



Multiple reproductive modes of *Myrcianthes coquimbensis* (Myrtaceae), an endangered shrub endemic to the Atacama Desert

Patricio García-Guzmán^{a,b,*}, Andrea P. Loayza^{b,c}, Francisco A. Squeo^{a,b,d}

^a Departamento de Biología, Universidad de La Serena, Casilla 554, La Serena, Chile

^b Instituto de Ecología y Biodiversidad (IEB)

^c Instituto de Investigación Multidisciplinario en Ciencia y Tecnología, Universidad de La Serena, La Serena, Chile

^d Centro de Estudios Avanzados en Zonas Áridas (CEAZA), La Serena, Chile

ARTICLE INFO

Edited by Fei-Hai Yu

Keywords:

Breeding system
Agamospermy
Selfing
Mix-Mating
Protandry
Reproductive assurance
Sexual interference

ABSTRACT

Many plants can produce seeds via multiple reproductive modes, such as selfing and outcrossing. Having multiple reproductive modes can be advantageous if it assures seed production when outcrossing fails, which is important for species inhabiting environments where pollinators are scarce or variable. However, it can also be disadvantageous due to the fitness costs associated to selfing. Consequently, plants have mechanisms to reduce the incidence of selfing. Here we examined the breeding system of *Myrcianthes coquimbensis*; this threatened Atacama Desert shrub is the last species to bloom in the community and exhibits low visitation rates per flower because pollinators are less abundant. Our aim was to determine whether this plant can produce fruits by modes other than outcrossing, and whether it possesses floral traits to prevent sexual interference. We conducted experimental flower treatments in two localities to determine whether fruits were produced by outcrossing, selfing, autonomous selfing and agamospermy. We also evaluated stigma receptivity and pollen viability during a flower's lifespan. *M. coquimbensis* developed fruits and seeds by all the reproductive modes assessed, including selfing and agamospermy. Flowers presented partial segregation of sexual functions, with the peak of pollen viability occurring before the peak of stigma receptivity. Selfing is unavoidable in *M. coquimbensis* and likely interferes with outcrossing. Coupled with possible early inbreeding depression, it probably results in a cost for seed production. Our results suggest that this species may be vulnerable in scenarios where pollinators are scarce; however, agamospermy may provide an alternative route of seed production in these scenarios.

1. Introduction

Understanding how different traits contribute to the reproductive output of plants is a central topic in ecology (Eckert et al., 2010). Plant traits that directly determine the means by which a plant produces seeds (i.e., the modes of reproduction) constitute its breeding system (Neal and Anderson, 2005; Barrett, 2014). For threatened and rare plants, breeding system studies are an essential first step to identify factors that reduce seed production (Johnson and Steiner, 2000; Davidson et al., 2014; Pérez et al., 2018), particularly for species living in harsh environments, where pollinators are scarce or their abundance variable (Castro et al., 2008). Moreover, knowledge of the breeding system of threatened plants can allow managers to infer how changes in the pollinator environment may affect their reproductive output (Larson and Barrett, 2000; Young et al., 2012), their risk of inbreeding depression (Bellanger et al., 2015) and ultimately, their population

viability (Richardson et al., 2016).

Plants exhibit remarkable variation in their reproductive strategies, and many species have multiple modes of reproduction including selfing and agamospermy (Vogler and Kalisz, 2001; Richards, 2003; Goodwillie et al., 2005; Moreira et al., 2017). Having multiple modes of reproduction is advantageous if it increases seed production when outcrossing fails (reproductive assurance) (Eckert et al., 2006). For example, lack of seed production in environments where pollinator abundance, activity or effectiveness is variable, can be compensated by autonomous agamospermy (Bierzychudek, 1987) or autonomous selfing (e.g., Fausto et al., 2001; Kalisz and Vogler, 2003). However, having multiple reproductive modes can also be disadvantageous due to the costs associated to agamospermy (asexuality) and selfing. In autonomous agamospermy, the egg cell that forms the embryo bypasses meiosis and recombination. This results first in seeds that are clones of the mother plant (Richards, 2003; Hörandl and Hojsgaard, 2012),

* Corresponding author at: Departamento de Biología, Universidad de La Serena, Casilla 554, La Serena, Chile.

E-mail address: pgarcia@userena.cl (P. García-Guzmán).

<https://doi.org/10.1016/j.flora.2020.151537>

Received 1 August 2019; Received in revised form 28 November 2019; Accepted 1 December 2019

Available online 07 January 2020

0367-2530/© 2020 Elsevier GmbH. All rights reserved.

which do not bring new allelic combinations to the population and hence make the species vulnerable to environmental changes (Richards, 2003) and second, in deleterious mutations accumulating over generations (Hojsgaard and Hörandl, 2015). Selfing (autonomous and facilitated) may also lead to reproductive costs because individuals resulting from several generations of selfing are homozygous and have higher chances of expressing deleterious mutations, which reduce the fitness of selfed progeny (i.e., inbreeding depression) (Kephart et al., 1999; Herlihy and Eckert, 2002), particularly during the seed development stage (i.e., early-acting inbreeding depression) (Husband and Schemske, 1996). Moreover, if autonomous selfing within a flower occurs in conjunction with outcrossing, it uses ovules and pollen that would otherwise be available for outcrossing (i.e., ovule and pollen discounting, respectively) (Eckert and Herlihy, 2004). Pollen and ovule discounting can also occur when selfing is mediated by pollinators (both within and among flowers) (Lloyd and Schoen, 1992).

Because of its cost, many plant species have mechanisms that prevent or reduce the incidence of selfing (Barrett, 2003), self-incompatibility being the main mechanism that prevents fecundation by self-pollen (Hiscock and McInnis, 2003; Busch and Schoen, 2008). Nonetheless, even in self-incompatible plants, self-pollen deposited on the stigmas can interfere with cross-pollination (i.e., sexual interference), ultimately decreasing seed production (Barrett, 2002). Therefore, plants also possess floral traits to prevent sexual interference. Two such traits are 1) the temporal separation in the maturation time of the stigma and pollen, which allows a segregation of the sexual functions within the flower (dichogamy) and 2) the spatial separation of the stigma and anthers (herkogamy), which prevents self-pollen from arriving to the stigma (either autonomously or mediated by pollinators) (Lloyd and Webb, 1986; Webb and Lloyd, 1986; Dai and Galloway, 2011).

From a conservation perspective, there is an urgent need to understand more about the reproductive dependency of endangered plant species on pollen availability (Wilcock and Neiland, 2002; Fernandez et al., 2012), particularly of outcross-pollen (Gonzalez-Varo and Traveset, 2010; Gélvez-Zúñiga et al., 2018). The first step in achieving this understanding is to elucidate their breeding system (Castro et al., 2008). In this study, we investigated the breeding system of *Myrcianthes coquimbensis* (Myrtaceae), a rare and endangered shrub endemic to a narrow strip of the Atacama Desert in Chile (García-Guzmán et al., 2012). Three lines of evidence suggest that seed production in *M. coquimbensis* is limited by pollen availability. First, this species exhibits one of the lowest visitation rates per flower of the plant community (3.6×10^{-3} visits / flower / min; 0.6 % of the community total) (Cortés, 2010). Second, it blooms during the late spring and early austral summer; that is, in the beginning of the driest period of the year (Peña, 2016), when all other plant species in the community are no longer flowering (Cortés, 2010). In harsh environments, pollinator activity often decreases towards the end of the blooming season (Arroyo et al., 1985; Totland, 1994). Third, individual plants have large floral displays; although this trait can attract more pollinators (e.g., Galloway et al., 2002), insects visiting plants with large floral displays are more likely to probe more flowers in sequence, but ultimately probe only a small proportion of the available flowers (Mitchell et al., 2004). Seed production in this species could also be restricted by sexual interference of self-pollen deposited by pollinators because its brush-type flower (characteristic of Myrtaceae) may facilitate self-pollination (Webb and Lloyd, 1986). Moreover, although fully developed flowers are herkogamous (i.e., the stigma is longer than the stamens), the distance between anthers and stigma is small in relation to pollinator movements, which leads to frequent contact between them in an unordered sequence (i.e., unordered herkogamy) (Webb and Lloyd, 1986). As a result, only dichogamy can restrict facilitated self-pollination, particularly if the stigma is receptive before pollen is shed from the anthers (i.e., protogyny) (Lloyd and Webb, 1986). In light of all this, in this study we address two specific questions: 1) Is *M. coquimbensis* able to

reproduce by modes other than outcrossing? and 2) is the sexual function within a flower segregated during its lifespan?

2. Materials and methods

2.1. Study species

Myrcianthes coquimbensis (Barnéoud) Landrum and Grifo is an evergreen shrub narrowly distributed along a coastal fringe of < 100 km² in the Atacama Desert (García-Guzmán et al., 2012). This species has hermaphrodite flowers that have one pistil surrounded by over fifty stamens. The ovary has two locules, each of which contains five to eleven ovules (Landrum and Grifo, 1988; Arancio et al., 2001). In contrast to co-occurring shrubs, flowers of *M. coquimbensis* are presented in large displays (> 100 flowers per shrub). The flowering season extends from October until February and peaks in November. During this period, flowers are visited by 17 species of insects (Cortés, 2010). Fruits are fleshy berries that develop over eight months and that typically contain a single, recalcitrant seed (Loayza et al., 2015). *M. coquimbensis* is classified as endangered due to habitat loss by urbanization (MINSEGPRES, 2008; García-Guzmán et al., 2012).

2.2. Study sites

We conducted this study in two localities within the distribution range of *M. coquimbensis* separated by approximately 50 km: Totoralillo (30°04'S - 71°22' W) and Juan Soldado (29°39'S - 71°18'W). In Totoralillo, *M. coquimbensis* grows up to 500 m inland from the coastline and is associated to rocks; in Juan Soldado, this species also grows associated to rocks, but within a ravine that extends five kilometers inland from the coastline. Mean annual temperatures in both sites range between 15 ± 0.5 °C and 14 ± 0.4 °C, and mean accumulated annual precipitation is 78 ± 45 mm and 57 ± 56 mm for Totoralillo and Juan Soldado, respectively (16 year means (CEAZAMET, 2019)). However, because *M. coquimbensis* blooms at the end of the spring and during the summer months, there is no rainfall during the flowering season.

2.3. Reproductive modes

We experimentally assessed the modes of reproduction of *M. coquimbensis* using standard protocols (Kearns and Inouye, 1993) during two flowering seasons: in December 2012 in Totoralillo (hereafter Totoralillo) and in Juan Soldado during January 2015 (hereafter Juan Soldado). On each occasion, we performed five floral treatments. First, to determine natural pollination levels, we randomly selected and marked buds and designated them as control flowers (C). Second, to determine outcrossing (O), we emasculated developing flowers (stage [2], see next section), hand pollinated them with pollen from plants at least 10 m away and then excluded each individual flower with a mesh bag (mesh size 0.3 mm). Third, to test for self-compatibility (S), we followed the same procedure described above, but pollinated flowers with pollen from the same plant. Fourth, to determine whether plants were able to autonomously self-pollinate (i.e., autogamy; AS), we excluded individual buds with mesh bags before they opened. Finally, to test for the ability to produce embryos without pollen (agamospermy; A), we emasculated individual flowers at the bud stage and then excluded them with mesh bags. Because we found evidence of agamospermy in Totoralillo during the 2012 flowering season, we added a new experimental treatment during the 2015 season in Juan Soldado, which consisted of carefully emasculating the flower at the bud stage, removing its style and excluding the bud with a mesh bag. Style removal, prevents casual arrival of pollen to the stigma; thus, seed development from this treatment would constitute additional evidence of autonomous agamospermy (AA; i.e., the ability to produce embryos and endosperm without pollen). Each flower treatment was applied to one flower within a single plant (N = 52 Totoralillo, N = 61 Juan Soldado).

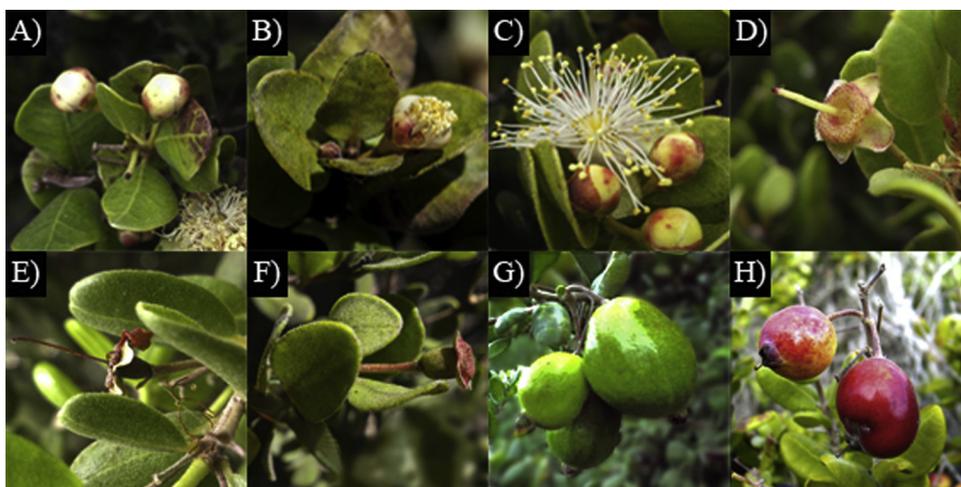


Fig. 1. Phenological stages of *Myrcianthes coquimbensis*. Flower stages: A) buds [stage 0], B) developing flower [stage 1], C) full open flower [stage 2], D) mature flower [stage 3], E) non-pollinated (dead) flower. Fruit stages: F) developing fruit, G) green fruits, and H) ripe fruits. Stage numbers, used for floral treatments, are indicated within square brackets. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

plants).

We first monitored flowers between four and six weeks after conducting the experimental procedures (i.e., in January 2013 in Totoralillo and in February 2015 in Juan Soldado); during this monitoring period we considered dead flowers (Fig. 1E, dried ovary) as evidence of reproductive failure and developing fruits (Fig. 1F, green and swollen ovary) as evidence of reproductive success. We considered developing fruits, instead of ripe fruits, as a more accurate indicator of reproductive success, because throughout their development (which expands for ca. 8 months) a high percentage of fruits abort, particularly in dry years (Peña, 2016); therefore, many fruits in their initial stage abort due to water shortage. Furthermore, given that partenocarpy (i.e., fruit production without seeds) has never been observed in this species, nor reported in other Neotropical Myrtaceae (Picarella and Mazzucato, 2019), it is unlikely that developing fruits are simply the result of ovary enlargement.

Developing fruits recorded during the first census were kept within mesh bags and monitored a second time in June 2013 in Totoralillo, and in June 2015 in Juan Soldado to determine the probability of fruit maturation (Fig. 1F) and the number of seeds per fruit. Finally, the percentage of aborted fruits was calculated for all treatments as the difference between the percentage of ripe and developing fruits in relation to the percentage of developing fruits.

To determine levels of self-compatibility and autogamy in *M. coquimbensis*, we calculated indices of self-compatibility (ISI) and autogamy (IAS), respectively (modified from Ruiz Zapata and Arroyo, 1978). The ISI index was calculated using developing fruits (ISI_{fruits}) and the number of seeds (ISI_{seeds}) per fruit as: $ISI = fS/fO$, where fS and fO are the percentage of fruits or the number of seeds per fruit formed by selfing and outcrossing, respectively. The IAS index was calculated using developing (IAS_{Dfruits}) and ripe (IAS_{Rfruits}) fruits as: $IAS = fAS/fS$, where fAS and fS are the percentage of developing or ripe fruits formed by autogamy and selfing, respectively. Both indices range from 0 to 1: ISI values close to 1 indicate self-compatibility, values ranging between 0.2 and 1 indicate incomplete self-compatibility, and values between 0 and 0.2 indicate self-incompatibility; in turn, an IAS value of 1 represents a fully autogamous plant, whereas values greater than 0 indicate partial autogamy (Ruiz Zapata and Arroyo, 1978).

2.4. Timing of the sexual functions within a flower

To determine whether sexual functions in *M. coquimbensis* are temporally segregated (dichogamy), we examined stigma receptivity and pollen viability at different times during a flower's lifespan. We calculated on which days to manually pollinate emasculated buds or flowers, by first determining the duration of each stage. In December 2012, we defined four floral stages based on the development of the

pistil, stamens and petals (Fig. 1A – D): buds [0]; developing flowers with partially elongated petals and stamens, but closed anthers [1]; open flowers with a fully elongated pistil and petals, and dehiscent anthers [2]; flowers without petals and stamens [3]. We then marked a single branch in each of 25 plants and daily monitored the transition of all flowers from stage [0] to stage [3].

2.4.1. Stigma receptivity

We determined whether stigmas were receptive via manual pollination of flowers in stages [0] through [3]. In December 2013, we selected 15 plants in Totoralillo and marked, emasculated and bagged four buds per plant. On the first day, we selected one of these emasculated buds (stage [0]) and pollinated it with pollen from two to three plants located at a distance of at least 10 m; following pollination, the bud was immediately bagged. We repeated this procedure on the following days with each of the remaining emasculated buds as they successively entered the following floral stage (i.e., [1] to [3]), which was calculated based on the previously determined floral schedule. Once they were manually pollinated, bud/flowers remained bagged for two days and then collected and stored in a formaldehyde, acetic acid and ethanol solution (FAA) (Dafni, 1992). To determine whether stigmas from collected buds/flowers were receptive at the time of manual pollination, their pistils were digested overnight in an 8 N solution of NaOH at room temperature, rinsed three times with distilled water and dyed with 0.2 % aniline blue diammonium salt (SIGMA) diluted in a 0.1 M phosphate buffer (Dafni, 1992; Shivanna and Tandon, 2014). Finally, dyed pistils were mounted and observed under a fluorescence microscope (Leica DM2500) with an ultraviolet filter (excitation: 320–380 nm, emission: 425 nm) and the number of germinated pollen grains (i.e., those with pollen tubes attached; hereafter pollen tubes) on each stigma was counted.

2.4.2. Pollen viability

To determine whether the percent of viable pollen varied during the days when anthers were dehiscent (stage [2]), in December 2013 we selected 17 plants in Totoralillo and marked and bagged three buds (stage [0]) per plant. On the day anthers started to desiccate, we began collecting a few stamens from each flower and deposited a sample of their pollen in Eppendorf tubes filled with 1 ml of 2,3,5-trifенил-2H-tetrazolium chloride (Tetrazolium hereafter), which dyes red the pollen's cytoplasm in the presence of dehydrogenase enzymes (Vieitez, 1952). After stamens were collected, flowers were once again bagged and the procedure was repeated daily until anthers had released all their pollen (typically occurring by the fourth day). Pollen samples were transported in a warm case (40 °C) to the laboratory for analysis. In the laboratory, samples were re-suspended and a drop of each suspension was deposited on a slide. Then, we recorded the total number

of pollen grains, as well as the number of dyed (i.e., viable) grains per sample using a light microscope (100X magnification). We observed five drops per sample, summed the counts and calculated the proportion of viable grains.

2.5. Statistical analyses

To assess 1) the effects of the flower treatments on fruit and seed formation and 2) differences in stigma receptivity among floral stages, we fitted generalized linear mixed models (GLMMs) (Bolker et al., 2009). For these analyses, floral treatments and floral stages were used as fixed factors and individual plants as the random factor. We used binomial error distributions and logit link functions for the fruit and seed formation response variables (success and failure) and a negative binomial error distribution with a log link function for counts of pollen tubes. To determine if the ISI and IAS indices were statistically different from 1, we used the respective comparisons between treatments (O v/s S and S v/s AS) from the GLMM's post hoc test (see below). Daily variation in the percent of viable pollen was examined by fitting a general linear model where sampling day was used as the fixed factor, and the percent data was square root arcsine transformed before the analysis.

The statistical inference used for the fixed factors was a Likelihood ratio test (LRT, χ^2) with single term deletions in the pairwise comparisons. To assess the significance of the random factor (i.e., each individual plant), we used the corrected Akaike's Information Criteria (AICc) to compare the full model (with the random variable) with a reduced model in which the random variable was removed. The random factor was considered relevant when the full model had a lower AICc than the reduced model and $\Delta AICc > 2$ (Burnham and Anderson, 2002). Multiple comparisons among levels of each fixed factor were assessed with Tukey post hoc tests. Statistical analyses were performed using the R statistical environment (Crawley, 2012; R Development Core Team, 2016).

3. Results

3.1. Reproductive modes

Developing *M. coquimbensis* fruits were formed by all modes of reproduction assessed, including autonomous selfing and agamospermy. However, in both localities the number of developing fruits produced differed among reproductive modes and individual plants (Table 1). In almost all cases, the best fit models, where those that considered the

Table 1

Results from the generalized mixed models examining the effects of flower treatments on the percentage of developing fruits and seeds. The effect of the random variable (the individual plant) was examined by comparing the full model (with the random variable) to the reduced model (without the random variable) using differences in Akaike's Information Criteria. Values of $\Delta AICc > 2$ indicate a significant effect of the variance between plants. Subscripts in parentheses indicate the degrees of freedom.

Response variable	Fixed factor		Random factor		
	χ^2	P	AICc Reduced model	AICc Full model	$\Delta AICc$
Developing fruits					
Totoralillo	(4) 11.8	0.01	(5) = 357.6	(6) = 351.8	5.7
Juan Soldado	(5) 65.2	< 0.001	(6) = 466.1	(7) = 440.9	25.1
Seeds					
Totoralillo	(4) 12.92	0.01	(5) = 254.4	(6) = 241.9	12.5
Juan Soldado	(1) 2.3	0.128	(2) = 110.8	(3) = 112.6	1.8

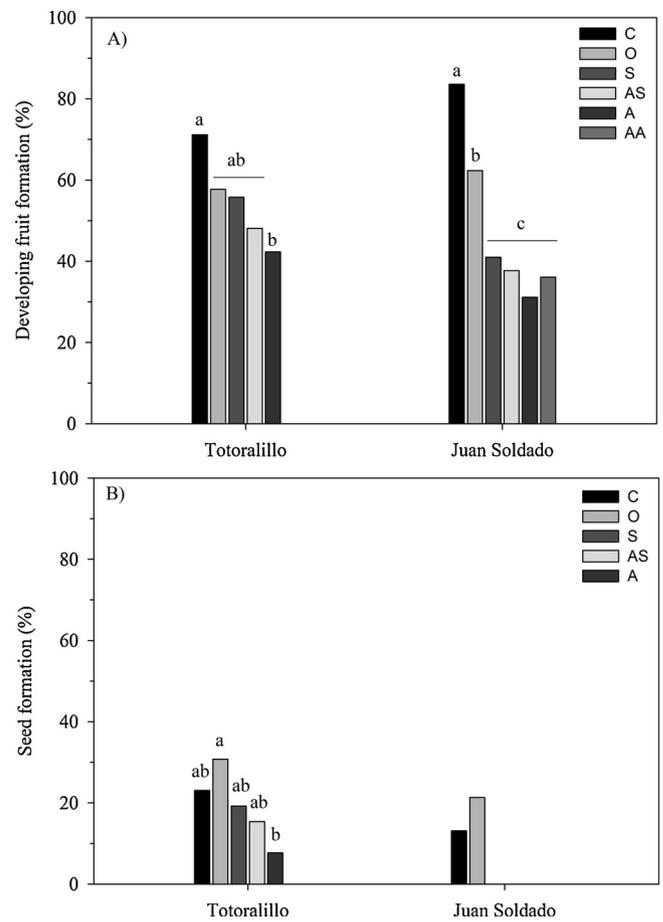


Fig. 2. Percentages of developing fruits (A) and seeds (B) (equivalent to ripe fruit formation) of *Myrcianthes coquimbensis*, formed by each mode of reproduction in Totoralillo and Juan Soldado. Percentages are relative to the total number of flowers per treatment (Totoralillo: 52; Juan Soldado: 60). Modes of reproduction: Control (C), outcrossing (O), selfing (S), autogamy (AS), agamospermy (A), and autonomous agamospermy (AA). Different lowercase letters show significant differences among treatments within a site ($P < 0.05$).

random factor (i.e., the individual plant) (Table 1). The only exception was for seed production in Juan Soldado, in which case the two models (full and reduced) were equally parsimonious ($\Delta AICc < 2$). In both localities, control flowers (i.e., those naturally pollinated; Fig. 2) produced the highest number of developing fruits. When ripe, all experimental fruits contained only one seed; therefore, the percentage of ripe fruits is equivalent to the percentage of seeds formed. In Totoralillo, the highest percentage of seed production was via outcrossing, but it did not differ significantly from the percentage of seeds produced by control, self-compatible and autogamous treatments; less than 10 % of the flowers produced seeds via agamospermy. In Juan Soldado, only outcrossed and control flowers ultimately produced seeds, with a higher percentage of seed production resulting from the former (Fig. 2). Fruits formed by outcrossing had the lowest probability of abortion in both sites (Table 2).

The ISI_{fruits} differed between study sites: in Totoralillo an $ISI_{fruits} = 0.9$ coupled with non-significant differences in fruit production between the self-compatible and outcross treatments, reveals complete self-compatibility; in contrast, in Juan Soldado an $ISI_{fruits} = 0.7$ coupled with significant differences between the self-compatible and outcross treatments, indicates only partial self-compatibility. In Totoralillo, $ISI_{seeds} = 0.6$ and was coupled with non-significant differences between the self-compatible and outcross treatments. The $IAS_{Dfruits}$ was equal to 0.9 in both sites and, coupled with non-significant differences between the self-compatible and autogamous treatments, indicates that selfing

Table 2
Percentage of abortions of developing fruits recorded from each of the reproductive modes assessed in both localities.

Reproductive mode	Fruit abortion (%)	
	Totoralillo	Juan Soldado
Control	67	84.3
Outcrossing	47	66
Selfing	66	100
Autogamy	68	100
Agamospermy	82	100
Autonomous agamospermy	–	100

can be carried out autonomously within a flower. The $IAS_{Rfruits}$ had value of 0.8 and was also coupled with non-significant differences between the self-compatible and autogamous treatments.

3.2. Timing of the sexual functions within a flower

The flowers of *M. coquimbensis* had a mean lifespan of 9.3 ± 3 s.d. days ($N = 167$; from the day a bud opened until the beginning of stage [4], when no stamens or petals remained. The majority of the anthers started to release pollen around the third day (stage [2]) and lasted until approximately the sixth day (Table 3).

The mean number of pollen tubes present per stigma (Fig. 3A) differed among flower stages and was better explained by the model that included the random factor (Table 3). Stigmas in the bud [0] and developing flower [1] stages had a very low number of pollen tubes; these increased in stage [2] and reached their maximum in stage [3] (Fig. 4B). The percent of viable pollen (Fig. 3B) also differed among days (Table 4); in general, pollen viability decreased as the days progressed, from a mean of 90 % of viable pollen on the first day to 24 % on the fourth day (Fig. 4B). These results reveal that during stage [2], which lasted approximately four days (Table 3), there is a temporal coincidence of the sexual functions within a flower. Nonetheless, pistil receptivity significantly increased once pollen viability began to decline (stage [2]) and disappear from anthers (stage [3]).

4. Discussion

Here we show that *M. coquimbensis* produces fruits and seeds by multiple modes of reproduction, including modes that not require pollinators; seeds, however, are produced mainly by outcrossing. We also observed a partial segregation of sexual functions within the flower, with the peak of pollen viability (male phase) occurring before the peak of stigma receptivity (female phase).

The high ISI_{fruits} values reported here are comparable to those reported in other Neotropical Myrtaceae (Lughadha and Proença, 1996; Arroyo and Humaña, 1999); likewise, ISI_{seeds} values were similar to those of *Eugenia uniciflora* ($ISI = 0.4$) and *E. punicifolia* ($ISI = 0.6$), a genus closely related to *Myrcianthes* (da Silva and Pinheiro, 2009). Values of the IAS index suggest that *M. coquimbensis* can produce seeds

Table 3

Mean \pm standard deviation (std) of flower life span (days) and estimated day of occurrence (from anthesis) of each flower stage of *Myrcianthes coquimbensis* ($N = 167$ flowers).

Floral stages	flower life span (days)		Day of occurrence
	mean	\pm std.	
0	–	–	1
1	1.5	0.9	1
2	3.7	1.4	3
3	4.0	2.4	7
4	–	–	11

via autonomous selfing and are similar to those of the *Eugenia* species mentioned above (Proença and Gibbs, 1994; da Silva and Pinheiro, 2009). We caution, however, to interpret with care the autogamy capacity of *M. coquimbensis* given by the IAS index (AS/S) because it may have been overestimated. This is because manual pollinations were conducted when stigma receptivity was not at its peak. Thus, fruit and seed production by manual selfing may have been under estimated, leading in turn to an overestimation of the IAS index.

Evidence of autonomous selfing in *M. coquimbensis* suggests that this species may ensure reproduction when outcross pollination fails; however, three conditions must be met for this to occur (Lloyd, 1992). First, selfing should boost seed production when outcrossing fails (i.e., reproductive assurance) (Eckert and Herlihy, 2004). In this study, we were unable to estimate values of reproductive assurance (RA) because it is necessary to compare fruit or seed production from emasculated open flowers (which lack the capacity to autonomously self) to open flowers (Eckert et al., 2006, 2010). For *M. coquimbensis*, however, this method can lead to a biased estimation of RA because removing large quantities of stamens from the flowers strongly reduces their attractiveness to pollinators (especially those looking for pollen) (Eckert et al., 2010). Nonetheless, we can approximate a value of RA by comparing seed production by autonomous selfing to seed production in control flowers. In Totoralillo, the difference in seed production between these two treatments was only 8 % (being lower for autonomously selfed flowers). This suggests that autogamy may indeed boost seed production in a scenario where pollinators are absent.

Second, progeny resulting from autogamy should have no or low inbreeding depression (ID) (Kalisz et al., 2004). Although we did not formally assess ID in this study, abortion of all the developing fruits produced by both selfing treatments in Juan Soldado, and a lower percentage (~ 20 %) of aborted fruits resulting from outcrossing in comparison to selfing and natural pollination in Totoralillo, suggest that ID in *M. coquimbensis* may express itself during the seed development stage. There are few reports of early-acting ID in Myrtaceae (Pound et al., 2003; Gonzalez-Varo and Traveset, 2010), however, some studies suggest ID as a factor in the significant reduction of fruit and seed set in this family (Kennington and James, 1997; Yates et al., 2007), which may partially result from the abortion of selfed fruits. Because angiosperms invest maternal resources into seed development following fertilization (Obeso, 2002), and *M. coquimbensis* inhabits an extremely water- and nutrient-poor environment, it is also possible that ID is expressed as outcross embryos having a higher competitive ability for the limited resources compared to self-produced ones (Korbecka et al., 2002).

The third condition for RA by autonomous selfing is that it must occur when outcrossing is no longer possible (i.e., delayed selfing) (Kalisz et al., 1999), thus preventing ovule discounting (Lloyd and Schoen, 1992). For *M. coquimbensis*, we suspect that self-pollination may occur during stage [1] (Fig. 1B and C), when some dehiscent anthers have contact with the slightly receptive stigma, and before all stamens are extended and anthers dehiscent. During stage [2] (when pollen is abundant), anther contact with the stigma is unlikely because of herkogamy and the probability of contact decreases even more in later stages because “old” stamens fall away from the pistil. Therefore, autonomous selfing likely occurs prior to, or at most, parallel with, outcrossing (i.e., prior and competing selfing, respectively), and not after this period. Moreover, for delayed autonomous selfing to occur, flowers should exhibit incomplete protogyny (Goodwillie and Weber, 2018). Nonetheless, considering jointly levels of pollen viability and stigma receptivity, the pattern of sexual functions within *M. coquimbensis*’ flowers indicate incomplete protandry, which is the common form of dichogamy in the Myrtoideae subfamily (Lughadha and Proença, 1996). Consequently, unless outcross pollen has higher growth rates than self-pollen in the style (i.e., pollen prepotency) (Lloyd and Schoen, 1992), in *M. coquimbensis* autonomous selfing probably results in ovule discounting (i.e., unavailable for cross-fertilization). In

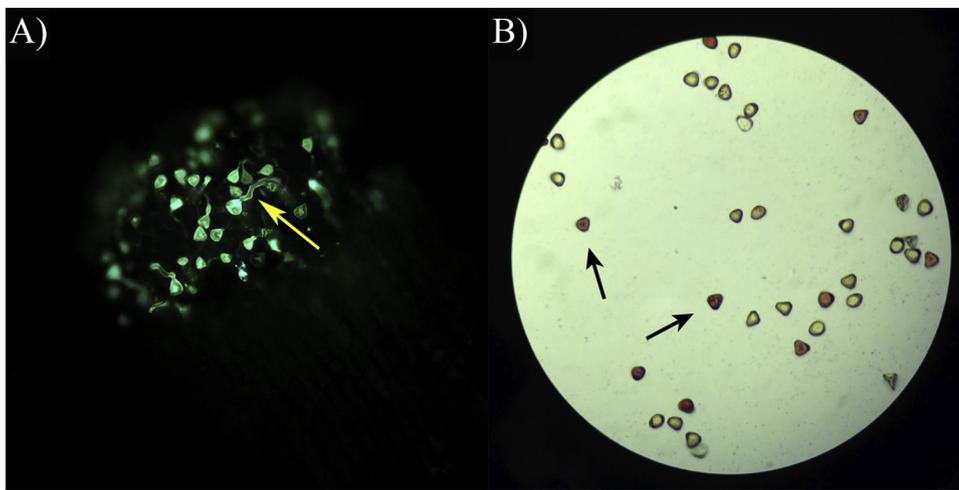


Fig. 3. (A) Fluorescent germinated pollen with pollen tubes attached (indicated by the arrow), observed on the stigma of a flower of *Myrcianthes coquimbensis* (100X). (B) Viable (red) and non-viable pollen of *M. coquimbensis* (100X) (indicated by arrows). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

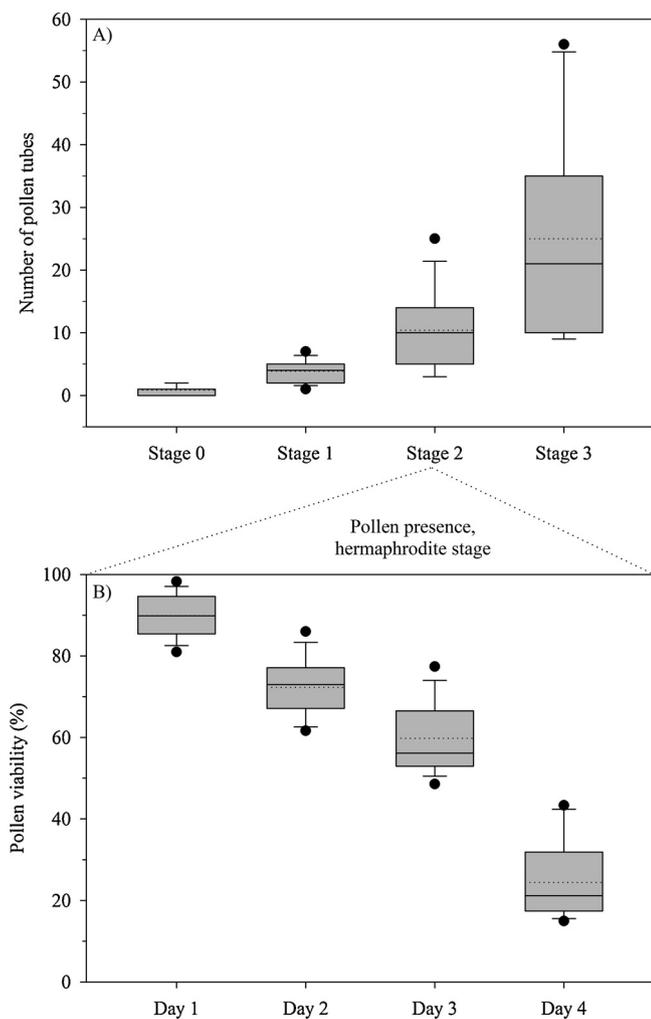


Fig. 4. A) Number of pollen tubes observed in the stigma of flowers at different stages. B) Percentage of viable pollen observed throughout a stamens' life span [stage 2] in a flower of *Myrcianthes coquimbensis*. There were significant differences in the number of pollen tubes among all the stages, and also in pollen viability for all days considered ($P < 0.05$).

summary, even though autonomous selfing in this species may boost seed production, the costs imposed by this reproductive mode (ID and ovule discounting) suggest it does not ensure reproduction when outcross pollination fails.

Our results suggest that facilitated selfing (Lloyd and Schoen, 1992) is an inevitable mode of selfing in *M. coquimbensis*. This is because incomplete protandry is unlikely to prevent pollinators from self-pollinating flowers (Lloyd and Webb, 1986). Facilitative selfing can negatively impact seed production via ovule discounting and sexual interference (Owen et al., 2007). The incidence and intensity of ovule discounting due to facilitated selfing may be low if pollinators deposit a mix of outcross and self-pollen on the stigmas (Wilcock and Neiland, 2002). In this scenario, having multiple ovules within a flower, such as the case of *M. coquimbensis* (Landrum and Grifo, 1988), can increase the chances of outcross fecundation (Rosenheim et al., 2016). Because this species is self-compatible, sexual interference can occur when pollinators deposit low viability pollen grains (Wilcock and Neiland, 2002), which can occur on the final days of stage [2]. Sexual interference can also occur if viable self-pollen is deposited on self-incompatible plants (Galen et al., 1989). This scenario may occur in the Juan Soldado population where plants had the highest self-incompatibility levels. In either case, sexual interference can result in a clogged stigma, and ultimately in the “loss” of a flower that would otherwise be available for outcross pollination (Barrett, 2002).

The formation of fruits and seeds by autonomous agamospermy was an unexpected result in this study. In Myrtaceae, agamospermy has been reported in *Syzygium jambos* and *S. cumini* (Lughadha and Proenca, 1996) and in the genus *Callistemon* (Hojsgaard et al., 2014), however, in all these species, seeds were formed by adventitious embryony, a type of agamospermy that requires pollination (selfing or outcrossing) to form a sexual embryo, which develops in parallel to an asexual embryo (from somatic cells) (Whitton et al., 2008; but see Mangla et al., 2015). Hojsgaard et al. (2014) reported gametophytic agamospermy by apospory for the *Syzygium* species mentioned above, but no details are given on whether it is autonomous or pseudogamous (i.e., when pollen is needed to form the endosperm). In our study, 36 % of the flowers with their style removed produced developing fruits, thus we discard the possibility of accidental pollination of bagged flowers. To our knowledge, this is the first study to provide empirical evidence of seed formation from autonomous agamospermy in Myrtaceae.

Reproductive assurance provided by agamospermy is not necessarily analogous to RA provided by selfing, because autonomous agamospermy can occur in parallel with outcrossing at three different scales: 1) at the population level, with populations composed only by outcrossed or by obligate agospermic individuals (Bierzychudek, 1990; Mráz et al., 2019); 2) at the individual level (within population), with plants being agospermic or outcrossed (Mangla et al., 2015); 3) within individuals, with flowers capable of reproducing by agamospermy and outcrossing (Whitton et al., 2008). In our study, we observed agamospermy alongside other reproductive modes within individuals,

Table 4

Results from the generalized mixed model (GLMM) examining the effect of manual pollination on the number of pollen tubes and from the linear model (LM) examining the daily change in the percent of viable pollen. In the GLMM, the effect of the random variable was examined by comparing the full model (with the random variable) to the reduced model (without the random variable) using differences in Akaike's Information Criteria. Values of $\Delta\text{AICc} > 2$ indicate a significant effect of the variance between plants. Subscripts in parentheses indicate the degrees of freedom.

Response variable	Fixed factors		Random factor					
	Manual pollination	Days	Individual plant			AICc Reduced model	AICc Full model	ΔAICc
Pollen tubes	χ^2 (GLMM) (3) 243.58	<i>P</i> < 0.001	F (LM)	<i>P</i>		(8) = 324.2	(9) = 319.6	4.6
Pollen viability	–	–	(3, 64) 190.3	< 0.001		–	–	–

however, the four plants that produced seeds by agamospermy, did so only via this reproductive mode (i.e., agamospermy at the individual level; see Table A.1 in the Appendix). Therefore, if sexual plants fail to reproduce due to pollinator scarcity, obligate and/or agamospermy facultative plants may boost seed production (Mangla et al., 2015). Finally, although agamospermy may provide RA in *M. coquimbensis*, fruit abortion was 35 % higher in fruits resulting from agamospermy relative to those resulting from outcrossing (Table 2). Abortion of agamospermic fruits and seeds may result from the expression of deleterious mutations (Hojsgaard and Hörandl, 2015; Hodac et al., 2019). However, in agamospermy facultative species, such as *M. coquimbensis*, the mutation load should be low because mutations are purged during the development of the gametophyte (pre-fecundation) (Hodac et al., 2019). Fruit abortions in our system likely result from resource limitation because approximately a half of the plants that aborted developing fruits from A and AA treatments, also aborted the developing fruits resulting from outcrossing (Totalillo: 39 % A; Juan Soldado: 47 % A, 50 % AA; see Tables A.1 and A.2 in the Appendix).

5. Conclusions

In this study we provide empirical evidence that *M. coquimbensis* can reproduce via at least three reproductive modes. Selfing is almost unavoidable in this species, and very likely interferes with outcrossing. Coupled with possible early inbreeding depression, selfing probably results in a cost for seed production. Our results suppose a vulnerability of this endangered species in scenarios where pollinators are scarce. However, agamospermy may provide an alternative route of seed production in these scenarios, but given that seed production by this reproductive mode was observed only in one site and in low frequency, further studies should explore its incidence along *M. coquimbensis* distribution range and the fitness of the resulting seeds.

CRedit authorship contribution statement

Patricio García-Guzmán: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Visualization. **Andrea P. Loayza:** Methodology, Writing - review & editing, Funding acquisition. **Francisco A. Squeo:** Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Danny Carvajal and Rodrigo Rios for their valuable comments on earlier versions of this manuscript. We also thank Paloma Gachón and Julio Caballero for helping with field experiments, and Rodrigo Vasquez (CEAZA) who aided with the fluorescence microscope. This work was supported by the Rufford Small Grant Foundation [9531-

2] and the Instituto de Ecología y Biodiversidad (IEB) [Project CONICYT PIA support CTE AFB170008]. PGG received a PhD Fellowship from CONICYT [21120854].

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.flora.2020.151537>.

References

- Arancio, G., Muñoz, M., Squeo, F.A., 2001. Descripción de algunas especies con problemas de conservación en la IV región de Coquimbo, Chile. In: Squeo, F.A., Arancio, G., Gutiérrez, J.R. (Eds.), Libro Rojo de Flora Nativa y de los Sitios Prioritarios para su Conservación: Región de Coquimbo. Ediciones Universidad de La Serena: La Serena, pp. 63–103.
- Arroyo, M.T.K., Armesto, J.J., Primack, R.B., 1985. Community studies in pollination ecology in the high temperate Andes of central Chile II. Effect of temperature on visitation rates and pollination possibilities. *Plant Syst. Evol.* 149, 187–203.
- Arroyo, M.T.K., Humaña, A.M., 1999. Breeding systems of two endemic rainforest species in Southern Chile: *Amomyrtus meli* (Phil.) Legr. et Kaus (Myrtaceae) y *Luzuriaga polyphylla* (Hook.) Macbr. (Phylsaceae). *Gayana Bot.* 56, 31–37.
- Barrett, S.C.H., 2002. Sexual interference of the floral kind. *Heredity* 88, 154–159.
- Barrett, S.C.H., 2003. Mating strategies in flowering plants: the outcrossing-selfing paradigm and beyond. *Philos. Trans. R. Soc. L. B Biol Sci* 358, 991–1004.
- Barrett, S.C.H., 2014. Evolution of mating systems: outcrossing versus selfing. In: Losos, J. (Ed.), *The Princeton Guide to Evolution*. Princeton University Press, pp. 356–362.
- Bellanger, S., Guillemin, J.-P., Touzeau, S., Darmency, H., 2015. Variation of inbreeding depression in *Centaurea cyanus* L., a self-incompatible species. *Flora - Morphol. Distrib. Funct. Ecol. Plants* 212, 24–29.
- Bierzychudek, P., 1987. Pollinators increase the cost of sex by avoiding female flowers. *Ecology* 68, 444–447.
- Bierzychudek, P., 1990. Demographic consequences of sexuality and apomixis in *Antennaria*. In: Kawano, S. (Ed.), *Biological Approaches and Evolutionary Trends in Plants*. Academic Press, London, pp. 293–307.
- Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., White, J.-S.S., 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.* 24, 127–135.
- Burnham, K.P., Anderson, D.R., 2002. *Model Selection and Multimodel Inference: a Practical Information-theoretic Approach*. Springer-Verlag, New York.
- Busch, J.W., Schoen, D.J., 2008. The evolution of self-incompatibility when mates are limiting. *Trends Plant Sci.* 13, 128–136.
- Castro, S., Silveira, P., Navarro, L., 2008. How flower biology and breeding system affect the reproductive success of the narrow endemic *Polygala vayredae* Costa (Polygalaceae). *Bot. J. Linn. Soc.* 157, 67–81.
- CEAZAMET, 2019. Red de estaciones meteorológicas. Available URL: <http://www.ceazamet.cl/>.
- Cortés, F., 2010. Diversidad y estructura de las asociaciones planta-polinizador del sitio prioritario Punta Teatinos – Juan Soldado, región de Coquimbo. Universidad de La Serena, Chile Unpublished Master Thesis.
- Crawley, M.J., 2012. *The R Book*. John Wiley & Sons Ltd, Chichester.
- da Silva, A.L.G., Pinheiro, M.C.B., 2009. Reproductive success of four species of *Eugenia* L. (Myrtaceae). *Acta Bot. Brasiliica* 23, 526–534.
- Dafni, A., 1992. *Pollination Ecology: a Practical Approach*. Oxford University Press Inc, New York.
- Dai, C., Galloway, L.F., 2011. Do dichogamy and herkogamy reduce sexual interference in a self-incompatible species? *Funct. Ecol.* 25, 271–278.
- Davidson, J.B., Durham, S.L., Wolf, P.G., 2014. Breeding system of the threatened endemic *Primula cusickiana* var. *maguirei* (Primulaceae). *Plant Spec. Biol.* 29, E55–E63.
- Eckert, C.G., Herlihy, C.R., 2004. Using a cost-benefit approach to understand the evolution of self-fertilization in plants: the perplexing case of *Aquilegia canadensis* (Ranunculaceae). *Plant Spec. Biol.* 19, 159–173.
- Eckert, C.G., Samis, K.E., Dart, S., 2006. Reproductive assurance and the evolution of uniparental reproduction in flowering plants. In: Harder, L.D., Barrett, S.C.H. (Eds.), *The Ecology and Evolution of Flowers*. Oxford University Press, Oxford, pp. 183–203.
- Eckert, C.G., Kalisz, S., Geber, M.A., Sargent, R., Elle, E., Cheptou, P.O., Goodwillie, C.,

- Johnston, M.O., Kelly, J.K., Moeller, D.A., Porcher, E., Ree, R.H., Vallejo-Marin, M., Winn, A.A., 2010. Plant mating systems in a changing world. *Trends Ecol. Evol.* 25, 35–43.
- Fausto Jr., J.A., Eckhart, V.M., Geber, M.A., 2001. Reproductive assurance and the evolutionary ecology of self-pollination in *Clarkia xantiana* (Onagraceae). *Am. J. Bot.* 88, 1794–1800.
- Fernandez, J.D., Bosch, J., Nieto-Ariza, B., Gomez, J.M., 2012. Pollen limitation in a narrow endemic plant: geographical variation and driving factors. *Oecologia* 170, 421–431.
- Galen, C., Gregory, T., Galloway, L.F., 1989. Costs of self-pollination in a self-incompatible plant, *Polemonium viscosum*. *Am. J. Bot.* 76, 1675–1680.
- Galloway, L.F., Cirigliano, T., Gremski, K., 2002. The contribution of display size and dichogamy to potential geitonogamy in *Campanula americana*. *Int. J. Plant Sci.* 163, 133–139.
- García-Guzmán, P., Loayza, A.P., Carvajal, D.E., Letelier, L., Squeo, F.A., 2012. The ecology, distribution and conservation status of *Myrcianthes coquimbensis*: a globally endangered endemic shrub of the Chilean Coastal Desert. *Plant Ecol. Divers.* 5, 197–204.
- Gélvez-Zúñiga, I., Neves, A.C., Teixido, A.L., Fernandes, G.W., 2018. Reproductive biology and floral visitors of *Collaea cipoensis* (Fabaceae), an endemic shrub of the rupestrian grasslands. *Flora* 238, 129–137.
- Goodwillie, C., Kalisz, S., Eckert, C.G., 2005. The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annu. Rev. Ecol. Syst.* 36, 47–79.
- Goodwillie, C., Weber, J.J., 2018. The best of both worlds? A review of delayed selfing in flowering plants. *Am. J. Bot.* 105, 641–655.
- Gonzalez-Varo, J.P., Traveset, A., 2010. Among-individual variation in pollen limitation and inbreeding depression in a mixed-mating shrub. *Ann. Bot.* 106, 999–1008.
- Herlihy, C.R., Eckert, C.G., 2002. Genetic cost of reproductive assurance in a self-fertilizing plant. *Nature* 416, 320–323.
- Hiscock, S.J., McInnis, S.M., 2003. The diversity of self-incompatibility systems in flowering plants. *Plant Biol.* 5, 23–32.
- Hodac, L., Klatt, S., Hojsgaard, D., Sharbel, T., Hörandl, E., 2019. A little bit of sex prevents mutation accumulation even in apomictic polyploid plants. *BMC Evol. Biol.* 19.
- Hojsgaard, D., Klatt, S., Baier, R., Carman, J.G., Hörandl, E., 2014. Taxonomy and biogeography of apomixis in angiosperms and associated biodiversity characteristics. *Crit. Rev. Plant Sci.* 33, 414–427.
- Hojsgaard, D., Hörandl, E., 2015. A little bit of sex matters for genome evolution in asexual plants. *Front. Plant Sci.* 6, 82.
- Hörandl, E., Hojsgaard, D., 2012. The evolution of apomixis in angiosperms: a re-appraisal. *Plant Biosyst.* 146, 681–693.
- Husband, B.C., Schemske, D.W., 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* 50, 54–70.
- Johnson, S.D., Steiner, K.E., 2000. Generalization versus specialization in plant pollination systems. *Trends Ecol. Evol.* 15, 140–143.
- Kalisz, S., Vogler, D., Fails, B., Finer, M., Shepard, E., Herman, T., Gonzales, R., 1999. The mechanism of delayed selfing in *Collinsia verna* (Scrophulariaceae). *Am. J. Bot.* 86, 1239–1247.
- Kalisz, S., Vogler, D.W., 2003. Benefits of autonomous selfing under unpredictable pollinator environments. *Ecology* 84, 2928–2942.
- Kalisz, S., Vogler, D.W., Hanley, K.M., 2004. Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating. *Nature* 430, 884–887.
- Kearns, A., Inouye, D.W., 1993. *Techniques for Pollination Biologists*. University Press, Niwot.
- Kephart, S.R., Brown, E., Hall, J., 1999. Inbreeding depression and partial selfing: evolutionary implications of mixed-mating in a coastal endemic, *Silene douglasii* var. *oraria* (Caryophyllaceae). *Heredity* 82, 543–554.
- Kennington, W.J., James, S.H., 1997. The effect of small population size on the mating system of a rare clonal mallee, *Eucalyptus argutifolia* (Myrtaceae). *Heredity* 78, 252–260.
- Korbecka, G., Klinkhamer, P.G.L., Vrieling, K., 2002. Selective embryo abortion hypothesis revisited - A molecular approach. *Plant Biol.* 4, 298–310.
- Landrum, L.R., Grifo, F.T., 1988. *Myrcianthes* (Myrtaceae) in Chile. *Brittonia* 40, 290–293.
- Larson, B.M.H., Barrett, S.C.H., 2000. A comparative analysis of pollen limitation in flowering plants. *Biol. J. Linn. Soc.* 69, 503–520.
- Lloyd, D.G., 1992. Self-fertilization and cross-fertilization in plants. II. The selection of self-fertilization. *Int. J. Plant Sci.* 153, 370–380.
- Lloyd, D.G., Schoen, D.J., 1992. Self-fertilization and cross-fertilization in plants. I. Functional dimensions. *Int. J. Plant Sci.* 153, 358–369.
- Lloyd, D.G., Webb, C.J., 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms I. Dichogamy. *New Zeal. J. Bot.* 24, 135–162.
- Loayza, A.P., Gachon, P.R., García-Guzmán, P., Carvajal, D.E., Squeo, F.A., 2015. Germination, seedling performance, and root production after simulated partial seed predation of a threatened Atacama desert shrub. *Rev. Chil. Hist. Nat.* 88, 10.
- Lughadha, E.N., Proenca, C., 1996. A survey of the reproductive biology of the Myrtoideae (Myrtaceae). *Ann. Mo. Bot. Gard.* 83, 480–503.
- Mangla, Y., Chaudhary, M., Gupta, H., Thakur, R., Goel, S., Raina, S.N., Tandon, R., 2015. Facultative apomixis and development of fruit in a deciduous shrub with medicinal and nutritional uses. *AoB Plants* 7, plv098.
- MINSEGPRES, 2008. Decreto supremo n° 50 de 2008. Aprueba y oficializa nómina para el Segundo proceso de clasificación de especies según su estado de conservación. Ministerio Secretaría General de la Presidencia de la República. Santiago.
- Mitchell, R.J., Karron, J.D., Holmquist, K.G., Bell, J.M., 2004. The influence of *Mimulus ringens* floral display size on pollinator visitation patterns. *Funct. Ecol.* 18, 116–124.
- Mráz, P., Zdvorák, P., Hartmann, M., Štefánek, M., Chrtěk, J., 2019. Can obligate apomixis and more stable reproductive assurance explain the distributional successes of asexual triploids in *Hieracium alpinum* (Asteraceae)? *Plant Biol.* 21, 227–236.
- Moreira, M.M., Miranda, A.S., Lima, H.A., 2017. *Agarista revoluta* (Ericaceae): a generalist plant with self-compatible and self-incompatible individuals. *Flora* 234, 7–14.
- Neal, P.R., Anderson, G.J., 2005. Are “mating systems” “breeding systems” of inconsistent and confusing terminology in plant reproductive biology? Or is it the other way around? *Plant Syst. Evol.* 250, 173–185.
- Obeso, J.R., 2002. The costs of reproduction in plants. *New Phytol.* 155, 321–348.
- Owen, K., Vaughton, G., Ramsey, M., 2007. Facilitated autogamy and costs of selfing in the perennial herb *Bulbine bulbosa* (Asphodelaceae). *Int. J. Plant Sci.* 168, 579–585.
- Peña, M., 2016. Fenología reproductiva de *Myrcianthes coquimbensis* y su relación con los patrones de precipitación en todo su rango de distribución (Barrancones - Totoralillo, Región de Coquimbo, Chile). Unpublished Licenciante Thesis. Universidad de La Serena.
- Pérez, M.E., Meléndez-Ackerman, E.J., Monsegur-Rivera, O.A., 2018. Breeding system and pollination of *Gesneria pauciflora* (Gesneriaceae), a threatened Caribbean species. *Flora* 242, 8–15.
- Picarella, M.E., Mazzucato, A., 2019. The occurrence of seedlessness in higher plants; insights on roles and mechanisms of parthenocarpy. *Front. Plant Sci.* 9, 11.
- Pound, L.M., Wallwork, M.A.B., Potts, B.M., Sedgley, M., 2003. Pollen tube growth and early ovule development following self- and cross-pollination in *Eucalyptus nitens*. *Sex. Plant Reprod.* 16, 59–69.
- Proença, C.E.B., Gibbs, P.E., 1994. Reproductive biology of eight sympatric Myrtaceae from Central Brazil. *New Phytol.* 126, 343–354.
- R Development Core Team, 2016. *R: a Language and Environment for Statistical Computing, Version 3.13*. R Foundation for Statistical Computing, Vienna. <https://www.r-project.org/>.
- Richards, A.J., 2003. Apomixis in flowering plants: an overview. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 358, 1085–1093.
- Richardson, M.L., Keathley, C.P., Peterson, C.L., 2016. Breeding system of the critically endangered Lakela's Mint and influence of plant height on pollinators and seed output. *Popul. Ecol.* 58, 277–284.
- Rosenheim, J.A., Schreiber, S.J., Williams, N.M., 2016. Does an ‘oversupply’ of ovules cause pollen limitation? *New Phytol.* 210, 324–332.
- Ruiz Zapata, T., Arroyo, M.T.K., 1978. Plant reproductive ecology of a secondary deciduous tropical forest in Venezuela. *Biotropica* 10, 221–230.
- Shivanna, K.R., Tandon, R., 2014. Pollen–Pistil interaction. In: Shivanna, K.R., Tandon, R. (Eds.), *Reproductive Ecology of Flowering Plants: A Manual*. Springer, India, New Delhi, pp. 97–105.
- Totland, Ö., 1994. Intra-seasonal variation in pollination intensity and seed set in an alpine population of *Ranunculus acris* in southwestern Norway. *Ecography* 17, 159–165.
- Vieitez, E., 1952. Use of 2,3,5 - trifeniltetrazolium to determinate vitality of pollen. *Ann. Edafol. y Fisiol. Veg.* 11, 297–308.
- Vogler, D.W., Kalisz, S., 2001. Sex among the flowers: the distribution of plant mating systems. *Evolution* 55, 202–204.
- Webb, C.J., Lloyd, D.G., 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms II. Herkogamy. *New Zeal. J. Bot.* 24, 163–178.
- Whitton, J., Sears, C.J., Baack, E.J., Otto, S.P., 2008. The dynamic nature of apomixis in the angiosperms. *Int. J. Plant Sci.* 169, 169–182.
- Wilcock, C., Neiland, R., 2002. Pollination failure in plants: why it happens and when it matters. *Trends Plant Sci.* 7, 270–277.
- Yates, C.J., Elliott, C., Byrne, M., Coates, D.J., Fairman, R., 2007. Seed production, germinability and seedling growth for a bird-pollinated shrub in fragments of kwongan in south-west Australia. *Biol. Conserv.* 136, 306–314.
- Young, A.G., Broadhurst, L.M., Thrall, P.H., 2012. Non-additive effects of pollen limitation and self-incompatibility reduce plant reproductive success and population viability. *Ann. Bot.* 109, 643–653.