and latitude (linear regression analysis) are shown in each graph. Population acronyms are explained in Table A1. Latitudinal ubication is indicated below each acronymous. Dotted lines representing significant regression analyses but are not in scale with the latitudinal increase. Different letters above the boxes indicate significant differences among populations (Steel–Dwass all pairs multiple comparisons *p* < 0.001). Boxes show the average (x), median (-) and quartiles.

### 3.2. Fruit and Seed Characteristics of Non-Native Populations

As in the native populations, fruit set in the two pollination treatments of the non-native populations was high and SCI was therefore also high in all of these populations (Table A2). The fruit weight was not different between hand-pollination treatments within the non-native populations; (Table A2). Meanwhile, the seed set did not differ between hand-pollination in all non-native populations. On the other hand, time to first germination was very similar and did not differ between the treatments in each population, although the percentage of final germination was higher in the seeds derived from cross-pollination in the populations MAH and MUI (Table A3).
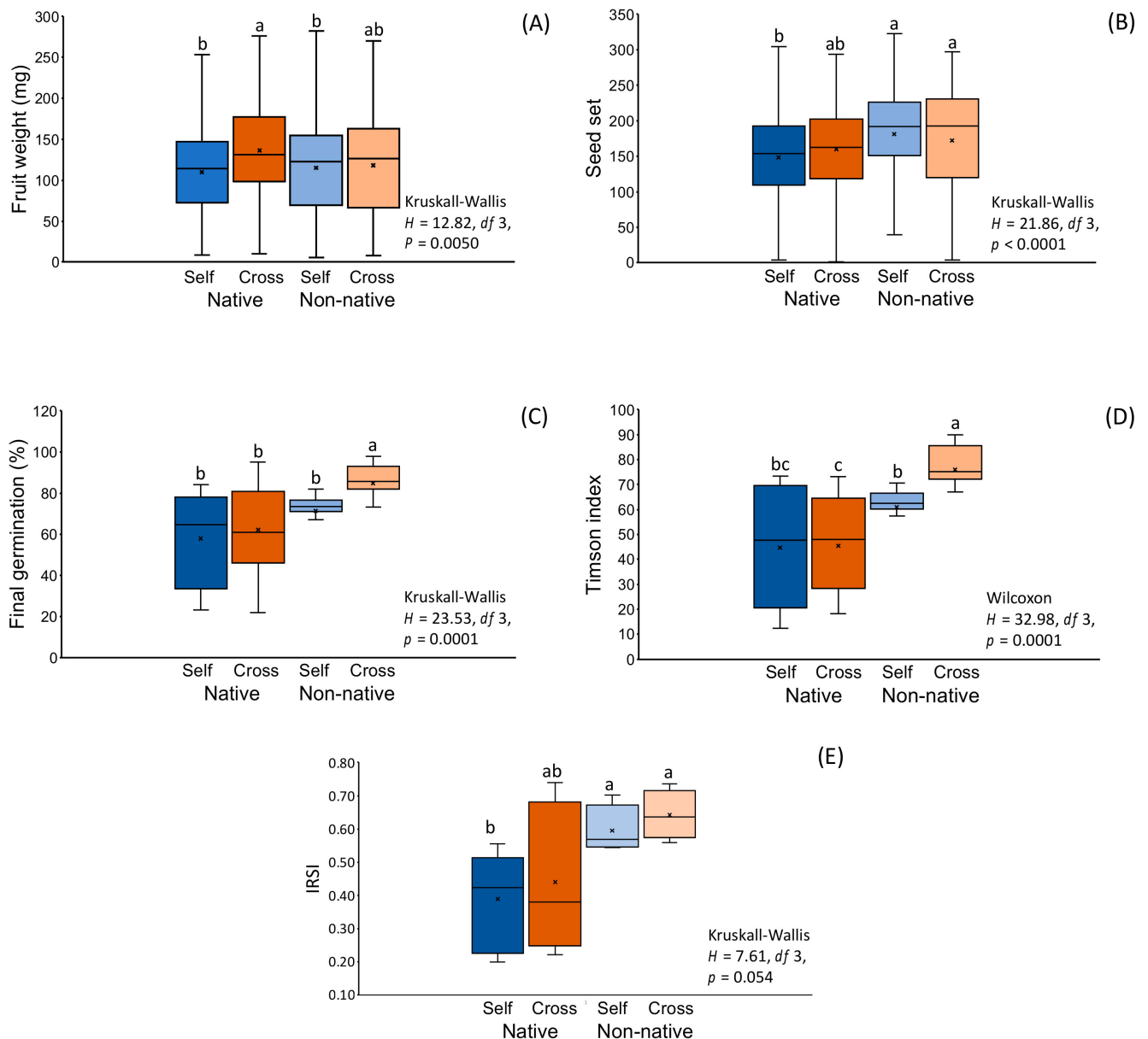
### 3.3. Comparison of Reproductive Characteristics between Native and Non-Native Populations

Fruit and seed characteristics differed significantly between the groups of native and non-native populations, but no clear general pattern was shown either by the origin of the populations (native and non-native) or by the pollination treatment (self- and cross-pollination) (Figure 4). The weight of the fruits from cross-pollination was significantly greater and was similar in the native and non-native populations, while fruit weight from selfing was lower but also similar among the two sets of populations (Figure 4A). However, seed set were both greater in the two pollination treatments of the non-native populations (Figure 4B), while the values of final germination and germination rate (Timson index) were higher in the cross-pollination treatment of the non-native populations (Figure 4C,D). Finally, the IRSI also were higher of both pollination treatments for the non-native group and the lowest was obtained for the self-pollination treatment of the native group (Figure 4E).
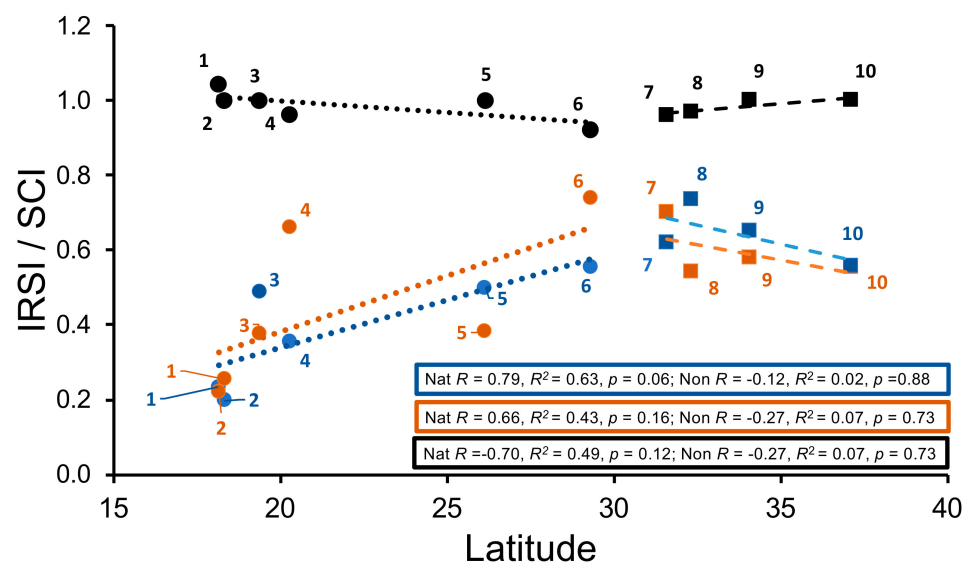
### 3.4. Integral Reproductive Success Index

The IRSI of the native populations show a positive tendency to latitudinal increase in both pollination treatments, although self-pollination was only marginally non-significant (Figure 5). This means that the populations located at a lower latitude (OJO and SON) presented the lowest reproductive success, while the population at the highest latitude (BOL) presented the highest reproductive success. Meanwhile, the compatibility index (SCI) presented a negative, but non-significant, relationship, being higher in the populations OJO and SON and decreasing to BOL (Figure 5). On another hand, the IRSI of both hand-pollination treatments and the SCI of all non-native populations were as high as of the native population located at the highest latitudinal-distribution (BOL).

**Figure 4.** Comparison between groups of native and non-native populations of *Oenothera drummondii* of the characteristics of fruit weight (**A**), seed set (**B**), final and rate germination (**C**,**D**) and the integral reproductive success (IRSI) (**E**), obtained by self- (Self) and cross-pollination (Cross). The result of the Kruskal–Wallis nonparametric ANOVA is shown on each graph. Different letters above the boxes indicate significant differences (Steel–Dwass all pairs multiple comparisons, *p* < 0.001). Boxes show the average (x), median (-) and quartiles.

**Figure 5.** Relationship between self-compatibility index (SCI) (symbols and line black) and the integral reproductive success index (IRSI) of self- (symbols and line blue) and cross-pollination (symbols and line orange) with the latitudinal location of native and non-native populations of *Oenothera drummondii*. The boxes show the results of linear regression for each index for native (Nat) and non-native (Non) populations. Circles indicate native populations and squares indicate non-native populations. Numbers indicate acronyms of populations described in Figure 1.

## 4. Discussion

We have shown that *O. drummondii* presents a mixed system (self- and cross-pollination) with a high degree of self-compatibility, both in its native distribution range and in non-native populations. We also showed that the characteristics of the fruits and seeds produced by both self- and cross-pollination vary between native populations, with no clear pattern. This is also found when comparing these characteristics between the groups of native and non-native populations. However, in the native range, final accumulated germination of seeds and germination rate (Timson index) of self-compatibility treatment were positively related to latitudinal increase, whereas the self-compatibility index did so slightly negatively. In the non-native populations, these two parameters had values as high as those recorded in the native populations located at the highest latitudes. We also showed that the integral reproductive success index (IRSI) provides a better perspective of the true reproductive success of the *O. drummondii* populations, both native and non-native, since it incorporates the values of the most useful parameters of reproductive success (fruit set, seed set and germination).

### 4.1. Mating System and Fruit and Seed Characteristics in Native Populations

Studies in natural populations indicate wide variability in mating systems, but also show that mixed mating (self- and cross-pollination) are more common than expected [5,38,39]. The genus *Oenothera* is no exception, since wide variation is recognized in the species mating systems [7] and both cross- and self-pollination can occur in Onagraceae species that present, positive herkogamy [32]. Gallego-Fernández and García-Franco [34] noted positive herkogamy in *O. drummondii*, but it has not relationship with latitude increase. Our results show that *O. drummondii* is a species with a mixed mating system because of the high values of fruit set and seed set both by self- and cross-pollination.

Field observations in most of the studied native populations of *O. drummondii* indicate high fruit production [29]. The low fruit set recorded by spontaneous self-pollination in the greenhouse suggests low capacity of autogamy, so the high fruit set recorded in the field could be result of assisted pollination. The flowers of *O. drummondii* produce abundant nectar and pollen [24,29] and they are visited by sphingids and crepuscular bees,

as occurs with other *Oenothera* species [28,40]. Observations made in one of the native populations studied (MAN) [29] show that bees (*Apis mellifera*) arrive to flowers shortly before sunset to collect pollen. The foraging activity is carried out in groups (2–5 bees) in one or two flowers of the same plant, limiting the pollination to selfing or geitonogamy. However, the sphingids (aff. *Manduca* sp.), which are nectar collectors with high energetic requirements, arrive shortly after dusk and fly around the population, visiting a large number of flowers of different individuals with no clear foraging sequence among plants, thus constituting potential outcrossing pollinators. This suggests that bees and sphingids both play important roles in the reproduction of *O. drummondii* in its native range of distribution. However, further observations and experiments are required in other native populations in the field in order to corroborate these findings.

Non-native populations also presented low fruit set and seed set by spontaneous self-pollination, but a high production of fruits and seeds in both hand-pollination treatments (self- and cross-pollination). Furthermore, opposed to the low number of fruits expected because of the alien environment in the non-native locations, it has been recorded a large production of fruits in one of our studied population (DIQ; [41]). On the other hand, in *O. drummondii* populations located in Europe and Israel (DIQ and LEZ), it has been recorded crepuscular anthesis and flower visits by local sphingids and bees [28,29,40]. This suggests that flowers of the non-native populations will have the same pattern of visits as those natives have. However, the flower lifespan in non-native populations is twice than in natives [29], which may be associated with limitation of pollinators [42]. Nevertheless, if pollinators are, in fact, limited, the constant coastal wind present probably shake violent the flowers and autogamy could thus be achieved.

The above indicates that *O. drummondii* presents those characteristics proposed by Baker [17] for colonizer species of new habitats. *Oenothera drummondii* has all of the benefits of being highly compatible and able to incorporate itself into the pollinator community, taking full advantage of its mixed mating system. Furthermore, there have been recorded low genetic variation in MAN (native) and DIQ (non-native) populations [43], which is evidence of colonization-extinction processes typical on coastal dunes [44,45].

### 4.2. Fruit and Seed Characteristics of Native Populations

The differences in most the characteristics of fruits and seeds among the native populations of *O. drummondii* showed a no relationship with latitudinal increase. However, there were a clear positive relationship in the fruit width and individual seed weight and a tendency in the seed-set weight to be smaller in lower latitudes and greater in those populations at higher latitudes. This pattern was also reflected in the final accumulated germination and in the germination rate (Timson index), since the populations located at low latitude had the lowest values of these parameters in the two pollination treatments studied.

Several studies have recorded floral trait variation patterns related to latitude increase, but rather than species following a general pattern, they respond differently to the conditions imposed by the latitudinal gradient [4]. In particular, the floral phenotypic features of *O. drummondii* differ among native and non-native populations and they do present a negative pattern with latitudinal increase [34]. The latitudinal positive relationship of individual seed weight in the two pollination treatments and of final germination and the germination rate in self-pollination suggest that the environmental conditions prevalent at higher latitudes can favour these reproductive characteristics of *O. drummondii*, since the wide contrasts found with the southern populations (OJO and SON). Reduction in seed set, seed size and germination are generally attributed to changes in local environmental conditions, due to the nutrient and water contents in the substrate, as well as pollen limitation (pollen load and quality) as a result of the absence or scarcity of pollinators and inbreeding [46,47]. Our study plants were maintained under controlled greenhouse conditions (in terms of nutrients, water, temperature and relative air humidity) throughout the experiment and all flowers were manually pollinated following a standardized method, so these factors did not influence the observed differences. The differences in seed size and

germination recorded in the studied populations may therefore be the result of inbreeding. Such results have been recorded in self-compatible species and can be important in small and isolated populations [48,49]. Although we do not have information regarding the size of all studied populations in the field, we do know that those located at lower latitudes (OJO, SON, MAN and TEC) are small and relatively isolated; therefore, our results possibly reflect the effects of inbreeding taking place in the field.

### 4.3. Comparison between Native and Non-Native Populations

The characteristics of the fruits and seeds obtained by the hand-pollination treatments varied between the sets of native and non-native populations and, although in general they do not show a particular pattern, some tendencies towards higher values were observed in the germination of the non-native populations. This indicates that, even though non-native populations are isolated in geographically distant sites, they maintain a high level of self-compatibility and have not modified their potential for a mixed pollination system. This also suggests that these populations can take advantage of local pollinators [29,50], which could allow them to invade new sites [7]. Several *Oenothera* species have become successfully established in Europe and in other regions of the world, where some taxa are considered to be actively invasive species [7,44,51], similarities in environmental conditions between their native habitats and invaded sites must be important factors in the success of these species.

### 4.4. Integral Reproductive Success Index (IRSI)

Reproductive success in plants usually is measured by seed set (e.g., [52]), number and weight of fruits (e.g., [53]), fruit set, seed set, seed development [54,55]) or germination [56]. However, the most commonly used parameters are fruit set, seed set and germination, since these represent the final product of one reproductive stage process (pollination, fertilization, or seedling emergence). In the studied populations of *O. drummondii*, we tested all of the parameters that indicate reproductive success (Tables A2 and A3) but found that the individual responses were inconsistent. If we had considered the most common reproductive success parameters (fruit set, seed set and total germination) separately, interpretation of the reproductive success of *O. drummondii* can be biased in different ways. The fruit set was very high in the two pollination treatments of all populations, the seed set showed some differences among populations, while final germination in the native populations followed the latitudinal gradient and the non-native seeds showed similarity to the northernmost native populations in terms of final germination. When considering the three most common reproductive parameters, the proposed Integral Reproductive Success Index (IRSI) shows the full success of *O. drummondii* in each of the pollination treatments studied in the different populations, since the IRSI integrates the effects of each of the parameters in a final unique reproduction value for the species. The two populations located in the southernmost part of the native distribution had the lowest IRSI, but this positively increased in the higher-latitude populations. In addition, the values of the latter were similar to those of the non-native populations. This suggests that the southern native populations can be subject to a different selection of reproductive factors than those of the northern, while the great integral reproductive success of non-native populations of *O. drummondii* can be an important factor in their establishment success.

To our knowledge, there has been no reproductive index that integrates the different components of plant reproductive success. In our study, the IRSI and final accumulated germination values presented the same pattern. The latter had a strong effect on the value obtained by the former since neither fruit set nor seed set clearly differed between populations. However, under other situations, where each parameter can change, the IRSI can help to more clearly understand reproductive success.

## 5. Conclusions

*Oenothera drummondii* is a coastal species, which has ecological characteristics that allow it to live and colonize these ecosystems with stressful environmental conditions [30,41]. Our results showed that *O. drummondii* presents a mixed mating system. This can confer reproductive advantages both in native and non-native ranges, allowing the flowers to interact with the local fauna (bees and sphinxes). However, the environmental and biotic conditions present throughout the entire range of *O. drummondii* can impose contrasting selection pressures on its reproductive characteristics. Self-compatibility and germination responses (rate and final germination) are reduced in low latitude populations, while they are increased in higher latitude populations, including native and non-native. These reproductive traits may contribute to the successful establishment of *O. drummondii* outside the native range. However, field studies evaluating pollinators, mating and the same reproductive parameters studied here, including more both native and non-native populations, will allow us to understand the adaptation process of *Oenothera drummondii* in the new environments.

## Appendix A

**Table A1.** Geographic location of native and non-native populations of *Oenothera drummondii* studied. Numbers corresponding with geographic location in Figure 1.

|   | Origin | Country | Population | Acronym | Latitude | Longitude |
|---|--------|---------|------------|---------|----------|-----------|
| 1 | Native | Mexico | Ojoshal | OJO | 18°15′ N | 93°59′ W |
| 2 | Native | Mexico | Sontecomapan | SON | 18°33′ N | 94°59′ W |
| 3 | Native | Mexico | La Mancha | MAN | 19°37′ N | 96°22′ W |
| 4 | Native | Mexico | Tecolutla | TEC | 20°29′ N | 97°01′ W |
| 5 | Native | USA | South Padre | SPA | 26°13′ N | 97°11′ W |
| 6 | Native | USA | Bolivar | BOL | 29°30′ N | 94°30′ W |
| 7 | Non-native | Israel | Rishon-Lezion | LEZ | 31°59′ N | 34°43′ W |
| 8 | Non-native | Australia | Mandurah | MAH | 32°32′ S | 115°41′ E |
| 9 | Non-native | South Africa | Muizenberg | MUI | 34°06′ S | 18°28′ E |
| 10 | Non-native | Spain | Dique | DIQ | 37°09′ N | 06°54′ W |

**Table A2.** Characteristics of the fruits and seeds (mean ± SD) obtained by spontaneous, self- and cross-pollination (Self and Cross, respectively) in flowers of native and non-native populations of *Oenothera drummondii*. The last row of each trait of fruits and seeds show the comparison (Wilcoxon test) between self- and cross-pollination data of each population (ns = not significant; * $p = 0.05$; ** $p = 0.01$; *** $p = <0.001$). Population acronyms provided in Figure 1 and Table A1.

| Chracteristics of Fruits and Seeds | Pollination Treatment | Native Populations | | | | | | | Non-Native Populations | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | OJO | SON | MAN | TEC | SPA | BOL | LEZ | MAH | MUI | DIQ |
| Fruit set (%) | Spontaneous | 0 | 12 | 20 | 15 | 2 | 19 | 5 | 4 | 0 | 7 |
| | Self | 96 | 100 | 96 | 96 | 100 | 92 | 96 | 97 | 100 | 100 |
| | Cross | 92 | 100 | 96 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Self-Compatibility Index | | 1.04 | 1.00 | 1.00 | 0.96 | 1.00 | 0.92 | 0.96 | 0.97 | 1.00 | 1.00 |
| Fruit weight (mg) | Spontaneous | 11.4 ± 2.1 | 18.1 ± 14.6 | 34.2 ± 29.4 | 24.3 ± 24.1 | 22.9 ± 17.2 | 35.8 ± 48.6 | 18.0 ± 12.2 | 22.9 ± 5.3 | 11.9 ± 12.9 | 17.8 ± 24.9 |
| | Self | 112.7 ± 28.5 | 104.6 ± 37.6 | 105.6 ± 56.8 | 112.4 ± 56.9 | 107.8 ± 68.5 | 117.5 ± 85.0 | 112.6 ± 64.0 | 139.0 ± 81.7 | 97.7 ± 35.5 | 109.0 ± 48.7 |
| | Cross | 131.3 + 51.4 | 102.8 + 41.5 | 149.2 + 50.7 | 127.9 + 70.9 | 113.8 + 44.0 | 192.8 ± 66.5 | 96.3 ± 61.6 | 176.1 ± 67.5 | 116.6 ± 37.3 | 84.4 ± 56.6 |
| | | ns | ns | *** | ns | ns | *** | ns | ns | * | ns |
| Seed set | Spontaneous | 0.0 ± 0.0 | 46.7 ± 38.8 | 42.0 ± 36.6 | 54.8 ± 83.2 | 62.0 ± 35.4 | 89.8 ± 109.9 | 58.0 ± 0.0 | 10.0 ± 0.0 | 0.0 ± 0.0 | 72 ± 100.4 |
| | Self | 165.5 ± 47.1 | 137.7 ± 34.7 | 159.6 ± 58.7 | 139.7 ± 61.8 | 137.8 ± 66.1 | 159.8 ± 95.2 | 215.7 ± 59.0 | 165.6 ± 96.8 | 161.5 ± 35.8 | 190.5 ± 60.3 |
| | Cross | 168.4 ± 67.5 | 120.2 ± 44.7 | 164.9 ± 65.4 | 164.6 ± 75.4 | 117.9 ± 62.8 | 227.1 ± 45.3 | 176.8 ± 94.8 | 175.2 ± 87.8 | 173.2 ± 49.7 | 161.7 ± 83.3 |
| | | ns | ns | ns | ns | ns | ** | ns | ns | ns | ns |

**Table A3.** Seed germination characteristics and the integral reproductive success index (IRSI) of self- and cross-pollination treatments in flowers of native and non-native populations of *Oenothera drummondii*. The last row of each germination parameter shows the comparison (Wilcoxon test) between self- and cross-pollination data of each population (ns = not significant; * $p = 0.05$). Population acronyms provided in Figure 1 and Table A1.

| | Pollination Treatment | Native Populations | | | | | | | Non-Native Populations | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | OJO | SON | MAN | TEC | SPA | BOL | LEZ | MAH | MUI | DIQ |
| First, germination (d) | Self | 4.0 ± 0.0 | 4.5 ± 1.0 | 5.0 ± 0.8 | 5.5 ± 1.0 | 5.0 ± 1.2 | 4.0 ± 0.0 | 4.5 ± 1.0 | 4.0 ± 0.0 | 4.0 ± 0.0 | 4.0 ± 0.0 |
| | Cross | 4.0 ± 0.0 | 7.3 ± 1.5 | 5.0 ± 0.8 | 4.0 ± 0.0 | 5.75 ± 0.50 | 4.0 ± 0.0 | 4.0 ± 0.0 | 5.0 ± 1.2 | 4.0 ± 0.0 | 5.3 ± 1.5 |
| | | ns | * | ns | * | ns | ns | ns | ns | ns | ns |
| Timson Index | Self | 18.8 ± 5.9 | 16.3 ± 3.9 | 49.0 ± 4.9 | 43.7 ± 5.6 | 68.8 ± 3.2 | 70.9 ± 2.2 | 61.6 ± 3.6 | 65.2 ± 1.8 | 62.9 ± 3.7 | 54.5 ± 15.4 |
| | Cross | 27.1 ± 5.3 | 20.9 ± 2.5 | 43.8 ± 6.7 | 69.2 ± 3.8 | 57.2 ± 2.3 | 63.6 ± 6.2 | 72.0 ± 3.8 | 87.6 ± 1.6 | 76.7 + 4.5 | 67.6 ± 12.9 |
| | | ns | ns | * | * | * | ns | * | * | * | ns |
| Final germination (%) | Self | 31.8 ± 8.9 | 31.0 ± 3.7 | 68.8 ± 2.6 | 57.0 ± 7.1 | 78.0 ± 2.8 | 81.3 ± 2.5 | 73.0 ± 4.3 | 72.8 ± 2.1 | 77.3 ± 4.4 | 62.8 ± 15.5 |
| | Cross | 32.3 ± 8.2 | 48.3 ± 6.1 | 53.8 ± 4.9 | 91.25 ± 4.4 | 73.8 ± 9.9 | 73.8 ± 9.9 | 79.8 ± 4.9 | 95.3 ± 1.9 | 85.3 ± 3.9 | 78.3 ± 15.8 |
| | | ns | * | * | * | ns | ns | ns | * | * | ns |
| Integral Reproductive Success Index (IRSI) | Self | 0.23 | 0.20 | 0.49 | 0.35 | 0.50 | 0.50 | 0.70 | 0.54 | 0.58 | 0.55 |
| | Cross | 0.22 | 0.26 | 0.38 | 0.66 | 0.38 | 0.74 | 0.62 | 0.74 | 0.65 | 0.56 |

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