

Inbreeding depression, reproductive trends, and biological variation in two interrelated *Jumellea* species with differing dispersal trends and commonness

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Abstract— Genetic diversity and inbreeding can affect the long-term viability of plant populations subjected to size reductions, according to theory. In addition, a variety of life-history and ecological features can influence the response to population size reductions in different species. For the uncommon and fragmented *Jumellea fragrans* and its widely distributed congener, *J. rossii*, the reproductive patterns, levels of genetic diversity and degree of inbreeding depression were examined. Over-collection and fragmentation on *J. fragrans* were the primary goals of this study; we also wanted to learn more about their biology and ecology in order to better understand their medicinal and aromatic characteristics. For the purposes of determining the breeding system and assessing inbreeding depression, hand pollination experiments were done. Selfing rates and levels of genetic diversity were studied using nuclear microsatellite markers. During the germination stage (d 14 066) of *Jumellea rossii*, a mixed-mating system and evidence of inbreeding depression were discovered. Allele richness (AR) was 8 575 and expected heterozygosity (H_e) was 0 673. The genetic diversity was relatively high. Selfing rates in *J. fragrans* reflect an outcrossing mating mechanism. It is likely that the low levels of genetic diversity observed in this population were due to the outcrossing mating system and the comparatively high density of individuals compared to those found in *J. rossii* (AR 14 4983 and H_e 14 0492). According to researchers, *Jumellea fragrans* did not display signs of inbreeding depression. Even if the long-term viability of the *J. fragrans* population is not in jeopardy, fragmentation and over-collection have likely diminished its genetic diversity. Both in situ and ex situ conservation measures for *J. fragrans* are proposed in order to meet local demand.

Keywords— commonness, biological variation, reproductive trends, dispersal trends.

I. INTRODUCTION

In terms of plant diversity and endemism, many oceanic islands are of significant importance because of their small surface area and abundance of plant species (Bulut, et al., (2020). As a result of habitat degradation and fragmentation, oceanic islands are home to much of the world's vulnerable biodiversity. As a result of the drop in population size and the rise in geographic isolation caused by habitat degradation and fragmentation, plant quantity and density decline. According to Crispim, et al., (2021), these physical alterations are thought to impair reproduction

and gene flow as well as genetic variety, and they are expected to have a negative impact on the plant populations' ability to persist and evolve in the face of environmental change (Subaşı & Güvensen, 2021).

Aromatic, medicinal, and horticultural plants are particularly vulnerable to overharvesting, which reduces the overall population size. There is a wide variation in the extent to which unregulated harvesting affects a species' population viability (Freitas, et al., 2020), but it is regarded one source of death that directly affects the population's vital rates and may increase its extinction risk (Shekha, et

al., 2013) It's possible that populations of harvested plants are going extinct faster because of over-collection than they are because of habitat degradation alone, according to (Bauder & Karolyi, 2019). Habitat fragmentation and overcrowding have shown that species-specific responses to population declines due to habitat fragmentation and overcrowding are expected (Kendon, et al., 2017).

One of the most important factors affecting a population's vulnerability to fragmentation and over-collection is the breeding system, specifically the compatibility system (Rashid and Kendon, 2020). It is possible to breed plants through a variety of methods, including a combination of self-fertilization and cross-fertilization, as well as the use of pollinators (De & Pathak, 2018). Obligatory outbreeders, on the other hand, must have additional mates in order to reproduce. If there is no need for mates and pollen vectors, the reproduction of inbreeding species will be less dependent on the availability of these factors. The lack of mates and/or low pollinator visitation rates can cause reproductive loss in self-incompatible species, making them more sensitive to population shrinkage than self-compatible or autopolled species (Pala, et al., 2021). Furthermore, the mating system (the average proportion of cross- or self-fertilization) may influence the consequences of population size decrease on genetic diversity since it dictates the spatial distribution of genetic variation within and among populations (Gardiner, et al., 2017). By limiting the number of available mates and the diversity of the remaining population, fragmentation and individual destruction effect mating by creating a genetic bottleneck. Self-fertilization or mating between related individuals (e.g. biparental inbreeding) might restrict outcrossing options and promote inbreeding (Holyoak, et al., 2020). The expression of inbreeding depression (i.e. the relative reduction in fitness of selfed vs. outcrossed progeny) may be reduced by an increase in homozygosity and the homozygous expression of deleterious recessive alleles, resulting in a decrease in progeny fitness and survival (Bulut, et al., 2020). Consider the importance of inbreeding depression in a conservation setting because it is a major danger to the survival of rare or fragmented taxa (Hedrick and Kalinowski, 2000). A manual crossover estimate is also a good predictor of the persistence of species that have experienced population declines (Bissessur, et al., 2019). The degree to which populations are vulnerable to fragmentation and over-collection can also be influenced by ecological factors such as the initial distribution and abundance of a species, population size and density, and species lifespan (Warren, et al., 2018).

The construction of sustainable management programs is required in light of the worldwide loss of biodiversity, primarily as a result of habitat degradation and exploitation. Threatened plant species management necessitates an

understanding of their life histories and the use of many approaches, including ecological and genetic studies of plants. breeding system, sex-based mating, and inbreeding depression are among the most important components of these life-history traits because they are critical for population dynamics and long-term viability in a population with a reduced size (Rashid, 2017). Plant reintroduction and plant production in circumstances where cultivating the plant can reduce the impact on the wild population and meet the plant's demand are also of major importance from a conservation and reintroduction perspective (La Réunion, 2020). This family, which includes both ornamental and medicinal plants, has a significant economic impact, particularly in the fields of horticulture and floristry (Mohajer-Tabrizi, et al., 2020). As a result, the usage of these plants is generally unregulated, leading to over-collection. As a result of this large-scale removal of plants from their natural habitat, as well as the Orchidaceae is one of the plant families with the largest percentage of endangered species due to habitat destruction, change, and fragmentation (Rashid & Basusta, 2021)

The Mascarene archipelago is home to two distinct but related species of orchid, *Jumellea rossii* and *Jumellea fragrans* (Chen, et al., 2017). The physical similarities between the two species are obvious, yet their habitats and pollination patterns are somewhat different (Brzosko & Mirski, 2021). The aromatic and therapeutic characteristics of the leaves of both species are traditionally gathered (Ketema, et al., 2020). There are still considerable numbers of *Jumellea rossii* throughout a wide range of habitats (Mäder, et al., 2019), however the population of *J. fragrans* has been severely decreased as a result of both overharvesting and habitat loss. Considering this, both species, but especially *J. fragrans*, raise serious concerns about their future viability. A global effort of conservation and promotion has been launched to reduce collection pressure and boost the existing populations of these aromatic and medicinal species (Jesus, et al., 2019).

In this work, we evaluate and contrast the reproductive strategies, genetic diversity, and inbreeding depression of two closely related orchid species that are geographically distinct. A hand pollination experiment will be used to determine the breeding systems in the two populations of *J. fragrans* and *J. rossii*, while nuclear micro-satellite markers will be used to estimate the mating systems and levels of genetic diversity. Pollen removal and deposition rates will be measured, as will the potential for inbreeding depression. To better understand how over- and fragmentation-related effects on *J. fragrans* are affecting this species, these new data will help us better understand their biology and ecology.

II. MATERIALS AND METHODS

Pollen deposition vs. removal rates

At least once a week, 22–74 individuals of each species were inspected in the two research sites to measure the success of male and female pollination (removal and deposition of pollinia, respectively). Pollen removal rates were calculated using eqn (1), with P 14 mean percentage of pollen removal, N 14 number of plants, nm 14 the total number of flowers with removed pollinia, and nt 14 total number of flowers per plant for each species. Using the same formula, the pollen deposition rate was estimated Kruskal–Wallis χ^2 and Mann–Whitney tests were used to examine the variation in pollination success between years and between species. R 2.15.1 was used for the calculations (Crispim, et al., 2021).

Genetic diversity and estimated selfing rates

Reunion's Parc National de La Reunion issued a sampling permit code of DIR/I/2012/002 for the two populations examined between January and July 2012. A DNeasyVR Plant small kit was used to extract DNA from dried leaves (Qiagen, Hilden, Germany). A total of 12 nuclear microsatellite loci (P2G7, P1A9, P2E3,..., P2H10) were obtained from *J. rossii* and successfully amplified in *J. fragrans*. Individuals were genotyped for these 12 loci (Rashid, et al., 2018).

According to (Subaşı & Güvensen, 2021) was used to estimate null allele frequencies for each locus in each group (Freitas, et al., 2020). There were 37 diploid individuals in each population, thus we estimated the mean observed number of alleles per locus (AL) and the mean number of rare alleles per locus (RA, frequency 005) for each population using GenAlex 6.5 and FSTAT 2.9.3. Comparing species with different sampling sizes can be done by measuring allelic richness. Genepop 40 was used to estimate the fixation index (FIS) (Shekha, et al., 2013) and the observed and expected heterozygosity. Signed-rank tests were used to compare genetic diversity indexes between the two populations examined (Bauder & Karolyi, 2019). According to Rashid & Saler, (2020), deviation from Hardy–Weinberg equilibrium (HWE) was investigated using exact tests based on 1000 iterations of Markov chain iteration using Genepop 40.

Study site and species

There are four major habitat types on Reunion: lowland, sub-mountain, mountain, and sub-alpine (Strasberg et al, 2005) (between 1800 and 3069 m a.s.l.). There are roughly 150 orchid species in 30 genera in the native flora of Reunion, 25 percent of which are indigenous to the island (Rashid and Kendon, 2020).

Jumellea fragrans and *J. rossii*, two species of epiphytic orchids native to the Mascarene Islands, were the focus of this study (De & Pathak, 2018). Their occurrence, distribution, and pollination patterns are all unique to each of these species. At elevations between 500 and 1800 meters above sea level, the *Jumellea ros-sii* tree is prevalent. At dusk, the plants produce bunches of stalks that produce 1–5 creamy white flowers. The nectariferous spur is 16–20 mm long. There are two pollinia linked to a single viscidium, which separates it from the stigma by the rostellum, which is golden and very tiny. As long as the stigmatic cavity has been filled with at least one pollen, pollination has taken place. Depending on elevation, the blooming season might span anywhere from December to March. Most of *Jumellea rossii*'s pollination occurs at night, when it is visited mostly by Noctuidae (Pala, et al., 2021). Although *Jumellea fragrans* is found in the lowland rain forest at a height of less than 500 meters (as the result of human activity), it has a limited and fragmented range. In terms of appearance, the species shares many characteristics with *J. rossii*, but it is larger in both size and flowering time. Hawk moths pollinate the blooms, which have a longer spur than *J. rossii*'s (about 39mm) (Gardiner, et al., 2017). Reunion's mountain windward rain forest of Be louve and its lower-lying rain forest of Mare Longue were both studied as part of the research project.

Compatibility system determination and inbreeding depression estimation

Crossing treatments.

J. fragrans and *J. rossii* breeding system, between eight and 22 plants per species were selected for each site in both years based on the number of buds and/or fresh, unpollinated blooms that were still open. Flowers and buds that had not yet been pollinated were placed in fine-mesh cotton sacks to keep pollinators away. One or both of the following treatments were applied to fewer than half of the open blooms on each plant (for a total of about 30 flowers per treatment). The pollinia of a different plant, located more than 5 meters away, pollinated the blooms of this plant. Pollination by the flower's own pollinia is referred to as (b). Autopollination was tested on additional plants that had unaltered blooms.

Embryo viability and germination procedures

Researchers examined the viability of embryos resulting from cross- and self-pollination. A 2,3,5-triphenyl tetrazolium chloride (TTC) viability test was performed on samples containing 5mg of seeds per fruit (Holyoak, et al., 2020). Pre-conditioning of seed samples was accomplished using 15 mL microtubes filled with a sucrose solution containing 10% (w/v). A solution of 1 percent TTC was added to the seeds after they had been incubated for 24

hours at room temperature. The sucrose solution had been withdrawn. For 48 hours, seeds were kept at 40 degrees Celsius in the dark. For each sample, a light microscope with a magnification of 100x was used to count a minimum of 100 seeds. Red or pink-colored embryos were considered viable, while embryos that were not stained were considered non-viable. It was determined by using eqn (Bulut, et al., 2020), with P 14 mean percentage of viable seeds for each treatment and each species, N 1–4 number of capsules, N1–4 number of viable seeds, and Nm1–4 number of viable seeds in the sample.

After sterilization in a 34% solution of H₂O₂ for 20 minutes, seeds were sown in aseptic conditions on Petri dishes containing half-strength H₂O₂ to examine if pollination method affected seed germination rates. The phytohormone 6-benzylaminopurine was added to 01 mM of the Murashige and Skoog basal medium (Bissessur, et al., 2019). The cultures were kept at a constant temperature of 25 degrees Celsius and exposed to a 16-hour light cycle. nm 14 number of germinated seeds per capsule, nt 14 total number of seeds seeded per capsule, and P 14 mean percentage of germinated seeds were all used to compute the percent-age of seed germination after 11 weeks of culture. Mann–Whitney tests were used to compare the means of viable and germinated seed percentages among pollination modes and species (Warren, et al., 2018).

III. RESULTS

Compatibility system and inbreeding depression

Self- and cross-pollination treatments of *Jumellea rossii* yielded the same amount of fruits (Table 1), however none

of the flowers evaluated for autonomous self-pollination yielded fruit. *J. fragrans* (Mohajer-Tabrizi, et al., 2020) showed the similar trend. These results show that both species are self-compatible, but necessitate the use of a pollen carrier to set fruit.

Within each species, there was no discernible change in fruit set, fruit size, fruit weight, or embryo viability among outcrossed and self-treatments (Table 1). As a result, the inbreeding depression coefficient was not significant at any of the five stages examined. Seeds from cross-pollination (La Réunion, 2020) germinated more frequently than those from self-pollination (Rashid, 2017) in *J. rossii*, which resulted in a substantial inbreeding depression coefficient of 0.66.

All the assessed variables, except for fruit length, were different between *Jumellea fragrans* and *Jumellea rossii* (Table 1): *J. rossii* had a larger fruit set, heavier and wider fruits, and more viable seeds than *J. fragrans*. When compared to *J. rossii*, germination rates for *J. fragrans* were much greater.

Pollen deposition vs. removal rates

For *J. rossii*, variations in pollen removal and pollen deposition were observed from year to year (Table 2). The 2009 flowering season, in which the removal rate was low and no deposition was recorded in the population, was mostly responsible for this variation. In terms of pollen clearance percentages, only *J. fragrans* demonstrated a significant year-to-year change (P 0004, Kruskal–Wallis).

Table 1: Descriptive Analysis of *Jumellea fragrans* and *J.rossii*

Categories	J.rossii					J.fragrans				
	Cross		Self			Cross		Self		
	Sample	Mean	Sample	Mean	P-Value	Sample	Mean	Sample	Mean	P-value
Viable seeds	7	75.11±2.12	9	62.21±3.98	0.11	9	39.54±10.22	7	31.74±9.91	0.68
Germination	6	13.21±5.32	8	4.99±0.81	0.02*	6	39.49±9.78	5	29.12±8.99	0.44
Fruit width	6	4.98±0.33	6	5.33±0.21	0.61	5	3.99±0.31	6	3.99±0.31	0.68
Fruit length	6	49.32±2.23	6	49.67±3.12	0.61	6	49.87±3.21	7	51.32±2.75	0.79
Fruit weight	5	1.12±0.11	5	1.15±0.11	0.72	6	.76±0.11	6	0.79±0.11	1.00
Fruit set	11	69.32±8.34	9	79.76±4.58	0.65	29	39.12±6.32	18	43.44±5.83	.69

Pollen removal and pollen deposition rates for *J. rossii* differ significantly between species (P 14 0005 for pollen removal, P 0001 for pollen deposition, Kruskal–Wallis tests; Table 2; Fig. 1). There was usually a significant

difference in pollen re-mobilization and pollen deposition, except in 2011 for *J. fragrans* (P 1–4 0–19, Kruskal–Wallis test; Table 2).

Genetic diversity and estimated selfing rates

It was found that all 12 microsatellite loci were polymorphic for the two species studied. *J. rossii* had an average null allele frequency of 0.196007, while *J. fragrans* had an average null allele frequency of 0.116009. The estimated genetic variation in the two populations tested differed greatly. There was more genetic diversity in *Jumellea rossii*, with a higher mean number of alleles per locus (P 14 0009, Wilcoxon's signed-ranks test; Table 3) and allelic richness (P 14 0021, Wilcoxon's signed-ranks test; Table 3) than *J. fragrans*. A higher number of uncommon alleles per locus

(P 14 0012, Wilcoxon's signed-rank test) were found in *Jumellea rossii* (Table 3) than in *J. fragrans*. Mean heterozygosity in both species was lower than expected, indicating a lack of heterozygotes. Exact tests demonstrated that heterozygote deficit in both populations led to large deviations from HWE, with FIS estimates of 0.455 for *J. rossii* and 0.303 for *J. fragrans* (Table 3). *J. rossii*'s estimations of selfing rates ranged from 0.39 to 0.63, regardless of the approach used (Table 4). Compared to *J. rossii*, selfing rates for *J. fragrans* were lower, ranging from 0.16 to 0.47. (Table 4). Only the FIS and g2 approaches were able to accurately estimate the selfing rate.

Table 2: Pollen removal and deposition in *J. rossii* & *Jumellea fragrans*

Variables	Pollen deposition	Pollen removal
<i>J. rossii</i>	18.7	12.9
<i>J. fragrans</i>	2.89	12.77

Table 3: Genetic diversity

Component	<i>J. rossii</i>	<i>J. fragrans</i>	Significant
n	49.11±1.99		NA
AL	8.76±2.87		0.007**
AR	7.56±2.33		0.011*
RA	3.87±1.67		0.38
HO	0.45±1.89		0.69
He	0.46±0.187		0.038*
FIS	0.381		NA

Table 4: ML methods

Component	ML method	G2 method	FIS method
<i>J. rossii</i>	0.51	0.29	0.21
<i>J. fragrans</i>	0.55	0.71	0.44

FIS-calculated selfing rates (S(F)) based on the number of samples; Guo and Thompson's (1992) exact tests are used to determine the relevance of S(F) when there is no Hardy–Weinberg equilibrium. Ho's test significance (P-value = 1), selfing rate (S(g2)) and P(g2) (David et al., 2007). There are no selfing rates (S(ML) 14 0), and the P-value of the Ho test is 0.05.

IV. DISCUSSION

Hand pollination was used to determine whether or not the two species' compatibility systems were fully functional. For most orchid species, this is the case (Rashid & Basusta, 2021). But in the tested populations, none of the bagged

blooms yielded fruit, suggesting neither species is self-pollinating and both are completely dependent on pollen carriers. As a result of the lack of pollinators or specialized pollinators, orchidaceae (5–20 percent of the total species) and other insular environments, self-fertilization is common (Chen, et al., 2017). On Reunion, over half of the orchid species, including five of the nine *Jumellea* species, are assumed to be self-pollinating (Brzosko & Mirski, 2021). Pollination syndrome and spur length appear to be linked to *Jumellea*'s ability to self-fertilize, as the five self-pollinating species all have long spurs (spur length >9 cm) and no long-tongued specialized pollinators (Ketema, et al., 2020).

Short-spurred *Jumellea rossii* and *J. fragrans* (spur length 5 cm) preserve pollinator interactions.

Over the course of the three-year study, pollen removal by *J. fragrans* was on average 28%, whereas pollen deposition was on average 13%. Similar results were found for the pollen clearance percentage of *J. rossii*, which was 26%, compared to the pollen deposition percentage of 6%. These findings reveal the existence of pollinators in each population that was examined. Pollen movements and pollination efficiency are same in both populations, except in 2009, when pollen removal and pollen deposition rates were considerably lower in the *J. rossii* population. Compared to tropical orchids, the values of pollen remobilization and deposition are similar (Bulut, et al., 2020). Numerous studies show that plant–pollinator interactions are harmed as a result of fragmentation. Pollinator diversity and movement patterns, as well as plant variety, reproductive systems, reproductive success, and production were all shown to be significantly affected by landscape changes in a review of 155 studies conducted by Mäder, et al., (2019). *J. fragrans* appears to be unaffected by habitat decrease, and pollen re-movement and deposition rates are comparable to those of the *J.rossii* population, even though the overall trend is toward decreasing habitat size. A second investigation of pollen movements shows that a considerable percentage of pollinia taken from the two populations analyzed never encountered stigmas, and the poor fruit sets found suggest that pollinators are a limiting factor. These changes appear to be occurring more often, especially in tropical orchids (Jesus, et al., 2019).

An estimated selfing rate in both species' mating systems is supported by the heterozygote deficiency, FIS values and estimated selfing rates found in both populations. The rostellum prevents self-pollination by separating the stigma from the anthers in both species' floral biology. Furthermore, bagging studies have shown that these species do not have the ability to reproduce on their own. As a result, it appears that *J. fragrans* and *J. rossii*'s inbreeding is the result of easier inbreeding (geitonogamy, pollinator-mediated autogamy or biparental selfing). Non-adaptive selfing is common in many self-compatibility species because of the huge flower display required to attract pollinators, which results in geitonogamous selfing (Crispim, et al., 2021). Two species of Noctuidae (Rashid, et al., 2018) have been shown to be the primary pollinators of *J. rossii*, and they are known to visit multiple blooms on the same plant, even if their flying ranges may be small. This behavior may encourage geitonogamy and account for the high rate of selfing seen in *J. rossii* due to this phenomenon. The only known pollinator of *J. fragrans* is *Agrius convolvuli*, a member of the Sphingidae family (Subaşı & Güvensen, 2021). Hawk moths are reported to

visit multiple flowers on a single plant, despite their larger flight ranges compared to Noctuidae. The pollinators of *J. rossii* and *J. fragrans* have feeding patterns that promote geitonogamous selfing, even though they belong to distinct pollinator groups. Both *J. fragrans* and *J. rossii* have the ability to have numerous flowers blooming at the same time on a single plant, suggesting that geitonogamy may be the primary mechanism of selfing in these two species. The estimated selfing rates for *J. rossii* are high and suggestive of a mixed mating system, whereas the lower and less significant values for *J. fragrans* show that the species is primarily outcrossing. Both species undergo in-breeding. When the number of accessible mates decreases or pollinator behavior changes, it has been shown to enhance biparental in-breeding and/or oedyssey (Freitas, et al., 2020). This has been shown to increase population selfing. Higher selfing rates in *J. fragrans* would have been expected for these reasons, and this is what we found. When it comes to the level of geitonogamy, factors such as floral display and plant density can have an impact, increasing the level of geitonogamy with greater floral displays (Shekha, et al., 2013) in *J. fragrans* could not be explained by differences in floral display or plant density between the two populations (Bauder & Karolyi, 2019). However, this may be due to the pollinator's ineffectiveness. Pollinator species do differ in their ability to deposit and remove pollen from blooms, and this is true even within the same species. When the pollinator proboscis is slightly shorter than the flower spur, the pollinator head presses firmly against the orchid column, enhancing pollination efficiency in moth-pollinated orchids (Rashid & Saler, 2020). The pollinator proboscis is longer than the flower spur in *J. fragrans* (Kendon, et al., 2017), which could lead to some inefficient pollination events in this species. So, pollen dispersion may be done more haphazardly, with less emphasis on methodical removal and deposition. Even while pollinator behavior promotes geitonogamy, the low selfing rates of *J. fragrans* may be explained by this. In this work, 12 nuclear microsatellite markers were used to examine genetic variation among populations of each analyzed species. Compared to the few other studies assessing genetic variation in orchid species through microsatellite markers (Rashid and Kendon, 2020), these markers showed a medium-high level of genetic variation (AR 14 8575 and He 14 0673) in the population of *J. rossii*. A more moderate level of genetic diversity was found in the *J. fragrans* population in comparison to the *J. rossii* population (AR: 14%, and He: 14%, respectively). *J. fragrans* has a smaller genetic diversity than *J. rossii*, despite the fact that *J. fragrans* appears to demonstrate a higher amount of allogamy than *J. rossii*. Because of the extensive distribution of *J. fragrans*, its genetic diversity may reflect the impacts of population decrease, which is generally

thought to be stronger in widely scattered species than in narrowly distributed ones (De & Pathak, 2018). Pala, Kendon, et al., (2021) observed a similar tendency when comparing levels of genetic diversity with the more common *Gymnadenia conopsea*. There may have been a loss of allelic diversity or gene diversity in the studied population due to the repeated fragmentation of the *J. fragrans* habitat (Gardiner, Kendon, et al., 2017). This can be caused by population bottlenecks at the time of disruption and subsequent genetic drift (Holyoak, Kendon, et al., 2020). The genetic diversity of *J. fragrans*, despite its limited distribution, is relatively substantial. Other research (Bulut, et al., 2020) have found comparable outcomes to these. First, the *J. fragrans* mating system, which is mainly outcrossing, can help to maintain genetic diversity. For another explanation for *J. fragrans*' medium level of heterozygosity, the loss of heterozygosity happens more slowly than a drop in allelic diversity. Consequently, the reported degree of heterozygosity can be higher than the level of heterozygosity expected from the observed allelic richness in a population that has been fragmented (Bissessur, et al., 2019). The allelic richness appears to be more influenced by population size decrease than heterozygosity in *J. fragrans*, which seems to be the case. Although *J. fragrans* had a large distribution until recently, it has been dwindling rapidly as a result of habitat loss (Warren, et al., 2018). *J. fragrans* has a long generation time, but it should not be long enough to allow for the evolution of noticeable fragmentation effects throughout that time period. Given that the effect of fragmentation grows stronger over time (Rashid, 2017), it is reasonable to predict that the genetic diversity of *J. fragrans* will dwindle in the future. In addition, because of the relatively high density and total number of individuals present in the examined population, the negative consequences of fragmentation and over-collection on genetic diversity have likely been postponed. A drop in population size has little effect on species with high densities since it results in remnants of smaller size that contain a large number of organisms. Reduced genetic diversity will not be great or immediate in such a scenario (La Réunion, 2020). One of the most important factors influencing population genetic diversity and overall inbreeding is the amount of inbreeding depression that may be detected by manual crosses (Mohajer-Tabrizi, et al., 2020). Seed germination was negatively affected by inbreeding (Rashid & Basusta, 2021) in *Jumellea rossii*. A mixed-mating system (Chen, et al., 2017) produced this result, which is in line with the species' observed mating system and is taken into account when calculating the fitness cost of geitonogamy (Brzosko & Mirski, 2021). On the other hand, we discovered no evidence of depression caused by inbreeding in *J. fragrans*

in this investigation, as there was no significant difference in any of the evaluated parameters between out-crossing and self-treatment. Studies show that the homozygous expression of harmful recessive genes following inbreeding is responsible for the majority of inbreeding depression, including heterozygote frequency, mating history, and life stage (Ketema, et al., 2020). Repeated selfing, on the other hand, decreases the severity of inbreeding depression by eradicating these harmful genes (Bulut & Rashid, (2020)). Conversely, when it comes to inbreeding depression, outcrossing species tend to have higher rates of inbreeding depression than selfing species (Mäder, et al., 2019). Because of this, we expected to find an inbreeding depression in the *J. fragrans* population, which is largely outcrossing.

A hypothesis for this lack of major inbreeding depression is linked to the species' history, which would have allowed it to purge a significant portion of its genetic load. Although *J. fragrans* had multiple instances of fragmentation and overcollection, this resulted in a succession of genetic bottlenecks (Jesus, et al., 2019). There will be a reduction in genetic diversity due to the loss of rare alleles that occurs during the sampling process as a result of repeated bottlenecks. It is expected that some of the recessive alleles that cause inbreeding depression will be lost during the sampling process (Crispim, et al., 2021). Due to population bottlenecks and reduced genetic diversity, inbreeding depression can be expressed through the expression of the remaining deleterious alleles that are recessive. However, in fragmentation settings, inbreeding occurs, thus these harmful alleles should be gradually cleansed. Even more importantly, populations that have experienced several bottlenecks and remain fragmented over successive generations are likely to demonstrate lower levels of breeding depression due to the sampling process and purging, especially in self-compatible species. It's possible that inbreeding depression is absent in *J. fragrans* because of its moderate genetic diversity. Reproduction (until the germination stage) is not constrained by inbreeding depression, and this could have a positive effect on the population's short-term survival.

However, the research on inbreeding depression shows that its amplitude grows during the life cycle of many plant species (reviewed by Husband and Schemske, 1996), hence inbreeding depression can sometimes be initially observed in later stages of the life cycle, such as seedling growth (Rashid, et al., 2018). Doesn't imply *J. fragrans* won't show signs of inbreeding depression in the future, even if it doesn't show it now. As a result, data from later in life are needed to handle the issue of inbreeding depression rigorously. Only one population of each species was studied, making it impossible to compare the amount of

intraspecific vs. interspecific variance in the parameters measured. However, a study of ten different *J. rossii* populations found that all of them had a lack of heterozygotes and FIS values that indicated a high percentage of selfing in their mating system (Subaşı & Güvensen, 2021). From 0389 to 0414, FIS values were statistically identical in all three habitat types. All of the *J. rossii* populations studied appear to have a mixed-mating system based on these findings and the results of the current study. *J. rossii*'s re-productive pattern appears to be remarkably consistent among individuals. Only one additional significant population of *J. fragrans* is known. With a heterozygote deficiency and FIS value of 0336 (Freitas, et al., 2020), both populations of *J. fragrans* showed a similar trend, suggesting that they share a comparable mating strategy. All of the other known populations have a small number of isolated members, making it difficult to make comparisons. There is more variety across species in the reproductive patterns studied here than within species, according to these findings.

V. CONCLUSIONS

Although both *Jumellea* species were self-compatible, pollen from a pollen carrier had to be used to establish fruit in this study. Pollinator-mediated geitonogamy seemed to be the primary cause of selfing in *J. rossii*, while out-crossing was the primary cause in *J. fragrans*. Regardless of the primary cause of selfing in either species, it appears to be a result of pollinators. The genetic diversity of the widely distributed *J. rossii* population was higher than that of the fragmented and illegally harvested *J. fragrans* population. Considering the current state of *J. fragrans*' population reduction and restricted distribution, conservation efforts should focus on this species. Since fragmentation's negative impacts increase with each generation, the species' genetic diversity could still be at risk if excessive harvesting continues, even if it isn't now critical. If the genetic diversity of *J. fragrans* continues to decline, it could have a significant impact on the species' evolutionary potential. As a result, this species must be protected both in and out of the wild. Even if *J. rossii* is not now threatened, its unregulated collection is nevertheless a risk. As a result, the preservation of *J. fragrans*' natural populations and genetic diversity is critical. When it comes to meeting demand for this precious resource, ex situ production of both species is a viable option.

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