Reproductive biology of flowering plants on Pohnpei

A Dissertation Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Viann Marie Harmony Yomai

December 2021

Abstract

Oceanic islands have long been considered natural laboratories of evolution. Present-day biotas of oceanic islands can provide better understanding of the effects of island age, size, and distance from continents on trait evolution. For plants, small, isolated oceanic islands should have the strongest establishment filter, relative to larger, less remote islands. A key step in understanding the establishment filter on such islands is to characterize the reproductive biology of current inhabitants relative to mainland source areas. In this dissertation, I investigate the reproductive biology of 84 angiosperm species that are considered indigenous (resident) or naturalized (recent colonist) on Pohnpei Island, Micronesia. I then ask how they differ from their continental mainland relatives. In the first chapter, I asked if there was a difference between breeding systems of native versus naturalized species on Pohnpei, and I found most taxa had some degree of autogamy and there were no significant differences between several determinants of island breeding systems. In the second chapter, I compared Pohnpei island breeding systems with those of their closest continental mainland relatives. I asked if there were differences in: (1) self-compatibility status, and (2) sexual systems. I found that Pohnpeian species had higher rates of autonomous self-pollination and self-fertilization, whereas their mainland relatives had more outcrossed breeding systems even though all were self-compatible. I also found that all Pohnpeian species and all but three continental mainland relatives had bisexual flowers and, when documented, hermaphroditic sexual systems. The high potential for self-pollination could be due to depauperate pollinators on Pohnpei island, as also indicated by my finding of high levels of pollen limitation. Finally, in the third chapter, I tested two hypotheses that arise from my finding of higher levels of autogamy on islands. First, floral sizes on islands should be smaller than on mainlands, due to less reliance on pollinator attraction. Second, floral allometry should shift such that island styles are proportionally shorter than styles of mainland flowers, due to relaxed sexual selection on style length on islands. Contrary to most studies, island flower size (petal length) was not smaller than that of continental mainland relatives. On the other hand, style length was significantly smaller in island species relative to their mainland sister taxa. These mainland sister taxa did not have unusual petal or style lengths compared to the species means of each of their mainland genera. The similar patterns across many unrelated plant colonizations of an island, strongly support the conclusion that smaller style lengths evolved independently of petal size post establishment.

Table of Contents

Introduction 1
Plant breeding systems on islands 2
Patterns of colonization and Baker's Law 2
Breeding and mating systems of island plants 4
Botanical history of Micronesian islands 4
Dissertation Aims 5
Aim 1: Characterize breeding system of flowering plants on Pohnpei 5
Aim 2: Understanding breeding system shifts on islands 5
Aim 3: Understanding floral size evolution on island 6
Chapter 1 – Breeding systems of naturalized versus indigenous species provide support for Baker's law on Pohnpei island 7
Abstract 8
Background 8
Methods 11
Study site 11
Sexual system 12
Herkogamy 12
Dichogamy 12
Pollinator attraction and dispersal traits 13
Pollen:ovule ratios 13
Experimental hand-pollinations 14
Statistical analyses 15
Results 15
Floral traits related to pollinator attraction and sexual function 15
P:O ratios 16
Hand-cross Pollination 16
Discussion 17
Floral traits as indicator of pollination systems of Pohnpei 17
Floral traits as indicators of breeding systems of plants of Pohnpei 18
Pollination efficiency based on P:O ratios 19
Self-compatibility, autofertility and pollen limitation of Pohnpei species 20

Conclusions 21
Acknowledgments 21
Data statement 22
References 23
Chapter 2 – Self-compatible plants on Pohnpei self-pollinate more than their outcrossing mainland relatives 29
Abstract 30
Introduction 31
Methods 33
Pohnpei-Mainland Comparisons 33
Sexual system 34
Experimental – Hand-pollination to determine self-compatibility 34
Statistics 35
Results 35
Sexual systems 35
Experimental pollinations on Pohnpei island 35
Mainland systems 36
Self-compatibility and autofertility are higher on Pohnpei than on mainlands 36
Discussion 37
Self-compatibility is prevalent among Pohnpeian species and their mainland relatives 37
Autonomously selfed flowers set more seeds than outcrossed flowers 38
Island species self more than their mainland relatives 38
Conclusions 39
Acknowledgement 39
References 40
Chapter 3: Evidence for relaxed selection on style length on an isolated, oceanic island 44
Abstract 45
Introduction 46
Methods 47
Island and mainland species sampling 47
Island-mainland and mainland-mainland relationships 48
Experimental design and statistics 48
Results 49

Style length, but not petal length, is shorter on Pohnpei than on mainlands 50
Are the mainland sisters to island species a non-random subset of their mainland genus? .. 51
Discussion 51
Pohnpei island flowers are not smaller than their mainland relatives 51
Style length deviates from petal length on islands 52
Conclusion 53
Acknowledgement 54
References 55
Conclusion 57
Vita 89

List of Tables

Table 1.0. Species list and authority, nativity status and collection sites. Families arranged in sections in order: Melastomataceae, Fabaceae, Malvaceae. Nativity status taken from Herrera et al. 2010, and indigenous/endemic species are in shaded rows……. 60

Table 1.1. Floral traits related to pollination system. All values are means of three flowers per species (± SD for petal length). Herkogamy is anther-stigma separation, positive if stigma height > anther height, negative if the reverse (± ½ 95% CI). Dichogamy was either incomplete protandry (IPDR) or incomplete protogyny (IPGY). For fragrance, D indicates fragrance detected, and N indicates fragrance not detected (Dafni 1992). Families (row shading) arranged in order: Melastomataceae, Fabaceae, Malvaceae. Shaded rows indicate native species…… 62

Table 1.2. Pollen and ovule numbers and pollen:ovule (P:O) ratio. Pollen grains are estimated number of pollen grains from 4 anthers per flower in Fabaceae and Melastomataceae and 10 anthers per flower in Malvaceae. Ovule numbers are estimated number of ovules for the same four flowers that the anthers were collected from. Values are means (± 1 SD). Shaded rows indicate native species…….... 64

Table 1.3. Hand-pollination experiments. Values represent the average number of seeds per N = 5 flowers (1 SE). In each row, values with different superscripts are significantly different. "Hand self," hand-self pollinations; "Autonomous self," bagged and unmanipulated; "Open pollination," emasculated without bagging; "Hand outcross," emasculated, cross-pollinated. Shaded rows indicate native species……. 66

Table 1.4. Summary of breeding indices and inferred breeding systems. If numerator and denominator an index were not significantly different, then "~1" or "~0", otherwise values shown. ISI, index of self-incompatibility; AI, autogamy index; AF, autofertility index; PL, pollen limitation index - if seed set of open (geitonogamous or outcross) pollination was lower than either outcross or hand-self seed set, "PL", or if non-significant "~0" (no PL); OCI, outcrossing index; P:O ratio, pollen to ovule ratio. See text for discussion of inferred breeding systems: FA, Facultative autogamy; FO, Facultative outcrossing; SC = species with self-compatibility based on crossing data. "Mixed" indicates either FA or FO. Shaded rows indicate native species….. 67

Table 2.0. Known sexual systems for mainland species…….. 68

Table 2.1. Hand-pollination experiments. Values represent the average number of seeds per N= 5 flowers. "Hand self", hand-self pollinations; "Autonomous self", bagged and unmanipulated;

"Hand outcross", emasculated, cross-pollinated; "Open", natural pollination of emasculated unbagged flowers. Data from Yomai and Williams (2021) are indicated by superscript A…… 69

Table 2.2. Autofertility, self-compatibility, and fruit set self-compatibility comparisons between island and mainland. SC, self-compatible; SI, self-incompatible; values in parenthesis under island self-compatibility rate are seed set self-compatibility rate……. 71

Table 2.3. Results of generalized least squares (No Phy) and phylogenetic generalized least squares analyses (lambda model) of autofertility index (AFI) and self-compatible index (SCI-F) for island and mainland species. A negative difference means mainlands had lower values. Say which models were thrown out – eg. OU, theta, etc. models had AIC values more than twice as high as those shown here. Models with AIC twice as much or more as the ones reported are not shown. N = 33 pairs…… 73

Table 2.4. Results of one-tailed t-tests on breeding system indices. *AFI,* autofertility index (based on seed set); *SCI,* self-compatibility index (based on fruit set)……... 74

Table 2.5. Inferred breeding systems of island and mainland species pairs. All island species are self-compatible (SC) with bisexual flowers. References are for mainland breeding systems. 7 = intra, 13 = sister, and 11 genus level…… 75

Supplementary Table 2.0. Categories of breeding systems derived from Dafni 1992…….. 77

Supplementary Table 2.1. Mainland species assumed floral types based on images viewed from website dataset…….. 78

Table 3.0. Data collected on Pohnpei island for species. Asterisked taxa are data reported in Yomai and Williams (2021)…….. 79

Table 3.1. Phylogenetic least squares regression results for island-mainland comparisons…….81

Table 3.2. Result of a one-sided T test for mainland sister taxa and mainland genus sisters. values…….82

Figure 1.1 Photos of flowers in the field: (A) bagged *Vigna hosei*, (B) fully open Melastoma malabathricum flower, (C) almost fully open *Hibiscus tiliaceus* and (D) *V. marina* in the field..83

Figure 2.1. Breeding system indices between mainland and island. A) Mean autofertility index (AFI) and B) mean self-compatibility index (SCI). Values above 0.8 (red dashed lines) indicate highly selfing breeding systems. Upper and lower bars represent mean ± standard deviation, and whisker bars represent highest and lowest values……..84

Figure 2.2 Breeding system interaction graph showing transitions between mainland (1) and island (2). Thickness of the line represents how many individuals have the same transition. 5 = SI

Outcrossing, 4 = Facultative outcross, 3 = Mixed mating, 2 = Facultative autogamy, 1 = Obligate autogamy, 0 = Cleistogamy. N = 33 pairs……85

Figure 3.0. Phylogenetic tree containing 392 species tips. Terminal branches and names highlighted in red are Pohnpei species, and the rest are mainland species……...86

Figure 3.1. Log-log differences in floral organ sizes between Pohnpei island and mainland sister taxa……87

Figure 3.2. Plot of log ratio (LR) of island-mainland petal length versus log ratio of island-mainland style length. ……..8

Introduction

Plant breeding systems on islands

In my dissertation, I focus on studying reproductive biology of angiosperms on Pohnpei – a small, oceanic island in the Federated States of Micronesia (FSM). Oceanic islands have long been considered "natural laboratories" and have provided many key insights into ecological and evolutionary processes. Because island biotas originate largely from the nearest mainland source populations, immigration and extinction rates are dependent on distances to mainland areas and island size, respectively (MacArthur and Wilson, 1967). Plant communities on remote islands should be increasingly biased towards species that have both the capacity for long distance dispersal and reproductive traits that allow the establishment of a breeding population from a single colonist (Baker 1955; Grossenbacher et al. 2017). Species on small and isolated oceanic islands are the most likely to display solutions to the critical problems faced during colonization: lack of mates and lack of pollinators. Patterns of plant reproductive biology have been studied in a number of island systems (Anderson et al., 2001; Lobo et al., 2005; Machado et al., 2006; Trusty et al., 2011; Santos et al., 2012), but studies on extremely isolated oceanic islands are relatively rare (Yorkston, 2005; Yorkston and Daehler 2006; Bramwell and Caujape-Castells 2011; Lord, 2015).

Patterns of colonization and Baker's Law

Colonization is divided into two steps: dispersal and establishment. A successful dispersal means an individual survives dispersal from one point to another, in this case, from a continental mainland to an island. Plant dispersals can occur via a biotic vector (e.g., birds, humans, bats, etc.,) or via abiotic vectors (e.g., wind and ocean currents). Most studies indicate that birds are the major dispersing agents of seeds to islands (Carlquist, 1974; De Queiroz, 2005; Bernardello et al., 2006; Bartish et al., 2011), but ocean currents and surface winds can also play a role in dispersing individuals (Carlquist,1974). Selection favors seeds that are particularly well equipped for specific types of long-distance dispersal, resulting in the vast array of elaborate solutions we see today on island species' seeds including plumes, hairs, wings, floats, nutritive tissues, and sticky surfaces (Traveset et al., 2014; Moeller et al., 2017).

While it appears that birds are common dispersal agents of propagules to islands (Carlquist, 1974; Anderson et al., 2001), one successful dispersal does not imply successful establishment. Successful dispersal is often determined by chance, but specialized morphological

structures of seeds can influence the likelihood of establishment (Traveset et al., 2014), if they confer a higher probability of finding suitable recruitment sites. A successful establishment may be more difficult than dispersal because establishment involves both reproduction and the propagation of descendant generations.

Successful establishment then is contingent on multiple environmental factors such as compatibility with soil nutrients, climate, topography, and availability of pollinators (Barrett et al., 1996; Emerson et al., 1996; Medrano et al., 2005) and mates (Pannell et al., 2015). An ideal establishment would involve a propagule that is self-compatible (SC), hermaphroditic, selfing or apomictic, and preferably pollinated abiotically (wind or water). This gives the propagule a higher chance of establishing a population because it is relying mostly on itself for establishment than a second or third party. Throughout the years, studies have consistently shown that self-compatibility moderately benefits island colonization, which supports Baker's Law. Baker's law states that self-compatible species will be more successful in establishing populations in new environments compared to self-incompatible species. However, almost a decade after Baker's Law was popularized by Stebbins (1957), Carlquist (1967) found an unusually high level of dioecy on islands, particularly in Hawaii. Carlquist's study contradicted Baker's law because by definition, dioecious species are self-incompatible. Baker (1969) responded to Carlquist's study explaining that Baker's law should only be evident at the early stages of establishment, but it should not apply to subsequent evolution. At the early stages of establishment, Baker's law should be apparent in order to establish a population. However, after populations have been established, long-term establishments should evolve to exhibit more outcrossing traits in order to avoid inbreeding depression.

Reproductive traits are filtered upon successful establishment, and there are two major establishment filter: mate limitation filter and pollinator limitation filter. Pollinator limitation filter favors species with traits that do not require specialized pollinators that may be absent on the island, or species that have low demands for pollinators (Barret 1998). Mate limitation filter favors species with some or high potential for selfing. These two factors form the establishment filter, which depends primarily on plant reproductive biology (Grossenbacher et al., 2017). With increasingly rare dispersal, lack of mates, and lack of pollinators become the predominant problems faced by new immigrants.

Breeding and mating systems of island plants

In plants, the breeding system refers to the morphological and physiological aspects of individuals that determine its breeding biology - these encompass sexual systems, pollination mechanisms, self-incompatibility/compatibility, and floral phenotype/functional factors (Neal and Anderson, 2005). Breeding systems respond to selection pressures in multiple ways, and these responses can directly constrain the mating system which controls the genetic transmission between generations. Thus, without genetic studies inbreeding or mating systems, understanding the breeding system can provide insight into the evolution of reproductive biology. Reproductive biology is a broad term referring to the general reproductive system of a species, which includes mating system and breeding system (Neal and Anderson, 2005). Plant reproductive biology is particularly interesting in island species because islands can be seen as areas of natural evolutionary experiments (Darwin and Wallace, 1858).

Island plants, on the one hand, tend to have elevated levels of hermaphroditism and self-compatibility (Crawford et al., 2011), whereas on the other, they can have high levels of dioecy and wind pollination (Grossenbacher et al., 2017). Wind pollinated plants are often accompanied by dioecy, and self-compatible plants are usually associated with hermaphroditism (Friedman and Barrett, 2009; Lord, 2015).

Botanical history of Micronesian islands

The Federated States of Micronesia (FSM) is a group of islands in the Micronesian region that stretches from the middle of the Pacific to Southeast Asia. The FSM is a global biodiversity hotspot just north of the equator, harboring some of the most biologically diverse forests and coral reefs in the world (Wortel 2010). Over 1,239 species of ferns and flowering plants have been described in the FSM. Approximately 782 species are native, including about 145 species of ferns, 267 species of monocots and 370 species of dicots (Falanruw, 2002). More than 200 of these plants are found nowhere else on earth (Wortel, 2009), with additional estimates that nearly 80% of the plant life in the FSM is likely to be endemic. FSM extremely isolated; in fact, the closest mainland is Papua, New Guinea which is 2175 km away. However, the majority of the flora and fauna of FSM is generally thought to have come from the Philippines which is 3,431 km away (Fosberg and Sachet, 1975). This could be explained by wind currents coming from Southeast Asia.

Although a fair number of studies have been conducted on mainland breeding systems at the community level (Lyons and Antonovics, 1991; Gribel et al., 1999; Oliveira and Gibbs 2000; Arroyo and Squeo, 2012; dos Santos et al., 2012; Goldberg and Igic, 2012), there are far fewer studies on island populations (Anderson et al., 2001; Lord, 2015; Traveset and Navarro 2018). Most published studies in FSM focus on biota demographic surveys. In my thesis research, I wanted to take it a step further by characterizing reproductive biology of plants on Pohnpei, capital of the FSM. I focus on comparing breeding systems between invasive and indigenous flowering plants.

Dissertation Aims

Aim 1: Characterize breeding system of flowering plants on Pohnpei

A key step in understanding colonization and evolution of plants is investigating their reproductive biology. Such investigations on islands are scarce, especially for the Micronesian islands. The existent studies on the Micronesian islands focus on surveys of terrestrial plant occurrences and their nativity status. Such studies are an important first step, however, a wide gap in understanding colonization and evolution of plants remains. I address this gap by characterizing and comparing breeding systems between native and invasive plant species. Altogether, we were able to provide the first ever, to our knowledge, study of reproductive biology of plants in the Micronesian region.

Aim 2: Understanding breeding system shifts on islands

Island biotas typically originate from an island's nearest mainland. Thus, to understand evolution on islands, it is vital to compare traits of island species to their closest continental mainland relatives. Most studies use meta-analysis to compare self-compatibility and other reproductive traits between global continental mainlands and global islands. This is a great method to understand the overview and clear consensus that island breeding systems are different from mainland breeding systems. However, there is still a gap in understanding the clear origins and evolution of plants on islands. We filled this gap by directly comparing breeding systems and self-compatibility rates of flowering plants on Pohnpei with their continental mainland relatives. This approach helps us understand clearly the degree to which plants colonize an island with their current breeding systems and reproductive traits, and the degree to which they evolve their traits and systems post-colonization. This type of comparative study is limited, and our study is,

to our knowledge, the first study to directly compare a large number of breeding systems between island taxa and their closest conspecific or congeneric mainland relatives.

Aim 3: Understanding floral size evolution on island

Studies have consistently shown that island and mainland flora differ significantly across phenotypic traits due to limited space, mate, pollinator resource, and change in temperature and other environmental factors. Island flowers tend to be smaller, less colorful, and lack pollinator rewards (nectar, resins, oils) and/or other expensive traits to maintain. One major comparative proxy is floral size; however, nothing is known about whether the change in size is due to allometric scaling of flower to plant size, to changes due to shifts to more autogamous breeding systems. This study aims to test the prediction that change in size is due to allometric scaling, that energy is allocated more toward selfing techniques rather than outcrossing techniques.

Chapter 1 – Breeding systems of naturalized versus indigenous species provide support for Baker's law on Pohnpei island

The materials in this chapter were published in *AoB Plants* in June 2021

Abstract

The factors that facilitate successful colonization of islands should be especially evident where the establishment filter is strongest. Colonizers of small, remote oceanic islands should be initially rare, extremely mate-limited and often without pollinators. Hence, plant communities on such islands should reflect an establishment history in which young "naturalized" species are most likely to display self-compatibility and autonomous selfing, whereas "indigenous" species may exhibit more diverse reproductive strategies. To test this prediction, we characterized breeding systems of 28 species on Pohnpei, in the Federated States of Micronesia, a group of remote Pacific islands that are considered a global biodiversity hotspot. Three families with both naturalized and indigenous species were selected – Fabaceae, Malvaceae, Melastomataceae. Measurements included field observations of dichogamy/herkogamy and floral attraction traits, pollen:ovule (P:O) ratios and experimental hand-pollinations for self0compatibility and pollen limitation. Phylogenetic generalized least squares analyses test for trait correlations between naturalized and indigenous species. Flowers of all 28 species were bisexual, and pollinator attraction features were common. Pollen:ovule ratios ranged from 9 to 557 (median = 87), and all 11 hand-pollinated species were self-compatible. All species had >5 ovules and <3500 pollen grains per flower. Indigenous species did not differ significantly from naturalized species for any trait. There is a dearth of data from remote islands bearing on the question of establishment history. In this study, we inferred all species to have some degree of autogamy and indigenous species were no more likely than naturalized species to display outcrossing mechanisms. On Pohnpei, high ovule numbers, and the inaccessibility of wind pollination and obligate outcrossing strategies, reflect the importance of retaining any reproductive assurance mechanisms in the face of pollinator uncertainty.

Background

Oceanic islands have long been considered 'natural laboratories' and have provided many key insights into ecological and evolutionary processes (Darwin and Wallace 1858; Barrett 1996). Because island biotas originate largely from the nearest mainland source populations, immigration and extinction rates are dependent on distances to mainland areas and island size, respectively (MacArthur and Wilson 1967). Plant communities on increasingly remote islands should have greater capacity for long-distance dispersal and reproductive traits that allow

establishment from single, rather than multiple concurrent, introductions (Baker 1955). Community-level studies of plant reproductive biology have provided insights into colonization history in a number of oceanic island systems (Anderson *et al.* 2001; Lobo *et al.* 2005; Machado *et al.* 2006; Trusty *et al.* 2011; Santos *et al.* 2012), but studies on extremely isolated oceanic islands are still relatively rare (Yorkston 2005; Yorkston and Daehler 2006; Bramwell and Caujape-Castells 2011; Lord 2015).

'Baker's law', as originally stated, indicates that successful establishment of 'sexually reproducing' colonies on isolated islands is much more likely to come from a single self-compatible founder than from two self-incompatible or dioecious founders (Baker 1955). The capacity for self-fertilization solves the immediate problems of lack of mates and lack of pollinators, but leads to inbreeding. Hence, Carlquist (1966) argued that the value of outcrossing is so great that many obligate outcrossers in modern oceanic plant communities are likely to have colonized as outcrossers. This led Baker (1967) to point out that 'Baker's law' neither rules out occasional establishment by outcrossers nor the subsequent evolution of outcrossing from self-compatible colonists. Instead, early immigrants 'are much more likely' to exhibit traits associated with self-pollination and self-fertilization, whereas longer term persistence must eventually involve shifts to more outcrossed mating systems. Consequently, older immigrants should begin to display modified physical traits that enforce both outcrossing (e.g. dichogamy, dioecy) and cross-pollination (e.g. wind or new local pollinators) (Baker 1967).

To date, tests of Baker's law have focused on whether or not self-compatibility is over-represented on islands relative to mainlands (Lord 2015; Grossenbacher *et al.* 2017; Razanajatovo *et al.* 2019). To the best of our knowledge, no study has yet explored Baker's (1967) corollary that there should be historical signal of the establishment filter within modern island plant communities. A comparison of recently naturalized and older, indigenous species might reveal common pathways to successful establishment and persistence. Such an approach has been useful in community studies of invasion biology on mainlands (Burns *et al.* 2011) or large, oceanic islands (references in Schlessman *et al.* 2014). Plant 'breeding systems' comprise the morphological and physiological functional traits that determine mating patterns, such as sexual systems, pollination mechanisms, self-incompatibility/compatibility and floral functional morphology (Neal and Anderson 2005). Taken together, such traits can provide insight into evolved pollination and mating patterns.

Under Baker's scenario, the early stages of establishment typically involve rare or unpredictable pollination environments (Olesen and Jordano 2002) and lack of mates. Thus, both self-pollination and self-compatibility are favoured. Plants in young immigrant populations will have either bisexual flowers and unassisted, intrafloral (autonomous) self-pollination (Crawford *et al.* 2011), or uni- or bisexual flowers and extrafloral (geitonogamous) self-pollination, assisted by gravity, wind or animal pollinators (see Schoen and Lloyd 1992 for different modes of self-pollination).

Later stages of establishment involve accommodation to, or escape from, chronic outcross pollen limitation. The former can occur by shifts to obligate autogamy and reduced inbreeding depression (Pannell *et al.* 2015). The latter, by shifts to new plant–pollinator interactions or to wind pollination (Culley *et al.* 2002; Friedman and Barrett 2009; Lord 2015). Wind pollination is favoured on islands when there are, (i) few animal pollinators (Anderson *et al.* 2001; Schueller 2004; Eckert *et al.* 2006; Kissling and Barrett 2013), or (ii) stronger wind currents (Carta *et al.* 2009), or when, (iii) wind is more effective than animals (Carlquist 1974; Rech *et al.* 2016).

In sum, plant communities on small, isolated islands should be comprised of both recent colonists that predominantly exhibit 'selfing syndrome' traits (Sicard and Lenhard 2011) and older indigenous species, that have either evolved stable autogamous mating systems or have transitioned to pollinator-assisted, mixed-mating or even outcrossed systems. Obligate outcrossing is expected to be rare on small, distant islands (for large islands, see Sakai *et al.* 1995; Schlessman *et al.* 2014), since establishment by multiple simultaneous colonists is rare (Baker 1955, 1967), and because small population sizes and high extinction rates (MacArthur and Wilson 1967) limit its evolution. Indigenous or endemic species on such islands should display a greater diversity of reproductive strategies than recent colonists.

Almost nothing is known about plant reproduction on Micronesian islands (Fosberg and Sachet 1975; Costion and Lorence 2012). The Federated States of Micronesia (FSM) stretches from the mid-Pacific almost to Southeast Asia, in an area just north of the equator. The FSM has been designated a global biodiversity hotspot, since the islands harbour some of the most biologically diverse forests and coral reefs in the world (Wortel 2010). Compared to many Pacific volcanic islands, Pohnpei is relatively old, with volcanic rocks of up to 8.7 million years in age (Rehman *et al.* 2013). Over 1239 species of vascular plants have been described in the

FSM. Approximately 782 species are considered native, including 145 species of ferns, 267 species of monocots and 370 species of dicots (Falanruw 2002). More than 200 of these species are endemic to the FSM (Wortel 2010). Islands in the FSM are extremely isolated—for example, the capital island of the FSM, Pohnpei, is 2175 km from the nearest large landmass, Papua New Guinea. However, the majority of the flora and fauna of the FSM is generally thought to have come from the Indo-Pacific region (Indonesia or south-eastern Asia extending out into the Pacific) (Fosberg and Sachet 1975; Keating *et al.* 1984).

Angiosperm breeding systems have not been studied in FSM, and to the best of our knowledge, this is the first study to compare breeding systems in the specific context of establishment history on any oceanic island. The plants of Pohnpei are well-known and the nativity status of each species has been well-documented (Herrera *et al.* 2010). Such knowledge allowed us to test the general prediction that recently arrived, 'naturalized' or 'invasive' species should be heavily invested in selfing syndrome traits, whereas long-established native 'indigenous' or 'endemic' species are likely to have evolved more variation in reproductive strategies. We restricted our study to three families of angiosperms that have both naturalized and indigenous species. Our main questions were, (i) what kinds of breeding systems and pollination systems occur on Pohnpei, and (ii) are naturalized species more likely to exhibit breeding system traits that reflect self-pollination and self-fertilization than long-term native species?

Methods

Study site

Pohnpei is the largest (335 km^2) and highest (~800 m) island in the FSM. Pohnpei has persistent warm, wet conditions year-round, with an average annual temperature of 27.2 °C, and annual precipitation of 5.03 m (Lander and Khosrowpana 2004). The island is divided into five municipalities: Madolenihm (eastern), U (north-eastern), Kitti (southern), Nett (northern) and Sokehs (western).

Fabaceae, Malvaceae and Melastomataceae were selected for study (Fig. 1) because each family has both native and naturalized species on Pohnpei. The majority of the flowers were sampled in the understory of forests in Kitti and in coastal areas of Nett. Nativity status (native or naturalized), names and authorities were taken from Herrera *et al.* (2010) (Table 1.0).

Sexual system

Observations of floral sexuality were made in the field. Plant sexual systems were classified as either hermaphroditic (bisexual flowers), monoecious (male and female flowers on the same plant), dioecious (male and female flowers on separate plants) or other rare sexual systems (Tree of Sex Consortium 2014).

Herkogamy

To determine spatial potential for self-pollination, we measured anther–stigma (A-S) separation. Flowers of each species were first observed at different stages of development, so that herkogamy within each species could be measured at an equivalent stage of sexual maturity. Fabaceae has several flower types that are morphologically distinct: in some species, the keel completely encloses the anthers and stigma, but in others the wings and keel do not completely enclose anthers. For species with complete enclosure by wings and keels, we collected different sizes of flowers in the field, transported them to the lab and dissected them to determine the size at which flowers displayed dehiscing anthers and receptive stigmas. Anther–stigma separation was measured as the minimum distance from the rim of the stigma to either, the top of anthers (if between ovary and stigma), to the bottom of anthers (if further from the ovary than the stigma) or to the middle of the anther if stigmas and anthers were at the same height but not completely touching (Medrano *et al.* 2005). Distances were evaluated in terms of the potential for stigma–anther contact and self-pollination, with positive values indicating stigmas extended significantly further than the anthers, and negative values the converse (Webb and Lloyd 1986; Opedal 2018). Significant A-S separation was determined using 95% confidence intervals, assuming a normal distribution.

Dichogamy

The duration of the period separating the presentation of the pollen and receptivity of stigmatic surfaces varies widely between species (Endress 1994). Plants can either have complete dichogamy, where there is no overlap in the presentation of the pollen and stigma receptivity, or incomplete dichogamy, where differences in timing occur, but with some overlap. In protandry, anther dehiscence begins prior to stigma receptivity, and in protogyny, stigma receptivity begins prior to anther dehiscence (Lloyd and Webb 1986; Bertin and Newman 1993).

To determine temporal potential for self-fertilization the timing and duration of floral sexual stages was recorded (closed bud, open flower, anthers undehisced, anthers dehisced and beginning and end of stigma receptivity). Receptivity was determined by observing stigma colour and/or stickiness (Kubitzki and Kurz 1984). Buds of different sizes were dissected to determine at which stage stigmas shifted from being green and dry (non-receptive) to sticky or wet with a translucent, white to neon green colour, which we scored as receptive. Older stigmas withered and became dark yellow to black (non-receptive)

Pollinator attraction and dispersal traits

Floral colour sometimes reflects the type of pollination vector (Muchhala *et al.* 2014), and we scored colour visually on living flowers (recognizing that insects may perceive colour differently). Floral size can also indicate investment in pollinator attraction and was measured as petal length (banner petal in Fabaceae) on living flowers. Floral reduction can indicate abiotic pollination and/or selfing (Wright *et al.* 2013). Presence or absence of fragrance was determined subjectively by smell on living flowers in the field.

Pollen:ovule ratios

Pollen:ovule (*P:O*) ratio provides information on breeding system and pollination vector (Cruden 1977). A rough guide, based mostly on mainland species (Cruden 1977), was followed: values < 6 indicate cleistogamy, 6–30 obligate autogamy, 31–180 facultative autogamy, 181–884 facultative outcrossing and >885 obligate outcrossing. Intermediate values were be interpreted in context with other factors, since they are more difficult to interpret in isolation (Cruden 1977; Michalski and Durka 2009). Pollen:ovule ratio was measured using methods from Cruden (1977). Four flower buds per species for members of all three families were sampled in the field and stored in 70 % ethanol. In the lab, four anthers per bud were counted for Fabaceae and Melastomataceae and 10 for Malvaceae. The 10 anthers for Malvaceae were sampled evenly from the bottom-most to the top-most anther. All anthers were placed on microscope slides and crushed with cover slips. Some Malvaceae pollen grains were clumped together, so detergent was used to separate clumps. From there, a subsample of known volume of the pollen–detergent mixture was placed on microscope slides and pollen grains were counted under the microscope. Pollen grain number per flower was calculated by scaling to the total number of anthers or

subsamples per anther for each flower. Ovaries were dissected and ovules were hand-counted [see Supporting Information—File 4]. Pollen diameter was measured along the longest equatorial axis on pollen preserved in fuschin gel [see Supporting Information—File 3] (Pacini and Hesse 2005).

Experimental hand-pollinations

Eleven species were selected for hand-pollination experiments to test for self-compatibility and pollen limitation. Four flowers on each of $N = 5$ plants were treated as follows: (i) *Hand-self*: to measure the degree of self-compatibility, pollen grains were deposited on stigmas of the same flower and bagged to exclude pollinators (Fig. 1). (ii) *Autonomous-self*: to measure unassisted, within-flower self-pollination success, flowers were bagged prior to anther dehiscence and stigma receptivity to completely exclude pollinators. (iii) *Open-pollination*: to measure extrafloral pollination success (by geitonogamy or outcrossing), flowers were emasculated prior to anther dehiscence and left unbagged for pollinators to visit. (iv) *Hand-outcross*: to measure outcross pollination success, flowers were emasculated, and stigmas were rubbed with open anthers of flowers from different plants. All treatments were visited 2–3 weeks later and developing seeds were counted. Seed set was scored as the number of developing seeds per flower [see Supporting Information—File 5].

A number of breeding system indices were calculated from relative seed set of the experimental treatments, as shown in Box 1. For ISI, negative values were set to zero. For OCI, flower width was doubled petal length for Malvaceae and Melastomataceae and wing petal length for Fabaceae. ‘Success’ in self- versus outcross comparisons was measured as seed set. Note that *OCI*, *ISI*, and *P:O* ratio range from zero (more autogamous) to one (more outcrossing), whereas AI and AF are the reverse (Box 1). Pollen limitation (*PL*) can be measured a number of different ways (Box 1). Our *PL* indices measured *pollination vector-assisted pollen limitation*, since the open-pollination treatment excluded autonomous self-pollination but allowed both geitonogamy and outcrossing. Thus, lower open seed set than either or both outcrossed or selfed seed set indicates pollen limitation due to lack of a pollination vector.

Statistical analyses

All statistical analyses were conducted in R version 3.6.2 (R Core Team 2020). We generated a phylogenetic tree by extracting species from Smith and Brown (2018) using the V.Phylo.Maker package in R with a single tip per species (Jin and Qian 2019). For each continuous trait, we tested for differences between recent colonists (species categorized as 'invasive' or 'naturalized') versus ancient colonists ('indigenous' or 'endemic' species) (as in Herrera *et al.* 2010). Values were $\log_{10}$-transformed for analysis. We used nativity status as a categorical predictor variable in phylogenetic generalized least squares (PGLS) under various models of evolution and in non-phylogenetic generalized least squares (GLS) in APE (Paradis *et al.* 2004). Models with lowest AIC_C were retained. Phylogenetic signal was assessed using Pagel's λ (Pagel 1999). To compare experimental pollinations, separate ANOVAs were done for each species, with seed number per flower as the response variable. Means were separated using Tukey's HSD test [see Supporting Information—File 1].

Results

Floral traits related to pollinator attraction and sexual function

All species had bisexual flowers. No floral trait was associated with nativity status. Floral sizes varied by family (Table 1.1), with petal length ranging from 0.5 to 29 mm in Fabaceae, and 8 to 108 mm in Malvaceae. Naturalized Melastomataceae species (*Dissotis rotundifolia* and *Melastoma malabathricum*) had longer petals than the endemic, *Astronidium ponapense,* but the difference was not significant. Petal length was not associated with nativity status (λ = 1.01; N = 17, 8; P = 0.743; see Supporting Information—File 2).

Floral colours tended to be bright—only two species had a non-showy (green) flower colour (Table 1.1)—however, fragrance was not only detected in three Fabaceae species (*Acacia auriculiformis*, *Adenanthera pavonia*, *Leucaena leucocephala*) and two Malvaceae species (*Abelmoschus moschatus* and *Commersonia bartramia*). Although we did not formally search for pollinators, during collection, only ants, grasshoppers and caterpillars were seen on the flowers, and these are classes of insects not generally known to be pollinators.

All flowers were more or less upright, and all but two species (Fabaceae) had significant A-S separation, or herkogamy. Most Fabaceae and Malvaceae had positive herkogamy (anthers positioned between ovary and stigma), whereas all Melastomataceae, one Malvaceae and six

Fabaceae had anthers extended beyond the stigma (Table 1.1). All species exhibited incomplete dichogamy: incomplete protogyny in all Melastomataceae and Fabaceae, and incomplete protandry in all Malvaceae (Table 1.1).

All three Melastomataceae had high *OCI* scores due to their wide flowers and significant, although slight, A-S separation (<0.5 mm in all) (Table 1.1). In Fabaceae, two species had very small petals (<1 mm, +0 points), two species were intermediate (+1 or 2 points) and the rest were >6 mm (+3 points). All but three scored +1 for herkogamy, but herkogamy was ≤0.5 mm in six of these, and all scored +0 for their partially overlapping female and male sexual phases. Malvaceae had the highest *OCI* scores due to their generally large flowers and strong herkogamy (all +4), though all had incomplete protandry.

P:O ratios

Pollen to ovule ratios across all species ranged from 8 to 557, with a median of 86 (Table 1.2). In Melastomataceae, flowers had c. 1600–2000 pollen grains of small size (<25 µm diameter) with many ovules (>100), resulting in very low *P:O* ratios (median *P:O* ratio = 16.4). On the other hand, Malvaceae flowers generally produced fewer numbers (80–2200) of very large pollen grains (>82 µm diameter) and fewer ovules, resulting in *P:O* ratios that ranged from 9 to 159 (median = 13.6). Fabaceae had small to medium-sized pollen grains of variable number (538–3344) and few ovules, and generally had much higher *P:O* ratios, ranging from 20 up to 557 (median 131.0). Pollen:ovule ratio was not significantly different between naturalized and indigenous species ($\lambda = 0.543$; $N = 23$; $P = 0.922$).

Ovule numbers did not differ between naturalized and indigenous species ($\lambda = 1.290$; $N = 23$; $P = 0.648$). Pollen size and pollen number also showed no significant difference for nativity status (for size, $\lambda = 0.608$; $P = 0.2713$; for number, $\lambda = 0.504$; $P = 0.377$; both $N = 23$). There was a significant negative correlation between pollen size and pollen number (log–log slope = −0.971; $\lambda = 0.503$; $N = 23$; $P = 0.001$), but that result that was not affected by nativity status ($\lambda = 0.055$; $N = 15, 8$; $P = 0.226$).

Hand-cross Pollination

Within all 11 species, the hand-self crosses produced a higher or similar number of seeds as the hand-outcrosses (1.4). In addition, all but one species produced seeds under autonomous self-

pollination. Six open-pollinated treatments produced more seeds than hand-outcross treatments, a result that could be not be due to autonomous selfing but may include geitonogamous pollinations (Table 1.3). Open-pollination seed numbers were lower than either one or both of the hand-self or outcross treatments, an indicator of self or outcross pollen limitation. All species were self-compatible, with *ISI* < 0.8, and all but two Fabaceae had *AF* values < 0.2.

Discussion

In this study, we addressed the corollary to Baker's law (Baker 1967)—if establishment on islands is more likely by self-pollinating, self-compatible species, then cross-pollination and outcrossing mechanisms in island plant communities should generally have evolved from self-fertilizing founders. The best cases for such a scenario should be found on remote oceanic islands. Thus, we tested the prediction that recently established species on Pohnpei would have high levels of selfing syndrome traits, whereas indigenous species might exhibit more diverse reproductive strategies. Instead, we found universal self-compatibility in self-crosses, and minimal differences between naturalized and indigenous species in breeding system traits. Almost all species had low *P:O* ratios and floral morphologies consistent with selfing syndromes (Cruden 1977; Sicard and Lenhard 2011; Cutter 2019). Autonomous self-pollination was also common, suggesting that the low *P:O* ratios reflect a general reliance on self-fertilization. We conclude that all 28 species have some degree of autogamy in their breeding system, which likely reflects strong selection for autogamy during early establishment and limited pathways to evolving highly outcrossed mating systems.

Floral traits as indicator of pollination systems of Pohnpei

A majority of the species studied had some pollinator attraction features, such as showy flowers with large petals and some had fragrance. Floral phenotypes may not perfectly predict specific pollinators of most plant species (Ollerton *et al.* 2015), but there are broad and consistent sets of traits that differentiate outcrossed animal-, wind- and self-pollinated flowers. Animal-pollinated flowers usually display bright colours, large showy petals, and strong fragrance or pollen rewards that attract potential pollinators such as bats, birds and insects (Stroo 2000; Kessler *et al.* 2008; Muchhala *et al.* 2014; Edger *et al.* 2015). Abiotic pollinated flowers are almost the exact opposite: non-showy flowers, no fragrance and small petals (Culley *et al.* 2002; Friedman

and Barrett 2009), but with many pollen grains. Habitually self-pollinated flowers are also typically less attractive and smaller (Hetherington-Rauth and Johnson 2020), but with few pollen grains, incomplete dichogamy and lack of herkogamy.

There were few signs of wind pollination on Pohnpei. In Malvaceae, pollen grains were 83–163 µm diameter, which is much larger than the 17–58 µm range seen in most wind-pollinated species (Friedman and Barrett 2009). In Melastomataceae and Fabaceae, pollen production was strikingly low and no species had fewer than six ovules per flower. Wind-pollinated species tend to produce high amounts of pollen, and one or only a few ovules per flower (Friedman and Barrett 2009). Furthermore, anthers of Melastomataceae are poricidal, which is typical of animal pollination. Finally, species with the highest *P:O* ratios were inconsistent with wind pollination—they had either high floral attraction scores (e.g. *Aeschynome*, *Derris*, *Vigna*), or strong indicators of obligate autogamy (*Abrus*), or both (*Dendrolobium*, *Hibiscus tiliaceous*, *Sida rhombifolia*, *Vigna marina*) (Table 1.4). Thus, the incomplete and variable retention of features like showy petals, large pollen grains and floral fragrance in a few species suggests either occasional animal pollination or the retention of ancestral outcrossing characters.

Floral traits as indicators of breeding systems of plants of Pohnpei

Flowers of all species were bisexual, which allows the potential for intrafloral, autonomous self-pollination. Melastomataceae and Fabaceae flowers had incomplete protogyny, which is usually associated with outcrossing species that have delayed selfing (Webb and Lloyd 1986; Goodwillie and Weber 2018), a good mechanism for reproductive assurance, since extrafloral pollination can occur prior to intrafloral selfing. All Malvaceae had incomplete protandry. Protandry is much more common than protogyny (Bertin and Newman 1993) and is thought to be a better solution to the problem of interference from self-pollen on the stigma when outcrossing is common (Lloyd *et al.* 1980; Lloyd and Webb 1986). In incomplete protandry the onset of stigma receptivity occurs before the pollen presentation period has ended. However, some Malvaceae species had reproductive assurance via delayed selfing despite incomplete protrandry, since the stigmas eventually curved over and touched still dehiscent anthers (*Sida acuta* and *S. rhombifolia*).

Flowers of all Melastomataceae and six (of 19 species) Fabaceae exhibited ‘reverse herkogamy’ in which a pollinator would contact the anther before the stigma. The converse, ‘approach herkogamy’ was more common, and is often seen in self-compatible species with more specialized pollination systems (Opedal 2018). Most species had low herkogamy: A-S distance was ≤ 1 mm in 18 species, whereas only two Malvids had A-S separation ≥ 3 mm. Given the partial overlap between anther dehiscence and stigma receptivity in all species, these results suggest substantial potential for selfing.

Pollination efficiency based on P:O ratios

Pollen to ovule ratios are a good indicator of long-term pollination conditions—extremely low *P:O* ratios reflect highly efficient pollination systems such as in autogamous systems, whereas extremely high *P:O* ratios are typical of more inefficient systems such as with high outcrossing, wind pollination or unpredictable pollination environments (Cruden 1977). Based on *P:O* ratio alone, all of our species were fully or partially autogamous (Table 1.4). Values in all three Melastomes are consistent with cleistogamy (*P:O* ratio < 30), and both cleistogamous and chasmogamous flowers were seen in all three species in the field. A survey of neotropical mainland Melastomataceae by Santos *et al.* (2012) found 80 % of species were either self-compatible or apomictic. We did not distinguish apomixis from cleistogamy (Uphof 1938); however, the high number of ovules (100+) in all three species is consistent with pollinator limitation (Burd *et al.* 2009; Burns *et al.* 2019).

In Fabaceae, 84% of species had *P:O* ratios between 30 and 189, values in the facultative autogamy range (Cruden 1977). Floral morphology of most legume species includes a keel that fully covers the stigma and anthers, and this special structure favours self-pollination (Agbagwa and Obute 2007) and the evolution of cleistogamy. In the field, we saw that the keel and wings completely enclosed reproductive organs in most species, and handling the flowers while anthers were dehiscing usually caused pollen grains to fall on the stigma or keels. Only *Senna obtusifolia* could be considered fully autogamous (*P:O* ratio of 20), and only *Derris trifoliata* (an indigenous species) had a *P:O* ratio (557) that even approaches facultative outcrossing. In Malvaceae, four of six species had *P:O* ratios consistent with autogamy, and the other two with facultative autogamy (both self-compatible).

A general pattern was for species to have many ovules and few pollen grains per flower. Animal-pollinated and wind-outcrossed flowers usually have on the order of tens of thousands of pollen grains per flower, whereas the maximum on Pohnpei was <3500. These general results point to the importance of investing in many ovules for rare cross-pollination events (Burd *et al.* 2009), while at the same time, the chronic pollinator limitation relaxes selection on pollen number. Thus, low *P:O* ratios here are likely a consequence of both pollinator uncertainty and long-term reproduction by self-fertilization. We did find evidence that pollen number evolution is constrained by a trade-off with pollen size. The high dependence on self-fertilization and the pollen size/number trade-off suggests that the high phylogenetic signal and lack of significant differences between ancient and recent colonizers for pollen and ovule traits is due to a lack of opportunity to evolve highly outcrossed mating systems in these species.

Self-compatibility, autofertility and pollen limitation of Pohnpei species

Hand-pollinations showed that all 11 study species were self-compatible, and 10 produced seed by autonomous selfing. Based on the high autogamy and autofertility indices, and the low *P:O* ratios and self-incompatibility indices, we concluded that nine species had facultatively or generally autogamous breeding systems and the other two were generally mixed-mating (Table 1.4). No species had all indicators point to outcrossing. For instance, *Crotalaria pallida* set more seeds with hand-self- than with open-pollination and so is pollen-limited by both geitonogamy and outcrossing, yet its *P:O* ratio of 87 and high *OCI* suggests that it retains some investment in outcross or geitonogamous pollination traits.

Of the four indigenous species, *Dendrolobium umbellatum* and *Vigna marina* had relatively high OCI and *P:O* ratios, suggesting some degree of outcrossing, yet even these had ISI, AI and AF values more consistent with autogamy. Both were also pollen-limited. In 6 of 11 species, open-pollinated flowers produced higher seed yields than hand outcrosses (Table 1.4), which could be due to geitonogamy or higher self- than outcross pollen viability. Flowers were emasculated in both open and outcross treatments, so differential handling effects should not affect this result. However, outcross pollen might have experienced greater viability loss during transfer of anthers (plants were within 500–1500 m of each other), relative to open-pollination, or to self-pollinations, in which anthers were taken from the same flower (further confirmation of incomplete dichogamy).

Conclusions

Plant communities on small, isolated oceanic islands represent a best-case scenario for the general predictions of Baker's law—that founders are few to one, mate and pollinator limitation is extreme and therefore that the establishment filter strongly selects against colonists with obligate outcrossing mechanisms. Baker (1967) indicated that a finding of a 'high proportion' of enforced outcrossing among recently established island species would be inconsistent with Baker's law. Among 19 species considered to have become naturalized on Pohnpei in human historical times, we found no evidence of regular outcrossing in any. Furthermore, if Baker's law is true, most outcrossing species on islands will have evolved from self-compatible founders (Baker 1967), via changes in floral function that favour outcrossing or by transitions to obligate outcrossing by dioecy, but not to self-incompatibility (since *de novo* origin of SI is exceptionally rare). Our sample of nine indigenous species did not differ in breeding system traits from their naturalized relatives. There was a universal ability to self-fertilize coupled with low *P:O* ratios, but also a diversity of individual pollinator attraction mechanisms. Thus, for the families we studied, our data are consistent with both Baker's law and its 1967 corollary—early establishment involved self-fertilized mating patterns, whereas long-term persistence involved retention of the ability to self and a mosaic of new and old floral mechanisms that incompletely promote pollinator attraction and cross-pollination.

Acknowledgments

We are grateful to Iakop Ioanis (Conservation Society of Pohnpei), Jerry Route, Ratul Yomai, Peipei Ikiuo and all the field assistants in Pohnpei and Chuuk. We would also like to thank Dana Lee Ling for advice and access to the College of Micronesia National Campus' lab and herbarium, and Dr. Donald Buden for directing us to offices with field resources. We also thank Dr. Jessica Budke and Margaret Oliver for editing a draft of this manuscript.

Data statement

All data and script for analyses are available as Supplementary files at AoBP (https://academic.oup.com/aobpla/article/13/4/plab038/6307697?login=true#supplementary-data)

References

Agbagwa IO, Obute GC. **2007**. Breeding system and pollination ecology of two Abrus species (Fabaceae) from tropical West Africa. *Acta Botanica Croatia* **66**: 205–216.

Anderson GJ, Bernardello G, Stuessy TF, Crawford DJ. **2001**. Breeding system and pollination of selected plants endemic to Juan Fernández Islands. *American Journal of Botany* **88**: 220–233.

Arroyo MTK, Squeo F. **2012**. Relationship between Plant Breeding Systems and Pollination In: *Biological Approaches and Evolutionary Trends in Plants*.205–227.

Baker H. 1955. Self-Compatibility and Establishment After ' Long-Distance ' Dispersal. *Society for the Study of Evolution* **9**: 347–349.

Barrett Spencer C.H. 1996. The Reproductive Biology and Genetics of Island Plants. *Philosophical Transactions of the Royal Society B: Biological Sciences* **351**: 725–733.

Barrett Spencer C H. **1996**. The reproductive biology and genetics of island plants - Discussion. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **351**: 725–733.

Bartish I V., Antonelli A, Richardson JE, Swenson U. **2011**. Vicariance or long-distance dispersal: Historical biogeography of the pantropical subfamily Chrysophylloideae (Sapotaceae). *Journal of Biogeography* **38**: 177–190.

Bernardello G, Galetto L, Anderson GJ. **2006**. The angiosperm flora of Archipelago Juan Fernandez (Chile): origin and dispersal. *Canadian Journal of Botany* **78**: 862–871.

Bramwell D, Caujape-Castells J (Eds.). **2011**. *The Biology of Island Floras*. Cambridge: Cambridge University Press.

Busch JW. **2005**. Evolution of Self - Compatibility in Geographically Peripheral Populations of Leavenworthia alabamica (Brassicaceae). *American Journal of Botany* **92**: 1503–1512.

Carlquist S. **1974**. Island Biology : We ' ve Only Just Begun. *Bioscience* **22**: 221–225.

Carta JA, Ramírez P, Velázquez S. **2009**. A review of wind speed probability distributions used in wind energy analysis. Case studies in the Canary Islands. *Renewable and Sustainable Energy Reviews*.

Costion CM, Lorence DH. **2012**. The Endemic Plants of Micronesia: A Geographical Checklist and Commentary. *Micronesia* **43**: 51–100.

Crawford DJ, Anderson GJ, Bernardello G. **2011**. The reproductive biology of island plants.

The Biology of Island Floras: 11–36.

Cruden RW. **1977**. Pollen-Ovule Ratios : A Conservative Indicator of Breeding Systems in Flowering Plants. *Society for the Study of Evolution* **31**: 32–46.

Culley TM, Weller SG, Sakai AK, Culley TM, Weller SG, Sakai AK. **2002**. The evolution of wind pollination in angiosperms. *Trends in Ecology and Evolution* **17**: 361–369.

Cutter AD. **2019**. Reproductive transitions in plants and animals: selfing syndrome, sexual selection and speciation. *New Phytologist* **224**: 1080–1094.

Dafni A. **1992**. *Pollination Ecology: A Practical Appraoch*. Oxford University Press.

Darwin, C., & Wallace, A. 1858. On the tendency of species to form varieties; and on the perpetuation of varieties and species by natural means of selection. *Journal of the proceedings of the Linnean Society of London. Zoology*, **3**: 45-62.

Eckert CG, Samis KE, Dart S. **2006**. Reproductive assurance and the evolution of uniparental reproduction in flowering plants In: *Ecology and Evolution of Flowers*.183–203.

Edger PP, Heidel-Fischer HM, Bekaert M, *et al.* 2015. The butterfly plant arms-race escalated by gene and genome duplications. *Proceedings of the National Academy of Sciences* **112**: 8362–8366.

Endress PK. **1994**. Diversity and evolutionary biology of tropical flowers. *Trends in Ecology and Evolution* **10**: 511.

Faegri K, Van Der Pijl L. **2013**. *Principles of Pollination Ecology*. Pergamon Press Ltd.

Falanruw M. **2002**. *Terrestrial Biodiversity of the Federated States of Micronesia. FSM National Biodiversity Strategy and Action Plan Project.*

Fosberg FR, Sachet M-H. **1975**. Flora of Micronesia, 1: Gymnospermae. *Smithsonian Contributions to Botany*: 1–15.

Friedman J, Barrett SCH. **2009**. Wind of change: New insights on the ecology and evolution of pollination and mating in wind-pollinated plants. *Annals of Botany* **103**: 1515–1527.

Goldberg EE, Igic B. **2012**. Tempo and mode in plant breeding system evolution. *Evolution* **66**: 3701–3709.

Goodwillie C, Weber JJ. **2018**. The best of both worlds? A review of delayed selfing in flowering plants. *American Journal of Botany* **2**: 16–19.

Gotzenberger L. **2008**. Comparative Biology of the Pollen-Ovule Ratio.

Gribel R, Gibbs PE, Queiróz AL. **1999**. Flowering phenology and pollination biology of Ceiba

pentandra (Bombacaceae) in Central Amazonia. *Journal of Tropical Ecology* **15**: S0266467499000796.

Grossenbacher DL, Brandvain Y, Auld JR, *et al.* 2017. Self-compatibility is over-represented on islands. *New Phytologist*: 11.

Hetherington-Rauth MC, Johnson MTJ. **2020**. Floral Trait Evolution of Angiosperms on Pacific Islands. *The American Naturalist* **196**: 000–000.

Keating BH, Mattey DP, Helsley CE, *et al.* 1984. Evidence for a hot spot origin of the Caroline Islands. *Journal of Geophysical Research* **89**: 9937–9948.

Kessler D, Gase K, Baldwin IT. **2008**. Field experiments with transformed plants reveal the sense of floral scents. *Science*.

Kissling J, Barrett SCH. **2013**. Variation and evolution of herkogamy in Exochaenium (Gentianaceae): Implications for the evolution of distyly. *Annals of Botany* **112**: 95–102.

Kubitzki K, Kurz H. **1984**. Synchronized Dichogamy and Dioecy in Neotropical Lauraceae. *Plant Systematics and Evolution* **147**: 253–266.

Lewis WH. **1986**. Airborne pollen of the Neotropics:Potential roles in pollination and pollinosis. *Grana* **25**: 75–83.

Lloyd DG, Webb CJ, Primack RB. **1980**. Sexual Strategies in Plants II. Data on the temporal regulation of maternal investment. *New Phytologist* **86**: 81–92.

Lobo AJ, Quesada M, Stoner EK. **2005**. Effects of pollination by bats on the mating system of Ceiba pentandra (Bombaceae) populations in two tropical life zones in costa rica. *American Journal of Botany* **92**: 370–376.

Lord JM. **2015**. Patterns in floral traits and plant breeding systems on Southern Ocean Islands. *AoB Plants* **7**: 1–11.

Lozada-Gobilard S, Weigend M, Fischer E, Janssens SB, Ackermann M, Abrahamczyk S. **2019**. Breeding systems in Balsaminaceae in relation to pollen/ovule ratio, pollination syndromes, life history and climate zone. *Plant Biology* **21**: 157–166.

Lyons EE, Antonovics J. **1991**. Breeding System Evolution in Leavenworthia - Breeding System Variation and Reproductive Success in Natural-Populations of Leavenworthia-Crassa (Cruciferae). *American Journal of Botany* **78**: 270–287.

MacArthur RH, Wilson EO. **1967**. Theory of Island Biogeography. *Princeton University Press, New Jersey*.

Machado IC, Lopes AV, Sazima M. **2006**. Plant sexual systems and a review of the breeding system studies in the Caatinga, a Brazilian tropical dry forest. *Annals of Botany* **97**: 277–287.

Medrano M, Herrera CM, Barrett SCH. **2005**. Herkogamy and mating patterns in the self-compatible daffodil Narcissus longispathus. *Annals of Botany* **95**: 1105–1111.

Michalski SG, Durka W. **2009**. Pollination mode and life form strongly affect the relation between mating system and pollen to ovule ratios. *New Phytologist* **183**: 470–479.

Muchhala N, Johnsen S, Smith SD. **2014**. Competition for hummingbird pollination shapes flower color variation in andean solanaceae. *Evolution* **68**: 2275–2286.

Neal PR, Anderson GJ. **2005**. Are "mating systems" "breeding systems" of inconsistent and confusing terminology in plant reproductive biology? Or is it the other way around? *Plant Systematics and Evolution* **250**: 173–185.

Olesen JM, Jordano P. **2002**. Geographic patterns in plant-pollinator mutualistic networks. *Ecology* **83**: 2416–2424.

Ollerton J, Alarcon R, Waser NM, *et al.* 2009. A global test of the pollination syndrome hypothesis. *Annals of Botany*.

Ollerton J, Rech AR, Waser NM, Price M V. 2015. Using the literature to test pollination syndromes-Some methodological caustions. *Journal of Pollination Ecology*.

Opedal ØH. **2018**. Herkogamy, a Principal Functional Trait of Plant Reproductive Biology. *International Journal of Plant Sciences* **179**: 667–687.

Pacini E, Hesse M. **2005**. Pollenkitt - Its composition, forms and functions. *Flora: Morphology, Distribution, Functional Ecology of Plants*: 399–415.

Pannell JR, Auld JR, Brandvain Y, *et al.* 2015. The scope of Baker's law. *New Phytologist* **208**: 656–667.

De Queiroz A. **2005**. The resurrection of oceanic dispersal in historical biogeography. *Trends in Ecology and Evolution* **20**: 68–73.

Ramírez, N., & Hokche, O. 2019. Outbreeding and inbreeding strategies in herbaceous-shrubby communities in the Venezuelan Gran Sabana Plateau. *AoB Plants*, **11:** plz032.

Razanajatovo M, van Kleunen M, Kreft H, *et al.* 2019. Autofertility and self-compatibility moderately benefit island colonization of plants. *Global Ecology and Biogeography* **28**: 341–352.

Rech AR, Dalsgaard B, Sandel B, *et al.* 2016. The macroecology of animal versus wind

pollination: ecological factors are more important than historical climate stability. *Plant Ecology and Diversity*.

Rehman H, Nakaya H, Kawai K. **2013**. Geological origin of the volcanic islands of the Caroline group in the Federated States of Micronesia, western Pacific. *South Pacific Studies* **33**: 101–118.

Renner SS. **1989**. A Survey of Reproductive Biology in Neotropical Melastomataceae and Memecylaceae. *Annals of the Missouri Botanical Garden* **76**: 496.

Robert BI, Newman CM. **1993**. Dichogamy in Angiospems. *Botanical Review* **59**: 112–152.

dos Santos APM, Romero R, Oliveira PE, Fracasso CM, Luciene dos Santos M, Sazima M. **2012**. Reproductive biology and species geographical distribution in the Melastomataceae: a survey based on New World taxa. *Annals of botany* **110**: 667–679.

Schoen DJ, Lloyd DG. **1992**. Self- and Cross-Fertilization in Plants. III. Methods for Studying Modes and Functional Aspects of Self-Fertilization. *International Journal of Plant Sciences* **153**: 381–393.

Schueller SK. **2004**. Self-pollination in island and mainland populations of the introduced hummingbird-pollinated plant, Nicotiana glauca (Solanaceae). *American Journal of Botany* **91**: 672–681.

Sicard A, Lenhard M. **2011**. The selfing syndrome : a model for studying the genetic and evolutionary basis of morphological adaptation in plants. **107**: 1433–1443.

Stout AB. **1928**. Dichogamy in Flowering Plants. *Torrey Botanical Society* **55**: 141–153.

Stroo A. **2000**. Pollen morphological evolution in bat pollinated plants. *Plant Systematics and Evolution* **222**: 225–242.

Tedder A, Carleial S, Go M, Kappel C. **2015**. Evolution of the Selfing Syndrome in Arabis alpina (Brassicaceae). *PLoS ONE*: 1–17.

Traveset A, Heleno R, Willson M. **2014**. The ecology of seed dispersal. *in CAB International 2014. Seeds: the ecology of regeneration in plant communities. 3rd Edition (ed. R.S. Gallagher)*.

Trusty JL, Kesler HC, Rodríguez J, Francisco-Ortega J. **2011**. Conservation status of endemic plants on Isla del Coco, Costa Rica: Applying IUCN Red List criteria on a small island In: *The Biology of Island Floras*.

Uphof JCT. **1938**. Cleistogamic flowers. *The Botanical Review* **4**: 21–49.

Webb CJ, Lloyd DG. **1986a**. The avoidance of interference between the presentation of pollen

and stigmas in angiosperms II. Herkogamy. *New Zealand Journal of Botany* **24**: 163–178.

Webb CJ, Lloyd DG. **1986b**. The avoidance of interference between the presentation of pollen and stigmas in angiosperms I Dichogamy. *New Zealand Journal of Botany* **24**: 163–178.

Wortel OL. **2009**. *Federated States of Micronesia Fourth National Report*.

Wright SI, Kalisz S, Slotte T. **2013**. Evolutionary consequences of self-fertilization in plants. *Proceedings of The Royal Society B* **280**: 1–10.

Yorkston M. **2005**. Experimental hybridization between mountain and coastal forms of Sida fallax walp., and between S. fallax and S. rhombifolia L. (Malvaceae).

Chapter 2 – Self-compatible plants on Pohnpei self-pollinate more than their outcrossing mainland relatives

Abstract

Plant communities on isolated, oceanic islands are expected to be dominated by species that are hermaphroditic, self-compatible, and that have the capacity for autonomous self-pollination. If so, it seems likely that such species are descended from species in mainland source communities that already had the capacity for self-fertilization. Alternatively, shifts in breeding systems may occur after dispersal, during the establishment phase. In this study, we sought to determine the degree to which island species have diverged from their mainland conspecific or nearest-relatives in their breeding and sexual systems. We performed hand-pollinations on 36 flowering plant species on Pohnpei, Federated States of Micronesia to characterize sexual systems and the degree of self-compatibility, autonomous self-fertilization, and outcross/geitonogamous pollen limitation. We then made comparisons to their nearest continental mainland phylogenetic relatives taken from the literature. All 36 Pohnpeian species and all but three of their mainland relatives were self-compatible. All Pohnpeian species were characterized as hermaphroditic, whereas all mainland counterparts had bisexual or rarely, some combination of bi- and/or uni-sexual, flowers on the same plant. Phylogenetic least squares analyses found that the self-compatibility index (SCI-F, based on fruit set) and autofertility index (AFI-S, based on seed set) were 27% ($P = 0.0015$) and 42% ($P < 0.0001$) higher in Pohnpeian than mainland species, respectively ($N = 33$ pairs). A Wilcoxon signed-rank test found that inferred breeding systems on Pohnpei were significantly more autogamous than their mainland counterparts ($P = 0.0251$; $N = 33$ pairs). Most Pohnpeian species also displayed high pollen limitation indices. These results suggest that present-day Pohnpei island plant species are most often descended from mixed mating to outcrossing mainland species that are self-compatible and have bisexual flowers. We conclude that successful colonists of this remote oceanic island arrive with the capacity to self-pollinate and subsequently evolve higher levels of self-compatibility and autonomous selfing rates during establishment.

Key words: Breeding system, Micronesia, sexual system, self-compatibility.

Introduction

The distribution of species on oceanic islands reflects the success of both long-distance dispersal and population establishment (MacArthur and Wilson 1967), as well as the duration of population persistence. Newly established island populations should generally persist, and evolve, for longer periods on larger than on smaller islands (MacArthur and Wilson 1967). Although identifying the specific determinants that form a successful colonization is difficult, geographical patterns in species traits can provide valuable insights into determinants of successful colonization. Baker (1955) suggested that species with uniparental reproduction should be more successful in establishing a population in a novel environment after a long-distance dispersal, a pattern now known as Baker's Law. When plant propagules arrive on a remote island, the resulting plants are isolated from their mainland source populations, and mates and pollinators are likely to be limited. In this instance, self-compatible, hermaphroditic species should have an advantage because they can reproduce from a single individual (Pannell *et al.* 2015), and they do not rely on a second or third party. In fact, many studies have shown high numbers of self-compatible, hermaphroditic plant species on islands (Crawford *et al.* 2011; Chamorro *et al.* 2012; Lord 2015). Furthermore, animal pollinator species are less diverse, and often at lower densities (Kawakami and Higuchi, 2002), on islands due largely to the introduction of more invasive species (Kawakami and Okichi, 2010) on islands compared to mainlands.

Mainland plants that can self-pollinate or be pollinated by wind should be common colonizers of islands (Barrett 1996). Cases of dioecy on large islands were initially attributed to in situ evolution after colonization in response to inbreeding, since obligate outcrossers are unlikely founders since they would have no mates (Baker (1955). However, this became questionable when it was found that some oceanic islands had unusually high levels of dioecy (Carlquist 1966), which could indicate dispersal of already dioecious species to islands. Subsequent studies sometimes find relatively high frequencies of self-incompatible (SI) and dioecious plants on some larger oceanic islands (Cássia-Silva *et al.* 2021), such as New Caledonia (Schlessman *et al.,* 2014) and Hawaii (Gilmartin 1968; Sakai *et al.* 1995). Baker (1967) clarified that even if self-compatible hermaphrodites are favored at the initial stages of colonization, mechanisms that favor outcrossing should evolve over time in response to inbreeding depression (Pannell *et al.* 2015). Obligate outcrossing colonizers would be more

likely to establish on near-mainland than on remote islands, and more likely to evolve in situ on large than on small islands. A recent test of this hypothesis on a small, remote island, found no significant differences in breeding system traits, such as pollen-ovule ratios, between recently-arrived species (invasive or naturalized species) and more ancient island colonists (endemic, or indigenous species) (Yomai and Williams 2021). All had self-compatibility and more or less highly autogamous breeding systems, an indication of the value of reproductive assurance on islands (Yamamoto et al. 2018), but perhaps also that rapid species turnover occurs before strict outcrossing mechanisms can evolve.

The Federated States of Micronesia (FSM), one of the oldest and most isolated groups of oceanic islands, is home to thousands of endemic species (Costion and Lorence 2012). The capital of the FSM is on the largest and oldest island in the archipelago, Pohnpei. Yomai and Williams (2021) found that 28 plant species in Fabaceae, Melastomataceae, and Malvaceae, all had bisexual, mostly small, flowers, with self-compatibility and very low pollen-ovule ratios (ranging from 8-557), which are all indicators of more or less autogamous breeding systems. If the advantage of self-pollination and self-fertilization in tropical island forests is so great, then a separate question arises. Are remote oceanic island colonists likely to have arrived with highly self-fertile breeding systems, or do some evolve it rapidly after establishment?

It seems obvious that species on remote islands should generally represent species drawn from mainland populations that already have selfing syndrome traits. Yet few studies of island floras have attempted to distinguish whether shifts in sexual or breeding systems occur before or after colonization. Much of the flora of Pohnpei is thought to have originated from southeast Asia, following ocean currents (Ragone, Lorence, and Flynn 2000, Tomizawa et al., 2017; Yamamoto et al. 2018). Because Pohnpei is relatively old and isolated, it is possible that its flora has been around long enough for evolutionary shifts in reproduction. There have been large-scale studies that compare breeding systems of island plants to global breeding systems (Grossenbacher et al., 2017; Razanajatovo *et al.*, 2019). A more powerful approach would be to compare individual island species or populations to closely-related species or populations on continental mainlands, pair by pair. Such an approach would allow a more specific comparison of island species to their progenitor-like mainland relatives, and hence a better characterization of determinants of successful long-distance colonization.

Here we report field hand-pollination experiments of 36 species of flowering plants from Pohnpei island that quantified levels of self-compatibility (SCI) and autonomous self-pollination and fertilization (autofertility index; AFI). We also characterized sexual systems. We then compare these to continental mainland conspecifics or nearest-relatives, as determined by phylogenetic analysis. We aim to answer two questions: 1) to what extent have Pohnpei island and mainland relative pairs of plant taxa diverged in their breeding systems, and 2) have Pohnpei island and mainland near-relatives diverged in their sexual systems?

Methods

Pohnpei-Mainland Comparisons

As a general overview of our approach, we first consulted the list of flowering plants on Pohnpei compiled by Herrera *et al.* (2010), excluding cultivated species. We then searched for published information on breeding and/or sexual systems on a continental mainland that matched any of these same species or related species at the genus level. Here we define mainland as any continental landmass or any landmass that was once connected to a continent. In this case, New Zealand, Papua New Guinea, Australia, and some landmasses that would be considered "islands" are mainlands in this study. A total of 36 species from Pohnpei were selected based on the availability of breeding system information for mainland relatives.

When multiple estimates of mainland breeding/sexual system values for a single taxon were found, we calculated the mean across all estimates. Each island species was compared to its nearest mainland relative, either a conspecific population or a phylogenetic sister species/clade. When there were multiple mainland species within an island genus, we used two strategies: 1) we compared island species to the mean of its mainland genus; or 2) we employed Phylotastic (Stoltzfus, Lapp, Matasci, et al., 2013) to choose the nearest phylogenetic relative of each island species.

All species names were cross-checked for synonymy, taxonomic validity, and authority using The Plant List (2013). Information on breeding systems of mainland conspecifics/near-relatives was obtained from continental eFloras such as World Flora Online, 2021, Flora of China, and via keyword searches on Google Scholar, Web of Science, and other internet search engines for journal articles, reports, discussions, and dissertations using genus, species, and

keywords such as “Breeding System,” “Reproductive biology,” “Self-compatibility,” “Sexual system,” and so forth.

Sexual system

Following methods detailed in Lord (2015), plant sexual systems were classified as either hermaphroditic (bisexual flowers), monoecious (male and female flowers on the same plant), dioecious (unisexual male or female flowers on separate plants), or other rare sexual systems (Goldberg et al., 2017). Sexual systems were determined in the field for Pohnpeian species. Information on mainland species was extracted from databases or the literature. We only recognized “Hermaphroditic” sexual systems when there was verification that bisexual flowers typically had fully functional male and female organs (eg. andromonoecy etc.).

Experimental – Hand-pollination to determine self-compatibility

Hand-pollination experiments were conducted on 26 Pohnpeian species, as in Yomai and Williams (2021). Briefly, for each species, 5 plants were selected and 4 flowers on each were treated as follows. 1) ‘Hand-self’, bagged, supplemental hand self-pollination; 2) ‘Autonomous-self’, bagged and unmanipulated; 3) ‘Open-pollination’, emasculated, no bag; ‘Hand-outcross’, emasculated, hand cross-pollinated. An additional 11 species were included from Yomai and Williams (2021). In all 36 species, fruit set and seed set were measured as the respective number of fruits or seeds set per $N = 5$ flowers (1 per each of 5 plants).

Breeding system indices were calculated as follows. The self-compatibility index was: SCI = *hand-self success /hand-outcross success,* where success was measured as fruit set and/or seed set. Fruit and seed set were also used to calculate autofertility indices: AFI = *autonomous self-success/hand self-success* (Schoen and Lloyd, 1992). Maximum values of both indices indicate high levels of self-pollen fruit or seed set. A pollen limitation index was also calculated for island species as: PL = 1 − (open-pollination success/supplemental pollination success; where supplemental pollination was with either self or outcross pollen (see Yomai and Williams 2021). Since open-pollination flowers were emasculated, higher values of PL indicate pollen limitation by lack of a pollination vector for either outcross or geitonogamous self-pollen transfer.

Breeding systems were ranked into five general breeding systems based on the pattern of seed set differences between manipulated versus unmanipulated experiments (Supplementary

Table 1), as also outlined in Dafni (1992). Breeding system indices and characterizations were compared to mainland breeding systems as characterized in the literature.

Statistics

All statistical analyses were conducted in R version 3.6.2 (R Core Team 2020). We created a phylogenetic tree by extracting species from Smith and Brown (2018) using R package V.Phylomaker (Jin and Qian 2019). Differences between island and mainland breeding system indices were assessed by phylogenetic generalized least squares regression (PGLS) using the APE package (Paradis *et al.* 2004), under different evolutionary models. These include Pagel's lambda, Blomberg's K, Brownian motion, Ornstein Uhlenbeck (using corMartins), and generalized least squares regression (GLS). Models were assessed using the lowest AIC value. Differences in breeding system ranks between island-mainland pairs were tested using a Wilcoxon signed rank test with the R package MASS (Venables and Ripley, 2002).

Results

Sexual systems

We found data on sexual system for 32 island-mainland relative pairs. All of the Pohnpei island species were hermaphroditic with functional bisexual flowers, whereas the sexual systems of mainland relatives were variable or not specifically reported (Table 2.1). Of 17 verified sexual systems, two were monoecious, two andromonoecious and 13 hermaphroditic, whereas the other studies all depicted bisexual flowers (Table 2.1).

Experimental pollinations on Pohnpei island

All 36 species on Pohnpei had high levels of seed set after hand self-pollination, when compared to open or outcross hand-pollinations (Table 2.2) and are therefore self-compatible. Based on seed set, all species had SCI values above 0.87, and all but one (*Bidens pilosa*) had 100% hand self-fruit set (data not shown).

Similarly, all but one species had non-zero levels of autonomous selfing, and 25 species set at least one autonomous seed/flower (Table 2.2). Based on seed set, most AFI values were near 1, and with the exception of two *Mimosa* species, the minimum was 0.48. 36 species had 100% autonomous self-fruit set.

Finally, emasculated open-pollinated flowers generally had lower seed set than either hand-self or hand-outcross pollinated flowers. Twenty-eight of 36 species had PL values of 0.2 or greater, evidence of pollen limitation caused by lack of pollination vector.

Mainland systems

Studies of mainland "sister" taxa comprised 9 conspecifics and 27 near-relative species (Table 2.3), as determined on a large phylogeny (Stoltzfus, Lapp, Matasci, et al., 2013). Twenty studies reported values for AFI and 23 for SCI. Since all mainland SCI values were based on fruit set and all mainland AFI were based on seed set, SCI and AFI values in Table 2.3 show fruit set, with seed set shown in parentheses.

Twenty-five of 28 mainland near-relatives (including all same-species matches) were self-compatible, based on high self-fruit set, whereas three mainland near-relatives were described as self-incompatible (SI; *Acacia pycnantha, Ipomoea asarifolia*) or at least with some SI populations (*Acacia paradoxa*)(Table 2.3).

Self-compatibility and autofertility are higher on Pohnpei than on mainlands

In the best model (Lambda in PGLS), levels of self-compatibility were significantly higher in Pohnpei island versus mainland taxa (based on fruit set, SCI-F; $P = 0.0015$) (Table 2.3). The GLS model had a similar AIC value, and a matched pair analysis found the same result: a significantly higher SCI for Pohnpei taxa (SCI = 0.92 ± 0.47) than mainland taxa (SCI = 0.50 ± 0.08) (Table 2.3).

The level of autonomous selfing, based on seed set (AFI-S), was also significantly higher in Pohnpei island versus mainland relatives, whether or not phylogeny was considered (Lambda model; $P < 0.0001$) (Table 2.4). The matched pair analysis recovered the same result: significantly higher AFI for Pohnpei than for mainland taxa (AFI = 0.95 ± 0.12 versus AFI = 0.68 ± 0.33, respectively) ($P = 0.0039$; Table 2.4).

Finally, inferred breeding systems were assigned ranks, based on our experimental work and results from the literature (Table 2.6; Supplementary Table 2.0). As shown in Figure 2.1, all but 4 of 33 island taxa have a similar or less outcrossed breeding system than mainland taxa. A Wilcoxon signed-rank test showed that inferred breeding systems on mainlands are significantly more outcrossed than those on Pohnpei ($P = 0.0251$; $N = 33$ pairs).

Discussion

In this study we investigated differences of autofertility, self-compatibility, and sexual systems between 36 Pohnpeian plant (13 indigenous, 14 naturalized, and 9 invasive) species and their continental mainland relatives. We hypothesized that most successful colonizations of remote, oceanic islands involve species that already have the capacity for autonomous self-pollination and fertilization and the ability to set inbred seeds. If source populations on mainlands were "pre-adapted" for such traits, then one prediction is that the closest mainland relatives of island species should generally have similar breeding system traits. Below we discuss our findings that show high levels of autonomous selfing in Pohnpeian plant species, and a high degree of correspondence in selfing ability between island-mainland species pairs.

Self-compatibility is prevalent among Pohnpeian species and their mainland relatives

All species on Pohnpei were SC and almost all of their continental relatives were also SC (5 mainland taxa were partially or fully self-incompatible). This result is consistent with a more general meta-analysis of "selfing ability" that found species with high SCI and AFI were slightly more likely to be island colonists than to be mainland species (Razanajatovo *et al.* 2019). Transitions from SI to SC are relatively easily accomplished (Igic, Lande, and Kohn, 2008), whereas the converse is highly unlikely (Baker, 1955; Igic et al. 2006). Three of the self-incompatible, mainland species were in the genus *Acacia. Acacia* is known to have a wide range of variation in self-incompatibility (Kenrick and Knox 1989). Most *Acacia* species are self-incompatible, but some species can be partially self-compatible. For instance, Kenrick and Knox (1989) reported *Acacia paradoxa* as partially self-compatible because their experiments found that *A. paradoxa* individuals ranged from completely self-compatible (SCI = 1) to completely self-incompatible (SCI = 0). They also noted many other *Acacia* species have a range of SCI values among individuals. This could mean that *Acacia* species arrived on Pohnpei as partially self-compatible but evolved to be completely self-compatible during establishment of a breeding population. Another prime example of the loss of self-incompatibility is that of *Leucaena leucacephala*, which occurs in an otherwise self-incompatible genus (Baker 1983), and is known to be self-incompatible in Australia (Panetta 2003), but self-compatible on Hawaii (Baker 1983) and on Pohnpei (this study).

Autonomously selfed flowers set more seeds than outcrossed flowers

Almost all species on Pohnpei had higher seed set after either autonomous or hand self-pollination than after hand-outcross or open-pollination of emasculated flowers. It could be argued that hand outcross pollinations set fewer seeds than self-pollinations because of flower handling effects or the loss of pollen viability during transport. Yet, inter-floral pollen transfer and flower handling did not prevent seed set in open-pollinated, emasculated flowers. In either case, the main result is unaffected – there were high levels of autonomous selfing relative to substantial manual self-pollinated seed set.

Island species self more than their mainland relatives

Pohnpei island species all had high levels of "selfing ability" – the capacity to self-pollinate without a pollination vector followed by the ability to set self seed. But they also had quantitatively higher levels of autonomous selfing and self-fertilization (seed set) than their more mixed-mating/outcrossing mainland counterparts. A comparison of mainland breeding systems as characterized in the literature also indicated a quantitative shift to more autogamous breeding systems on Pohnpei. Taken together then, our results suggest that Pohnpei island colonists are likely to be drawn from self-compatible mainland species with bisexual flowers, but dispersal is then followed by shifts to higher levels of self-compatibility and autonomous selfing during establishment.

Widespread capacity for autonomous self-fertilization suggests pollinator limitation was prevalent during establishment and has persisted. Thus, it was not surprising to find evidence of pollen limitation, by geitonogamy or outcrossing, in almost all species. There are not enough studies on pollinators or potential pollinators on Pohnpei. Existent studies focus mainly on the destructive effects of invasive species on native plants and animals (Buden 2009; Pelep and Hadfield, 2011).

Although, some have argued that lack of mates is a reason why island species have difficulty establishing viable populations after long-distance dispersal. All of the Pohnpeian species were self-compatible with bisexual flowers and the capacity to evolve regular self-pollination, which is consistent with their having experienced a lack of mates at some point in history. It is striking that the sexual systems of all 30 mainland counterparts of island species could be described as hermaphroditic, with either bi-sexual, or some combination of uni- and bi-

sexual, flowers. Thus, our results indicate that successful colonists of a remote oceanic island must arrive with the capacity to self-pollinate and subsequently evolve greater selfing ability.

Conclusions

Island biota typically originate from continental mainland source populations. Thus, when comparing island taxa to their nearest mainland relatives, it is much more likely that the island taxon has evolved relative to its mainland sister taxon. Our results suggest that plants on Pohnpei generally arrived as self-compatible, hermaphrodites with more outcrossed breeding systems, which then rapidly shifted towards more autogamous systems during the establishment phase of colonization. The shift to more autogamous systems was often accompanied by a shift to higher levels of autonomous self-pollination in Pohnpeian species and is consistent with our finding of pollen limitation for both geitonogamous and outcrossed modes of pollination. We did not investigate pollinator visitation and studies on the diversity and density of pollinators on Pohnpei would be really informative.

Acknowledgement

We would like to thank Jerry Route for assistance in the field, Alexandra Scearce for literature search on mainland species, and Margaret Oliver for allowing us to use specimens from the University of Tennessee, Knoxville herbarium (TENN). We also like to the Bill Raynor Scholarship Fund for funding this research.

References

Agrawal A, Erwin A, Cook S. 2008. Natural selection on and predicted responses of ecophysiological traits of swamp milkweed (*Asclepias incarnata*). *Journal of Ecology* **96:** 536-542.

Aluri JSR, Kumar R, Chappidi PR. 2016. Reproductive biology of mangrove plants Clerodendrum inerme, Derris trifoliata, Suaeda maritima, Suaeda monoica, Suaeda nudiflora. *Translyvanian Review of Systematical and Ecological Research* **18:** 31-68.

Atlas of living Australia. Published on the internet. Available from http://www.ala.org.au. (Last accessed on May 20, 2021).

Baker HG. 1955. Self-compatibility and establishment after "Long-Distance" dispersal. *Society for the Study of Evolution* **9**: 347–349.

Baker HG. **1967**. Support for Baker's law - as a rule. *Evolution* **21**: 853–856.

Baker HG, Cox PA. **1984**. Further thoughts on dioecism and islands. *Annals of the Missouri Botanical Garden* **71**: 244–253.

Barrett SCH. **1996**. The Reproductive biology and genetics of island plants. *Philosophical Transactions of the Royal Society B: Biological Sciences* **351**: 725–733.

Buden DW. **2009**. Carlia ailanpalai (Reptilia: Scincidae): An invasive species of lizard in the Federated States of Micronesia 1. *University of Hawaii Press* **63**: 243–251.

Carlquist S. **1966**. The biota of long-distance dispersal. I. Principles of dispersal and evolution. *The Quarterly review of biology* **41**: 247-270.

Cássia-Silva C, Freitas CG, Jardim L, Bacon CD, Collevatti RG. **2021**. In situ radiation explains the frequency of dioecious palms on islands. *Annals of Botany* **128**: 205-215.

Chamorro S, Heleno R, Olesen JM, McMullen CK, Traveset A. **2012**. Pollination patterns and plant breeding systems in the Galápagos: A review. *Annals of Botany* **110**: 1489–1501.

Costion CM, Lorence DH. **2012**. The endemic plants of Micronesia: A geographical checklist and commentary. *Micronesia* **43**: 51–100.

Crawford DJ, Anderson GJ, Bernardello G. **2011**. The reproductive biology of island plants. In: Bramwell D, Caujape-Castells J, eds. *The biology of island floras.* New York: Cambridge University Press, 11–36.

Dafni A. 1992. *Pollination ecology: a practical approach.* Oxford, UK: Oxford University Press.

eFloras. 2008. *Missouri Botanical Garden.* St. Louis, MO & Harvard University Herberia, Cambridge, MA. Published on the internet. Available from http://www.efloras.org. (last accessed May 20, 2021).

Etcheverry AV, Protomastro JJ, Westerkamp C. 2003. Delayed autonomous self-pollination in the colonizer *Crotolaria micans* (Fabaceae: Papilionoideae): stuctural and functional aspects. *Plant systematics and evolution* **239:** 15-28.

Gilmartin AJ. **1968**. Baker's law and dioecism in the Hawaiian flora: an apparent contradiction. *Pacific Science* **22**: 285–292.

Goldberg EE, Otto SP, Vamosi JC, Mayrose I, Sabath N, Ming R, Ashman TL. 2017. Macroevolutionary synthesis of flowering plant sexual systems. *Evolution* **71:** 898-912.

Grof B, Flores AJ, Mendoza PE, Pizarro EA. 1990. Regional experience with *Centrocema:* Northern south America. In: Shultze-Kraft R, Clements RJ. eds. *Centrocema: Biology, agronomy, and utilization.* Cali, CO: CIAT, 391-420.

Grossenbacher DL, Brandvain Y, Auld JR, Burd M, Cheptou PO, Conner JK, Grant AG, Hovick SM, Pannell JR, Pauw A, Petanidou T, Randle AM, de Casas RR, Vamosi J, Winn A, Igic B, Busch JW, Kalisz S, Goldberg EE. 2017. Self-compatibility is over-represented on islands. *New Phytologist:* **11:** 1-10.

Hamon S, Koechlin J. 1991. The reproductive biology of okara. 1. Study of the breeding system of four *Abelmoschus* species. *Euphytica* **53:** 41-48.

Herrera K, Lorence DH, Flynn T, Balick MJ. **2010**. Checklist of the vascular plants of Pohnpei, Federated States of Micronesia with local names and uses. *Allertonia* **10**: 1–192.

Igic B, Bohs L, Kohn JR. 2006. Ancient polymorphism reveals unidirectional breeding system shifts. *PNAS* **103:** 1359-1363.

Igic B, Lande R, Kohn JR. 2008. Loss of self-incompatibility and its evolutionary consequence. *Internation Journal of Plant Science* **169:** 93-104.

Jaimes I, Ramirez N. 1999. Breeding systems in a secondary deciduous forest in Venezuela: The imporatance of life form, habitat, and pollination specificity. *Plant systematics and evolution.* **215:** 23-36.

Jin Y, Qian H. **2019**. V.PhyloMaker: an R package that can generate very large phylogenies for vascular plants. *Ecography* **42**: 1353–1359.

Kawakami K, Higuchi H. 2010. Bird predation by domestic cats on Hahajima Island, Bonin

Islands, Japan. In: Kawakami K., Okochi I. eds. *Restoring the Oceanic Island Ecosystem*. Springer, Tokyo. 65-66.

Kawakami K, Okichi I, 2010. *Restoring the oceanic island ecosystem: impact and management of invasive alien species in the Bonin Islands.* Springer Science & Business Media. 11-103.

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

Lord JM. **2015**. Patterns in floral traits and plant breeding systems on Southern Ocean Islands. *AoB Plants* **7**: 1–11.

MacArthur RH, Wilson EO. **1967**. *Theory of island biogeography.* Princeton, NJ: Princeton University Press.

Olivera PE, Gibbs PE. 2000. Reproductive biology of woody plants in cerrado community of Central Brazil. *Flora* **195:** 311-329.

Ragone D, Lorence DH, Flynn T. **2001**. History of plant intorductions to Pohnpei, Micronesia and the role of the Pohnpei agriculture station. *Economic Botany* **55**: 290–324.

Razanajotovo M, van Kleunen M, Kreft H, Dawson W, Essle F, Pergl J, Pysek P, Winter M, Weigelt P. 2018. *Autofertility and self-compatibilty moderately benefit island colonization of plants.* **28:**341-352.

Pannell JR, Auld JR, Brandvain Y, Burd M, **Busch** JW, **Cheptou** PO, **Conner** JK, **Goldberg** EE, **Grant** AG, **Grossenbacher** DL, **Hovick** SM, **Igic** B, **Kalisz** S, **Petanidou** T, **Randle** AM, **de Casas** RR, **Pauw** A, **Vamosi** JC, **Winn** AA**. 2015**. The scope of Baker's law. *New Phytologist* **208**: 656–667.

Panetta FD. **2003**. The biology of Australian weeds: volume 3. *Plant Protection Quarterly* **18**: 90–98.

Paradis E, Claude J, Strimmer K. **2004**. APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**: 289–290.

Plant List. **2013**. *The Plant List. Version 1.1*. Published on the internet. Available from http://www.theplantlist.org (last accessed March 19, 2021).

Pelep PO, Hadfield MG. 2011. The status of the endemic snails of the genus *Partula* (Gastropoda: Partulidae) on Pohnpei, Federated States of Micronesia. *Micronesica* **41:** 253-262.

Ripley B, Venebles B, Bates DM, Hornik K, Gebhardt A, Firth D, Ripley MB (2013).

Package "MASS". CRAN Repository.

Sakai AK, Wagner WL, Ferguson DM, Derral HR. **1995**. Origins of dioecy in the Hawaiian flora. *Ecological Society of America* **76**: 2517–2529.

Schlessman MA, Vary LB, Munzinger J, Lowry PP. **2014**. Incidence, correlates, and origins of dioecy in the island flora of new caledonia. *International Journal of Plant Sciences* **175**: 271-286.

Schoen DJ, Lloyd DG. **1992**. Self- and Cross-Fertilization in Plants. III. Methods for Studying Modes and Functional Aspects of Self-Fertilization. *International Journal of Plant Sciences* **153**: 381–393.

Smith SA, Brown JW. **2018**. Constructing a broadly inclusive seed plant phylogeny. *American Journal of Botany* **105**: 302–314.

Stoltzfus A, Lapp H, Matasci N, Deus H, Sidlauskas B, Zmasek CM, Vaidya G, Pontelli E, Cranston K, Vos R, Webb CO, Harmon LJ, Pirrung M, O'meara B, Pennel MW, Mirarab S, Rosenberg MS, Balhoff JP, Bik HM, Heath TA, Midford PE, Brown JW, McTavish EJ, Sukumaran J, Westneat M, Alfaro ME, Steele A, Jordan G. 2013. Phylotastic! Making tree-of-life knowledge accessible, reusable and convinent. *BMC Bioinformatics* **14:** 1-17.

Tomizawa T, Tsuda Y, Saleh MZ, Wee AKS, Takayama K, Yamamoto T, Yllano OB, Salmo III SG, Sungkaew S, Adjie B, Ardli E, Suleiman M, Tung NX, Soe KK, Ardli E, Suleiman M. 2017. Genetic strucutre and population demographic history of a widespread mangrove plant *Xylocarpus granatum* J. Koenig across the Indo-West Pacific region. *Forests* **8:** 2-18.

Wanntorp L, Puglisi C, Penneys D, De-Craene LPR. 2011. Multiplications of floral organs in flowers: A case study in *Conostegia* (*Melatomataceae, Myrtales*). In: *Flowers on the Tree of Life.* Cambridge: Cambridge University Press. 218-235.

WFO. 2021. *World Flora Online.* Published on the internet. Available from http://www.worldfloraonline.org. (last accessed April 21, 2021).

Yamamamoto T, Yoshiaki T, Koji T, Reiko N, Yoichi T, Tadashi K. 2018. The presence of a cryptic barrier in the West Pacific Ocean suggests the effect of glacial climate changes on a widespread sea-dispersed plant, *Vigna marina* (Fabaceae). *Ecology and Evolution* **9:** 8429-8440.

Yomai VMH, Williams JH. 2021. Breeding systems of naturalized versus indigenous species provide support for Baker's law on Pohnpei island. *AoBP* **13:** 1-13.

Chapter 3: Evidence for relaxed selection on style length on an isolated, oceanic island

Abstract

Theory predicts that plants on oceanic islands should have smaller floral sizes than their mainland relatives, and all else being equal, these reductions should involve proportional (isometric) size reductions of all floral organs, as a consequence of developmental correlations. There is mixed evidence for flower size reduction on islands, whereas the hypothesis of isometric shifts in organ sizes has received little attention. Petal length is thought to be maintained by selection for pollinator attraction, whereas style length may be maintained by sexual selection, when pollen competition is strong. In this study, we asked three questions: 1) Do plants on the small, isolated island of Pohnpei, Micronesia have shorter style and petal lengths than mainland sister taxa? 2) Are differences in style length reflective of allometric scaling of flower size (petal length) or do they deviate, suggesting post-pollination style interaction? 3) Are floral organ sizes of each mainland sister of an island taxon similar or extreme relative to the average of its mainland genus? We collected petal length and style length data for 73 plant species in 61 genera on Pohnpei, and from 15 online flora databases and the primary literature for data on mainland taxa. We used phylogenetic generalized least squares to test for petal length and style length correlations between island and mainland relatives. Our results showed that flowers on Pohnpei were not smaller relative to their mainland sister taxa (N=50 pairs, P=0.255), but styles were significantly shorter on Pohnpei relative to their mainland sisters (N=43 pairs, P=0.005). Furthermore, there was no difference in petal or style lengths between mainland sister taxa and the mean of their mainland genus. Flower sizes changed unpredictably, but style lengths were about 40% smaller on Pohnpei island relative to mainland sister taxa. Previous work has shown that plant species on Pohnpei have shifted to more autonomous breeding systems relative to their outcrossing mainland sisters. Thus, our results are consistent with the hypothesis that relaxed sexual selection on style length due to shifts to reliance on self-fertilization has caused shorter style lengths to evolve.

Key words: Flower size, petal length, style length, pollination, allometry, island-mainland, pollen competition.

Introduction

Island flowers are often characterized as small, non-showy, bisexual, and self-compatible compared to their mainland relatives. These characteristics are associated with the "selfing syndrome," a suite of traits that are associated with the evolution of self-pollination, self-fertilization, or alternatively, with wind pollination (Bernardello et al. 2001, Sicard and Lenhard 2011). Because there are few pollinators on islands (Elmqvist et al. 1992, Bernardello et al. 2001, Shueller 2004) island floras tend to be enriched for species with abiotic pollination (Friedman and Barret 1992) or species that can self (Razanajatovo and Maurel et al. 2016, Razanajatovo and van Kleunen et al. 2019, Yomai and Williams 2021). Floral traits involved in pollinator attraction and reward, such as floral size and color, nectar, fragrance, and others, are expensive to maintain (Southwick 1984, Obeso 2002, Jong et al. 2008, Whitehead et al 2012). When selfing or abiotic pollination replaces outcrossing and biotic pollination, natural selection on pollinator attraction and reward traits is relaxed (Whitehead et al 2012). Consequently, the main attraction organs, petals, are expected to become reduced in size with susbsequent reallocation of resources. All else being equal, if petal size is correlated with other floral organ sizes, then smaller flowers with proportionally smaller organs are expected to evolve, since sepals, petals, stamens and carpels all arise from a common meristem.

Yet, some traits involved in male or female function, might evolve differences in allometric scaling of parts due to changes in the strength of sexual selection (Jong et al. 2008). For example, under relaxed natural selection on floral size, pollen numbers (anther size), ovule number (ovary size), and style length should decrease proportionally to other organ sizes, such as petals and sepals, without changes in allometry. Under sexual selection, styles are expected to be longer if length intensifies pollen competition, and competition intensity is a function of the typical size and genetic diversity of the stigmatic pollen load (Duncan and Rausher 2013). On islands, outcross pollination is expected to be rare and genetic diversity is expected to be low (Rick and Fobes 1975, Alvarez and Wendel 2006, Browne et al. 2003), so pollen competition will be weak. Since the style has reduced function as an arena for sexual selection, style length is predicted to be shorter than or equal to that expected under allometric scaling. This prediction should be the same irrespective of mainland mating systems, if islands experience pollinator limitation and reduced genetic diversity. Abiotically pollinated species might show the opposite pattern – styles longer than or equal to allometric scaling if wind pollination is effective.

An alternative scenario is that in many species herkogamy will be lost on island species, such that automatic selfing can occur. In such cases, style length and/or filament length can become modified beyond the allometric prediction (proportional scaling) and exhibit decreased herkogamy. Here we ask three questions: 1) do plants on small, isolated islands have shorter style lengths than mainland species? 2) if so, are differences in style length reflective of allometric scaling of flower size (petal length) or do they deviate, suggesting post-pollination style interaction? and 3) are mainland sister taxa of island colonists a non-random subset of their mainland genus? We hypothesize that petal will be smaller and style lengths will be shorter on islands but not reflective of allometric scaling.

Methods

Island and mainland species sampling

Pohnpei is the largest island in FSM, covering 138 mi^2 including islets and mangrove swamps, surrounded by an outer barrier reef about two miles offshore (Herrera et al. 2010). It is considered a high (approximately 800 meters) volcanic island, with rocks measuring up to 8.7 million years old (Rehman et al. 2013). In this study, 50 species of angiosperms on Pohnpei were studied (Table 3.0), based on their classification as native (endemic or indigenous) or naturalized (including invasive) (Herrera 2010; Lorence 2010). A prior study (Yomai and Williams 2021) added data on petal length for an additional 23 species. Cultivated/ornamental plants were excluded, unless they also had naturalized populations. In sum, data on Pohnpei island came from 73 species in 61 genera.

Flowering plants were sampled in the field from September to December 2019 and January to March 2020. A total of 10 flowers (two each from five plants) per species were sampled. Petal length was measured as the distance from petal base to petal tip, and style length was measured as the distance from the top of the ovary to the top of the stigma.

We searched mainland floras and primary literature for comparable data on petal or style length for each island species and/or other species within its genus. We defined mainland as a landmass that could serve as a source area, such as a continent or large island that was previously connected to a continent (eg., Papua New Guinea and Australia). The main floras consulted were Flora of North America (FNA), Flora of China (CHINA), Flora of East Africa (FTEA), Flora of India, Flora d'Afrique Centrale, KEW Plants, United States National Herbarium (NA), Australia

Tropical Rainforest Plants (ATRFP), Flora of Zambia (ZAM), Flora of Pakistan (PAKI), Plants of World Online, World Flora Online (WFO), Jepson Herbarium (JEPS), Flora of Mesoamerica (MESO), and New South Wales Flora (NSW). There were petal and/or style length data for 418 mainland species that were either conspecific or congeneric to an island species (see Supplementary Data File 1). There was an average of 7.6 mainland species per island genus in the mainland dataset.

Island-mainland and mainland-mainland relationships

To test hypotheses on the evolution of floral sizes on islands relative to mainlands, we compared island species to their nearest mainland relative, which was either a population of the same species, "conspecific mainland sister", or the nearest congener, based on phylogenetic analysis, "congeneric mainland sister." To test hypotheses on the evolution of floral sizes within the mainland source genera, we also compared each congeneric mainland sister to its mainland relatives in the same genus, hereafter, "congeneric mainland relatives."

We generated a phylogenetic tree with 392 species from the large Smith and Brown (2018) tree, using the R package V.Phylomaker (Jin and Qian 2019; Figure 3.0). Using that phylogeny, we assigned the most closely related species to each island species as its sister. In the case of conspecific island-mainland (I-M) pairs, we manually split each island species into two sister taxa (I and M) with arbitrary terminal branch lengths of 0.000001, and with their subtending branch adjusted by subtracting 0.000001. When a congeneric mainland sister to an island species was a clade with more than one species in either of the petal length (N = 9) or style length (N = 6) datasets, we took the mean of these taxa and arbitrarily assigned it to one of the several equally long sister branches and removed the others. Each analysis used different subsets of the phylogeny based on the availability of data on petal length and style length on islands and mainlands. Thus, the island taxa sometimes had different sisters. To answer the question of whether island colonizers are drawn from a non-random subset of mainland species, we constructed paired mainland-mainland comparisons between each mainland sister taxon (defined as above) and the species mean of the remainder of its mainland genus.

Experimental design and statistics

Petal and style lengths were means or midpoints of the range for mainland taxa and means for island taxa. Taxon values were log-transformed for all analyses. In general, the response variable was the log ratio (LR) of island to mainland values for matched pairs: $LR_{island-}$

$_{mainland}$ = $\log_{10}$(mean island petal or style length) – $\log_{10}$(mean mainland petal or style length)]. We also used the same formula to calculate the log ratio of conspecific mainland sisters to mainland generic relatives (genus mean). In island-mainland analyses, LR values < 0 indicate island flowers have smaller petals/styles relative to their mainland relatives. Similarly, in mainland-mainland analyses, LR < 0 indicates mainland sisters have smaller floral parts than their mainland genus relatives.

All statistical analyses were conducted in R version 3.6.2 (R Core Team 2020). We used model-based analyses to test for differences between trait values between island-mainland or mainland-mainland pairs. For island-mainland analyses, we compared non-phylogenetic generalized least squares (GLS) and phylogenetic generalized least squares (PGLS) under different evolutionary assumptions, including Pagel's lambda (Pagel 1999), Blomberg's K (Blomberg et al 2003), Brownian motion, and Ornstein Uhlenbeck (OU, Hansen 1997) models, run using the APE package (Paradis et al. 2004). We retained models with the lowest AIC_c or within ΔAIC_c of 4.

For the question of whether island colonizers are drawn from a non-random subset of mainland species, we used a two-tailed paired t-test to compare log petal lengths or log style lengths of each mainland counterpart of an island taxon to the mean of the rest of its mainland genus. This approach can be considered as using phylogenetically independent comparisons, yet without adjusting for variation in the degree of shared ancestry among lineages (eg. Felsenstein 1985).

Results

Sixty-seven of 73 island species in 55 genera, from 22 families of eudicots, were included on the phylogenetic tree (Figure 3.0). On Pohnpei island, petal and style lengths were measured for 58 and 43 species, respectively (Table 3.0). For petals, there was corresponding data for 50 mainland counterparts, of which 21 were conspecific sisters and 29 congeneric sisters to Pohnpei island taxa. For style lengths, the 43 island-mainland pairs comprised 16 conspecific and 27 congeneric pairs. There were 33 species on Pohnpei and 17 species on mainlands that had data on both petal and style length, and of these, there were 13 conspecific sister pairs with both petal length and style length data for mainlands and island.

Style length, but not petal length, is shorter on Pohnpei than on mainlands

In all tests for island-mainland differences in petal or style lengths, PGLS under Pagel's model always had the lowest AIC_C ($\Delta AIC_C > 4$ for all other models) For petals, 23 of 50 sister pairs had petal length log ratios (LR) <0, whereas 24 pairs had LR > 0 (Figure 3.1). The PGLS analysis resulted in an intermediate lambda and found no correlation between island and mainland petal lengths (P=0.26, N=50 pairs). The results of no petal size differences and intermediate phylogenetic signal were the same whether the analysis was restricted to conspecific pairs (P=0.76, N=21 pairs) or to congeneric sister species (P=0.32, N=29 pairs) (Table 3.1).

For styles, 29 of 43 taxon pairs had negative log ratio values, whereas only 13 were positive (Figure 3.1) The PGLS analysis showed relatively high phylogenetic signal and found a significant negative correlation between island and mainland style lengths (P=0.005, N=43 pairs). These results held for conspecific sister pairs (P = 0.009, N = 16), but were only marginally significant for congeneric sister pairs (P = 0.070, N = 27 pairs; Tale 3.1) Based on backtransformed means, Pohnpei styles were 40% shorter than mainland styles.

We also compared island-mainland LRs for petals to those of styles (Figure 3.2). As can be seen in Figure 3.2, most LRs for island-mainland petal length (x-axis) and style length were < 0. The magnitude of change between island and mainland petal lengths was positively correlated with the magnitude of change in style lengths (log-log slope = 0.47, P = 0.002; Table 3.2, but the slope was less than one (upper limit of the 95% confidence interval was 0.751). As can be seen in Figure 3.2, when I-M petal size differences were small; styles were proportionally longer than petals (points above the 1:1 line), but as petal size differences increased, changes in style lengths were increasingly unpredicted (Figure 3.2).

We also analyzed relationships between style length and petal length separately for island taxa and for mainland sister taxa. The slopes of the log style – log petal GLS regressions were positive, but significantly <1 in both cases (island slope = 0.82, $CI^{.95}${0.34, 0.97}; mainland slope = 0.68, $CI^{.95}${0.33, 0.90}). Thus, a 10-fold increase in petal length predicted an 8.2-fold and 6.8-fold increase in style length on Pohnpei and on mainlands, respectively. Mainland styles were longer than island styles in flower with petals up to about 10mm long, whereas for petals more than about 10mm in length, petal size was similar to style size on both mainland and island.

Are the mainland sisters to island species a non-random subset of their mainland genus?

The mainland taxa most closely related to Pohnpei colonists had similar style and petal lengths as the average of the other mainland species in their genus. For these mainland-mainland comparisons, two-tailed paired *t-tests* were non-significant for petal length (t = 1.9855; P = 0.275; N = 50 pairs) and style length (t = 1.9886; P = 0.6491; N = 43 pairs) (Table 3.2).

Discussion

Theory predicts that plants on oceanic island should have smaller floral sizes than their mainland relatives, and all else being equal, size reduction should involve proportional (isometric) size reduction of all floral organs. In this study, we hypothesized floral sizes, as measured by petal length, should be reduced on Pohnpei relative to those of mainland near-relatives, but also that island styles should be proportionally shorter than their associated petals. We did not find a pattern of petal size reduction on Pohnpei, but their styles were proportionally shorter than predicted by their associated petals. Below we discuss these two patterns in light of shifts in pollination ecology and mating patterns on Pohnpei island. We also address the question of whether island floras are non-randomly drawn from a subset of mainland populations or species that had already evolved smaller floral organs relative to their mainland genus.

Pohnpei island flowers are not smaller than their mainland relatives

Our results showed that contrary to predictions, plants on a remote, oceanic island did not on average have smaller petal lengths than their mainland sisters, and flowers of mainland sisters in turn were no smaller than their mainland relatives in the same genus. A possible explanation for this is that since self-compatibility is overrepresented on islands (Grossenbacher et al 2017) and small flowers are usually associated with self-compatibility (Goodwillie 2010), plants that arrived on Pohnpei had already undergone reduction. But our date contradict that explanation, since island plants became increasingly autogamous, whereas their mainland sisters did not have unusually small flowers, relative to their mainland genus. On the other hand, our results are consistent with a meta-analysis which found that on average, plants on seven Pacific islands did not have smaller flowers relative to their mainland sister taxa (Hetherington 2020). That study did find that plants on two of the seven islands, Galapagos Islands and Revillagigedo Islands, had evolved smaller flowers relative to their mainland sister taxa. This suggests that there may be factors influencing evolution of flower size are diverse (e.g., nutrient availability, island age,

island size, pollinator type, etc). In the plant species from Pohnpei, there was no evidence for wind pollination, which almost always leads to smaller flowers, due to loss of pollinator services. It seems most likely that plants on Pohnpei are retaining mainland-sized flowers because they are still investing in pollinator attraction traits that support uncommon outcrossed pollination events (Yomai and Williams 2021).

When a plant with a specialized flower form disperses to an island, chances are it will do so without its pollinator. Under this circumstance, more generalized relationship with animal pollinators may be advantageous. This is because flowers with more generalized morphologies are more likely to attract other pollinator from within depauperate pollinator faunas (Spears 1987; Marten-Rodgrizues et al., 2015). For instance, Inoue and Amano (1986) found that islands tended to lack large-bodied pollinators and in their absence, species within *Campanulaceae* produced smaller flowers compared to their mainland relatives. Thus, absence of pollinators can drive floral size reduction. It has also been suggested that that wind pollination on island should be apparent since it is the next most reliable pollen transport with the absence of animal pollinators. Friedman and Barrett (2009) explained that wind pollination provides reproductive assurance when pollinators are limited. However, this idea is not supported for plant species on Pohnpei since there was no evidence of wind pollination (Yomai and Williams 2021). Flowers of most species on Pohnpei have not entirely lost their pollinator attracting traits. Yomai and Williams (2021) found that 28 species on Pohnpei retained many animal pollinator attraction traits like large showy petals, nectar, and fragrance, as well as pollenkitt (the sticky coating on pollen that is associated with animal pollen dispersal), even when all of these species had shifted to increased selfing relative to their more outcrossing mainland relatives (Yomai, unpublished, see chapter 2 above).

Style length deviates from petal length on islands

In contrast to the lack of petal length differences between Pohnpei island flowers and their near and distant relatives, island style lengths were on average 40% shorter than those of their mainland sisters. Since those mainland sisters had similar style lengths to their congeneric relatives, the data strongly suggests that island plants evolved shorter style lengths after dispersal, during the establishment process. One of our motivations for this study was to test the hypothesis that mainland populations maintain long styles due to the interplay between pollen

competition as a driver of faster pollen tube growth and maternal responses of sustaining ever longer style lengths to maintain fertilization timing or promote choice of higher quality pollen (Longlotz 2005; Williams and Reese 2019). If so, then passing through a strong establishment filter on remote, oceanic islands should dramatically weaken the strength of sexual selection, and relaxed selection should cause an allometric shift to proportionally shorter style lengths relative to other floral organs. Our results are strikingly consistent with our admittedly naive hypothesis.

Production of floral organs are costly and since plants on Pohnpei have shifted from outcrossing to selfing (Yomai, unpublished, see chapter 2 above), the selective pressure for longer style lengths is relaxed. A literature review of 16 paired comparisons of outcrossing and selfing sister taxa (including 7 closely-related species pairs, 5 of known sister species, and 4 of conspecific population pairs) found that the selfers had 41% shorter styles than the outcrossers (Wyatt 1984; Kerwin and Smith-Huerta 2000, Georgiady et al., 2002; Sicard and Lenhard 2011; Taylor and Williams 2012; Duncan and Rausher 2013; Hove and Mazer 2013). Thus, the reduction of style length in recently evolved selfing populations or species suggests that a similar shift to selfing in Pohnpei species has had the same effect. Yomai and Williams (2021) reported that 36% of plants on Pohnpei exhibited reverse herkogamy, and the reduction in style length could assist the shifts to greater selfing in these plants maintain a better chance of receiving pollen by gravity. The study also found reported that 57% of plants on Pohnpei exhibited approach herkogamy which could mean that the reduction in style length enables generalized pollinators that crawl over stigmas on shorter styles near nectaries at the base of ovaries to transport pollen grains to stigma, especially since plants on Pohnpei are mostly selfers (Yomai, unpublished, see chapter 2 above). Furthermore, Yomai and Williams (2021) reported that 86% of plants on Pohnpei had anther and stigma distances of ≤ 1mm. A shorter distance between anther and stigma would make it easier for pollen grains to land on or touch stigmas.

Conclusion

Isolated oceanic islands are good candidates for understanding the maximal effects of the establishment filter on plant evolution on islands. Flower sizes are predicted to be smaller on islands, and if so, then floral organs should be proportionally smaller as well. In this study we showed that contrary to predictions, island species did not have smaller flowers relative to their mainland sister taxa, but the flowers did have much shorter styles. Shorter styles on islands can

be attributed to shifts to more autogamous mating systems, a pattern we have previously documented on Pohnpei. Since autogamy generally reduces both the number of (Yomai and Williams 2021), and the relatedness among, pollen grains on a stigma (Williams and Reese 2019), the intensity of pollen competition on islands should be reduced. Sexual selection theory predicts that style length is positively correlated with the intensity of pollen competition. Thus, our findings are consistent with relaxed selection on style length on Pohnpei, and support the idea that sexual selection mediated by outcross pollen transfer via pollinators plays a large role in maintaining longer styles in mainland populations.

Acknowledgement
We would like to thank Jerry Route and Iakop Ioanis for collecting data on Pohnpei species, to Alexandra Scearce [University of Tennessee, Knoxville (UTK)] and Jacob Edwards (Columbia U., NY) for gathering data on mainland species from the literature. We would also like to thank Tamara Greenstone Alefaio and Bertha Reyuw for their help in organizing and scheduling field seasons. We are also grateful for statistical advice on phylogenetic analyses from Orlando Schwery and Jacob Moutouama. Research funding was provided by UTK Office of Undergraduate Studies (JHW) and a Bill Raynor Micronesia Challenge Scholarship (VMHY).

References

Alvarez I and Wendel JF. 2006. Cryptic interspecific introgression and genetic differentiation within *Gossypium aridium* (*Malvaceae*) and its relatives. *Evolution* **60:** 505-517.

Bernardello G, Anderson GJ, Stuessy TF, Crawford DJ. 2001. Breeding system and pollination of selected plants endemic to Juan Fernandez Islands. *American Journal of Botany* **88:** 220-223.

Blomberg SP, Garland Jr T, Ives AR. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57:** 717-745.

Brown RA, Anderson DJ, White MD, Johnson MA. 2003. Evidence for low genetic divergence among Galapagos' *Opuntia* cactus species. *Noticias Galapagos* **62:** 11-13.

Chankaew S, Isemura T, Naito K, Chankaew S, Isemura T, Naito K, Ogiso-Tanaka E, Tomooka N, Somta P, Kaga A, Vaughan DA, Srinives P. 2014. QTL mapping for salt tolerance and domestication-related traits in Vigna marina subsp. oblonga, a halophytic species. *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik* **127**: 691–702.

Duncan TM and Rausher MD. 2013. Evolution of the selfing syndrome in *Ipomoea. Frontiers in Plant Science* **30:** 1-8.

Elmqvist T, Cox PA, Raney WE, Pierson ED. 1992. Restricted pollination on oceanic islands: pollination of *Ceiba pentandra* by flying foxes in Samoa. *Biotropica* **24:** 15-23.

Erbar C and Longlotz M 2005. Pollen to ovule ratio: standard or variation - compilation *Bot. Jahrb. Systm.* **126:** 71-132.

Friedman J, Barrett SCH. **2009**. Wind of change: New insights on the ecology and evolution of pollination and mating in wind-pollinated plants. *Annals of Botany* **103**: 1515–1527.

Felstein J. 1985. Phylogenies and the comparative method. *Am. Nat.* **125:** 133-151.

Goodwillie C, Sargent RD, Eckert CG, Elle E, Geber MA, Johnston MA, Kalisz S, Moeller DA, Ree RH, Vallejo-Marin M, and Winn AA. 2010. Correlated evolution of mating system and floral display traits in flowering plants and its implications for the distribution of mating system variation. *New Phytologist* **185**: 311–321.

Hansen TF. 1997. Stabilizing selection and the comparative analysis of adaptation. *Evolution* **50:** 1404-1417.

Herrera K, Lorence DH, Flynn T, Balick MJ. **2010**. Checklist of the Vascular Plants of Pohnpei, Federated States of Micronesia with Local Names and Uses. *Allertonia* **10**: 1–192.

Hetherington-Rauth MC, Johnson MTJ. **2020**. Floral trait evolution of angiosperms on pacific islands. *American Naturalist* **196**: 87–100.

Jurgens A, Witt T, Gottsberger G. 2002. Pollen grain numbers, ovule numebers and pollen-ovule ratios in *Caryophylloideaae:* correlation with breeding system, pollination, life form, style number, and sexual system. *Sex Plant Reprod* **14:** 279-289.

Inoue K and Amano M. 1986. Evolution of *Campanula punctata* Lam. in the Izu Islands: Changes of pollinators and evolution of breedings systems. *Plant Species Biology* **1:**89-97.

Marten-Rodriguez S, Quesada M, Castro AA, Lopezaraiza-Mikel M, and Fenster CB. 2015. A comparison of reproductive strategies between island and mainland Caribbean *Gensneriaceae. Journal of Ecology* **103:** 1190-1204.

Martins PE, Hansen FT. **1977**. Phylogenies and the comparitive method: A general approach to incorporating phylogenetic information into the analysis of interspecific data. *American Naturalist* **149**: 645–667.

Obeso JR. 2002. The costs of reproductin in plants. *New Phytologist* **155:** 321-348.

Pagel M. 1999. The maximum likelihood approach to reconstructing ancestral character states of

discrete characters on phylognies. *Systematic Biology* **48:** 612-622.
R Core Team. 2020. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. www.R-project.org.
Razanajotovo M, van Kleunen M, Kreft H, Dawson W, Essl F, Pergl J, Pysek P, Winter M, Weigelt P. 2019. Autofertility and self-compatibility moderately benefit island colonization of plants. *Global Ecology and Biogeography* **28:** 341-352.
Reese JB and Williams JH. 2019. How does genome size affect the evolution of pollen tube growth rate, a haploid perforamce trait? *American Jouranl of Botany* **106:** 1011-1020.
Rehman H, Nakaya H, Kawai K. **2013**. Geological origin of the volcanic islands of the Caroline group in the Federated States of Micronesia, western Pacific. *South Pacific Studies* **33**: 101–118.
Rick CM and Fobes JF. 1975. Allozymes of Galapagos tomatoes. In Bownman RI, Berson M, Leviton Ae (eds). *Patterns of Evoution in Galapagos Organisms*. San Francisco, American Association for Advancement of Science. pp 97-106.
Sicard A and Lenhard M. 2011. The selfing syndrome: a model for studying the genetic and evolutionary basis of morphological adaptation in plants. *American Jouranl of Botany* **107:** 1433-1443.
Smith SA, Brown JW. **2018**. Constructing a broadly inclusive seed plant phylogeny. *American Journal of Botany* **105**: 302–314.
Southwick EE. 1984. Photosynthate allocation to floral nectar: A neglected energy investment. *Ecology* **65:**1775-1779.
Spears Jr EE. 1987. Island and mainland pollination ecology of *Centrocema virginianum* and *Opuntia stricta. Journal of Ecology* **75:** 351-362.
Whitehead MR, Phillips RD, Peakall R. 2012. Pollination: The price of attracition. *Current Biology* **22:** 680-682.
Williams JH, and Reese JB. 2019. Evolution of development of pollen performance. *Current Topics in Developmental Biology* 131: 299-336.
Yomai VMH, Williams JH. 2021. Breeding systems of naturalized versus indigenous species provide support for Baker's law on Pohnpei island. *AoB Plants* **13:** 1-13.

Conclusion

My dissertation research demonstrated that Baker's law is evident on Pohnpei. I showed that plant species undergo an establishment filter after a successful long-distance dispersal, but only species with capacity for uniparental reproduction and potential for selfing pass the filtration system. Thus, mainland species that successfully disperse to Pohnpei are not pooled from a random subset. My dissertation further supports this by showing that shorter styles on islands. Furthermore, I showed that mating system shift from outcrossing to selfing occurs post establishment, but subsequent evolution to outcrossing does not occur. I conclude that unlike Hawaii and New Zealand, Pohnpei is small and isolated, thus species extinction occurs before evolution can happen.

Box 1. Breeding system indices. S, self; OC, outcross.

Abbreviation	Formulas and rationale	References
OCI	*Floral outcrossing index* OCI = sum of scores below *Range*: 0 (cleistogamy) to 5 (partially self-compatible, requires pollinators)	Cruden 1977; Dafni 1992
	Diameter of floral "target" (flower or head): <1mm = 0, 1-2mm =1, 2-6mm = 2, >6mm = 3 Herkogamy: absent* = 0, reverse or approach herkogamy = 1 *Consider degree of anther-stigma separation that prevents selfing Dichogamy: absent, overlapping, or protogyny = 0, protandry = 1	 Webb and Lloyd 1986; Opedal 2018 Lloyd and Webb 1986
	Strength: rough field-measure of floral attractiveness to pollinator-assisted outcrossing *Weakness*: does not preclude pollinator-assisted selfing among flowers (geitonogamy)	
Sexual system	*Sexual system* Description of distribution of floral sex organs in flowers/individual plants All flowers on plant cosexual (hermaphrodite); flowers unisexual and both sexes on same plant (monoecy), or each sex on separate plant (dioecy); and 8 other combinations or variants	Tree of Sex Consortium 2014; Lord 2015
ISI	*Index of self-incompatibility* ISI = 1 – (hand-self success/hand-outcross success[A]) *Range*: ≤ 0.2 autogamy; ≤ 0.8 self-compatible, and > 0.8 highly outcrossing	Lloyd 1965 Raduski et al. 2012
	Strength: rough measure of self-sterility; correlated with mating system *Weakness*: does not indicate mechanism of self-sterility; cut-off values are arbitrary; values < 0 can indicate outbreeding depression, outcrossing with relatives; handling errors; small sample size	 Gibbs 2014 Raduski et al. 2012
AF	*Autofertility index* AF = autonomous self-success/hand self-success[A] *Range*: 0 (low) to 1 (high) autofertility (values > 1, high autofertility, but handling errors or imperfect timing of hand-pollination)	Schoen and Lloyd 1992
	Strength: measures magnitude of unassisted, intra-floral self-fertilization (by unassisted self-pollination) relative to maximum possible *Weakness*: only useful if substantial self-success is found; apomixis not excluded	

Box 1.0 continued

	Alternative tests for mechanisms of selfing:	See Schoen and Lloyd 1992
P:O ratio	*Pollen to ovule ratio* P:O ratio = total pollen grains to total ovules per flower Range: values near zero indicate selfing and/or stable pollination ecology, higher values outcrossing and/or inefficient pollination	Cruden 1977
	Strength: measures relative allocation to male and female function per flower, an evolved response to long-term pollination efficiency; simple to measure and roughly correlates with degree of selfing or outcrossing, especially if controls are considered (taxonomic group, ecology, etc.) *Weakness:* pollination environment and degree of outcrossing often co-vary	Michalski and Durka 2009
PL	*Pollen limitation index* (floral level) *PL* = 1 – (open-pollination success/supplemental pollination success[A]) **[1]Open: no emasculation = autonomous (a) + geitonogamous (g) + OC pollinations allowed** [2]Open: emasculation = g + OC (only assisted open-pollination allowed) **[3]Suppl: no emasculation/bagging = a + g + OC allowed; S or OC applied** [4]Suppl: emasculation, no bagging = g + OC allowed; S or OC applied [5]Suppl: emasculation, bag = only S or OC hand-pollination allowed Notes: Bolded most common ($^{1}/_{3\ OC}$) – general *PL* $^{2}/_{5}$ s or oc (this study) – pollinator-assisted *PL* Range: 0 (no pollen limitation) to 1 (severe pollen limitation); values < 0 can indicate handling or over-pollination effects, or statistical error	Larson and Barrett 2000
	Strength: measures the degree to which seed set is limited by self or outcross pollen reception at the floral level (whole plant level more meaningful) *Weakness:* subject to variation in pollination environment, resource re-allocation among flowers within the plant, and within and among years; confounds actual pollination limitation with siring success	Burd 1994; Knight et al. 2005; Burns et al. 2019
	Alternative: actual per flower natural stigmatic pollen-load-to-ovule-number	Snow 1986

Tables

Table 1.0. Species list and authority, nativity status and collection sites. Families arranged in sections in order: Melastomataceae, Fabaceae, Malvaceae. Nativity status taken from Herrera et al. 2010, and indigenous/endemic species are in shaded rows.

Species	Nativity status	Collection sites	GPS coordinates
*Astronidium ponapense (*Kaneh) Markgr.	Endemic	Forest top	6°54`12``N 158°10`24 E
*Dissotis rotundifolia (*Sm) Triana.	Naturalized	Roadway by stream	6°49`51``N 158°10`28 E
Melastoma malabathricum L.	Indigenous	Open canopy	6°49`51``N 158°10`28 E
Abrus precatorius L.	Naturalized	Understory, roadway	6°49`03``N 158°10`09 E
Acacia auriculiformis Cunn ex. Benth.	Naturalized	Hilltop by road	6°54`26``N 158°09`31 E
Adenanthera pavonina L.	Naturalized	Hilltop by road, abandoned roadway	6°49`08``N 158°14`00 E
Aeschynome indica L.	Naturalized	Open canopy, abandoned land	6°49`11``N 158°11`23 E
Centrosema molle Mart. ex Benth.	Naturalized	Side of the road	6°53`42``N 158°17`51 E
Crotalaria pallida Aiton.	Naturalized	Open canopy on pepper farm	6°47`52``N 158°14`53 E
Crotalaria spectabilis Roth.	Naturalized	On the side of the road	6°47`52``N 158°14`53 E
Dalbergia candenatensis (Dennst.) Prain.	Indigenous	On the side of the road	6°49`51``N 158°10`28 E
Dendrolobium umbellatum (L.) Benth.	Indigenous	Roadway by stream, open canopy near stream	6°49`51``N 158°10`28 E

Table 1.0 continued

Derris trifoliata Lour.	Indigenous	Coast, abandoned roadway	6°49`22``N 158°10`06 E
Leucaena leucocephala (Lam.) de Wit.	Naturalized	Roadway by stream, open canopy	6°49`14``N 158°11`30 E
Mimosa diplotricha Suavalle.	Invasive	Coast	6°50`27``N 158°09`15 E
Mimosa pudica L. var hispida Brenan.	Invasive	Coast	6°50`27``N 158°09`15 E
*Senna alata (*L.) Roxb.	Naturalized	Roadside	6°49`11``N 158°11’23 E
Senna obtusifolia (L.) H.S Irwin & Barneby	Naturalized	Abandoned roadway	6°49`20``N 158°09`54 E
Senna occidentalis (L.) Link	Naturalized	Understory	6°47`52``N 158°14`53 E
Tephrosia candida (Roxb.) DC.	Naturalized	Abandoned roadway	6°49`11``N 158°11`23 E
Vigna hosei (Craib) Becker	Naturalized	Coast	6°58`43``N 158°13`27 E
Vigna marina (Burm) Merr.	Indigenous	Coast, beachline	6°58`43``N 158°13`27 E
Abelmoschus moschatus (Medik).	Naturalized	Coast	6°50`27``N 158°09`15 E
Commersonia bartramia (L.) Merr.	Indigenous	By stream, private property	6°50`02``N 158°11`06 E
Hibiscus tiliaceus (L.)	Indigenous	Forest, roadway	6°48`44``N 158°13`02 E
Sida acuta Burm. f.	Invasive	Abandoned roadway, roadside	6°49`13``N 158°11`20 E

Table 1.0 continued

Sida rhombifolia L.	Naturalized	Abandoned roadway	6°49`13``N 158°11`20 E
Thespesia populnea (L.) Soland ex Correa.	Indigenous	Coast	6°50`27``N 158°09`15 E

Table 1.1. Floral traits related to pollination system. All values are means of three flowers per species (± SD for petal length). Herkogamy is anther-stigma separation, positive if stigma height > anther height, negative if the reverse (± ½ 95% confidence interval). Dichogamy was either incomplete protandry (IPDR) or incomplete protogyny (IPGY). For fragrance, D indicates fragrance detected, and N indicates fragrance not detected (Dafni 1992). Families (row shading) arranged in order: Melastomataceae, Fabaceae, Malvaceae. Shaded rows indicate native species.

Species	Petal length (mm)	Herkogamy (mm)	Dichogamy	Fragrance	OCI index
Astronidium ponapense [E]	7 ± 0.3	-0.47±0.02	IPGY	N	4
Dissotis rotundifolia [N]	14 ± 0	-0.31±0.02	IPGY	N	4
Melastoma malabathricum [I]	14.7 ± 0.2	-0.27±0.01	IPGY	N	4
Abrus precatorius [N]	0.5 ± 0.09	-2.98±0.39	IPGY	N	1
Acacia auriculiformis [N]	0.7 ± 0.8	1.00±0.00		D	1
Adenanthera pavonina [N]	1.7 ± 0.6	0	IPGY	D	1
Aeschynome indica [N]	7 ± 0	0.1±0.05	IPGY	N	4
Centrosema molle [N]	29 ± 1.9	-0.3±0.02	IPGY	N	4
Crotalaria pallida [N]	12 ± 0.8	0.2±0.04	IPGY	N	4
Crotalaria spectabilis [N]	11.3 ± 1	0.3±0.05	IPGY	N	4
Dalbergia candenatensis [I]	8 ± 1.2	0.5±0.03	IPGY	N	4
Dendrolobium umbellatum [I]	4 ± 0.7	1±0.05	IPGY	N	3
Derris trifoliata [I]	7 ± 0.9	1.00±0.00	IPGY	N	4
Leucaena leucocephala [N]	17 ± 0.2	1.00±0.00	IPGY	D	4
Mimosa diplotricha [INV]	11.7 ± 2	-3±0.22	IPGY	N	4
Mimosa pudica [INV]	13 ± 1.3	-2±0.08	IPGY	N	4
Senna alata [N]	17 ± 2.1	1±1	IPGY	N	3
Senna obtusifolia [N]	23 ± 1.5	-0.5±0.13	IPGY	N	4
Senna occidentalis [N]	16.4 ± 0.3	0	IPGY	N	3
Tephrosia candida [N]	10 ± 0	-0.7±0.10	IPGY	N	4

Table 1.1. continued

Vigna hosei[N]	13 ± 0.1	2.3±0.14	IPGY	N	4
Vigna marina[I]	12 ± 0.1	2±0.05	IPGY	N	4
Abelmoschus moschatus[N]	108 ± 2.3	8±0.55[a]	IPDR	D	4
Commersonia bartramia[I]	13 ± 0.6	-1±0.04	IPDR	D	4
Hibiscus tiliaceus[I]	76 ± 0.07	2±0.59[a]	IPDR	N	4
Sida acuta[INV]	8 ± 0.02	1±0.18	IPDR	N	4
Sida rhombifolia[N]	6.3 ± 0.34	0.5±0.3	IPDR	N	4
Thespesia populnea[I]	100 ± 2.1	11±1.41[a]	IPDR	N	4

[a] Anthers were same height as stigma but separation distance was lateral.
[E]Endemic, [I]Indigenous, [N]Naturalized, and [INV]Invasive.

Table 1.2. Pollen and ovule numbers and pollen:ovule (P:O) ratio. Pollen grains are estimated number of pollen grains from 4 anthers per flower in Fabaceae and Melastomataceae and 10 anthers per flower in Malvaceae. Ovule numbers are estimated number of ovules for the same four flowers that the anthers were collected from. Values are means (± 1 SD). Shaded rows indicate native species.

Species	Pollen size (μm)	Pollen grains	Ovules	P:O ratio
Astronidium ponapense[E]	24 ± 1.0	1993.1 ± 12.6	100 ± 3.8	19.93 ± 0.48
Dissotis rotundifolia[N]	23 ± 0.0	1605.3 ± 48.7	103 ± 3.5	15.6 ± 0.6
Melastoma malabathricum[I]	20 ± 0.2	1670.0 ± 80.8	101.8 ± 4.1	16.4 ± 0.4
Abrus precatorius[N]	41 ± 0.0	1388.3 ± 36.9	8.0 ± 0	173.5 ±1.7
Acacia auriculiformis[N]	38 ± 0.2	1292.5 ± 45.0	26.5 ± 4.0	49.4 ± 4.9
Adenanthera pavonina[N]	41 ± 3.8	1653.9 ± 17.3	25.0 ± 0.0	66.2 ± 0.2
Aeschynome indica[N]	25 ± 0.0	1508.7 ± 35.7	8.0 ± 0.0	188.6 ± 2.4
Centrosema molle[N]	32 ± 6.2	1322.8 ± 22.3	20.0 ± 0.0	66.1 ± 0.9
Crotalaria pallida[N]	42 ± 0.1	1394.8 ± 32.3	16.0 ± 2.0	87.2 ± 1.1
Crotalaria spectabilis[N]	27 ± 1.0	1500.8 ± 31.4	10.0 ± 0.0	150.1 ± 3.2
Dalbergia candenatensis[I]	14 ± 0.0	1830.3 ± 23.1	25.0 ± 0.0	73.2 ± 0.2
Dendrolobium umbellatum[I]	27 ± 0.2	1788.1 ± 54.1	6.0 ± 0.0	298.0 ± 8.2
Derris trifoliata[I]	13 ± 0.8	3343.3 ± 278.0	6.0 ± 0.0	557.2 ± 35.9
Senna alata[N]	31 ± 3.0	±	19.3 ± 3.1	±
Senna obtusifolia[N]	25 ± 3.8	538.0 ± 17.4	26.8 ± 2.4	20.2 ± 1.4
Senna occidentalis[N]	28 ± 4.8	2500.0 ± 48.7	29.0 ± 1.4	86.3 ± 0.4
Tephrosia candida[N]	N/A	1342.3 ± 33.3	10.0 ± 0.0	134.2 ± 1.0
Vigna hosei[N]	17 ± 2.6	1331.6 ± 47.0	6.0 ± 0.0	222.0 ± 6.0
Vigna marina[I]	20 ± 3.0	1298.1 ± 50.8	6.0 ± 0.0	216.4 ± 1.0

Table 1.2 continued

Abelmoschus moschatus[N]	163 ± 6.4	NA	142.3 ± 0.81	NA
Commersonia bartramia[I]	94 ± 8.6	NA	25.0 ± 0.0	NA
Hibiscus tiliaceus[I]	173 ± 5.0	NA	23.3 ± 2.7	NA
Sida acuta[INV]	83 ± 0.8	NA	10.0 ± 0.0	NA
Sida rhombifolia[N]	128 ± 0.4	1587.8 ± 69.7	10.0 ± 0.0	158.8 ± 2.6
Thespesia populnea[I]	172 ± 0.2	NA	25±3.21	NA

[E]Endemic, [I]Indigenous, [N]Naturalized, and [INV]Invasive.

Table 1.3. Hand-pollination experiments. Values represent the average number of seeds per N = 5 flowers (1 SE). In each row, values with different superscripts are significantly different. "Hand self," hand-self pollinations; "Autonomous self," bagged and unmanipulated; "Open pollination," emasculated without bagging; "Hand outcross," emasculated, cross-pollinated. Shaded rows indicate native species.

Species	Hand self	Autonomous self	Open pollination	Hand outcross
Crotalaria pallida^{N}	20.6 (1.78)^{A}	18.8 (2.06)^{A}	5.2 (2.33)^{B}	1.2 (1.2)^{B}
Dendrolobium umbellatum^{I}	3 (0.32)^{A}	2.6 (0.51)^{A}	0.2 (0.2)^{B}	0.6 (0.4)^{B}
Leucaena leucocephala^{N}	13.6 (1.72)^{A}	11.4 (1.17)^{A}	6 (3.29)^{AB}	0.8 (0.8)^{B}
Mimosa diplotricha^{INV}	7.6 (0.4)^{A}	0.4 (0.4)^{B}	6.8 (0.8)^{A}	0 (0)^{B}
Mimosa pudica^{INV}	11.2 (1.62)^{A}	0 (0)^{B}	9.2 (0.8)^{A}	0 (0)^{B}
Senna alata^{N}	5.6 (0.4)^{A}	6.2 (0.66)^{A}	4 (1.05)^{A}	6 (0.32)^{A}
Vigna marina^{I}	4.6 (0.24)^{A}	4 (0.55)^{A}	1 (0.32)^{B}	3.8 (0.49)^{A}
Hibiscus tiliaceus^{I}	53 (1.05)^{A}	52.2 (0.86)^{A}	17.4 (2.54)^{B}	47.8 (0.86)^{A}
Sida acuta^{INV}	51.2 (2.33)^{A}	24.4 (5.68)^{B}	40.4 (5.17)^{AB}	52.6 (3.03)^{A}
Sida rhombifolia^{N}	3 (0.45)^{A}	6.8 (0.8)^{B}	6.4 (0.4)^{B}	1.4 (0.68)^{A}
Thespesia populnea^{I}	14.2 (0.8)^{A}	5.8 (1.07)^{B}	13 (2)^{A}	11.2 (0.58)^{A}

^{E}Endemic, ^{I}Indigenous, ^{N}Naturalized, and ^{INV}Invasive

Table 1.4. Summary of breeding indices and inferred breeding systems. If numerator and denominator an index were not significantly different, then "~1" or "~0", otherwise values shown. ISI, index of self-incompatibility; AI, autogamy index; AF, autofertility index; PL, pollen limitation index - if seed set of open (geitonogamous or outcross) pollination was lower than either outcross or hand-self seed set, "PL", or if non-significant "~0" (no PL); OCI, outcrossing index; P:O ratio, pollen to ovule ratio. See text for discussion of inferred breeding systems: FA, Facultative autogamy; FO, Facultative outcrossing; SC = species with self-compatibility based on crossing data. "Mixed" indicates either FA or FO. Shaded rows indicate native species.

Species	ISI	AI	AF	PL	OCI	P:O Ratio	Inferred Breeding system
Astronodium ponapense[E]					4	20	Autogamous
Dissotis rotundifolia[N]					4	16	Autogamous
Melastoma malabathricum[I]					4	16	Autogamous
Abrus precatorius[N]					1	174	FA
Acacia auriculiformis[N]					1	50	FA
Adenanthera pavonia[N]					1	66	FA
Aeschynome indica[N]					4	189	Mixed
Centrosema molle[N]					4	66	FA
Crotalaria pallida[N]	<0	>1	~1	PL	4	87	SC: FA
Crotalaria spectabilis[N]					4	150	FA
Dalbergia candenatensis[I]					4	73	FA
Dendrolobium umbellatum[I]	<0	>1	~1	PL	3	298	SC: Mixed
Derris trifoliata[I]					4	557	FO
Leucaena leucocephala[N]	<0	>1	~1	~0	4		SC: FA
Mimosa diplotricha[INV]	NA	NA	0.05	PL	4		SC: FA
Mimosa pudica[INV]	NA	NA	0	PL	4		SC: FA
Senna alata[N]	~0	~1	~1	~0	3	51	SC: FA
Senna obtusifolia[N]					4	20	Autogamous
Senna occidentalis[N]					3	86	FA
Tephrosia candida[N]					4	134	FA
Vigna hosei[N]					4	222	Mixed
Vigna marina[N]	~0	~1	~1	PL	4	217	SC: Mixed
Abelmoschus moschatus[N]					4	17	Autogamous
Commersonia bartramia[I]					4	9	Autogamous
Hibiscus tiliaceus[I]	<0	>1	~1	~0	4	107	SC: FA
Sida acuta[INV]	~0	0.46	0.48	~0	4	8	SC: Autogamous
Sida rhombifolia[N]	~0	>1	>1	~0	4	159	SC: FA
Thespesia populnea[I]	~0	0.52	0.41	~0	4	10	SC: Autogamous

[E]Endemic, [I]Indigenous, [N]Naturalized, and [INV]Invasive.

Table 2.0. Known sexual systems for mainland species.

Mainland species	Sexual system	Floral type	Reference
Abelmoschus moschatus	Hermaphrodite	Bisexual	Hamon and Koechlin 1990
Acacia paradoxa	Andromonoecy	Uni/Bi	Kenrick and Knox 1989
Acacia pycnantha	Andromonoecy	Uni/Bi	Kenrick and Knox 1989
Asclepias incarnata	Hermaphrodite	Bisexual	Agrawal, Erwin, Cook 2008
Bidens pilosa	Hermaphrodite	Bisexual	Jaimes and Ramirez 1997
Centrosema virginanum	Hermaphrodite	Bisexual	Shultze-Kraft et al., 1990.
Crotalaria micans	Hermaphrodite	Bisexual	Etcheverry et al., 2003
Dalbergia miscolobium	Hermaphrodite	Bisexual	Oliveira & Gibbs 2000
Datura metel	Not known	Bisexual	See supplementary Table 2
Derris trifoliata	Hermaphrodite	Bisexual	Aluri, Kumar, and Chappidi 2016
Desmodium canum	Hermaphrodite	Bisexual	Jaimes and Ramirez 1997
Hibiscus tiliaceus	Not known	Bisexual	See supplementary Table 2
Ipomoea alba	Not known	Bisexual	See supplementary Table 2
Leucaena leucocephala	Not known	Bisexual	See supplementary Table 2
Ludwigia nervosa	Hermaphrodite	Bisexual	Ramirez and Brito 1990
Melastoma malabatrichum	Hermaphrodite	Bisexual	Luo, Chen, and Zhang 2012
Mimosa albida	Hermaphrodite	Bisexual	Jaimes and Ramirez 1997
Mimosa camporum	Monoecy	Unisexual	Ramirez and Brito 1990
Senna obtusifolia	Not known	Bisexual	See Supplementary Table 2
Sida spinosa	Hermaphrodite	Bisexual	Jaimes and Ramirez 1997
Sida cordifolia	Hermaphrodite	Bisexual	Raju & Rani 2016
Solanum americanum	Not known	Bisexual	See supplementary Table 2
Thespesia populnea	Hermaphrodite	Bisexual	Aluri et al., 2020
Xylocarpus granatum	Monoecy	Unisexual	Tomlinson 1986

Table 2.1. Hand-pollination experiments. Values represent the average number of seeds per N = 5 flowers. "Hand self", hand-self pollinations; "Autonomous self", bagged and unmanipulated; "Hand outcross", emasculated, cross-pollinated; "Open", natural pollination of emasculated unbagged flowers. Data taken from Yomai and Williams (2021) are indicated by superscript A.

Species	Hand self	Autonomous self	Hand outcross	Open
Abelmoschus moschatus	123.6 ± 14.2	121.8 ± 5.8	100.8 ± 3.6	80.2 ± 4.8
Acacia auriculiformis	6.0 ± 2.4	5.0 ± 3	1.6 ± 1.4	1.0 ± 0.8
Acacia confusa	8.2 ± 2	6.2 ± 0.9	1.2 ± 1.7	1.8 ± 1.8
Adenanthera pavonina	5.8 ± 3.5	6.4 ± 4.3	2.0 ± 2	1.6 ± 2.6
Aeschynomene indica	5.8 ± 1.8	5.6 ± 1.9	2.2 ± 1.4	1.8 ± 2.2
Asclepias incarnata	1.8 ± 0.5	1.8 ± 0.5	0.8 ± 1.1	1.0 ± 1
Bidens pilosa	NA	20.2 ± 9.2	NA	30.4 ± 9.6
Centrosema mole	12.2 ± 3.5	8.0 ± 3	6.4 ± 3.8	7.6 ± 1.4
Crotalaria pallida^{A}	20.6 ± 4	18.8 ± 4.7	1.2 ± 2.7	5.2 ± 5.3
Crotalaria spectabilis	6.0 ± 1.5	3.6 ± 1.6	5.2 ± 1.8	3.0 ± 2.4
Dalbergia canadensis	5.8 ± 1.1	2.8 ± 1.5	4.0 ± 1	2.0 ± 1.6
Datura metel	182 ± 40.7	190.4 ± 55.7	210.4 ± 39.7	208 ± 35
Dendrolobium umbellatum^{A}	3.0 ± 0.8	2.6 ± 1.2	0.6 ± 0.9	0.2 ± 0.5
Derris trifoliata	1.0 ± 0.8	1.2 ± 0.5	0.2 ± 0.5	0.8 ± 0.5
Desmodium heterocarpon	3.8 ± 0.9	3.6 ± 0.6	1.0 ± 0.8	1.4 ± 0.6
Dissotis rotundifolia	11.6 ± 2.2	8 ± 2.5	2.8 ± 3.4	4.0 ± 4
Hibiscus tiliaceus	51.2 ± 5.3	24.4 ± 12.8	52.6 ± 6.8	40.4 ± 11.6
Impatiens balsamina	8.4 ± 1.6	9.4 ± 1.9	2.0 ± 2.6	3.2 ± 2.6
Ipomoea indica	13.8 ± 2.9	10.2 ± 2.3	1.2 ± 1.4	2.8 ± 2.5
Ipomoea littoralis	3.6 ± 0.6	3.6 ± 0.6	0.4 ± 0.6	0.6 ± 0.6
Leucaena leucocephala^{A}	13.6 ± 3.9	11.4 ± 2.7	0.8 ± 1.8	6.0 ± 7.4
Ludwigia octovalis	11.2 ± 3.1	10.0 ± 1.5	2.8 ± 3.8	2.2 ± 3.9
Melastoma malabathricum	17.0 ± 5.6	16.6 ± 7	3.2 ± 3.7	8.6 ± 3.9
Mimosa diplotricha^{A}	7.6 ± 0.9	0.4 ± 0.9	0 ± 0	6.8 ± 1.8
Mimosa pudica^{A}	11.2 ± 3.7	0 ± 0	0 ± 0	9.2 ± 1.8
Senna alata^{A}	5.6 ± 0.4	6.2 ± 0.7	4.0 ± 1.1	6.0 ± 0.3
Senna obtusifolia	6.0 ± 3.8	7.6 ± 3.9	2.0 ± 2	2.6 ± 1.7
Senna occidentalis	13.6 ± 1.7	10.8 ± 5.9	7.0 ± 3.4	7.4 ± 1.4
Sesbania grandiflora	11.6 ± 4.6	14.2 ± 5.1	12.6 ± 6.6	11.4 ± 2.8
Sida acuta^{A}	5.6 ± 0.9	6.2 ± 1.5	6.0 ± 0.8	4.0 ± 2.4
Sida rhombifolia^{A}	3.0 ± 1	6.8 ± 1.8	1.4 ± 1.6	6.4 ± 0.9
Solanum americanum	10.8 ± 8.7	13.0 ± 2.5	7.0 ± 4.4	4.4 ± 3.7
Tephrosia candida	5.2 ± 1.1	3.6 ± 2.7	1.0 ± 1	1.4 ± 0.9
Thespesia populnea^{A}	14.2 ± 1.8	5.8 ± 2.4	11.2 ± 1.4	13.0 ± 4.5
Vigna marina	4.6 ± 0.6	4.0 ± 1.3	3.8 ± 1.1	1.0 ± 0.8
Xylocarpus granatum	7.8 ± 0.9	8.4 ± 0.9	5.4 ± 1.9	4.8 ± 1.4

Table 2.2. Autofertility, self-compatibility, and fruit set self-compatibility comparisons between island and mainland. Asterisks indicate self-incompatibility and P indicates partial self-incompatibility. Values in parentheses under island self-compatibility rate are

Taxa		Autofertility Index		Self-compatibility rate	
Island	Mainland	Island	Mainland	Island	Mainland
Abelmoschus moschatus	*A. moschatus*	0.985	NA	1 (1.356)	NA
Acacia auriculiformis	*A. paradoxa*^{P}	0.870	NA	1 (4.230)	0.847 (range: 0-1.4)
Acacia confusa	*A. pycnantha**	0.971	NA	1 (4.8)	0.024
Aeschynomene indica	*A. amorphoides*	1.115	NA	1 (3.389)	NA
Asclepias incarnata	*A. incarnata*	1.538	NA	0.8 (2.85)	0.542
Bidens pilosa	*B. pilosa*	0.619	1	1 (0.644)	1
Centrosema molle	*C. virginanum*	0.941	0.0002	1 (1.443)	1
Crotalaria pallida	*C. micans*	0.913	0.807	1 (6.156)	1
Crotalaria spectabilis	*C. retusa*	0.815	0.980	1 (1.170)	NA
Dalbergia canadensis	*D. miscolobium*	1.167	NA	1 (1.433)	0.138
Datura metel	*D. wrightii*	0.899	0.525	1 (0.890)	1
Derris trifoliata	*D. trifoliata*	1.200	0.085	1 (2.2)	0.026
Desmodium heterocarpon	*D. canum*	0.947	1.270	0.8 (3.083)	1
Hibiscus tiliaceus	*H. trionum*	0.477	0.770	1	NA
Impatiens balsamina	*I. parviflora*	1.119	0.853	1 (0.812)	1
Ipomeoa indica	*I. alba*	0.739	0.724	1 (6)	0.724
Ipomoea littoralis	*I. asarifolia**	1	0	1 (7.2)	0
Leucaena leucocephala	*L. leucocephala*	0.838	NA	1 (3.423)	NA
Ludwigia octovalis	*L. nervosa*	0.893	0.290	1 (4.240)	0.600
Melastoma malabathricum	*M.malabathricu*	0.976	0	1 (2.847)	0.588
Mimosa diplotricha	*M. albida*	0.053	0.316	1 (1.17)	0.868
Mimosa pudica	*M. camporum*	0	0.091	1 (1.217)	0.690
Senna obtusifolia	*S. didymobotrya*	1.267	0.110	0.8 (2.957)	0.883
Senna occidentalis	*S. hebecarpa*	0.794	NA	1 (1.694)	0.526
Sesbania grandiflora	*S. punicea*	1.224	0.726	1 (1.075)	1

Table 2.2 continued

Sida acuta	*S. spinosa*	1.107	0.778	1 (1.180)	0.963
Sida rhombifolia	*S. cordifolia*	2.267	0.970	1 (1.256)	1.070
Solanum americanum	*S. americanum*	1.204	0.375	0.8 (2.088)	0.6565
Tephrosia candida	*T. purpurea*	0.692	NA	1 (3.667)	NA
Thespesia populnea	*T. populnea*	0.408	NA	1 (0.826)	NA
Vigna marina	*V. caracalla*	0.870	0.110	1 (1.791)	0.540
Xylocarpus granatum	*X. granatum*	1.077	0.600	1 (1.588)	0.267

Table 2.3. Results of generalized least squares (No Phy) and phylogenetic generalized least squares analyses (lambda model) of autofertility index (AFI) and self-compatiblility index (SCI-F) for island and mainland species. A negative difference means islands had lower values. Models with AIC values > 4 points higher than the lowest one are not shown. N = 33 pairs.

Trait	AIC	Lambda	Difference	Standard error	P value
AFI	52.86687	0.2889845	-0.422551	0.117414	<0.0001
AFI (No Phy)	53.54975	----------	-0.421863	0.132204	0.0028
SCI-F	13.94117	-0.3743866	-0.264958	0.078199	0.0015
SCI-F (No Phy)	14.49939	----------	-0.271455	0.074997	<0.0001

Table 2.4. Results of one-tailed t-tests on breeding system indices. *AFI*, autofertility index (based on seed set); *SCI*, self-compatibility index (based on fruit set).

	AFI (N =20 pairs)		*SCI* (N = 22 pairs)	
	Island	Mainland	Island	Mainland
Mean	0.922895	0.501032	0.95	0.678545
SD	0.467317	0.362176	0.122474	0.329761
$T_{0.95,1}$ value	1.685954		1.681952	
P value	0.001421		0.000394	

Table 2.5. Inferred breeding systems of island and mainland species pairs. All island species are self-compatible (SC) with bisexual flowers. References are for mainland breeding systems.

Taxon		Mainland area	Inferred breeding system		References
Island	Mainland		Island	Mainland	
Abelmoschus moschatus	*A. moschatus*	Thailand	FA	A	Hamon and Koechlin 1991
Acacia auriculiformis	*A. paradoxa*	Australia	OA	O	Kenrick Knox1989
Acacia confusa	*A. pycnantha*	Australia	OA	O	Kenrick Knox1990
Aeschynomene indica	*A. amorphoides*	Mexico	FA	FO	Carleial et al., 2015
Asclepias incarnata	*A. incarnata*	USA	FA	MX	Kephart 1981
Bidens pilosa	*B. pilosa*	China	O	MX	Ya-ting et al., 2019
Centrosema molle	*C. virginanum*	Brazil	FO	A	Suede 2019
Crotalaria pallida	*C. micans*	Argentina	OA	AS	Etcheverry et al., 2003
Crotalaria spectabilis	*C. retusa*	Brazil	FA	MX	Jacobi et al 2006
Dalbergia canadensis	*D. miscolobium*	Brazil	FA	O	Gibbs and Sassaki 1997
Datura metel	*D. wrightii*	USA	O	FO	Bronstein etal 2009
Derris trifoliata	*D. trifoliata*	India	FA	FO	Aluri et al 2016
Desmodium heterocarpon	*D. canum*	Venezuela	OA	A	Jaimes and Ramirez 1997
Hibiscus tiliaceus	*H. trionum*	Australia	O	AS	Seed et al., 2005
Impatiens balsamina	*I. parviflora*	Belgium	FO	AS	Vervoort etal 2011
Ipomeoa indica	*I. alba*	South Africa	FA	A	Rambuda Johnson 2004
Ipomoea littoralis	*I. asarifolia*	Brazil	OA	O	Kill and Ranga 2003
Leucaena leucocephala	*L. leucocephala*	China	OA	SI	Brewbaker 1983
Ludwigia octovalis	*L. nervosa*	Venezuela	OA	A	Ramirez and Brito 1990
Melastoma malabathricum	*M. malabathricum*	China	OA	FO	Lu et al. 2009
Mimosa diplotricha	*M. albida*	Venezuela	O	O	Jaimes and Ramirez 1997
M. pudica	*M. camporum*	Venezuela	O	FO	Ramirez and Brito 1990
Senna obtusifolia	*S. didymobotrya*	South Africa	OA	O	Rambuda Johnson 2004
S. occidentalis	*S. hebecarpa*	USA	FA	O	Berry et al., 2013

Table 2.5 continued

Sesbania grandiflora	*S. punicea*	South Africa	FA	A	Rambuda Johnson 2004
Sida acuta	*S. spinosa*	Venezuela	FA	O	James and Ramirez 1997
S. rhombifolia	*S. cordifolia*	Brazil	FA	A	Domingos-Melo et al 2018
Solanum americanum	*S. americanum*	South Africa	OA	FA	Rambuda Johnson 2006
Tephrosia candida	*T. purpurea*	India	OA	A	Kumari and Sharma 2017
Thespesia populnea	*T. populnea*	India	O	FO	Aluri et al., 2020
Vigna marina	*V. caracalla*	India	OA	MX	Gopinathan and Babu 1986
Xylocarpus granatum	*X. granatum*	India	FA	MX	Raju 2019

Supplementary Tables

Supplementary Table 2.0. Categories of breeding systems derived from Dafni 1992.

Level - Breeding system	Explanation
0 – Cleistogamy	SC; flowers are closed, only selfs
1 - Obligate autogamy	SC; flowers are open, only selfs
2 - Facultative autogamy	SC; mostly selfs and selfing individuals have higher seed yield but will outcross when pollinators are available.
3 – Mixed system	SC; selfs and requires pollinators for high seed yield
4 – Facultative outcrossing	Partially SC, sometimes SI; will self but demand for pollinators is high.
5 – Obligate outcrossing	SI or Dioecy; always requires pollination vector, demand for pollinators is high

Supplementary Table 2.1. Mainland species assumed floral types based on images viewed from website dataset.

Mainland species	Sexual system	Floral type	Website
Datura metel	Not known	Bisexual	Atlas of Living Australia. September 30, 2021. *Datura*. *https://bie.ala.org.au/species/https://id.biodiversity.org.au/node/apni/2888664#gallery*
Hibiscus tiliaceus	Not known	Bisexual	Atlas of Living Australia. September 30, 2021. *Hibiscus.* *https://bie.ala.org.au/species/https://id.biodiversity.org.au/taxon/apni/51288506*
Ipomoea alba	Not known	Bisexual	Atlas of Living Australia. September 30, 2021. *Ipomoea.* https://bie.ala.org.au/species/https://id.biodiversity.org.au/node/apni/2913990
Leucaena leucocephala	Not known	Bisexual	Atlas of Living Australia. September 30, 2021. *Leucaena* https://bie.ala.org.au/species/https://id.biodiversity.org.au/node/apni/2909789
Senna obtusifolia	Not known	Bisexual	Atlas of Living Australia. September 30, 2021. *Senna.* *https://bie.ala.org.au/species/https://id.biodiversity.org.au/node/apni/2915623#gallery*
Solanum americanum	Not known	Bisexual	Atlas of Living Australia. September 30, 2021. *Solanum.* https://bie.ala.org.au/species/https://id.biodiversity.org.au/node/apni/2913140

Table 3.0. Data collected for species on Pohnpei island. Asterisked taxa are data reported in Yomai and Williams (2021). Nativity status abbreviations are: C, Cultivated; I, Indigenous; N, Naturalized; and INV, Invasive. Mainland genus indicates mainland species numbers for that genus minus the mainland sister. All values are in mm.

Island taxon	Status[A]	Petal length	Style length	Mainland sister	Mainland genus [B]
*Abelmoschus moschatus**	N	108	NA	*A. enbeepeegearensis*	6
*Abrus precatorius**	N	0.5	NA	*A. precatorius*	3
Acacia auriculiformis[C]*	N	0.7	NA	*A. confusa*	8
Acmella uliginosa	N	1.33	0.52	*A. uliginosa*	4
*Adenanthera pavonine**	N	1.70	NA	*A. colubrina*	5
Adenostemma lanceolatum	N	0.30	0.51	*A. mauritianum*	5
Ageratum conyzoides	N	1.60	0.86	*A. conyzoides*	5
Aglaia mariannensis	I	0.33	2.50	*A. rimosa*	5
Atuna racemosa	I	6.40	5.80	*A. racemosa*	4
Bidens alba	INV	0.65	1.83	*B. pilosa*	9
Calophyllum inophyllum	I	5.75	6.36	*C. inophyllum*	9
Chromolaena odorata	N-INV	1.50	4.53	*C. odorata*	20
Cleome speciosa	N	2.70	9.8	*C. sulfurea*	20
Cleome viscosa	N	14.00	10	*C. viscosa*	9
*Commersonia bartramia**	I	13.00	NA	*Commersonia sp.*	4
Cordia subcordata	I	34.96	18.84	*C. cochinchinensis*	25
*Crotalaria spectabilis**	N	11.30	NA	*C. retusa*	5
*Dalbergia candenatensis**	I	8.00	NA	*D. candenatensis*	11
*Datura metel**	N	50.00	NA	*D. discolor*	7
*Derris trifoliata**	I	7.00	NA	*D. elliptica*	4
*Dissotis rotundifolia**	C-INV	14.00	NA	*D. angustifolia*	4
Elephantopus mollis	N	1.63	1.77	*E. mollis*	7
Emilia sonchifolia	N	0.86	1.02	*E. reddyi*	7
Ficus prolixa	I	NA	0.28	*F. benguetensis*	10
Ficus copiosa	I	NA	0.24	*F. carica*	10
Ficus tinctoria	I	NA	0.62	*F. citrifolia*	10
Ficus virens	I	NA	0.74	*F. microcarpa*	10
Garcinia ponapensis	E	0.70	0.2	*G. paucinervis*	16
*Hibiscus tiliaceus**	I	76.00	NA	*H. tiliaceus*	43
Hypserpa polyandra	I	0.48	0.36	*H. polyandra*	6
*Ipomoea alba**	N	5.40	1	*I. inaccessa*	10
Ipomoea indica	I	0.70	1.72	*I. prolifera*	10
*Ipomoea littoralis**	I	15.89	1.71	*I.littoralis*	10
Lumnitzera littorea	I	3.35	3.96	*L. littorea*	6
Mammea odorata	I	7.80	1.86	*M. suriga*	8
Melanthera biflora	I	5.75	3.08	*M. nivea*	6
Merremia pacifica	I-INV	16.89	7.27	*M. peltata*	10
Merremia peltata	I-INV	3.72	2.15	*M. umbellata*	10

Table 3.0 continued

Pachygone ledermannii	I	4.50	2.24	*P. ovata*	4
Psidium cattleyannum	N-C	3.27	4.59	*P. cattleyannum*	7
*Senna alata**	C-N	17.00	NA	*S. alata*	22
*Senna obtusifolia**	N	23.00	NA	*S. obtusifolia*	22
*Senna occidentalis**	N	16.40	NA	*S. occidentalis*	22
*Sida acuta**	N-INV	8.00	NA	*S. acuta*	8
*Sida rhombifolia**	N	6.30	NA	*S. microcephalus*	8
Sonchus oleraceus	N	0.30	0.80	*S. campanulata*	5
Spathodea campanulata	N-INV	32.61	7.00	*S. nodiflora*	5
Synedrella nodiflora	N	4.73	4.60	*S. pyneei*	10
Syzygium carolinensis	I	5.31	2.85	*S. grande*	10
Syzygium stelechanthum	I	2.40	2.20	*T. brachystemma*	10
Terminalia carolinensis	I	2.10	2.10	*T. complanata*	7
Terminalia catappa	I	2.56	8.33	*T. catappa*	7
Xylocarpus granatum	I	1.75	0.29	*X. granatum*	3

Table 3.1. Phylogenetic least squares regression results for island-mainland comparisons. ALL, full analysis of all species pairs; CONSP, analyses using only conspecific pairs; CONGEN, analyses using only congeneric sister taxa (i.e. conspecific pairs excluded). A positive mean difference indicates mainland values were larger than island values.

Trait (N pairs)	Comparison	Lambda	Mean difference (*SE*)	P value
Petal length (50)	ALL	0.5312090	0.0935233 (0.081754)	0.2555
Petal length (21)	CONSP	0.6061297	0.0331532 (0.108779)	0.7621
Petal length (29)	CONGEN	0.4196441	0.1289707 (0.127279)	0.3154
Style length (43)	ALL	0.7321317	0.2280466 (0.079309)	**0.0051**
Style length (16)	CONSP	0.8615001	0.2412019 (0.085576)	**0.0085**
Style length (27)	CONGEN	0.5071433	0.2112295 (0.114106)	0.0698
Petal & Style (28)	ALL	0.7322958	0.4652058 (0.145914)	**0.0024**
Petal & Style (10)	CONSP	0.9543137	0.2255256 (0.117470)	**0.0709**
Petal & Style (18)	CONGEN	0.5157707	0.5925270 (0.216967)	**0.0420**

Table 3.2. Result of a two-tailed t-test testing for differences between mainland source taxa (sisters to island species) and means of their mainland genus.

Trait (N = pairs)	Source taxa (log mm)	Rest of Genus (log mm)	t Stat	P value
Petal length (50)	0.7003	0.8004	1.661	0.2748
Style length (43)	0.5431	0.6001	1.989	0.6491



Figure 1.1 Photos of flowers in the field: (A) bagged *Vigna hosei*, (B) fully open *Melastoma malabathricum* flower, (C) almost fully open *Hibiscus tiliaceus* and (D) *V. marina* in the field.

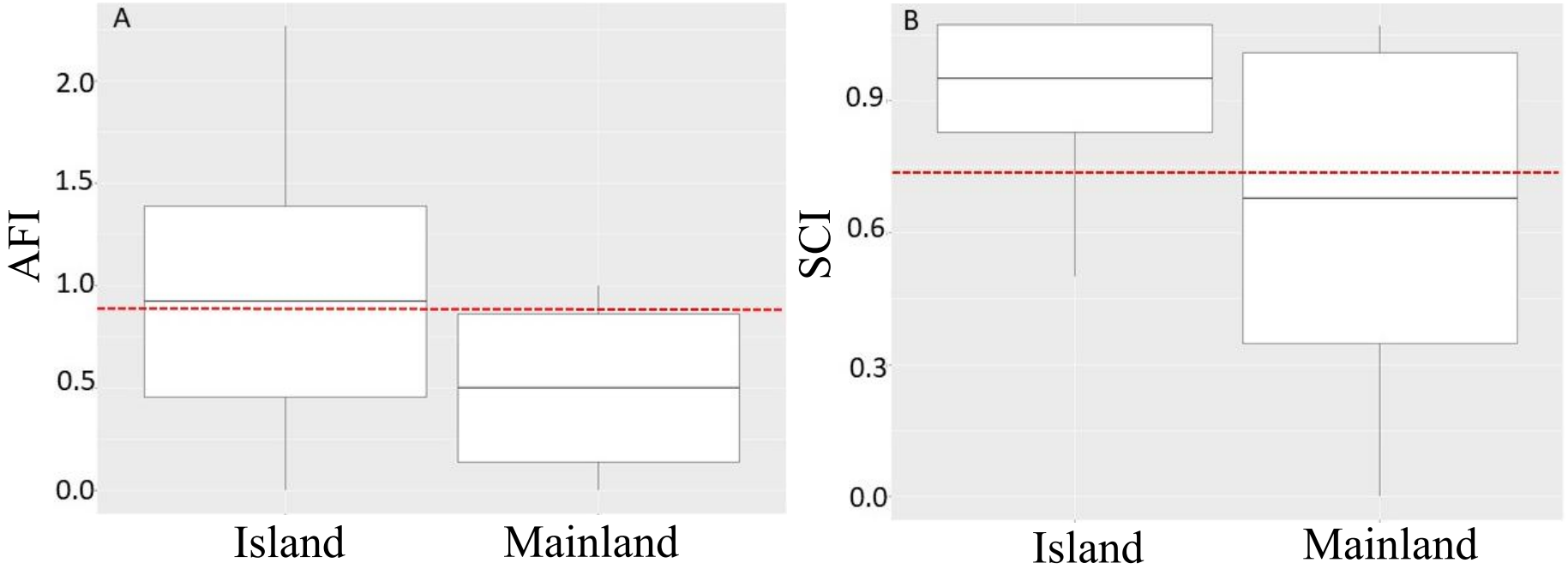


Figure 2.1. Breeding system indices between mainland and island. A) Mean autofertility index (AFI) and B) mean self-compatibility index (SCI). Values above 0.8 (red dashed lines) indicate highly selfing breeding systems. Upper and lower bars represent mean ± standard deviation, and whisker bars represent highest and lowest values.

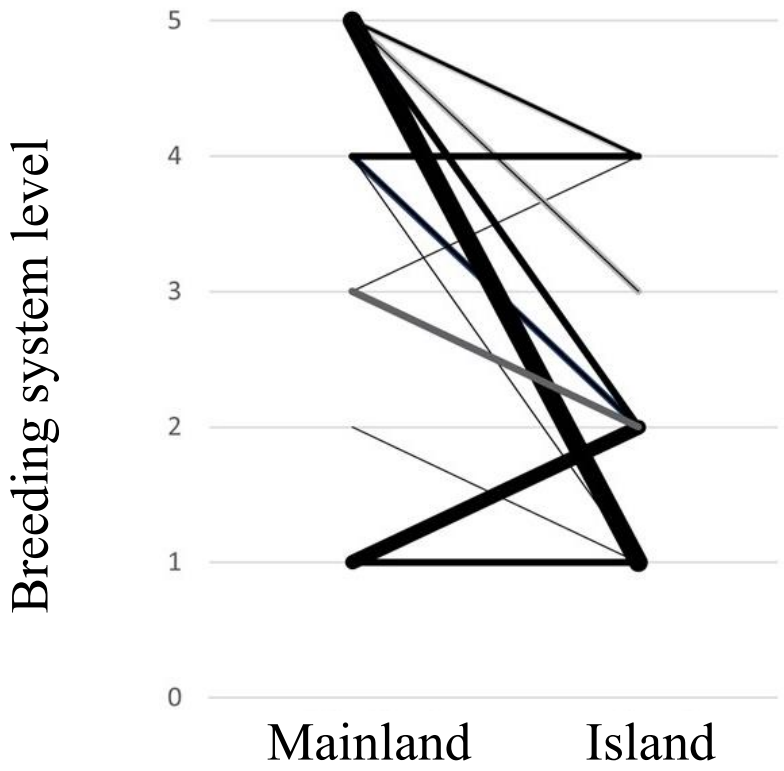


Figure 2.2 Breeding system interaction graph showing transitions between mainland (1) and island (2). Thickness of lines represents number of taxa with the same pattern (range: 1-6). 5 = SI Outcrossing, 4 = Facultative outcross, 3 = Mixed mating, 2 = Facultative autogamy, 1 = Obligate autogamy, 0 = Cleistogamy. N = 33 pairs.

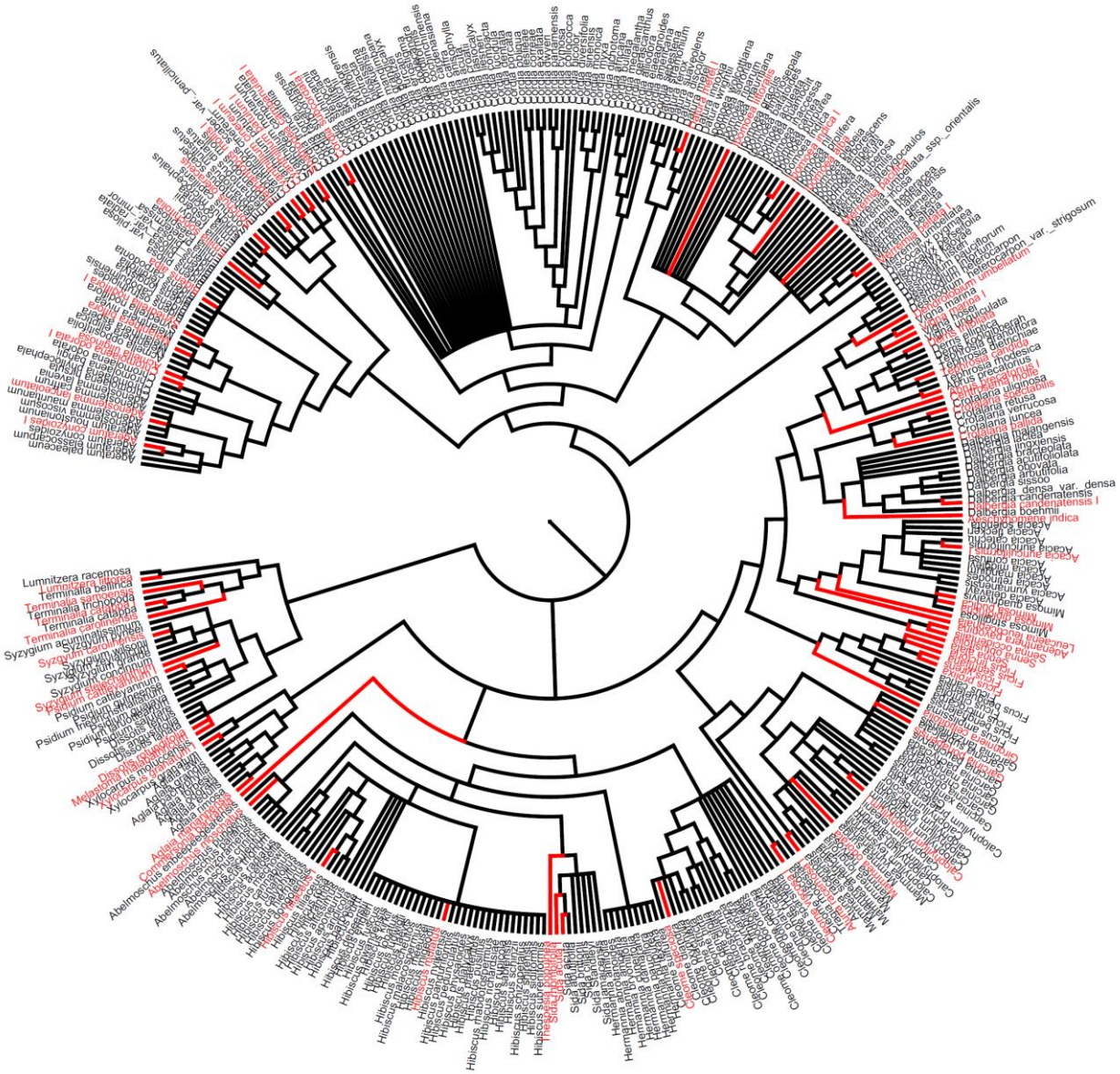


Figure 3.0. Phylogenetic tree containing 392 tips. Terminal branches and names highlighted in red are Pohnpei island species and in black are mainland species from island genera. Taxa with an I are island taxa conspecific with a mainland taxon and were added manually. Topology from Smith and Brown (2018).

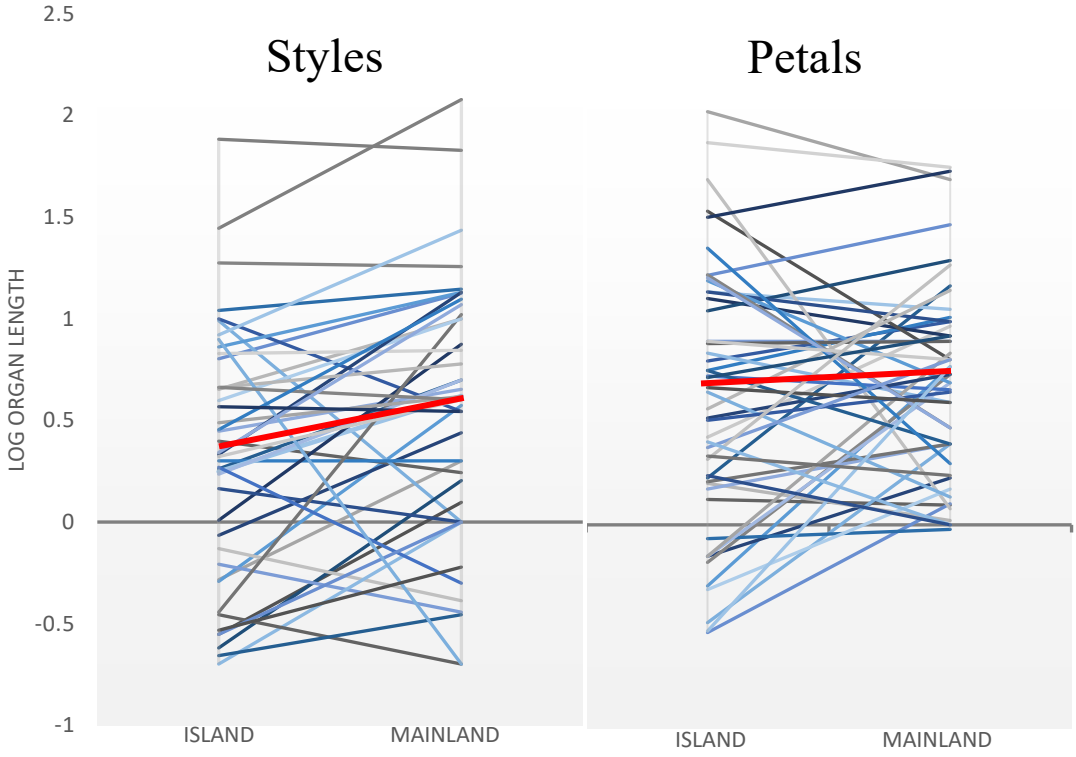


Figure 3.1. Log-log differences in floral organ sizes between Pohnpei island and mainland sister taxa. A) Style lengths (N = 43 pairs), B) Petal lengths (N = 50 pairs). Red lines indicate mean slope for styles (P = 0.008) and for petals (P = 0.256).

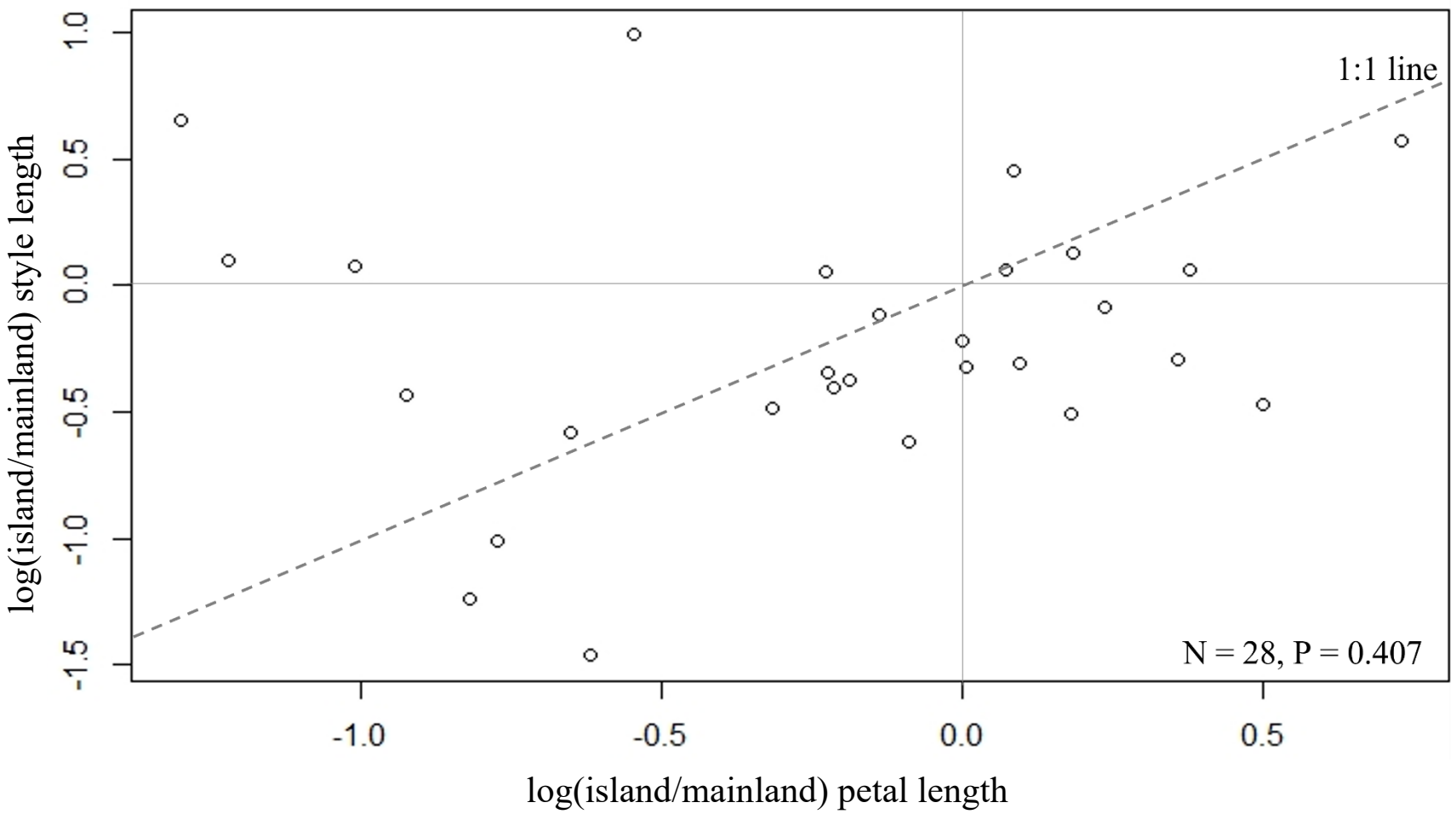


Figure 3.2. Plot of log ratio (LR) of island-mainland petal length versus log ratio of island-mainland style length. LR < 0 indicates organ size is smaller on island than on mainlands. X axis is island petal length – mainland sister petal length, Y axis is island style length – mainland sister style length.

Vita

Viann Marie Harmony Yomai was born and raised in Chuuk, Federated States of Micronesia to a Chuukese mother and a Yapese outer islander father. After graduating from Xavier High School in 2011, she moved to Hawaii for her undergraduate education at Chaminade University of Honolulu, majoring in Environmental Science. Immediately after graduating, Yomai moved to Knoxville, Tennessee to pursue her PhD in Ecology and Evolutionary Biology. All of this would have not been possible without the love, help, and support from Yomai family (Papa Felix, Mama Nian, Lani, Charlotte, Harvest, Tane, Ratul, Stella, and Skybo) and Nam Nguyen).