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Mutualism effects of within and among-tree flowering in a desert fig Ficus petiolaris

Daniel James Gates
Iowa State University

Mutualism effects of within and among-tree flowering in a desert fig *Ficus petiolaris*

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Daniel J. Gates

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Program of Study Committee:
John D. Nason, Major Professor
Kirk A. Moloney
Kevin J. Roe

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TABLE OF CONTENTS

CHAPTER 1. GENERAL INTRODUCTION

Background

Sexual reproduction is nearly ubiquitous among flowering plants, and most plants rely upon some amount of outcrossing among conspecifics. The abilities of plants to transfer genes to neighbors, however, are often limited by a dependence upon some form of pollen vector (Knight et al. 2005). Because of the importance of sexual reproduction for individual fitness, the timing and duration of flowering play a crucial role in the sexual success of an organism. Often, however, the selective forces driving the evolution of floral timing are difficult to determine resulting in a limited understanding of their ecological and evolutionary impacts (Ollerton and Lack 1992). In plants that rely solely upon insects as their pollinating vectors, it has been noted that selection upon flowering phenology is often influenced by peaks in pollinator activities but may be variable across taxonomic and ecological changes (Elzinga et al. 2007).

In obligate pollination mutualisms, plants are dependent upon a specific insect species for pollination, while the pollinator offspring in turn may only develop upon the host plant. In the obligate pollinator mutualism of figs and fig wasps, flowering is highly coevolved and may dictate the fate of both of the mutualists involved (Janzen 1979, Bronstein 1990). The small (2-3 mm) short-lived (2-3 d) pollinator wasps require that fig populations flower highly asynchronously among trees to ensure that periods of pollinator release are accompanied by periods of pollinator receptivity within the short lifespan of the pollinators. In the tropics, populations of figs are observed to accomplish the requirement of asynchronous among tree flowering by initiating syconia synchronously within trees and asynchronously among trees (Milton et al. 1982, Windsor et al 1989). Although this reproductive strategy is expected to

provide increased outcrossing, wasp predator satiation, and increased pollinator and seed disperser attraction, simulations show that large population sizes are required to avoid local pollinator extinctions and mutualism failure. In this thesis we will be investigating how selective pressures to maintain pollinators and subsequent male fitness of plants may change the flowering synchrony of individual fig trees and how the ecology surrounding fig trees is involved in influencing pollen dispersal and reproductive limitations for both pollen receptive and pollen donating trees.

Thesis Overview

Chapter 2

In the chapter two we investigate how an observed change in flowering phenology may contribute to reproductive assurance in a desert fig, *Ficus petiolaris*. In figs that are typically found in small population sizes or those near range margins, asynchronous within tree flowering is often observed and is hypothesized to increase reproductive assurance via allowing for wasps to cycle upon their natal trees (as discussed in Janzen 1979), via increased reproductive overlap of conspecifics within a population (Bronstein et al. 1992), or by a combination of the two. For determination of within-tree floral asynchrony's role in natal tree wasp cycling, we utilize molecular methods to calculate selfing rates for figs of two different populations. In the end, we use detailed censuses from eight different populations to determine where the selective advantages of asynchronous within tree flowering lie and how phenological changes within trees may increase mutualism stability in small populations.

Chapter 3

In the third chapter we set out to quantify how aggregated growth, consistent with that of vegetative reproduction, may influence the expected number of mating individuals in populations of *F. petiolaris*. If clonal growth does occur and flowering of ramets is synchronized by clonal control, numbers of putative mating individuals may be overestimated due to ramets within genets functioning more as a single tree. The same would be true if clonal growth was absent or did not account for all individuals within aggregations but flowering phenologies at short distances were influenced by correlated environmental or genetic factors. If clonal growth occurs and results in independently flowering ramets, as observed in *Ficus prolixa* (Compton and McCormack 1999), vegetative growth may adaptively increase trees ability to extend reproductive phases, increasing their probability of overlap with other sexual individuals within a population. We examine a combination of molecular and ecological data to determine that *F. petiolaris* is indeed capable of clonal growth. We then investigate how flowering is correlated at spatial scales consistent with those expected of vegetative growth.

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CHAPTER 2. ALLEE EFFECTS AND MUTUALISM STABILITY IN FRAGMENTED FIG-FIG WASP POPULATIONS: THE INFLUENCES OF FLOWERING ASYNCHRONY ON WASP DISPERSAL, PLANT MATING SYSTEM, AND REPRODUCTIVE ASSURANCE

Daniel J. Gates and John D. Nason

Abstract

Plants as well as animals may experience reproductive Allee effects in fragmented populations, and obligate pollinations may be especially sensitive to extinction risk via this density dependent process. In this study we examine how a shift from within-crown reproductive synchrony to asynchrony in the Sonoran Desert rock fig, *Ficus petiolaris*, influences reproductive assurance through contributions to selfing and/or outcrossing in small, spatially-isolated populations. Phenological censuses of eight populations distributed along Mexico's Baja California peninsula revealed within-crown asynchrony to be common (44% or reproducing trees) with 16% having sufficient sexual-phase overlap to permit selfing via pollinating fig wasps cycling within trees. Mating system analyses of two populations, however, estimated multilocus outcrossing rates (t_m) indistinguishable from 1, indicating selfing to be absent or at best a minor contributor to reproductive assurance. In contrast, within-crown reproductive synchrony results in pollinator-receptive trees being significantly more frequent (15%) than in related Neotropical figs in which reproduction is synchronous within crowns (3-8%). This suggests that the fitness benefits of within-crown asynchrony lie not with selfing, as commonly asserted, but with increased opportunities for outcrossing,

changing our understanding of how reproductive assurance is enhanced and extinction risk minimized in naturally fragmented populations occurring at the environmental limits of their species' ranges. Given the role of fig fruit as keystone resources vital to the maintenance of frugivore populations in many tropical habitats, our results have broader ecosystem-level implications.

Introduction

As a field, conservation biology is concerned with the biological phenomena that amplify extinction risk as a species becomes rarer and its population sizes smaller. Receiving particular attention from conservation biologists are the effects of demographic variability, the fragmentation and isolation of populations, loss of fitness from reduced genetic heterozygosity, and loss of adaptive potential due to reduced genetic diversity. Inherent in each of these four effects are potential positive feedbacks that may drive an "extinction vortex" leading to the demise of viable populations (Gilpin and Soulé 1986). Moreover, these effects are not independent processes and positive feedbacks among them are likely and may further exacerbate the likelihood of extinction risk.

Less well appreciated for its ability to contribute to extinction vortices are Allee effects. An Allee effect is defined as a positive correlation between population density and the *per capita* growth rate. As pointed out by Allee (1931, 1938), decreasing density or numbers may reduce individual fitness through a variety of possible mechanisms, and more recent theoretical and empirical studies indicate their occurrence to be widespread in natural populations (reviewed by Stephens and Sutherland 1999 and Courchamp et al. 1999). Allee effects are thus of concern with respect to extinction risk both because they are forms of

positive density dependence, reinforcing declines in fitness with density, and because they are not uncommon. Of further concern, in many organisms the lower critical density below which Allee effects limit recovery may be well above the extreme case of a few surviving individuals (Courchamp et al. 2008).

Among many potential Allee effects, a decreased likelihood of mate acquisition as the population density decreases is especially commonly cited. Although primarily studied as a mechanism limiting reproduction in small populations of animals (e.g., Kuussaari et al. 1998; Tcheslavskaia et al. 2002), this reproductive Allee effect is also manifested in plants as reductions in outcross pollination when populations become scarce (e.g., Moeller & Geber 2005; Wagenius et al. 2007). Most plants rely at least partly on sexual reproduction via outcrossing, which is dependent on the movement of pollen from the anthers of one plant to the stigmas of another. Often as population densities or sizes decline, relative rates of pollination, seed set, and, hence, fitness, also decline (Knapp et al. 2001), an Allee effect that has been observed in species in which pollen dispersal relies on animal vectors or wind (reviewed in Courchamp et al. 2008). Many plants are self-compatible, however, and have the capability for outcrossing as well as selfing (the autonomous fertilization of ovules). Although subject to various genetic fitness costs (e.g., inbreeding depression: Charlesworth and Charlesworth 1987; Husband et al. 1996) and benefits (automatic transmission advantage: Nagylaki 1976; Lloyd 1979), the evolution of mating systems mixing selfing and outcrossing is common (Vogler & Kalisz 2001; Goodwillie et al. 2005) and, at the intraspecific level, populations of self-compatible plants can exhibit substantial variation in the relative proportions of selfing and outcrossing (e.g., Holtsford and Ellstrand 1989; Waller and Knight 1989). Theoretical work indicates that mixed mating systems may represent

locally stable optima, with differences among populations arising in part as a consequence of variation in the dependability of pollination services. These pollination services, in turn, often covary with plant population size or density (e.g., Hendrix & Kyhl 2000; Moeller & Geber 2005), suggesting that as density declines and opportunities for outcrossing become limited, traits that favor increased reproduction via selfing may potentially mitigate extinction risks attributable to reproductive Allee effects.

Figs (genus *Ficus*, family Moraceae) provide an unusually interesting system in which to evaluate Allee effects associated with pollinator-limited reproduction in small or low-density populations. Characteristic of all figs, each species is a symbiont in an obligate pollination mutualism with a small (2 mm), short-lived (2-3 d), and species-specific fig wasp (family Agaonidae; superclass Chalcidoidea) (Janzen 1979; Weiblen 2002; Herre et al. 2008). In this highly coevolved interaction, the fig is dependent upon its specific fig wasp for pollination services, while the wasps, in turn, develop within the seeds of their fig host. Although *Ficus* is diverse (>750 species) and a common component of tropical forest ecosystems, individual species often occur at extraordinarily low population densities, creating a challenge for successful fig wasp dispersal between hosts and pollination. Exacerbating this problem, in most monoecious figs reproduction is highly synchronized within the crown of an individual tree with a temporal separation of male and female function (dichogamy) that precludes self-pollination (Nason et al. 1996, 1998). Further, reproductive activity is typically highly asynchronous among trees (Milton et al. 1982; Windsor et al. 1989; Milton 1991) so that at any given point in time the proportion of the population that may interact as potential mates is low. Despite these challenges, reproduction

occurs frequently, apparently as a result of long-distance fig wasp dispersal among trees within very diffuse host populations (Nason et al. 1998; Ahmed et al. in press).

Potential fitness benefits of the unusual reproductive phenology of figs (reviewed in Herre et al. 2008) include enhanced attraction of pollinators and seed dispersers as a result of larger crops on individual trees, satiation of the predators of pollen-bearing fig wasps and seeds, and absence of inbreeding depression due to selfing. Where stressful environmental conditions limit fig population size, however, these benefits may be outweighed by costs incurred by Allee effects associated with reduced reproductive assurance. Theoretical studies suggest that the reliance of figs on highly-specific, short-lived pollinators and asynchronous flowering among trees leads to a high risk of pollinator extinction and mutualism breakdown unless host populations consist of hundreds of individuals (Kjellberg & Maurice 1989; Bronstein et al. 1990; Anstett et al. 1995). Reproductive failure and extirpation of a mutualist may be particularly likely in harsh environments with isolated habitable patches, small local host populations, and hostile conditions for between-patch pollinator migration (Bronstein 1989). In such environments, it has been argued that reproductive assurance may be enhanced, and pollinator extirpation averted, by extending the duration of floral receptivity to pollinators (Khadari et al. 1995; Anstett et al. 1996) or by increasing the extent of intra-crown asynchrony in flowering, the latter enabling wasps to disperse within their natal tree and trees to gain reproductive fitness through selfing (Ramirez 1970; Janzen 1979). Alternatively, increased flowering asynchrony within trees may increase reproductive assurance by increasing the period of flowering (and wasp release and receptivity) within trees and, consequently, sexual-phase overlap between trees and opportunities for outcrossing (Bronstein & Patel 1992; Compton & McCormack 1999). While within-crown asynchrony is

known in a few monoecious figs (Ramirez 1970; Bronstein & Patel 1992; Smith & Bronstein 1996; Cook & Power 1996) and one species has been shown to be self-compatible (*F. aurea*, Hoessart-Mckey & Bronstein 2001), these alternative hypotheses for the benefits of within-tree asynchrony, both of which are expected to alleviate Allee effects in small populations, have yet to be formally tested.

In this paper we investigate within-crown flowering asynchrony, its mating system consequences, and their affects on reproductive assurance in the Sonoran Desert rock fig *F. petiolaris*. *Ficus petiolaris* has the northernmost range of New World figs and is the only species widely distributed across xeric environments in northwestern Mexico where it typically occurs in small, naturally fragmented populations. Other figs occur in warm deserts on other continents making *F. petiolaris* a model system well suited for understanding fig-fig wasp mutualism dynamics at the edge of its environmental limits. Our study had two major objectives. First, we quantified the degree of within-crown reproductive asynchrony and male-female flowering phase overlap in eight *F. petiolaris* populations distributed across Mexico's Baja California peninsula (Figure 1). Smith and Bronstein (1996) previously documented the occurrence of flowering asynchrony and phase overlap asynchrony in this species at two locations in Sonora, Mexico, but did not establish the extent to which this phenological pattern was a local or general phenomenon. Second, at two sites where we found within-tree flowering asynchrony and phase overlap sufficient to permit pollinators to cycle within trees, we used to genetic markers to quantify the relative contributions of selfing and outcrossing.

By quantifying both variation in reproductive phenology and its mating system consequences, we effectively address whether flowering asynchrony in *F. petiolaris*

enhances reproduction assurance via selfing, outcrossing or both. We also gain new insight into whether successful dispersal of the fig wasp is enhanced by the opportunity to cycle within the natal tree. These results, taken together, illustrate the mechanisms by which flowering asynchrony may mitigate potential Allee effects and local mutualism stability in naturally fragmented populations of figs and their wasp pollinators.

Fig Biology

Ficus petiolaris (subgenus *Urostigma*, section *Americana*) is widely distributed across Sonoran Desert habitats in northwestern Mexico and is the only one of ca. 120 New World fig species occurring naturally in Baja California (Felger et al. 2001). Unlike related hemiepiphytic "strangler" figs that form relatively continuous populations in tropical forests, *F. petiolaris* typically occurs in small, isolated populations growing on large rocky outcrops, canyon walls, and cliff faces where reproducing trees range in size from 3 to 15+ m. Reproduction appears to be aseasonal with fig fruit (technically syconia: specialized hollow infloresence/infructesences) occurring at various stages of development at any time of year (Smith & Bronstein 1996; J. Nason, pers. obs.). Like all New World figs, *F. petiolaris* is monoecious with syconia containing both male and female flowers. At receptivity the female flowers release volatile cues that attract pollen-bearing female fig wasps (Borges et al. 2008) which pollinate these flowers while ovipositing into a subset of them. Based on mitochondrial cytochrome oxidase I sequence data (K. Bernhard, unpubl. data), this pollinator is a single, undescribed species of *Blastophaga*.

Study Sites

Locations in southern Baja are more mesic (>25 cm ave. annual precipitation),
receiving primarily summer rains (July-September) and grading from South Gulf Coast
Desert into Cape Arid Tropical Forest phytogeographic areas (Roberts 1989). Locations in
northern Baja are drier (<15 cm ave. annual precipitation), receiving primarily winter rains,
and grading to the north from South Gulf Coast Desert to the drier San Felipe Desert region.
Populations to the north, where environmental conditions are more extreme (lower rainfall;
higher and lower average maximum and minimum temperatures), are generally smaller and
more isolated than to the south.

Methods

We censused the reproductive phenology of individual trees in eight populations
separated by up to 740 km along the Baja California peninsula (Table 1; Figure 1). Twenty
one phenological censuses were conducted at seven time points spanning a period of three
years, with the number of times a population was surveyed ranging from 1-6 (some sites are
in remote roadless areas and difficult to access). The number of trees censused per survey per
site was subject to population size/density constraints and ranged from 4-87 (mean 42; Table
2). Also due to these constraints, overlapping sets of trees were censused at sites surveyed
multiple times such that these surveys were not statistically independent. For each tree
censused, we estimated its reproductive output as being none, ≤5%, 5-25%, 25-50%, 50-
75%, 75-95%, or ≥95% of its maximum potential. To determine the extent of a tree's
reproductive synchrony/asynchrony we sampled syconia classifying them into five
developmental stages: pre-female phase (pistillate and staminate flowers immature), female
phase (pistillate flowers mature; syconia receptive to pollinating fig wasps), inter-floral phase

(post-pollination; seeds and larval fig wasps developing), male phase (staminate flowers and seed mature; adult fig wasps present within the syconium), post-male phase (syconium expanding, softening, and sweetening; wasps have exited via hole chewed by male fig wasps). We sampled a minimum of 30 syconia per tree, when we were not able to sample the defined 30 syconia we censused from as many syconia as we could gather. From these censuses we determined the developmental stages present as well as their relative proportions.

A tree was considered to have reproductive asynchrony if more than one reproductive stage was present at the time of census. Reproductive asynchrony at the population level was measured as the proportion of asynchronous trees. We also calculated the proportion of trees bearing pollinator receptive female-phase syconia that also had pollinator releasing male-phase syconia present. This is the specific form of sexual-phase overlap necessary for reproductive assurance via self-fertilization.

Within-crown reproductive asynchrony may also enhance reproductive assurance via outcrossing by increasing male and female sexual-phase overlap with other members of the population. One means of assessing whether this asynchrony is an adaptation enhancing the potential for outcrossing is to compare the extent of sexual-phase overlap between populations in which reproduction is synchronous or asynchronous within trees. As a measure for comparison, we focus on the expected proportion of trees in female phase at any given time, a key indicator of population-level pollen and pollinator receptivity. Following the approach of Nason et al. (1998) for tropical figs, we conservatively assumed that individual trees reproduce aseasonally twice per year (Windsor et al. 1989; Milton 1991) and that the pistillate flowering phase lasts seven days (Anstett et al. 1997). Given these

assumptions we estimated the expected proportion of trees in female-phase and receptive to pollinators at any given time to be 0.038 ([2 x 7 d] / 365 d). Although not generally observed in Neotropical figs, allowing for an increase in the duration of female phase (Khadari et al. 1995) to 14 days, this probability doubles to 0.077. These expectations given within-crown synchrony were used as null hypotheses against which we tested the observed proportion of trees bearing female-phase syconia in *F. petiolaris*. Specifically, for each population we calculated the average proportion of trees observed in female phase for census date and over dates as well as their 95% binomial confidence limits (Zar 1999). We concluded that within-crown asynchrony enhances reproductive assurance via outcrossing when the lower confidence limit was greater than the expected proportion of trees in female phase given within-crown synchrony.

We also conducted two additional analyses of the phenological data. First, the three southernmost *F. petiolaris* populations are located in Baja's Cape Region (Figure 1) where there is a pronounced monsoon season in July-September. For these populations we employed contingency table analysis (using JMP 8, SAS Institute) to test whether the incidence of within-crown asynchrony in censuses conducted prior to the wet season (May-June) differed from those conducted after (Oct.-Dec.). Second, because populations to the north, where environmental conditions are more extreme, are generally smaller and more isolated than to the south, within-crown asynchrony may increase with latitude. We tested this hypothesis by conducting a weighted linear regression of population mean flowering asynchrony on latitude. A commonly used weight is the inverse of the variance, but, because of small population sample size (Site 178) or very low overall reproductive activity (Site 172), in two northern sites the censused flowering asynchrony was zero, in which case the

inverse variance is undefined. Consequently, we weighted by the number of trees sampled, allowing us to include data from these populations. This analysis was conducted on May-June census data only as fall censuses were conducted only for the three southernmost populations.

Mating system analysis

In order to evaluate whether within-crown asynchrony enhances reproductive assurance via self-fertilization we conducted genetic analyses of the mating system. We selected two populations for analysis based on the availability of mature fruit. Both populations, La Paz Summit (Site 96) and San Bartolo (Site 70), are located in southern Baja California Sur ca. 40 km apart in the relatively mesic Cape Arid Tropical Forest (Roberts 1989).

In order to quantify the mating system at each site, we collected mature, male-phase fruits, split them into quarter sections, and dried them in the field. Seeds were later removed for germination in 3x3 inch pots filled with 2 inches of potting soil covered by 0.5 inch of coarse sand. Up to 30 seeds per fruit, were sprinkled over the top of the sand. The pots were place on a mist bench for 4 weeks before removal to a greenhouse bench. Pots were then hand-watered daily and fertilized weekly. Approximately 10 weeks after germination leaf material was removed from seedlings for allozyme gel electrophoresis. We extracted fresh leaf tissue in the buffer of Wendel and Parks (1983), transferred extracts onto 2x7 mm chromatography paper wicks, and stored these wicks in 96 well plates at -80 ˚C until the electrophoresis step.

Following the methods of Nason et al. (1996) and Nason and Hamrick (1997) for

other New World *Ficus*, we assayed seedlings for 15 polymorphic allozyme loci: Aconitate

hydratase (ACN1, 2; EC 4.2.1.3), aspartate aminotranferase (AAT1, 2,3; EC 2.6.1.1,

diaphorase (DIA; EC 1.8.1.4), fluorescent esterase (FE, 3.1.1. -), glucophosphate isomerase

(GPI; EC 5.3.1.9), isocitrate dehydrogenase (IDH, EC 1.1.1.42), malate dehydrogenase

(MDH1, 2; EC 1.1.1.37), phosphoglucomutase (PGM; EC 5.4.2.2), 6-phosphogluconate

dehydrogenase (PGD; EC 1.1.1.49), shikimate dehydrogenase (SKD; EC 1.1.1.25), and

triosephosphate isomerase (TPI; EC 5.3.1.1). These loci were resolved in 15% starch gels

(Starch Art Corp.) using the following gel tray/buffer systems: AAT, DIA, and FE in System

8 (Soltis et al. 1983), PGM, SKD, and TPI in System 11 (Soltis et al. 1983), and ACN, IDH,

MDH, and PGD in a 0.04 M morpholine-citrate buffer system adjusted to pH 7 (Murphy et

al. 1996). Staining procedures for all loci followed Soltis et al. (1983). As in other figs,

segregation patterns in progeny arrays were consistent with Mendelian inheritance expected

subunit structures (monomeric or dimeric).

To quantify the mating system of *F. petiolaris* based on the molecular marker data we

used the methods of Ritland and Jain (1981) and the program MLTR (Ritland 2002), which

employs maximum likelihood procedures to obtain a multilocus estimate of the rate of

outcrossing (t_m) (with selfing rate $s = 1 - t_m$). We used the Newton-Raphson (NR) method to

estimate t_m at both the individual tree and population levels because it corrects for missing

data and is recommended for species with low selfing and inbreeding (Ritland 1986).

Estimates with low standard error are generally obtained using ca. $N = 200$ progeny (Ritland

2002), as a result population-level estimates (with $N > 290$) are quite robust while tree-level

estimates (with $N < 100$) are necessarily noisy. We obtained a second population-level

estimate of t_m using the expectation-maximization (EM). The EM estimates range $t_m \leq 1$ but

may underestimate the true outcrossing rate in primarily outcrossing species. The population-level estimates are obtained assuming all maternal trees outcross at the same rate from a homogeneous pollen pool. For both estimation methods we allowed MLTR to simultaneously estimate the population-inbreeding coefficient (F). Since these estimates of F were not significantly different from zero (nor are estimates for adult trees based on three microsatellite loci; D. Gates, unpublished data), final estimates of t_m were obtained constraining $F = 0$ with standard errors obtained via bootstrapping (100 replicates).

Results

Phenological data

Phenological data collected through censusing of the *F. petiolaris* study populations are summarized in Table 2. The number of individuals within population censuses varied, but with one exception (Site 178) we were able to detect reproductive individuals and within-crown asynchrony, even where census sample sizes were small. These smaller samples are included because populations of a few isolated fig trees trees may be of greater interest to reproductive assurance than large expansive populations (Compton et al. 1994).

Reproductive activity within populations was generally high with the proportion of trees bearing syconia ranging from zero to 0.83 with an overall mean of 0.48 across census dates and sites (Table 2). The mean over sites (calculated using a single mean value per site) was lower, 0.38, primarily due to down weighting contributions from Site 96, which was censused seven times and generally had high reproductive activity relative to the overall mean. The probability of within-crown asynchrony given reproduction was also high ranging from zero to 0.80 with mean 0.48. The mean over sites, 0.44, was in this case similar. Those

reproductive trees that were not asynchronous typically bore interphase syconia. The overall

mean and means over sites were similar for the frequencies of reproductive trees bearing

female-phase syconia (0.40 and 0.39) and for male-phase syconia (0.18 and 0.17). Because of

the relatively low frequency of trees bearing male-phase syconia, the probabiltity of sexual-

phase overlap within-trees was also low, with an overall mean and mean over sites of 0.13

and 0.10, respectively.

The conditional probability that an individual tree bearing male-phase, pollinator

releasing syconia also bore female-phase, pollinator receptive syconia, had an overall mean

of 0.24 and mean over sites of 0.16 (Table 2). This probability measures the sexual-phase

overlap necessary for selfing to occur and was highly variable across census dates and sites

(range 0 to 1), suggesting that while phenological opportunities for selfing are not uncommon

in *F. petiolaris*, they are relatively sporadic within trees. While often low, this conditional

sexual-phase overlap was at times quite common, exceeding 0.35 in eight of 21 population

censuses (Table 2). Relative to related New World *Ficus*, the proportion of trees bearing

female-phase syconia in *F. petiolaris* was substantially higher: 0.19 and 0.15 (overall mean

and mean over sites) versus the 0.038 to 0.077 expected with within-crown synchrony. Of the

17 individual population censuses, in 11 the lower 95% binomial confidence limit was

greater than 0.077. At the population level, five of eight had means significantly greater than

0.038 with 4 greater than 0.077 (Figure 2).

Pooling together data from the three Cape Region populations (Sites 70, 96, and

119), contingency table analysis identified significantly higher within-crown asynchrony at

the end of the dry season (mean 0.73) than following the wet season (mean 0.45; $x_1^2 = 29.9$,

$P<0.0001$). Tests conducted separately for each population were significant at Sites 96

(dry/wet means 0.73/0.41; $\chi^2_1 = 24.74$, $P<0.001$) and 119 (dry/wet means 0.75/0.38; $\chi^2_1 = 4.00$, $P=0.045$), and, though not significant, Site 70 trended in the same direction (dry/wet means 0.68/0.56; $\chi^2_1 = 0.91$, $P=0.340$). In contrast, we did not obtain significant results for regressions of population mean within-crown asynchrony on latitude ($F_{1,5} = 1.09$, $P=0.344$) or population mean proportions of trees bearing female-phase syconia on latitude ($F_{1,6} = 3.74$, $P=0.101$).

Mating system analysis

Maximum likelihood estimates of outcrossing rates are presented in Table 3 for individual trees and for each of the two study populations (Sites 70 and 96). Eight maternal trees were analyzed at each site with fruits (syconia) per tree ranging from 1-10 (mean 4.7) and progeny per eight maternal trees ranging from 11-96 (mean 38.1). At the population-level, the mean numbers of fruits and progeny per site were 37.5 and 305, respectively. Multilocus and single locus estimates of the outcrossing rate t for individual trees were generally similar and in all cases statistically indistinguishable from one (though with the high variance typical of individual-level estimates). Most of these estimates were between 1 and 1.2, as expected with outcrossing using the NR method. Only for tree "unknown1" at Site 70 was t_m less than 0.9 (0.70±0.33). As a further indication of no or only very low selfing, all fruits assayed contained at least one offspring whose multilocus genotype contained an allele incompatible with that of the mother.

Population-level estimates using the NR and EM methods were similar and, like the individual tree estimates, were indistinguishable from complete outcrossing at both sites.

These estimates were statistically unaffected by fixing the inbreeding coefficient at $F = 0$ or allowing it to vary. In the latter case, F estimated by MLTR was not significantly greater than zero (Site 70 – NR: -0.20±0.09, EM: 0.001±0.001; Site 96 – NR: -0.20±0.00, EM: 0.004±0.002).

In Neotropical figs, there is no pollen carryover and female fig wasps (foundresses) die within the syconia they pollinate. 31 of the 75 syconia used in the mating system analysis contained the corpse of single pollinator and so must have received pollen from a single donor. Inspection of progeny genotypes showed that for all 31 syconia at least one offspring had an allele not found in the mother, indicating that all offspring in each of these syconia must have been sired by a single, outcross pollen donor (a result consistent with the MLTR analyses). This simple procedure to exclude selfing could not be applied to the remaining fruit (containing two or more foundresses), but it is notable that they too all contained at least one seed with a genotype that could only have been produced through outcrossing.

Discussion

The fig/fig wasp mutualism, by its nature would seem to be subject to high levels of extinction risk where the number of hosts is small, as in extreme environments or at the margins of a species' range. With a single, species-specific pollinator and the synchronous within-crown reproductive development typical of wet tropical figs, pollinator-limited reproduction is expected to increase as host population size decreases, underlying an Allee effect that may threaten the local stability of the mutualism at relatively high host population size thresholds (Anstett et al. 1995). Nonetheless, small, apparently viable fig populations occur in a variety of marginal habitats, suggesting they possess adaptations counteracting

expected pollen limitation affects. One such adaptation that is often cited is within-tree reproductive asynchrony (e.g., Janzen 1979; Bronstein 1990; Smith and Bronstein 1996), which may contribute to reproductive assurance via two mechanisms: (1) it may increase the overlap of male- and female-flowering phases within a tree, increasing the opportunity for self-fertilization via cycling of pollinating wasps within the natal host, or (2) it may increase the extent of a trees' flowering-phase overlap with other members of the population, thereby providing increasing opportunities for outcrossing and fitness benefits via both male (pollen donation) and female (pollen receipt) function.

Within-tree reproductive asynchrony in Ficus petiolaris

Our results indicate that within-tree asynchrony is common and widespread in *F. petiolaris*. If within-tree asynchrony acts as a mechanism to increase reproductive assurance in figs, we would expect to see greater asynchrony when and where pollen is more limited. Two situations where we expect this to be the case are in association with dry, as opposed to wet, seasons and with increasing latitudes. When we examined southern Baja populations subject to strong wet/dry seasonality, we found 10-30% higher levels of asynchrony in the dry versus the wet season (Table 2). This suggests adaptive plasticity with respect to seasonal environmental conditions. These results are similar to those of Smith and Bronstein (1996) who reported higher asynchrony in *F. petiolaris* and *F. insipida* at a drier site in Sonora, Mexico, where populations are smaller, than at a wetter one, where populations are larger, though they did not find distinct wet/dry season trends within sites. In contrast to significant wet/dry season effects, we did not find a trend of increasing asynchrony with latitude.

Asynchrony was quite variable among sites (and among replicate censuses within sites; Table 2), however, suggesting that our power to detect any potential latitudinal effect was low.

Sexual-phase overlap within trees is required for self-fertilization, which is commonly regarded as a mechanism of reproductive assurance where fig populations are small and susceptible to reproductive Allee effects (Ramirez 1970; Janzen 1979). In our study, this overlap was highly variable within and among censuses and sites but was not uncommon, with an overall mean of 0.24 and mean over sites of 0.16 (Table 2). These results are consistent with those of Smith and Bronstein (1996) who reported sexual-phase overlap of 0.06 and 0.38 for *F. petiolaris* at a wetter and drier site, respectively. The incidence of overlap has also been investigated in three of the four other fig species with ranges nearing the latitudinal limits of *Ficus* in N. America. Overlap in northern populations of these species is similar to that in *F. petiolaris*: *F. aurea* (0.20; Bronstein & Patel 1992), *F. insipida* (0.15 and 0.27 at a wetter and drier site, respectively; Smith & Bronstein 1996), and *F. pertusa* (0.08 at a wetter site; Smith & Bronstein 1996).

Although the frequency of sexual-phase overlap is relatively low, one might imagine it could still play a significant role in reproductive assurance by promoting selfing while contributing to the maintenance of pollinator populations by permitting them to cycle within trees. Further, we might expect these benefits to be especially important during times of year when environmental conditions are not conducive to successful fig wasp dispersal. Our mating system analyses, however, indicate that these benefits to sexual-phase overlap are little or none.

Limits to selfing in Ficus petiolaris

Although previous research has described within-crown flowering asynchrony in monoecious figs (e.g., Bronstein 1989, Bronstein and Patel 1992, Smith and Bronstein 1996, Cook & Power 1996), to understand fitness costs and benefits associated with this mode of reproduction we need studies separating the extent of reproductive assurance gained through selfing via increased overlap of sexual phases within trees, versus those gained through outcrossing via increased overlap of sexual phases with other members of the population. This study is the first to address this issue.

Based on the multilocus genetic analysis of nearly 600 progeny, we found the production of selfed progeny and inbreeding in two *F. petiolaris* populations to be absent or so low as to be undetectable. While this result is unexpected with respect to the predicted relationship between within-crown reproductive asynchrony and selfing (Janzen 1979), it is generally consistent with the low selfing rates characteristic of tropical trees (Nason and Hamrick 1997), as well as the high outcrossing rates, unusually long-distance pollen movement, and absence of inbreeding found in other New World *Ficus* (Nason et al. 1996, 1998). Although one species of monoecious fig, *F. aurea*, has been shown to be self-compatible (Hossaert-McKey and Bronstein 2001), inbreeding depression is usually strong in long-lived woody plants, including tropical trees (e.g., Nason and Hamrick 1997). In figs, the successful development of selfed-fruit may well be limited by the abortion of seeds (potentially killing seeds or wasps) or entire fruit (killing both).

Seed and fruit abortion would also impose strong selection on pollinators to disperse from, and not pollinate within, their natal tree, this is an additional, possible explanation for why we failed to detect selfed syconia. Although we did not directly investigate the cycling of pollinating wasps within crowns of natal trees, we have not observed any behavior suggest

this is at all common. When pollinating wasps emerge from their natal fruits they are first attracted to light and immediately fly upwards (Anstett et al 1996), a behavior we have observed on *F. petiolaris* (J. Nason pers. obs.). This is expected to have fitness benefits in terms of reduced risk of predation and increased dispersal. In terms of predation, this flight behavior immediately removes emerging fig wasps from predatory ants, which are common in fig trees, and dispersing from the vicinity of the natal tree decreases densities that would attract predators like birds (Bronstein 1988). Second, by entering the air column they are able to drift passively for long distances before intercepting and responding to the plume of volatile chemical attractant produced by a receptive host tree (Ware and Compton 1994; Nason et al. 1998). Because this behavior is stereotypical of fig wasps and potentially canalized, it may simply not be conducive to the cycling of wasps within trees, as is required for selfing and potential benefits for reproductive assurance to occur.

Instead of promoting selfing, it appears that within-tree flowering asynchrony enhances reproductive assurance via outcrossing. In figs with within-tree synchrony, gaps in male- and female-phase overlap among individuals are expected to be substantial in populations of small size. Under conditions of small population size, within-tree asynchrony appears to enhance outcross reproduction by increasing overlap in the period of a tree's flowering activity with conspecifics. Further, it increases the number of reproductively active trees at any point in time. These conclusions are supported by our phenological data for *F. petiolaris*, including the observation that the proportion of trees in female phase at any given time, an indicator of population-level pollinator receptivity, was at most sites significantly higher than in Neotropical figs in which reproduction is synchronized (Figure 2). Indeed, trees bearing female-phase syconia were commonly 2-5 times more frequent than expected

under reproductive synchrony. Our interpretation of the benefits of asynchrony for outcrossing are consistent with the results of two simulation studies (Bronstein 1990; Anstett et al 1995) focusing on the unique pollination biology of the fig-wasp mutualism and Allee effects associated with limited mate availability as population size decreases. These studies found that increasing the duration of female and male flowering phases *within* syconia decreased the minimum critical population size required to maintain viable pollinator populations because it increased the probability of temporal overlap in the production of male- and female-phase syconia on conspecific trees. An extended duration of female-phase syconia from the typical 1 wk to up to 3 wks has been reported for some fig species at the latitudinal or elevational margins of their ranges (Bronstein 1988; Khadari et al. 1995; Anstett et al. 1996), and appears to occur in *F petiolaris* as well (J. Nason pers. obs.). While physiological constraints limit how long individual female-phase syconia can wait for pollinators, within-crown asynchrony can greatly extend the duration of a trees receptivity to pollinators, as well as its production of pollen carrying wasps, by spreading the development of female- and male-phase syconia through time.

Broader implications of mutualism persistence

Some species have effects on community structure – species abundance and diversity – that greatly exceed that of other species of similar or greater local density. These species have been termed keystone species and because of their multifold affects on ecosystem form and function may be of special concern for conservation (Mills et al. 1994; Paine 1995). Fig trees are considered to be keystone species in many tropical and subtropical forest ecosystems. Indeed, because of generally aseasonal reproduction and large fruit crops they

contribute heavily to the diets of many specialist and non-specialist vertebrates, especially

during seasons in which fruiting activity of other tree species is low (Terborgh 1986;

Lambert & Marshall 1991; Shanahan *et al*. 2001; Harrison 2005; Kissling *et al.* 2007).

In desert and other environments stressful to figs and their wasp pollinators, fig

species diversity is low and the importance of fig fruit in maintaining frugivore populations is

little studied. Given the lower productivity of deserts, however, those figs that do occur may

yet play an important ecosystem function in supporting both frugivore density and diversity.

The contribution of within-tree reproductive asynchrony to this function is at least two-fold.

First, in species like *F. petiolaris* it appears to be widespread and important adaptation for

mutualist survival, especially in the face of small population sizes. In the absence of within-

tree asynchrony any potential ecosystem services provided by figs and fig wasps in extreme

environments would be moot: fig/fig wasp populations would not persist there to provide

them. Second, within-crown asynchrony increases the time period over which fruit are

available to frugivores within individual trees. Coupled with general pattern of aseasonal

reproduction, fruit should be locally available to frugivores year round, even where fig

populations are small. In sum, within-tree reproductive asynchrony appears to be a key

adaptation to survival of the fig/fig wasp mutualism in stressful environments, which, in turn,

may have important implications for ecosystem function in these areas.

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Table 1. Latitude and longitude of eight *Ficus petiolaris* study populations located along peninsular Baja California, Mexico. Populations are arranged from south to north. *N* denotes the total number of trees censused at a location.

Site number	Site name	Latitude	Longitude	Elevation (m)	*N*
70	San Bartolo	23.73652	-109.84383	362	72
119	El Potréro	23.81622	-110.16687	375	34
96	La Paz Summit	24.02061	-110.09076	553	180
178	La Trinidad	26.75476	-112.13293	167	8
113	Santa Agueda	27.24018	-112.37004	186	20
112	Mesa de La Caguama	27.57151	-113.07616	1104	36
172	La Purificación	28.29230	-113.11473	665	32
158	Aguajito Higuera	29.26153	-114.01678	852	62

Table 2. Reproductive census data for eight *Ficus petiolaris* study populations located in Baja California, Mexico. For each census, *N* is the number of different trees censused, $P(R|C)$ is the frequency of reproduction among censused trees, $P(A|R)$ is the frequency of flowering asynchrony among reproductive trees, $P(F|R)$ and $P(M|R)$ are the frequencies of trees bearing pollinator receptive female- and pollinator releasing male-phase syconia, respectively, among reproductive trees, $P(F|M)$ is the probability a tree bears female-phase syconia given that male phase syconia are also present, and $P(F)$ is the proportion of trees bearing female phase-syconia. The overall mean is calculated over censuses whereas the mean over sites is calculated using a single mean value per site. Population names and locations are given in Table 1.

Site	Year	Month	*N*	$P(R\|C)$	$P(A\|R)$	$P(F\|R)$	$P(M\|R)$	$P(F\|M)$	$P(F)$
70	2005	May	69	0.23	0.69	0.44	0.44	0.38	0.10
70	2006	May	37	0.43	0.64	0.31	0.31	0.40	0.14
70	2006	Dec	44	0.41	0.44	0.39	0.06	0.00	0.16
70	2007	Oct	41	0.83	0.65	1.00	0.00	0.00	0.83
70	2008	Nov	43	0.47	0.50	0.80	0.05	1.00	0.37
70 mean:			46.8	0.47	0.58	0.59	0.17	0.36	0.30
119	2006	Dec	7	0.57	0.00	0.00	0.00	-	0.00

Table 2. (Continued)

Site	Year	Month	N	P(R|C)	P(A|R)	P(F|R)	P(M|R)	P(F|M)	P(F)
119	2008	June	35	0.57	0.80	0.25	0.65	0.00	0.14
119	2008	Nov	24	0.33	0.38	0.63	0.00	0.00	0.21
119 mean:			22.0	0.49	0.39	0.29	0.22	0.00	0.15
96	2005	May	48	0.75	0.56	0.39	0.17	0.53	0.29
96	2006	May	87	0.74	0.77	0.47	0.11	1.00	0.34
96	2006	Dec	85	0.44	0.35	0.51	0.00	0.00	0.22
96	2007	Oct	69	0.78	0.61	0.03	0.49	0.00	0.02
96	2007	Nov	55	0.64	0.46	0.38	0.00	0.38	0.12
96	2008	Nov	81	0.32	0.58	0.62	0.38	0.00	0.47
96	2008	June	17	0.77	0.51	0.40	0.19	0.53	0.21
96 mean:			63.1	0.63	0.55	0.39	0.17	0.32	0.15
178	2008	June	8	0.00	-	-	-	-	0.00
113	2008	May	20	0.35	0.71	0.43	0.00	0.00	0.15

Table 2. (Continued)

Site	Year	Month	N	$P(R|C)$	$P(A|R)$	$P(F|R)$	$P(M|R)$	$P(F|M)$	$P(F)$
112	2006	June	4	0.75	0.00	0.00	0.00	-	0.00
112	2008	May	36	0.69	0.36	0.00	0.28	0.00	0.00
112 mean:			20.0	0.72	0.36	0.00	0.14	0.00	0.00
172	2008	May	32	0.06	0.00	0.50	0.00	0.00	0.03
158	2008	May	60	0.33	0.65	0.50	0.45	0.44	0.17
Overall mean:			41.6	0.48	0.48	0.40	0.18	0.24	0.19
Mean over sites:			33.9	0.38	0.44	0.39	0.17	0.16	0.14

Table 3. Tree- and population-level mating system analyses of *Ficus petiolaris* at San Bartolo

and La Paz Summit, located in southern Baja California. Fruits/tree and offspr./tree are the

number of fig fruit (syconia) and total number of offspring genetically assayed per tree. t_m

and t_s are multilocus and single locus outcrossing rate estimates. Tree- and population-level

estimates obtained using the Newton Raphson (NR) method with the latter also estimating

using the expectation maximization (EM) algorithm.

Population name	Tree number	Fruits/ tree	Offspr./ tree	t_m	t_s
San Bartolo (70)					
misc1	1	12		1.20 (0.58)	1.20 (0.57)
	2	8	67	1.20 (0.56)	1.16 (0.55)
	35	5	11	1.02 (0.48)	0.99 (0.45)
	37	2	15	1.20 (0.53)	1.20 (0.52)
	6	4	38	1.20 (0.55)	0.96 (0.45)
	1a	9	58	1.20 (0.57)	1.05 (0.50)
	1b	10	96	1.20 (0.55)	1.04 (0.48)
	unkown1	3	20	0.70 (0.33)	0.70 (0.33)
Population-level: NR	42	317		1.15 (0.06)	1.049 (0.04)
EM	42	317		1.00 (0.00)	0.963 (0.004)

La Paz Summit (96)

Table 3. (Continued)

Population name	Tree number	Fruits/ tree	Offspr./ tree	t_m	t_s
96	113	4	41	1.20 (0.58)	1.20 (0.59)
	115	3	43	0.91 (0.43)	0.85 (0.43)
	116	4	31	1.20 (0.59)	1.07 (0.49)
	39	5	59	1.20 (0.58)	1.12 (0.52)
	45B	4	21	1.20 (0.52)	1.20 (0.53)
	46	5	31	1.20 (0.57)	1.20 (0.57)
	51	3	28	1.18 (0.56)	1.20 (0.55)
	98	5	39	1.18 (0.56)	1.20 (0.51)
Population-level:	NR	33	293	1.01 (0.09)	1.00 (.049)
	EM	33	293	1.00 (.005)	0.965 (.007)

Figure 1. Eight *Ficus petiolaris* study population distributed along peninsular Baja

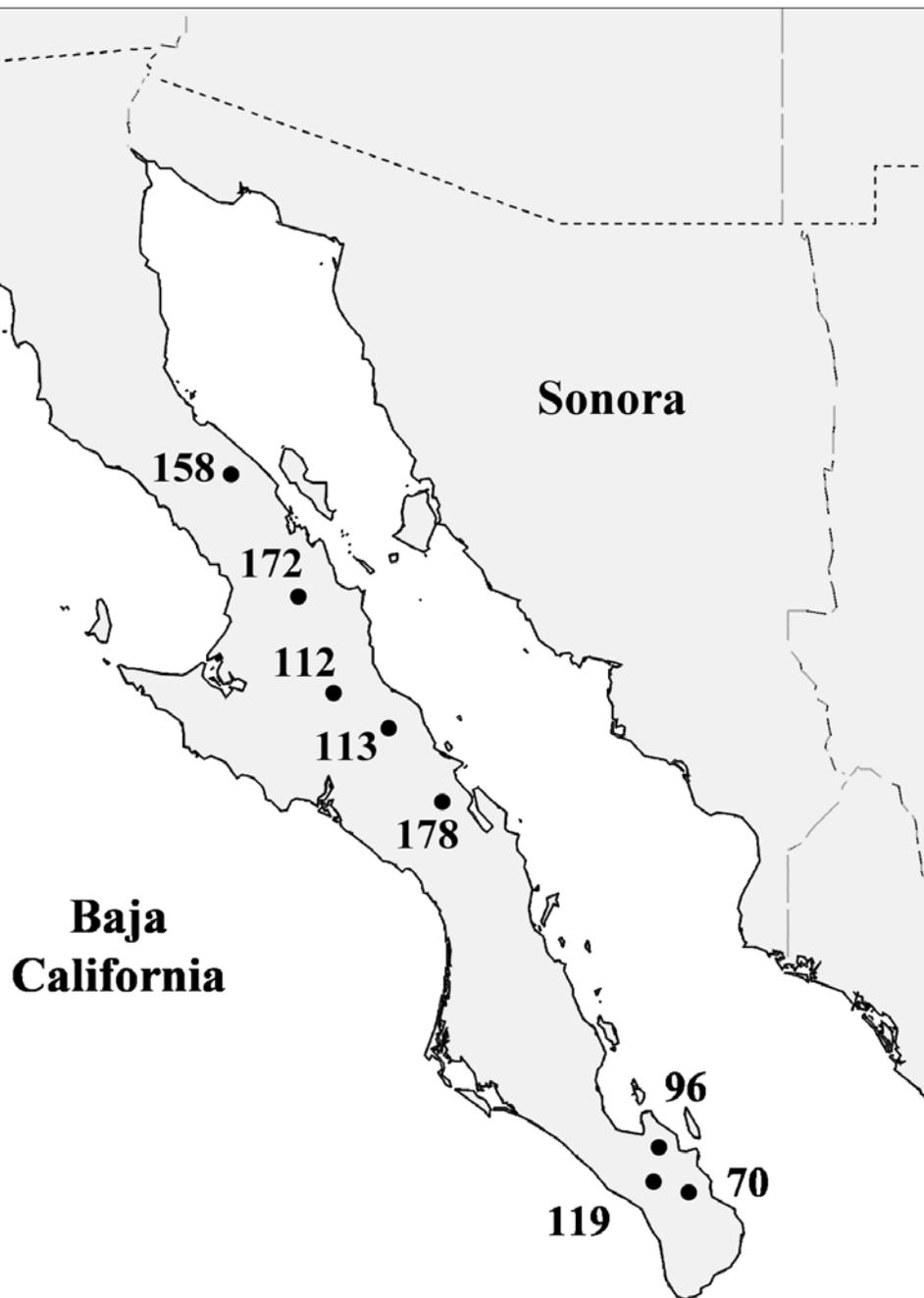California, Mexico. Population names are as in Table 1.

Figure 2. The proportion of trees bearing female-phase syconia in eight *Ficus petiolaris* study populations. Dashed lines represent expected values given within-tree flowering synchrony (see text). Values for Sites 70, 119, 96, and 112 are weighted means over multiple censuses. Error bars are 95% binomial confidence limits. The total number of census observations is given for each site.
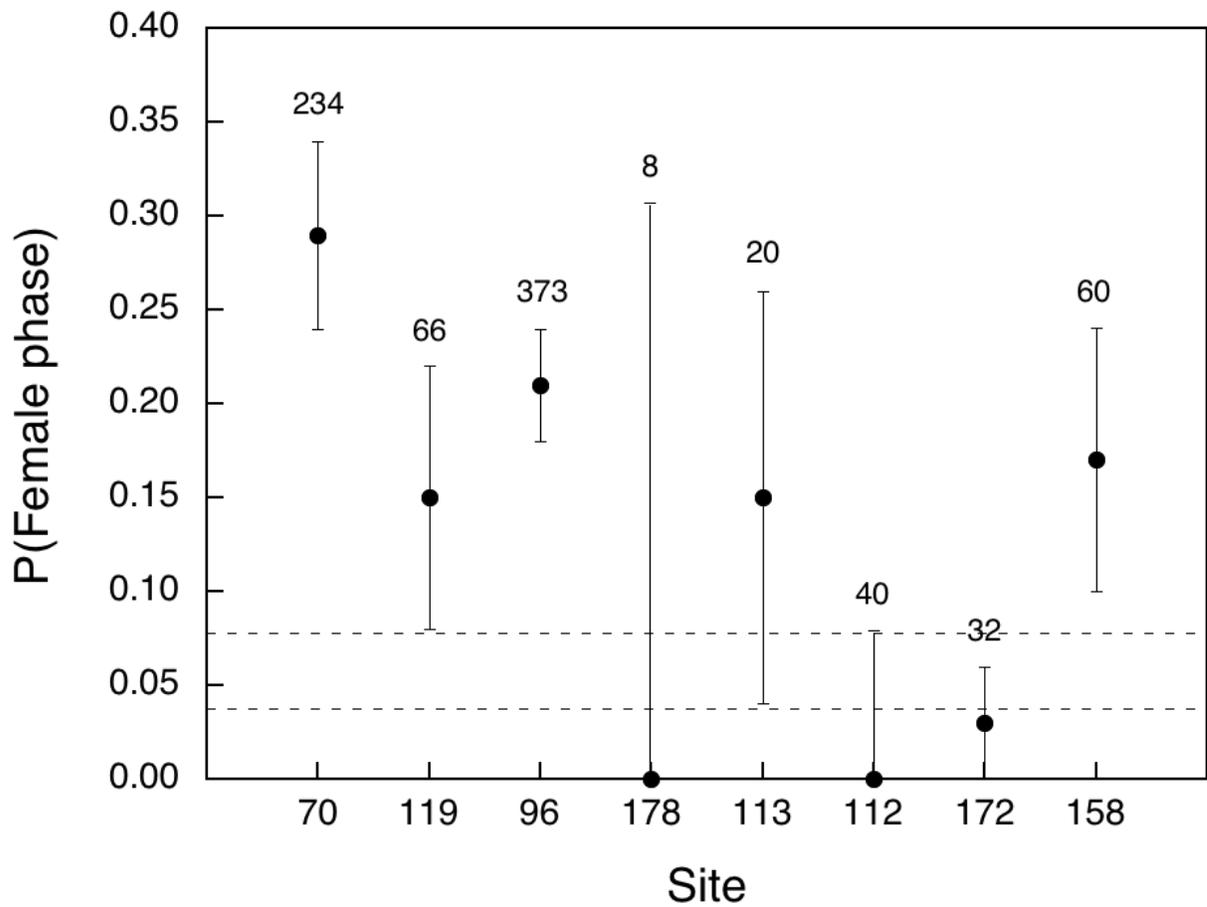
CHAPTER 3. EFFECTS OF ASEXUAL REPRODUCTION ON AMONG-TREE FLORAL PHENOLOGY AND MUTUALISM FUNCTION IN PERIPHERAL FIG AND FIG WASP POPULATIONS

Daniel J. Gates and John D. Nason

Abstract:

Most plants are capable of both sexual and asexual reproduction with allocation to each often determined by local environmental conditions. Because of their smaller size, peripheral populations are often sexually limited by pollen or mate availability, conditions under which asexual reproduction may provide fitness benefits through reproductive assurance, persistence of genotypes, and buffering of environmental dynamics. Given aseasonal flowering and the short lifespan of their species-specific fig wasp pollinators, clonal spread may also enhance reproductive assurance via sexual reproduction in small populations of figs if ramets are phenologically independent and increase overlap in flowering activity among genets. We test this hypothesis in naturally fragmented populations of the Sonoran Desert rock fig, *Ficus petiolaris*, the northernmost of New World figs. Clonal structure was quantified in nine populations from peninsular Baja California, Mexico. Although clonal diversity was low, the incidence of clonal spread was common (7 of 9 populations) with identical multilocus genotypes significantly more common at each site than expected under pure sexual reproduction. Flowering within genets was not always synchronized and in three out of six phenological censuses was no more synchronized than among randomly selected ramets. Where significant floral synchrony was observed within clones, spatial autocorrelation analysis revealed significant correlations in phenology among

neighboring genets as well. We consider the extent to which the observed clonal structure and flowering phenology may contribute to reproductive assurance in small, isolated populations. We test, as well, to determine if correlated phenologies may result in lowered inbreeding by forcing wasps to disperse out of patches of related individuals.

Introduction

Sexual reproduction is the predominant mode of reproduction in virtually all eukaryotes. Given the potential fitness benefits of asexual reproduction, including its inherent two-fold genetic transmission advantage, the maintenance of sex is a problem of substantial interest in evolutionary biology. Plants provide an interesting twist on this problem, as many species are capable of both sexual and asexual reproduction (Klimes et al. 1997). Consequently, there has been much research investigating the individual fitness costs and benefits of resource allocation to sexual function versus asexual reproduction via vegetative spread or apomixies (Loehle 1987, Gardner et al. 1999, Pan and Price 2001). At the population level as well, substantial effort has been devoted to quantifying the occurrence and extent of asexual reproduction (reviewed by Ellstrand and Roose 1987, Silvertown 2008). At both individual and population levels, local biotic and abiotic conditions are responsible for the ecological and evolutionary mechanisms selecting for or against asexual reproduction and often require consideration of landscape level processes. Indeed, in a recent review, Silvertown (2008) found clonal structure in plants to be related to time since last disturbance or population age, local population size (rare plants are more likely to be clones), the local environment of the organism, and where the population is located relative to its range margins.

Numerous models have been proposed to explain the extent of asexual reproduction within plant populations that are ancestrally, and often primarily, sexually reproducing. Fitness benefits that accrue to sexual genotypes by investing in clonal spread often come in the form of increased sexual reproduction. For example, increased size and/or longevity resulting from clonal spread can increase a plant's male and female sexual function over its lifetime (Armstrong 1982, 1983, 1984, Zhang and Zhang 2007, Mori et al. 2009). In environments or species where reproduction is pollinator limited, it can also enhance reproductive assurance by increasing the opportunity for sexual reproduction via selfing, though with potential costs due to inbreeding depression (Eckert 2000, Charpentier 2001). In their review of reproductive assurance in the genus *Solanum*, Vallejo-Marin and O'Brien (2007) found that although many species were capable of selfing or of clonal reproduction, there exists a very strong negative correlation between clonality and self-compatibility. These findings suggest that instead of contributing to reproductive assurance via facilitation of selfing, clonal reproduction may provide its own form of reproductive assurance by allowing the persistence of genotypes through times of pollen limitation without a lowered fitness from inbreeding via selfing. Unfortunately the role or asexual reproduction remains quite understudied in the context of uniparental gene transmission and reproductive assurance (Eckert et al. 2006).

In this study we examine an additional dimension to the potential costs and benefits of vegetative reproduction in sexually reproducing plants. Specifically, we focus on the potential benefits of clonal spread to the maintenance of obligate pollination mutualism. Obligate pollination mutualisms are well known in plants, such as figs and fig wasps and yuccas and yucca moths, providing textbook examples of coevolution (Thompson 1994). In

these obligate systems, the plant species is reliant upon one or a few pollinator species for pollination services, while during their development these pollinators feed solely on the seeds of the plant species they pollinate. A key ecological challenge in such systems is often pollinator-limited reproduction, which centers on the maintenance of flowering plant densities sufficient to both permit successful outcross pollination and to support locally viable pollinator populations (Bronstein et al. 1990, Anstett et al. 1995, Anstett et al. 1997). Clonal spread may potentially influence outcross pollination dynamics by affecting not only the density of flowering plants but also the timing of reproduction. If ramets within a clonal genet become partially or completely decoupled with respect to their flowering phenologies, that genet should be less likely to experience reproductive failure owing to temporal variation in the availability of pollinators (Snyder and Richards 2005).

The obligate pollination mutualism involving figs and their seed-eating pollinator wasps is unusual both in its exceptional diversity (750+ species of figs and species-specific fig wasps) and the exceptionally low population densities characteristic of many species (Nason et al. 1998). Moreover, reproduction in figs is typically highly synchronized within trees but highly asynchronous at the population level (Milton et al. 1982, Windsor et al. 1989), a reproductive phenology with important consequences for the viability of fig and fig wasp populations. Theoretical studies suggest that reliance on highly-specific, short-lived pollinators and asynchronous flowering among trees, typical of figs, leads to a high risk of pollinator extinction and mutualism breakdown unless fig populations are large (Kjellberg & Maurice 1989; Bronstein et. al. 1990; Anstett et al. 1995). Mutualism breakdown may be particularly likely in harsh environments with isolated habitable patches, small local host populations, and hostile conditions for between-patch pollinator migration. In such

environments, local pollinator extinction may be averted through several means, including increased intra-crown asynchrony in flowering (Janzen 1979) and/or extended duration of floral receptivity to pollinators (Khadari et al. 1995; Anstett et al. 1996).

In this study we investigate the occurrence and extent of clonal structure in figs and its potential influences upon the stability of fig and fig wasp populations in harsh environments. Our work focuses on the Sonoran Desert rock fig, *F.petiolaris*, the northernmost ranging of all Neotropical figs with populations and primarily located in desert habitats where high temperatures and low precipitation limit overall population sizes (Smith and Bronstein 1996). *Ficus petiolaris* typically grows on large boulders, canyon walls, and cliff-faces where spatial aggregates of trees (<15 m apart) are consistent with vegetative spread. If clonal spread does occur and is coupled with phenological independence among vegetative subunits (as observed for *Ficus prolixa* in the South Pacific; Compton & McCormack 1999), then genetically identical ramets may function as ecologically distinct individuals with potential benefits for figs via increasing opportunities for outcrossing and for fig wasps via increased probability of successful dispersal to a new host. To assess the potential for these benefits in *F. petiolaris*, we used genetic markers (microsatellites) to quantify clonal structure within nine sites distributed along the Baja peninsula. In turn, we coupled these results with reproductive censuses to determine whether clonal ramets are synchronized or independent in terms of their flowering activity.

Fig Biology:

The genus *Ficus* (family; Moraceae) is best known ecologically for the obligate pollination mutualism it forms with typically species-specific fig wasps (family Agaonidae,

super order Chalcidoidea). Fig species exhibit a variety of growth forms, including canopy trees, shrubs, vines, lianas, and hemiepiphytic stranglers. Strangler figs typically grow on other trees or, as in *F petiolaris,* rock surfaces, and have complex rooting and sprouting often capable of forming multi-trunked individuals (Zhekun and Gilbert 2003). Within these multi-trunked genets, individual ramets may function as independent trees if connections with other ramets are severed. Little information is available, however, on the incidence and ecological impacts vegetative growth within the genus *Ficus.*

Study Sites:

  Ficus petiolaris populations were studied on the Baja California peninsula (the Mexican states of Baja California Sur and Baja California Norte). *Ficus petiolaris* is the only fig native to this region and occupies a variety of dry-tropical to Sonoran Desert habitats. We quantified clonal structure at nine study sites separated by up to 740 km along the Baja peninsula (Figure 1), with latitude, longitude, elevation, and total marked ramets (*N*) given in Table 1. The study sites range from more mesic Cape Arid Tropical Forest in the south to more xeric Vizcaino and San Felipe Desert in the north (Roberts 1989).

Methods

Collection of field data and material:

  To investigate the synchrony of flowering among clonal ramets, we censused fig phenology at three of our nine sites, one in the south (Site 96), one in central Baja (Site 112), and one in the North (Site 158). We also censused two additional sites but because clonal structure was very low (Site 70), or not detected (Site 119), the effects of clonal structure on

the synchrony of flowering are not reported for these sites. Floral phenology data were collected over a series of seven visits to Baja California during the period May 2006 to November 2008. For each tree censused we estimated the amount of reproduction, the flowering phases present, and the relative abundance of these phases. We classified reproductive output upon trees censused as being none, ≤5%, 5-25%, 25-50%, 50-75%, 75-95%, or ≥95% of estimating maximum potential. For comparisons of flowering within a tree we divided a syconium's complete reproduction into five developmental phases: pre-female (floral initiation), female (receptivity to wasp pollination, pistillate flowers in anthesis), interfloral (developing seeds and pollinator larvae), male (staminate flowers at anthesis, seed and pollinator wasps mature), and post-male (pollen-bearing pollinators departed, the syconium expanding and sweetening to attract frugivores). We sampled as many as 30 syconia per tree by breaking them open and observing floral phases within. In trees bearing syconia of two or more floral phases we also estimated the relative abundance of each floral phase.

In the summer of 2008 we collected and silica gel dried leaf material from all nine sites for the purpose of quantifying clonal structure and genotypic diversity. At each site we sampled all available trees within spatial aggregates representing potential clones. These aggregates were on the scale of <15 m whereas individual trees were otherwise generally farther apart. For characterizing population allele frequencies we also sampled material from additional individuals up to a minimum of 19 trees per site. At Site 96 we sampled a total of 68 trees to provide a more powerful preliminary test of microsatellite scoring errors (see below).

Molecular analysis of clonal structure:

Plant DNA extractions (100 mg of dried leaf material) were carried out at Iowa State University's DNA Facility using a Genogrinder 2000 (SPEX sample prep) and an Autogen 740 (Autogen incorporated). After extraction, we used a Nanodrop ND-1000 (Thermo Fischer Scientific) to quantify the concentration of template DNA.

Since no nuclear microsatellite (simple sequence repeat or SSR) primers have been developed specifically for the Neotropical figs of section *Americana* (which includes *F.petiolaris*), we screened 23 different primer combinations developed for other members of the genus *Ficus*. The majority of these primers amplified in *F. petiolaris* and we settled upon 3 primer sets (MFC2, MFC3, MFC4 of Khadari et al. 2001) based upon their reliable amplification, amount of polymorphism, non-overlapping allele lengths, and similar optimum PCR conditions. These latter two features allowed us to multiplex all three into a single PCR reaction. We utilized the same PCR concentrations and conditions as Khadari et al. 2001 with the modification that we used 3 mM of $MgCl_2$ for all PCRs. Our primers were tagged with M13 dye and eight of the nine populations were visualized by using an ABI 3730 (Applied Biosystems) at Iowa State University's DNA Facility. The exception was population 96, which was genotyped with an ABI3100 (Applied Biosystems) before the DNA facility's switch to the ABI 3730 occurred. We resubmitted 5 random samples from population 96 for genotyping on the 3730 in order to coordinate the scoring 100% of alleles between machines. For visualization of alleles, we used the program Genemapper 4 (Applied Biosystems) and scored all genotypes blind with respect to the identity of putative clones. We resubmitted approximately 10% of individuals to determine genotype scoring error rates (DeWoody et al. 2006). Finally, we checked our dataset for potential scoring errors due to null alleles, large

allele dropout, or stuttering using the program Microchecker (van Oosterhout et al 2004), and

for deviations from Hardy-Weinberg equilibrium and linkage disequilibrium using the

program Genepop '007 (Rousset 2008). For each site we report total alleles (N_a), average

effective alleles per locus (N_e), observed heterozygosity (Ho), expected heterozgosity (H_e),

the fixation-index (F), and probability of multilocus genotypic identity (PI; see Foster et al.

2007 for implications of PI in clonal studies). These values were calculated using the

program GenAlEx 6 (Peakall and Smouse 2006).

Testing clonal diversity:

To characterize extent of clonal diversity within a site, we used the Stoddart and

Taylor's (1988) measure of observed genotypic diversity (G). This measure is calculated as

$G=1/\Sigma(p_i)^2$, where p_i is the frequency of the *i*th genotype. This statistic ranges from 1 to *n*

where G=1 when all *n* sample individuals share the same multilocus genotype (MLG) and

G=*n* when all individuals have different MLGs. Since there is a chance sexual reproduction

may generate two non-clone individuals sharing the same MLG, for each population we used

simulation methods to construct the expected (null) distribution of genotypic diversity under

pure sexual reproduction (G_e) given the total ramet sample size and the observed allele

frequencies (N_{Sim} = 1000 replicates per population). We then compared the observed G (G_o)

to this expected distribution, rejecting the hypothesis of no clonal structure at a site if G_o was

lower than the N_{Sim} * 0.05 lowest value of this distribution (program available by J. Nason

upon request).

We simulated the expected distribution of G for sexual reproduction under two

different spatial null hypotheses: (1) the "spatial random" null hypothesis and (2) the "spatial

aggregates" null hypothesis. The spatial random hypothesis makes no assumption about the spatial locations of identical multilocus genotypes within a population and has been used in previous studies to quantify clonal structure (Ceplitis 2001; Jesse et al. 2009). Because clonal ramets are typically spatially aggregated, we also evaluated a "spatial aggregates" null hypothesis where we first assigned individuals the coordinates of mapped *F. petiolaris* individuals. We then simulated genotypes for these marked individuals. For two individuals to be considered clones in this analysis and contribute to a reduction in G_e they must not only have drawn the same multilocus genotype but also be located in aggregations expected by vegetative reproduction. Because the odds should be low that two sexually produced individuals will by chance both have identical multilocus genotypes and be neighbors, the "spatial aggregates" null hypothesis provides a more powerful test of clonal structure than the traditional "spatial random" null hypothesis. We tested each hypothesis by comparing G_o to the distribution of G_e simulated according to the specific hypothesis. For each test the total ramet sample size equaled the census number of trees at a site.

In addition to genotypic diversity G (Stoddart and Taylor 1988), we report clonal diversity in each population using the complement to Simpson's Diversity Index (D; Beatty et al 2008, Arnaud-Haond and Belkhir 2007). We also conducted a weighted regression of D on latitude to examine if the proportion of asexual to sexual growth increases as we move up the Baja peninsula and away from the range center (as suggested in Silvertown 2008). Although weighting for this sort of analysis is the inverse of the variance, we used weights equal to the population sample size since two populations (119 and 172) had variances of 0, which would result in an infinite number for an inverse of the variance. All regression analyses were conducted in JMP 8 (SAS institute).

Analysis of phenological effects of cloning:

We tested the null hypothesis that ramets within genets are independent with respect to reproductive phenology, versus the alternative that they are positively phenologically correlated. This test was based on randomization procedures and pairwise phenological distances among all ramets within a census. For individuals *i* and *j*, a Euclidean phenological distance was calculated as

$$d_{p \cdot ij} = \sum_{k=1}^{s} \left(p_{ik} - p_{jk} \right)^2$$

where *k* indexes the five floral phases and p_{ik} and p_{jk} measure the phenology of *i* and *j*, respectively, at stage *k*. We used this distance in studying *F. petiolaris* because the asynchronous manner that syconia develop within trees does not allow for the more standard phenological comparisons of peak flowering or durations (as in Almeida-Neto and Lewinsohn 2004). Instead, our method treats the flowering phase of individual syconia as units of observation upon plants and allows us to compare plants with varying floral synchronies to each other. We used two different measures of phenology to construct Euclidean distances. In the first, p_{ik} and p_{jk} were binomial (1,0) designations of the presence or absence of floral phase. In the second, p_{ik} and p_{jk} were proportional to the amount of floral phase *k* where the sum of all phases within a tree equals one.

We used the program GenAlEx (Smouse and Peakall 2006) to determine the pairwise phenological distances as described earlier as well as construct autocorrelation coefficients (*r*) for pairwise distance values. The *r*-values of within-clone distances were considered

significant and the null hypothesis was rejected if probability (p random r > actual r) was less than .05 based upon a 1000 permutation test.

We finally used the program Genalex (Smouse and Peakall 2006) to determine if there is any level of spatial autocorrelation (SA) of phenological characters and if incorporation or exclusion of clonal ramets will result in fine scale changes in phenology. A positive spatial autocorrelation at a relatively short distance scale would be expected if clones were more similar to each other or if flowering may be controlled by micro-climate factors. To separate a potential interaction between these two possible factors we ran the spatial analysis twice, once including clones and once without clones. If significant SA exists with clones included and then disappears when they are excluded we can conclude that clonal growth was causing the correlation. Likewise, if we observe significant amounts of SA when only one ramet has been chosen to represent a clonal patch, SA is most likely caused by other factors such as microclimate controls or fine-scale spatial genetic similarities. We should note that binomial data were used exclusively for the analysis of SA, this was decided because we were more interested in what phenological phases were present and less interested in their relative proportions (and quantifying our field estimates of abundances may cause a subjective bias).

We analyzed pooled values of phenological spatial autocorrelation for the single population we sampled multiple times (96) as well as across all censuses. We created an unweighted mean r for all values across all sites and assuming a normal distribution constructructed 95% confidence intervals about r.

Results:

Altogether, we genotyped 259 ramets across the 9 populations at three microsatellite loci. Genotypes revealed no signs of null alleles, large allele dropout, or scoring error due to stutter, and we were able to rescore 94% of alleles correctly in resubmitted blindly scored individuals. No populations exhibited any significant levels of linkage disequilibrium and only a single population (95) had a fixation index significantly different than zero (-.218). The results of our genetic analyses are available in Appendix A.

Our molecular quantification of cloning revealed small amounts of clonal architecture at six of the nine sampled populations. The information regarding our observed genotypic diversities and the outcomes of our G_o vs. G_e simulations is located in Table 2.

In seven out of nine populations we identified clonal growth resulting in a total of 18 genotypes associated with 46 clonal ramets. Population 96 contained the most individual ramets associated with clonal patches but was also, by far, the largest population we sampled from. Site 112 in the north had the greatest reduction in genotypic diversity calculated by the complement of Simpson's index D (.9952). Two populations (70 and 113) had multiple individuals sharing multi-locus genotypes in an amount less than expected (G_e) based upon simulations of population level allele frequencies. When we analyzed values of G_o using the spatially explicit model, all populations (including site 70) with clones had significantly lower genotypic diversity than expected and all sites with clones were highly significant (p= 0.001 or less). A regression of Simpson's D for all nine populations regressed across latitude showed no significant signs of a decrease in D across latitude (9 populations, 1df, F = 0.9960, p > F = 0.3515).

After establishing that clonal growth occurs in *F. petiolaris*, we asked what effect it has, if any, upon the phenological traits that ultimately dictate the persistence of the fig/fig wasp mutualism. In Table 3 we present the results of permutation tests of phenological data gathered in six different floral censuses of three out of the seven different populations containing clones. We report the results of averaged pairwise correlations of reproductive phases within clones using two types of phenology data, presence absence data and proportion total reproduction. The correlation values are represented by *r* and *p* is the probability of randomly drawing a greater *r*. All but one of our censuses have within-clone phenological correlations greater than zero with three out of six census observations displaying significant amounts of within clone phenological correlations. Population 158 in May 2008 represents the only census where the *r*-values are lower than 0 (-.057 for pres/abs, -.093 for proportional analysis. When we compare across the different data analyses, significance levels are unchanged in all censuses regardless of whether we use presence/absence data or binomial data.

Since it appears that clonal reproduction often results in ramets with more similar phenologies than randomly drawn individuals within a population, we set out to determine if correlated phenologies are a product of local levels of autocorrelation by using spatial autocorrelation (SA). Figure 2 displays the correlograms for spatial autocorrelations of flowering phenology with clones removed. The correlograms report distance classes, the number of individuals within classes, upper and lower confidence envelopes around 0 based upon permutations, and upper and lower confidence values around *r* based upon bootstrapping (except for pooled estimates). Specific values of *r* and probability of randomly drawing a greater *r* (p) for the 15 and 50m distance classes for each autocorrelogram are

reported in the last column of Table 3. All census periods showed significant amounts of SA at either the 15m or 50m interval. In addition, all censuses except one showed the exact same levels of significance whether or not we were including clones in the analysis. The only census that changed significance based upon inclusion/exclusion of clones was population 158 June 2008, there the 15m *r*-value changed from 0.17 to 0.15 ($p= 0.03$ with clones, $p= 0.09$ without clones). This population, however, still shows significant signs of autocorrelation at 50m, which seems to be exhibiting stronger levels of autocorrelation ($p= 0.01$) with or without the clones included.

Discussion:

Clonal architecture in figs has been observed at least three times in varying genera of *Ficus* (Gardner and Early 1994, *F. rubiginosa*, Compton 1999, *F. prolixa*, Zhekun and Gilbert 2003, subgenera *Urostigma*). To our knowledge, however, no one has tested the effects that vegetative propagation may have upon the delicate fig fig-wasp mutualism. Honnay and Bossuyt (2005) predict even in the face of negative growth rates in less than ideal environments, clonal plants should have an advantage over strictly sexual plants because they have a prolonged lifespan which allows a greater period of waiting in unsuitable periods. In addition to selection favoring increased duration through periods of unfavorable environments, we predict that in seasonal populations near range margins, asexual reproduction may aid overall mutualism stability by producing semi-independent reproductive units (as observed by Compton 1999) and adding functional individuals into the population. This may allow for reproductive assurance in two different ways: through increasing the number of randomly flowering individuals, and facilitation of within genet

geitonogamous selfing (Janzen 1979, Cook and Power 1996, Hossaert-Mckey and Bronstein 2002). In the previous chapter Gates and Nason explicitly tested offspring arrays of *F. petiolaris* to determine if there were detectable amounts of selfing and found that *F. petiolaris* has multilocus outcrossing rates no different from 1 (no evidence of selfing). These results are confirmed in this study by our microsatellite generated F values that are either no different than 0 or significantly less than 0.

Clonal growth seems to occur consistently across populations of *F. petiolaris* in Baja but is always quite rare. Although it was not the purpose of this study, our tests of genotypic diversity across latitude did not suggest that clonal growth increases as populations approach the peripheries of their range (See Silvertown 2008). We believe that much of the observed variability in clonal diversity is more likely to result from the range and differences in the fig-sustaining habitats in different sites. Population 95 and 112 show the greatest decreases in *D* and as the names of each site indicate (Fig Canyon, Mesa de la Caguema), they contain steep rock faces and sheer canyon walls that figs growth nearly straight out of. Often figs growing out of these canyon walls are situated directly above others in cracks and fissures where their roots are strangling. There is no doubt that growing directly out of a sheer rock face puts severe limitations on the maximum size of figs growing in this habitat. Selection favoring more creeping and expansive growth forms would be consistent with the growth constraints placed upon figs strangling canyon walls.

In addition to proliferation outwards upon cliffs and rock faces, asexual reproduction can be expected to provide reproductive assurance through persistence of genotypes via uniparental gene transmission. Given the nature of the fig/fig-wasp mutualism, clonal reproduction may act to further reproductive assurance if ramets allow for an amount of

within genet asynchrony not attained within any single ramet, or, undoubtedly, if ramets are functioning as independent trees.

Results of permutation tests to determine phenological correlations within clones showed some degree of floral asynchrony among ramets. In three out of six censuses we concluded that clones were not significantly different than randomly drawn individuals within a population. The observation of different phenologies within clones as well as some evidence of independence when compared to other trees in the population allows us to consider some clonal growth as more than just expansion of a single tree outward instead of upward. There still exists, however, three censuses where clones did have significant amounts of correlated phenologies, which may be due to some genet-level control over flowering.

When we applied SA to the six census samples we found signs of SA in flowering phenology at either 15 or 50m distances within every census. This is meaningful when we remove clones because it provides evidence that flowering is not independent across all trees in the population. Among-tree correlations of phenology at short distances would result in wasps being forced to disperse pollen away from nearest neighbors. The result of this should be fewer matings between individuals at close proximity, which, in turn, should be expected to reduce the risk of biparental inbreeding through mating with nearby relatives. Although it is not the focus of this paper, we conducted preliminary tests of the spatial genetic autocorrelations of genotyped individuals at the three different populations where phenological censuses took place. The results of spatial genetic autocorrelation analyses should not only inform us as to if pollen dispersal takes place primarily at levels greater than near neighbor, as expected given among-tree phenological correlations, it should also give us

insight as to whether trees at short distances are phenologically similar because they also have similar genotypes.

The results of spatial genetic autocorrelation are not consistent across populations but give confidence in the power of our markers and allow us to tease out genetic versus environmental effects of correlated flowerings. At population 96 we found no significant evidence of spatial autocorrelation at any distance intervals, this suggests that correlated phenologies are not due to genetically similar individuals responding identically to global environmental cues and that correlated flowering phenologies are most likely a response to microclimate signals. Since our genetic data only consists of three microsatellite loci there are questions regarding whether we even have enough power to identify significant correlations. In populations 112 and 158 we ran fewer total individuals than at population 96 and found significant signs of genetic autocorrelation (population 112 50m $r = .162$, p = .036; population 158 15m $r = .128$, p = .039 and 50m $r = .090$, p = .031), which means we should also have enough marker power to reject the null in population 96 but simply didn't.

The fact that populations 112 and 158 contain individuals which are more genetically similar than expected due to chance at short distances does allow us to investigate whether pollination occurs between near neighbors. Genetic similarities in these two populations are most likely caused by correlated seed dispersal events or limited dispersal resulting in the recruitment of seeds near their maternal trees. By making the assumption that near neighbors are genetically similar, we can infer that if pollen was being transferred between genetically similar individuals some amount of inbreeding would take place as a result (Kalisz 2001). Across all loci and populations of the same genetic markers we observed no evidence of inbreeding amounts greater than 0. There is a possibility that pollinators disperse within

genetically similar trees and even slightly inbred seeds suffer from inbreeding depression and are not recruited. Our observation of correlated phenologies within these genetically similar individuals, however, suggests that the opportunity for pollen transfer between these related trees in population 158 and 112 are quite rare.

In populations 158 and 112 where we observe significant amounts of spatial genetic structure, we cannot rule out the possibility that correlated phenologies at short distances are still a result of genetic similarities. Since it would be quite difficult to remove the effects of environmental cues upon related individuals, our best estimate to determining the contributions of genetic structure upon flowering phenology would be to compare them to a population where genetic correlations are absent. Our population 96 data show correlated phenologies still exist when individuals at short distances are unrelated. The comparison of phenological correlations between populations with correlated genetic data at the same scale (112 and 158) and those with unrelated individuals at the scales of phenological correlations (96) allows us to conclude that local environmental effects upon the *F.petiolaris* phenologies can be quite great. Future studies should focus more explicitly upon the interface of genetic and environmental factors in *Ficus* since their impacts upon flowering phenology should heavily influence genetic structure and mating events in fig populations.

Although postitive SA at short distances may favor outcrossing by forcing trees to mate with distant members of the population, it may also have conservation implications if it reduces the potential mates by excluding near neighbors. Although within-tree asynchrony and semi-independent ramet flowering would expected to increase the functional number of mates in a population, spatial autocorrelation of floral phases would reduce the number of functional mates by causing what we assume to be independent trees to act more as a single

unit. Spatial autocorrelation of flowering in figs may represent a serious but overlooked concern in a dynamic obligate-pollinator system. Based upon the spatial scale of correlations and structuring of trees in a population, spatial autocorrelation of flowering may drastically reduce our expected number of mates in a population at any given time. *F. petiolaris* populations have a considerable amount of trees located within short distances of each other (population 112, 16 out of 36 ramets are associated with aggregates of trees). Trees within aggregates are unlikely to be capable of mating with each other because of correlated flowering, thereby reducing the number of potential mates for trees in aggregated populations. Consideration of this phenomenon should be further investigated especially since *Ficus* is often of conservation concern (Lambert and Marshal 1991).

Although clonal growth may not provide short-term benefits for wasps through floral independence of ramets, persistence of individuals within a population is expected to play an important role in the metapopulation dynamics of the pollinator wasps. If fig populations exchange migrants even infrequently, small populations of figs that suffer local pollination extinction may not suffer extinction due to mutualism failure. Bronstein and Hossaert-Mckey (1995) documented a rapid recolonization of *Ficus aurea* in Florida following Hurricane Andrew, which they attributed in part to the high vagility and recolonization abilities of the wasp pollinators. In other studies, fig wasps have been documented traveling 30+ km (Harrison 2003), 50+ km (Shanahan 2001), and 100 km (Pemberton 1934). Gardner and Early (1996) even have unconfirmed reports suggesting pollinating wasps of *F. rubiginosa* even made a ~3000 km jump from mainland Australia to New Zealand with the aid of some suitable meteorological conditions. Once these pollinators were first documented as arriving in New Zealand, it was only a short time before they had well-established

populations. Colonizing fig wasps are also highly fecund and females that singly founder fruits may skew the sex ratio of offspring to more female biased, allowing for even more syconia to be occupied by the next generation (Herre 1985). If recolonization is important in reestablishment of extirpated wasps, then extending tree lifespan through cloning should be advantageous in increasing the total time individuals and populations are able to wait for recolonization.

Given the delicate balance of the obligate pollinator mutualism between figs and fig wasps, *F.petiolaris* populations in Baja are expected to not only be limited by abiotic conditions but biotic influences of pollinator demographics as well. Local extinctions of the highly ephemeral pollinating wasps, therefore, become the most likely cause of mutualism failure (Bronstein et al. 1990). Evolutionarily derived flowering conditions such as increased duration of receptive syconia (Khadari et al. 1995) and asynchronous within-tree flowering (Bronstein 1992) are both proposed examples of selection within smaller fig populations acting to decrease the amounts of pollen limitation and increase plants overall fitness. Analyses of spatially correlated flowering, however, imply that ecological effects of flowering at short distances may minimize the opportunities for independence in clonal or non clonal aggregations of figs.

The vegetative growth in figs may provide greater selection advantages by allowing for persistence of genotypes between periods of pollen limitation and persistence throughout periods environmental hardships. These benefits of clonal reproduction are less specific to the obligate-pollination system of figs but are well documented in plants capable of both sexual and asexual propagation (Honnay and Bossuyt 2005). Future research of the metapopulation dynamics of pollinating wasp communities may reveal benefits of clonal

reproduction if extinctions and recolonizations of fig communities translate into selection for persistent genotypes.

The results of this study further illustrate how combinations of ecological and evolutionary features may influence the amounts of sexual and asexual reproduction in plants. Uniparental transmission of genes, in this case via asexual reproduction, should provide short-term benefits to plant fitness via allowing for persistence and a small amount of proliferation of clonal genotypes. On an evolutionary time-scale, however, *F. petiolaris* appears to be primarily sexual. Ecological effects of clonal reproduction and flowering phenology, especially upon the obligate pollination mutualism, are still not well understood but should receive further attention as they may weigh heavily than into the balance of sexual and asexual reproduction in the genus *Ficus*.

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Table 1. Latitude and longitude coordinates of nine *Ficus petiolaris* study populations located along peninsular Baja California, Mexico. Populations are arranged from south to north. *N* denotes the total number of trees genotyped at a location.

Site	Name	Latitude	Longitude	Elevation (m)	*N*
70	San Bartolo	23.73652	-109.84383	362	94
119	El Potréro	23.81622	-110.16687	375	34
96	La Paz Summit	24.02061	-110.09076	553	180
179/19	Nopolo	25.91582	-111.34837	6	23
95	Fig Canyon	26.35778	-111.80384	280	25
113	Santa Agueda	27.24018	-112.37004	186	19
112	Mesa de La Caguama	27.57151	-113.07616	1104	36
172	La Purificación	28.29230	-113.11473	665	32
158	Aguajito Higuera	29.26153	-114.01678	852	63

Table 2. Results of clonal diversity analyses at 9 different populations. N is number of trees marked within each site. N(ag) is number of potential clonal aggregates. Ramets | ag is number of ramets within potential clonal aggreagates. Ramets|genets is total number of ramets associated within clonal genets. p(pop) is the p-value for the population level G_o simulations. p(spatial) is the p-value for the spatially explicit G_o simulations. D is the complement of Simpson's Diversity Index of genotypes within populations.

Population	N	N(ag)	Ramets | ag	Clonal genets	ramets|genets	G_o	p(pop)	p(spatial)	D
70	94	8	20	2	4	90.2	> 0.2000	< 0.001	0.9995
119	34	2	4	0	0	34.0	1.000	1.000	1.0000
96	173	15	49	8	22	133.0	0.003	< 0.001	0.9983
179/19	23	2	5	1	2	21.2	0.016	0.001	0.9960
95	25	2	5	2	4	21.6	0.017	< 0.001	0.9933
113	19	1	3	1	2	17.2	0.164	0.001	0.9942
112	36	6	16	3	7	28.2	0.027	< 0.001	0.9921
172	32	2	4	0	0	32.0	1.000	1.000	1.0000
158	63	5	15	2	5	55.9	0.015	< 0.001	0.9980

Table 3. Permutation tests and spatial autocorrelation analyses of phenological independence among clonal ramets of *Ficus petiolaris*. For each of six censuses, *N* is number of trees censused, (C) is the number of comparisons involving clonal ramets, Bi refers to the analyses using binomial presence/absence data, and Pr refers to the analyses using proportional data. For the spatial autocorrelation analyses, *D* is the distance at which spatial autocorrelation analyses were conducted, (C) refers to the bin sizes at each distance when clones are included, and (NC) refers to bin sizes at each distance with out clones. Columns C and NC contain the results of analyses including and excluding clones, respectively. Results of the permutation and spatial autocorrelation tests are reported as *r*, the correlation coefficient for within-clones. All *p*'s refer to the probability of choosing a random *r* of greater value than observed *r*.

			Permutation Tests		Spatial Autocorrelation		
Site	Year	*N* (C)	Bi	Pr	D	C	NC
96	May-06	77 (9)	*r* 0.478	*r* 0.549	15m	*r* 0.405	*r* 0.405
			p 0.020	*p* 0.020		*p* 0.010	*p* 0.010
					50m	*r* 0.128	*r* 0.128
						p 0.130	*p* 0.090
96	Dec-06	81 (4)	*r* 1.013	*r* 1.013	15m	*r* 0.533	*r* 0.479
			p 0.020	*p* 0.020		*p* 0.010	*p* 0.010
					50m	*r* 0.068	*r* 0.087
						p 0.200	*p* 0.180

Table 3. (Continued)

Site	Year	N (C)		Bi		Pr	D		C		NC
96	Nov-07	56 (13)	r	0.479	r	0.295	15m	r	0.455	r	0.396
			p	0.060	p	0.110		p	0.010	p	0.050
							50m	r	0.116	r	0.128
								p	0.20	p	0.24
96	Nov-08	77 (13)	r	0.647	r	0.768	15m	r	0.35	r	0.31
			p	0.050	p	0.020		p	0.01	p	0.02
							50m	r	0.23	r	0.17
								p	0.01	p	0.03
112	May-08	36 (5)	r	0.564	r	0.501	15m	r	0.68	r	0.68
			p	0.060	p	0.110		p	0.01	p	0.01
							50m	r	0.17	r	0.12
								p	0.18	p	0.26
158	Jun-08	60 (4)	r	-0.057	r	-0.093	15m	r	0.17	r	0.15
			p	0.590	p	0.610		p	0.03	p	0.09
							50m	r	0.21	r	0.21
								p	0.01	p	0.01

Figure 1. Nine sampled populations distributed up the Baja peninsula. Latitude and

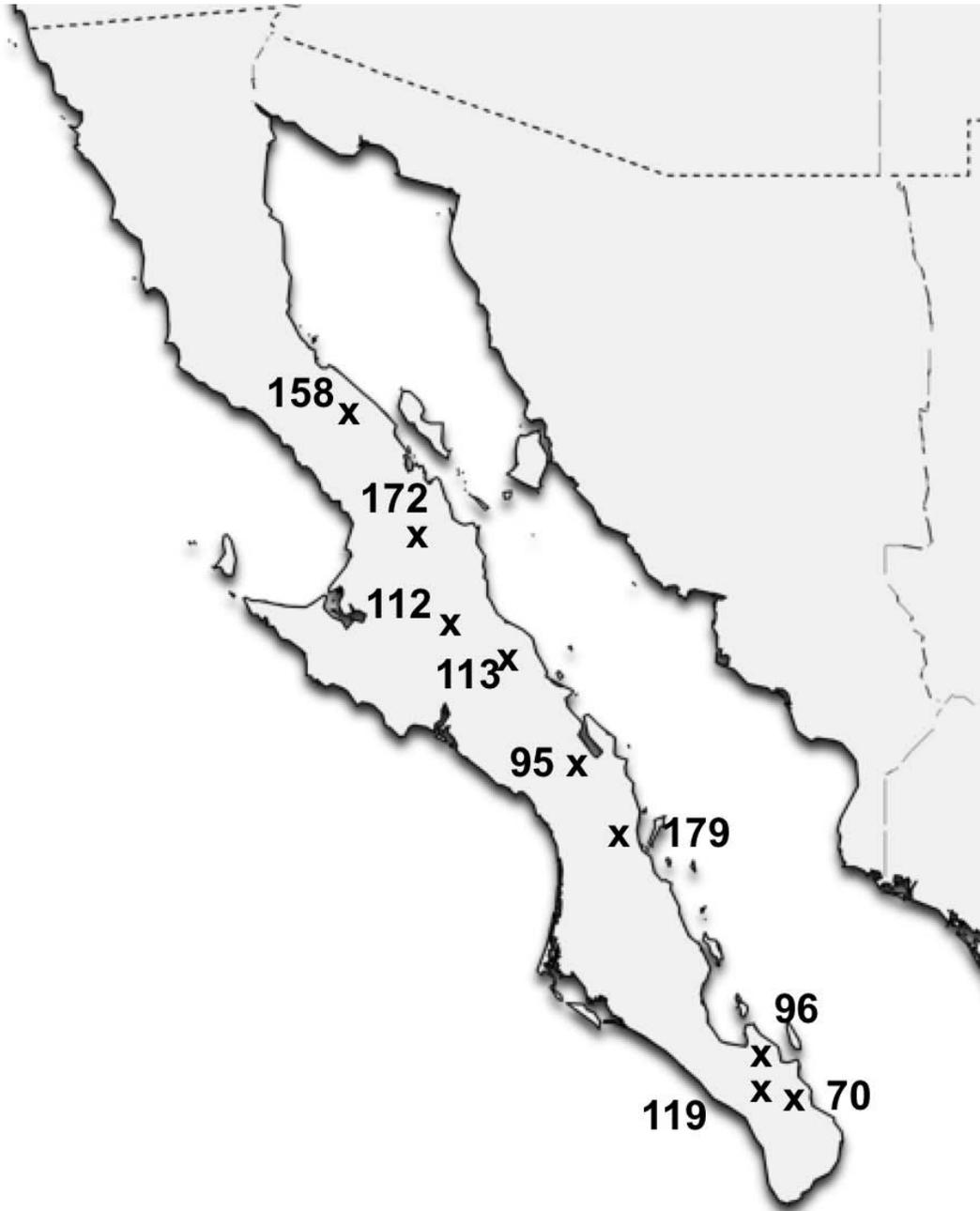Longitude coordinates for each population can be found in Table 1.

Figure 2. Correlograms of spatial autocorrelation of flowering phenologies of four different censuses at three different populations. The final two autocorrelations are pooled in two different manners: over the four censuses within population 96, and one pooled over all censuses using unweighted averages. Distance in meters is the x-axis, the autocorrelation coefficient (r) is the y-axis, the red dashed lines represent 95% confidence intervals around the $r = 0$, the blue line is the value of r for each distance class with 95% confidence intervals around r generated by bootstrapping, the numbers above the graph represent how many values of r are in each distance class.
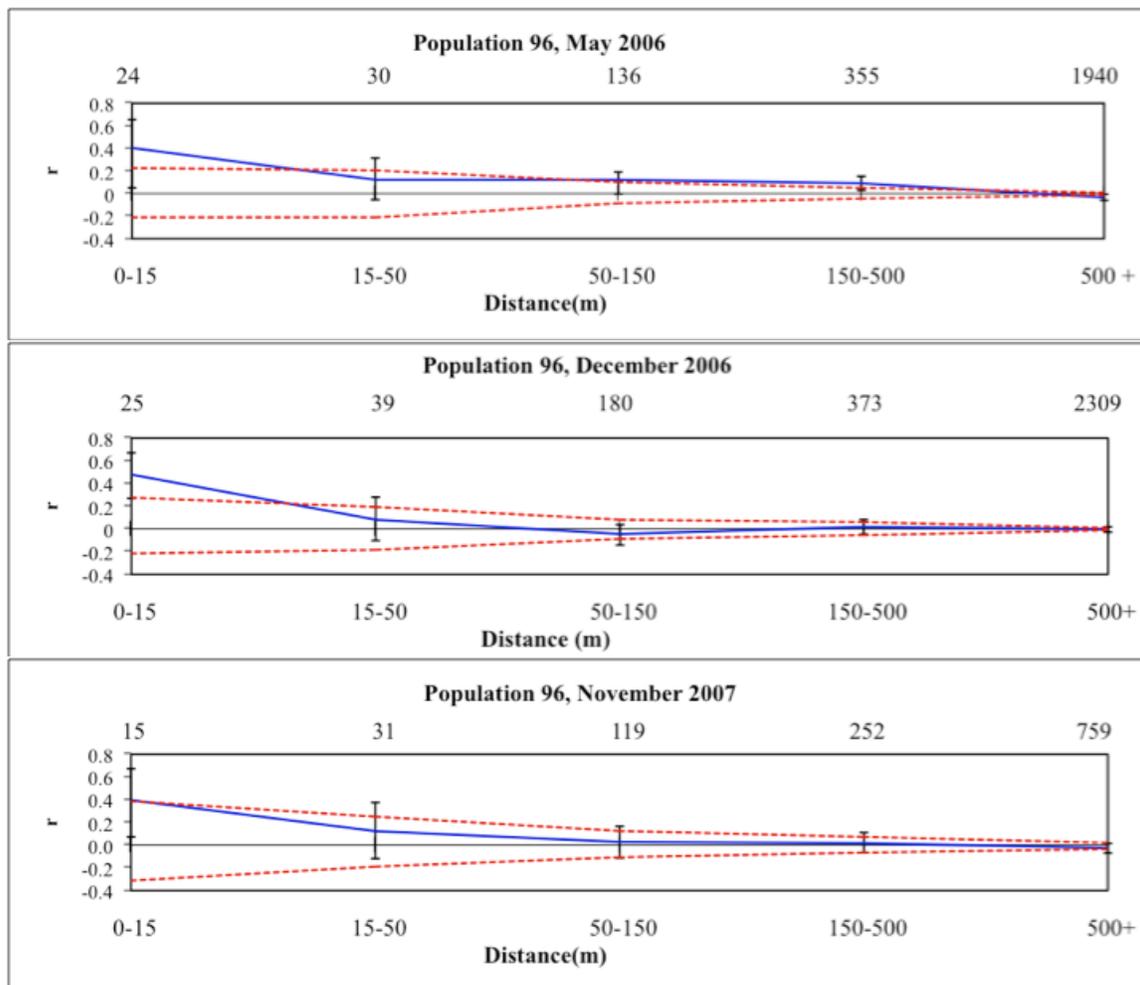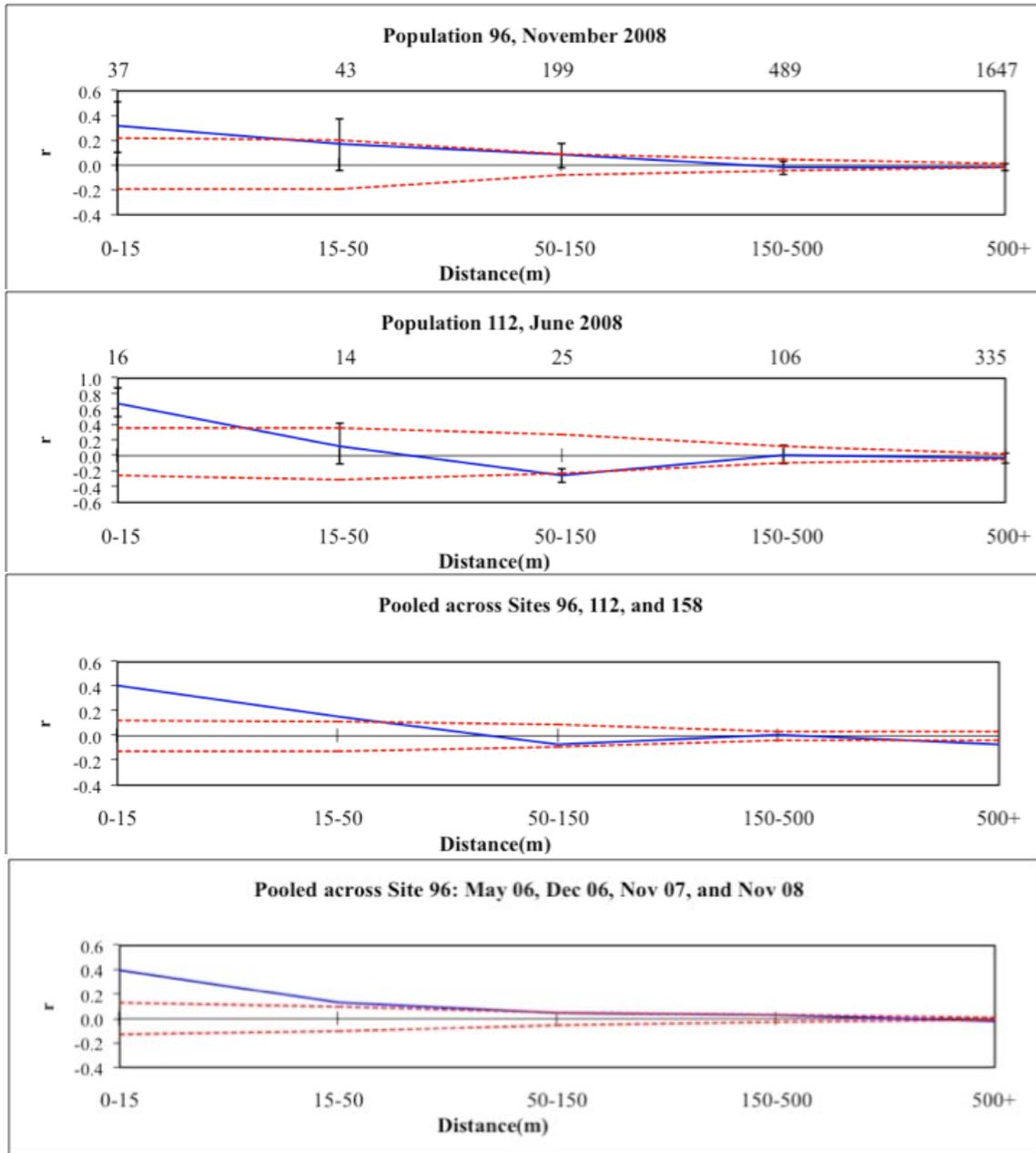
Figure 2. (Continued)

CHAPTER 4. GENERAL DISCUSSION

Conclusions

In this thesis, we attempt to determine how flowering phenologies within and among fig trees influence fig-wasp mutualism function. Within-tree flowering asynchrony has been observed numerous times in multiple different fig lineages and is hypothesized to have evolved in smaller populations or in more seasonal areas as way to provide reproductive assurance (Janzen 1979). There are two ways in which within-tree asynchrony may act to increase reproductive assurance: by allowing for pollinators to cycle within trees (Ramirez 1970, Janzen 1979), or by increasing the probability that periods of pollen release and receptivity will overlap among trees (Bronstein et al. 1992). We determined that self-fertilization was an unlikely agent of reproductive assurance because overlap of reproductive phases within trees is often sporadic and inconsistent and progeny of trees appear to be completely outcrossed. Fig populations exhibiting within-tree flowering asynchrony also had a greater amount of females than synchronously flowering tropical species, which is evidence that asynchronous flowering increases the functional number of mates within populations.

In fig populations where trees occur in patches within short distances of each other, flowering phenology among trees will be influenced by amount of clonal spread, phenological dependences of ramets within genets, and local environmental cues. We find that asexual growth is common across populations of *F.petiolaris* but only represented in small quantities within given populations. Within clones, we also find signs of correlative flowerings suggestive of some amounts of correlative flowerings within clones. Trees not involved in clonal growth, however, also display significant amounts of spatial autocorrelation of flowering phenologies at distances < 50m, which is likely to explain the

phenological correlations within clones. Although it is capable of maintaining high levels of genetic diversity by restricting mating among nearby relatives, spatial autocorrelation of phenological characters may also have critical implications for mutualism function if it decreases the amount of potential mates of individuals through floral coordination of nearby trees.

In both studies we have observed how flowering phenology, either within or among trees, may influence mutualism function in *F.petiolaris*. Within-tree flowering asynchrony and among-tree phenological spatial autocorrelation may bias estimates of functional mates and pollen limitations if they are not accounted for in future phenological studies. Although the fig and fig wasp mutualism is relatively unique as both species are entirely dependent upon the other, all outcrossing plants still require transmission of pollen and flowering phenologies within and among plants influence will influence genetic structure, mating systems, pollen limitations, and overall sexual success of individuals.

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APPENDIX. RAW POPULATION GENETIC DATA

Results of genetic analyses of nine Baja populations where N is the number of genets genotyped for three microsatellite loci, N_a is the total number of alleles, N_e is the effective number of alleles, I is an estimate Shannon's information index, PI is the probability of genotypic identity, H_o is heterozygosity observed, H_e is effective heterozygosity, and F is the fixation index.

Site	N	Na	N_e	I	PI	H_o	H_e	F
70	35	24	15.49	1.59	7.2E-04	0.74	0.71	-0.04
95	22	21	14.23	1.44	1.5E-03	0.82	0.68	-0.22
96	67	27	15.68	1.57	8.5E-04	0.63	0.67	0.07
112	27	24	10.30	1.43	2.6E-03	0.67	0.67	0.01
113	18	15	10.37	1.23	4.3E-03	0.67	0.64	-0.05
119	20	17	9.25	1.25	5.0E-03	0.68	0.61	-0.12
158	27	23	14.54	1.63	6.2E-04	0.77	0.74	-0.02
172	22	17	10.92	1.31	3.0E-03	0.59	0.66	0.10
179/19	21	21	14.14	1.58	7.6E-04	0.83	0.73	-0.15