Influence of gene dispersal and environmental heterogeneity on spatial and genetic patterns of the understory herb *Heliconia acuminata* across a fragmented landscape in central Amazon, Brazil

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ABSTRACT

Influence of gene dispersal and environmental heterogeneity on spatial and genetic patterns of the understory herb *Heliconia acuminata* across a fragmented landscape in central Amazon, Brazil

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Understanding how plants are spatially and genetically distributed in the environment can be a challenging task given the difficulty to characterize ecological processes, such as gene flow, and to disentangle the relative importance of multiple factors underlying the generation of distinct patterns. In this dissertation, I study different populations of the understory plant *Heliconia acuminata* L.C. Richard (Heliconiaceae) distributed across 1-ha fragments and continuous forest sites in the Biological Dynamics of Forest Fragments Project (BDFFP), an experimentally fragmented landscape in central Amazonia. I characterize a set of ten microsatellite markers developed for *Heliconia acuminata* to first evaluate gene flow, which is one of the main processes influencing genetic structure and spatial patterns of plants, and second to assess the potential influence of endogenous (e.g., seed dispersal) and environmental factors on spatial patterns of plants and genetic relatedness distribution. I combine genetic and ecological data in a novel and comprehensive Bayesian model to estimate parentage to more fully characterize the contribution of pollen and seed dispersal to *H. acuminata* gene flow. I then compare metrics of gene flow between fragments and continuous forest, while taking in consideration the variation in abundance of reproductive plants in each population. We tested the conservation genetics prediction that gene flow is interrupted in fragmented landscapes. Contrary to this hypothesis, we found that that both fragmentation and low population densities were associated with greater

immigration rates and longer pollination and seed dispersal distances. My results are one example of how fragmentation does not limit gene dispersal. I suggest that conservation genetics predictions are reformulated by taking in consideration the variation in the behavior of pollinators and seed dispersers across heterogeneous landscapes in response to habitat configuration and to the spatial and temporal availability of food resources. To investigate the influence of endogenous factors (plant - plant interactions) and environmental covariates (light, slope and soil characteristics) on spatial patterns of seedlings and adults, we use a new statistical methodology to model marked point patterns. Using this flexible approach, we also evaluate whether local spatial genetic structure is associated to spatial distribution of plants. The results show that *H. acuminata* seed dispersal is contagious, but not distance - restricted or genetically structured (presence of highly related plants). The absence of an association between spatial pattern and local genetic structure for adults also suggest the absence of genetic structuring in seedlings over time. Light and zinc availability are positively associated with spatial patterns of seedlings and adults, which may indicate carryover effects of seedlings on recruits over time. Carbon is negatively associated with adults, which may be evidence of competition with large dominant trees. I finally propose a new mechanistic framework to the studies of frugivore – mediated seed dispersal. I conduct a qualitative analysis of existent studies explicitly linking frugivores, fruiting plants and seed shadows and propose a frugivore - centered, process-based view of seed dispersal that integrates animal movement and seed dispersal ecology across multiple spatio -temporal scales. This critical analysis provides the empirical foundation over which we can build a more comprehensive, multi-scaled, research approach to the study of seed dispersal, process which is known to play a crucial role in the dynamics and evolution of plant populations.

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INTRODUCTION

The fine-scale spatial distribution of individuals and genotypes in natural plant populations reflects a variety of ecological processes (Chung 2008; Jacquemyn *et al.* 2009; Oddou-Muratorio *et al.* 2004). Disentangling the influence and relative importance of different factors to spatial structure of plants is, however, difficult because these processes are often intertwined and because different combinations of processes may result in similar patterns.

Genetic structure is primarily affected by pollination and seed dispersal, which are mostly mediated by animals in tropical plants (Jordano 2000; Ollerton *et al.* 2011). Gene flow or gene dispersal refers to the movement of genes in populations that effectively alters spatial genetic distribution and is the result of migration between discrete sub-populations and dispersal of both pollen and seeds within populations (Neigel 1997). Pollination is the result of mating between plants and thus reflects directly in genetic diversity and maintenance of populations (Dick *et al.* 2008). Seed dispersal allows for the movement of biparental genotypes and results in colonization of new habitats and spatial distribution of genotypes in the environment (Hamrick & Trapnell 2011). In animal mediated pollinated and dispersed plants, gene flow depends on the foraging behavior, physiological constraints, and cognitive abilities of pollinators and seed dispersers, which are, in turn, influenced by multiple endogenous and environmental factors (Côrtes & Uriarte in press; Hadley & Betts 2011).

Fine-scale spatial genetic structure (SGS) is the non-random distribution of genotypes in the environment (Vekemans & Hardy 2004). It is pronounced when genetic relatedness between plants is high at short distances, indicating formation of family structures (aggregation of kin) (Hamrick *et al.* 1993; Vekemans & Hardy 2004). High SGS is primarily attributed to seed dispersal limitation, meaning that progeny is dispersed in clumps or close to the maternal plant.

Besides pollen and seed dispersal, plant abundance is another important population characteristic that may impact levels of SGS (Vekemans & Hardy 2004). Populations with low population size and short seed dispersal distances are expected to have little seed and gene shadow overlap, which results in high genetic relatedness of neighboring plants (García & Grivet 2011). Conversely, populations with large population size and long seed dispersal distances are expected to have large seed and gene shadow overlap, increasing mixture of maternal genotypes and thus decreasing genetic relatedness between neighboring individuals (Hamrick *et al.* 1993; Hamrick & Trapnell 2011).

After seed deposition occurs, myriad ecological and environmental factors shape initial seed and gene shadows and modify the SGS of populations. Density-dependent mortality due to competition or pathogens and inbreeding depression may result in the non-random removal of individuals and genotypes from the population (i.e., thinning processes) (Bagchi *et al.* 2011; Collevatti & Hay 2011), whereas local factors favoring survival and growth of particular individuals or genotypes may facilitate recruitment of plants in particular microsites with favorable abiotic conditions (Comita *et al.* 2009; Santiago *et al.* 2012). These processes unfold over time leaving their signature in the spatial patterns and SGS of different age cohorts within populations (Comita *et al.* 2007; Gomez-Aparicio 2008; Troupin *et al.* 2006). A decline in SGS from seedling to subsequent life history stages may be attributed to a number of negative density dependent factors, including competition between neighboring individuals and density-dependence predation (Choo *et al.* 2012; Chung *et al.* 2003; Steinitz *et al.* 2011; Zhou & Chen 2010). Conversely, an increase in SGS through a plant population's life history stages may result from overlapping of recruits from related successive generations at favorable sites (Collevatti & Hay 2011; Jones & Hubbell 2006).

Fine-scale environmental heterogeneity of abiotic conditions such as light and soil fertility and biotic factors, such as distribution of food resources for animals, can influence directly and indirectly where plants establish and reproduce, as well as how and where pollinators and seed dispersers feed, move and disseminate pollen and seed. Processes occurring at larger scales, such as fragmentation of forested landscapes, can influence plant and animal dynamics at smaller scales, such as within forest stands, providing an extra layer of complexity in ecological systems. Proceeding at unprecedented rates in the tropics (FAO 2011; Whitmore 1997), deforestation and habitat fragmentation are well – known to impact the ecology and genetic structure of natural populations (Aguilar *et al.* 2008; DiBattista 2008; Laurance *et al.* 2002). One of the main predictions of conservation genetics, for instance, is that forest fragmentation disrupts gene flow by reducing dispersal distances and immigration of propagules from outside populations (Ouborg *et al.* 2006; Young *et al.* 1996). Fragmentation is also predicted to reduce effective population size, which can decrease overlap of seed and gene shadows, increase genetic drift and inbreeding, accelerating the loss of genetic variation (Aguilar *et al.* 2008). Assessing how different factors and processes occurring at small, as well as landscape level, helps to elucidate how natural plant populations are spatially and genetically structured.

The focal aim of this dissertation was to investigate how gene flow via pollen and seeds and environmental heterogeneity influences genetic and spatial distribution of the understory plant *Heliconia acuminata*. This herb constitutes an ideal study system because it is very abundant in the terra firme forest of Central Amazon, where the study was conducted, it has very limited clonal growth, and it is self-incompatible. Moreover, it is pollinated by hummingbirds

and seed-dispersed by manakins and thrushes, making it a representative example of animal - dispersed tropical plants.

The study was carried out in the experimentally fragmented landscape Biological Dynamics of Forest Fragments Project (BDFFP), Manaus, Brazil. The BDFFP is a 1000 km^2 landscape with several forest fragment reserves, ranging in size from 1 - 100 ha, as well as continuous forest. These fragments were isolated from 1980 - 1984 by clear - cutting the trees surrounding the patches and, in some cases, burning the felled trees (Gascon & Bierregaard 2001). Since isolation, fragments have undergone structural deterioration (Laurance *et al.* 2002), and secondary forests have colonized the surrounding matrix (Mesquita *et al.* 2001). Within this landscape, *Heliconia acuminata* has been the subject of an ongoing long term demographic project in which all plants have been mapped and characterized (height, number of shoots, presence of inflorescences) every year since 1998 (Bruna 2003). The plants have been monitored within 50 x 100 plots located in 1 – ha fragments and continuous forest.

For this research, I took advantage of the long term demographic database for information on seedlings recruitment and reproductive plants. In 2009, I collected leaf samples from all individuals in two plots located in continuous forest sites and three plots in separate 1 – ha fragments. All plants were genotyped at ten nuclear microsatellite markers. The development, characterization and protocols used for genotyping *Heliconia acuminata* are described in **chapter 1** of this dissertation. In **chapter 2**, I use a Bayesian approach to estimate parentage (pedigree) and parameters of dispersal kernels to quantify pollen and seed dispersal distances, immigration of propagules from outside populations, and reproductive dominance among parents. I then compare these metrics among fragments and continuous forests, while taking in consideration variation in plant abundance in these populations. In this chapter, I discuss the

prediction of conservation genetics that posits that gene flow is disrupted in fragmented landscapes. In **chapter 3**, I implement a new and flexible approach to point pattern analysis to assess the potential influence of seed dispersal and environmental factors on spatial distribution of seedlings and adults of *H. acuminata*. Jointly, I examine if relatedness of plants with nearby neighbors (local SGS) is associated with spatial location of plants. In this chapter, I combine data of all five plots into a single analysis, and thus incorporate, not only within – plot, but also across – landscape variation in environmental factors. And finally, in **chapter 4**, I conduct a qualitative analysis of existent studies explicitly linking frugivores, fruiting plants and seed shadows and propose a frugivore - centered, process-based view of seed dispersal that integrates animal movement and seed dispersal ecology across multiple spatio -temporal scales. This critical analysis provides the empirical foundation over which we can build a more comprehensive, multi-scaled, research approach to the study of seed dispersal, process which is known to play a crucial role in the dynamics and evolution of plant populations. Chapter 1 was already published (Côrtes *et al.* 2009), whereas chapter 4 is currently in press (Côrtes & Uriarte in press). Chapters 2 and 3 will be submitted to scientific journals in the near future.

CHAPTER 1 – CHARACTERIZATION OF TEN MICROSATELLITE MARKERS FOR THE UNDERSTORY AMAZONIAN HERB *HELICONIA ACUMINATA*

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ABSTRACT

We characterized ten microsatellite loci for the plant *Heliconia acuminata* from the Biological Dynamics of Forest Fragments Project (Manaus, Brazil). Markers were screened in 61 individuals from one population and were found to be polymorphic with an average of eight alleles per locus. We found moderate to high levels of polymorphic information content, and observed and expected heterozygosities. All ten markers are suitable for spatial genetic structure and parentage analyses and will be used for understanding *H. acuminata* dynamics across a fragmented landscape.

INTRODUCTION

Heliconia acuminata (Heliconiaceae) is a common understory species of the non-flooded tropical forest of central Amazonia and the Guyanas (Berry & Kress 1991). *Heliconia acuminata* is a perennial, self-incompatible hermaphroditic species with limited vegetative reproduction (EM Bruna and WJ Kress, unpublished data). The flowers are visited by hermit hummingbirds that "trapline" from one plant to the next (Kress 1985) and the seeds of Heliconia species are exclusively bird-dispersed (Berry & Kress 1991).

Heliconia acuminata has been the subject of a long-term investigation at the Biological Dynamics of Forest Fragments Project (BDFFP), located 70 km north of Manaus, Brazil (Bruna 2003). In the early 1980's fragments of 1-ha, 10-ha and 100-ha were isolated from surrounding forest by cattle pastures to study the effects of forest fragmentation on Amazonian ecosystems (Laurance *et al.* 2002). In 1997 5000 m^2 permanent plots were established in continuous forests (n = 6), 10-ha (n = 3) and 1-ha fragments (n = 4) and all *H. acuminata* individuals were monitored to investigate how its population dynamics responds to fragmentation (Bruna 2003). As part of an ongoing project, we selected ten microsatellite loci to evaluate the spatial pattern of genetic fine-scale structure of *H. acuminata* and to disentangle the contribution of pollen and seed dispersal to plant recruitment in both continuous forest and 1-ha fragments.

MATERIAL AND METHODS

Microsatellite libraries were enriched by Genetic Identification Services (GIS, http://www.genetic-id-services.com/) following Jones *et al.* (2002). Genomic DNA was partially restricted with a cocktail of seven blunt-end cutting enzymes and fragments were subjected to magnetic bead capture (CPG, Inc.). In parallel, libraries were prepared using Biotin-CA(15),

Biotin - AAC(12), Biotin - AAG(12) and Biotin - ATG(12) as capture molecules. Captured molecules were amplified and restricted with HindIII to remove the adapters. Fragments were ligated into the HindIII site of pUC19 and recombinant molecules were electroporated into *E. coli* DH5alpha. Clones were randomly selected and sequenced using the ABI Prism Taq dye terminator cycle sequencing methodology. GIS designed primers using DesignerPCR version 1.03 (Research Genetics, Inc.) from 58 microsatellite-containing clones.

Samples of leaf tissue were collected in 1997 and 1998 from 1-ha fragments (n = 3 plots) and continuous forest (n = 1 plot) at the BDFFP, and frozen samples (- 80° C) were used for DNA extraction using AutoGenprep 965 robot (AutoGen Inc.). Twelve individuals from the continuous forest were selected to test for amplification of 40 primer pairs and, sequentially, to determine optimal annealing temperature for the successful primers. Polymerase chain reactions (PCR) were performed in a 20-µl volume as follows: 10 ng DNA, 2 µl 10X PCR buffer (670mM Tris-HCI, pH 8.8, 160 mM (NH4)2SO4), 0.8 µl 50mM MgCl2, 1 µl dNTP (0.25 mM), 1 µl 10 µM unlabeled forward and reverse primers, 0.25 5U/ µl Taq polymerase (Bioline USA Inc.). Amplifications were performed in a thermal cycler (MJ Research) using the following conditions: initial denaturation at 95° C for 4 min, 30 cycles of denaturation at 93° C for 40 s, 54 - 64° C annealing temperature for 40 s, extension at 72°C for 30 s, and a final extension 72°C for 4 min. Reaction products were separated on a 1.5% agarose gel staining with ethidium bromide. Polymorphisms were analyzed using the Agilent 2100 Bioanalyzer (Agilent Technologies) and DNA 500 LabChip kit.

RESULTS AND DISCUSSION

Out of 40 candidate primer pairs, 14 were selected based on successful amplifications and

evidence of polymorphism. Forward primers were end-labeled at 5´end with one fluorescent phosphoramidite (6-FAM or HEX). Markers were screened on 61 individuals selected randomly from a single population from the continuous forest. PCR of 10 µl contained 10 ng DNA, 1 µl 10X PCR buffer, 0.3 µl 50mM MgCl2, 1 µl dNTP (0.25 mM), 0.2 µl 10 µM forward primer and 0.3 µl 10 µM unlabeled reverse primer, 0.03 µl 5U/ µl Taq polymerase (Bioline USA Inc.). Thermocycler programmes were the same as described above and specific annealing temperatures for each locus are given in Table 1.1. Fragments were sized on an ABI PRISM 3130xl DNA Analyzer (Applied Biosystems) using ROX-labeled size standard prepared as described in DeWoody *et al.* (2004). Fragments were scored using GeneMapper version 4.0 (Applied Biosystems). An average of 12 homozygote individuals per locus were selected for sequencing using BigDye Terminator (Applied Biosystems) to accurately verify the repeat motif. Four markers with low polymorphism level or inconsistent repeat sequences were excluded. Genotyping error rates were determined by re-extracting and re-running 28 samples (46%) for the ten markers.

The number of alleles per locus ranged from four to 13 with an average of eight. Polymorphic information content, and expected and observed heterozygosity presented moderate to high levels of variation, from 0.3 to 0.8 (Table 1.1). All loci were in Hardy-Weinberg equilibrium. Null allele frequencies were generally lower than 0.05, however Hac-B4 displayed a high frequency of 0.2 (Table 1.1). The combined non-exclusion probability of all ten loci was low, 0.0303 for the first parent and 0.0018 for the second parent. All these tests were conducted using Cervus version 3.0.3 (Kalinowski *et al.* 2007). Linkage disequilibrium was determined using GENEPOP version 4.0.7 (Rousset 2008) and loci were not linked after a Bonferroni correction ($p > 0.001$), indicating that markers are independent. Total genotyping error rate was

2.9%.

Based on our results, all markers will be useful in population-level studies including analysis of individual relatedness and parentage analysis. We are currently using these microsatellite loci to evaluate the effects of forest fragmentation on the fine-scale spatial structure of *Heliconia acuminata*. Ecological and demographic information will complement the current genetic investigation. We anticipate the integration of these data will provide a more comprehensive understanding of the dynamics of this model system in this experimentally fragmented landscape.

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Table 1.1. Characterization of ten microsatellite loci for *Heliconia acuminata*. Listed are locus name, sequences for forward (F) with respective fluorescent label (FAM or HEX) and reverse (R) primers, repeat motif, number of genotyped individuals (*N*), annealing temperature (T_a), size range, number of alleles (*k*), polymorphic information content (PIC), observed (H_o) and expected heterozygosity (H_e), null allele frequency and GenBank accession number.

Locus	Primer sequence 5'- 3'	Repeat motif	N	*T*a (°C)	Size range (bp)	*k*	PIC	*H*o	*H*e	Null allele frequency	GenBank accession no.
Hac-A103	F: ^{FAM}GCATTGGCTTCCTTTCTC R: ACTTGCTTGGTTCCTGTTG	T_9...$(CA)_{13}(GA)$	61	60	233-263	13	0.841	0.869	0.863	-0.0075	FJ644651
Hac-A116	F: ^{FAM}GGTTCTGGAGATTGGAAATG R: GTTGGAGGTGAGTTTAGGACTG	$(TC)_{13}(AC)_{10}(GCAC)_2$ (AT)2	61	57	248-278	10	0.632	0.623	0.663	0.034	FJ644652
Hac-A12	F: ^{HEX}CATCGTCTTTGCTGTAATCTTC R: GTCGTAATGCTTCTTGTGATTG	$(CT)_4(GT)_{13}$	61	60	145-201	12	0.84	0.869	0.862	-0.009	FJ644653
Hac-A5	F: ^{FAM}TGGTCAAATCACCTTTTCAAC R: GGACACCCACTCAGTCAAA	$(AT)_6(GT)_{14}$	61	60	161-175	8	0.684	0.754	0.732	-0.019	FJ644654
Hac-B117	F: ^{HEX}TTGCGACAGTTAAAATGAGTG R: ACATACCCACTGCACGAGTAC	$T(TTG)_7$-TGG-$(TTG)_2$	61	54	199-217	6	0.663	0.623	0.711	0.066	FJ644655
Hac-B4	F: ^{HEX}CCTCCCTTTCCTACCAGTT R: GGACAGCGATAACAAGAAGA	$(GCC)_5(TCC)_5$…$(TTG)_4$	61	57	211-217	4	0.425	0.311	0.473	0.204	FJ644656
Hac-B6	F: ^{FAM}AACCAAGACCACCTCCACTC R: AGGAACGAACGGCAGATAAG	$(CAA)_7$	61	62	266-275	4	0.464	0.492	0.514	0.01	FJ644657
Hac-C114	F: ^{HEX}ACCTCCAAAAGGAGTAAAGCTA R: AAGGTAAGGGACTGTCCTACAC	$(AGA)_9$	61	58	217-250	7	0.532	0.607	0.58	-0.035	FJ644658
Hac-C7	F: ^{HEX}GAAGCCTCCATCATCTCTTG R: GGCAGAAACTGAGTGGTGA	$(CTT)_7$	61	57	184-205	7	0.62	0.656	0.662	-0.011	FJ644659
Hac-D1	F: ^{HEX}CGCGAAGAAGATGAAGAGC R: CCCGACAGAAGCCCTAATC	$(ATG)_9$	61	62	158-177	7	0.304	0.295	0.328	0.048	FJ644660
Mean						8	0.600	0.609	0.638		

CHAPTER 2 – FRAGMENTATION AND LOW POPULATION DENSITY ENHANCE GENE FLOW IN AN AMAZONIAN HERB

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ABSTRACT

Forest fragmentation is predicted to reduce pollen and gene dispersal and effective population size, leading to increased genetic drift and inbreeding. Despite these expectations, little is known about the mechanisms that influence gene flow in human-modified landscapes. Here we rely on Bayesian genetic analyses to estimate parentage and characterize pollen and seed dispersal kernels for the Amazonian plant *Heliconia acuminata* L.C. Richard (Heliconiaceae), a common species pollinated and dispersed by birds. The study was conducted in two continuous forest sites and three 1 - ha fragments in the experimentally - fragmented landscape of Brazil's Biological Dynamics of Forest Fragments Project. We genotyped flowering plants and established seedlings using ten microsatellite markers to (1) quantify pollen and seed dispersal distances; immigration of propagules from outside populations, and reproductive dominance among parents; and (2) assess whether these metrics differed between continuous forests and fragments. Contrary to the usual assertion that forest fragmentation disrupts gene flow, we found that both fragmentation and low population densities were associated with greater immigration rates and longer pollination and seed dispersal distances. We suggest that continuous forest sites with high density of plants present elevated local availability of flowers and fruits leading to more spatially - limited bird foraging and shorter gene dispersal distances. Although all populations presented relatively low reproductive dominance, the high-density population exhibited the most equal parental contribution to seedlings' genotypes. A greater number of flowering plants, flowering asynchrony and high fruit removal observed for *H. acuminata* are likely to homogenize parental contributions to seedlings, which indicates low biparental inbreeding. Our results are one example of how fragmentation does not limit gene dispersal. It is timely that conservation genetics predictions are reformulated by taking in consideration the variation in the behavior of

pollinators and seed dispersers across heterogeneous landscapes in response to habitat configuration and to the spatial and temporal availability of food resources.

INTRODUCTION

Gene flow via pollen and seeds is critical in determining the genetic structure of plant populations (Dick *et al.* 2008). For most tropical plants, both pollen and seeds are dispersed by animals (Jordano 2000; Ollerton *et al.* 2011). Therefore, gene flow for these species will depend on the foraging behavior, physiological constraints, and cognitive abilities of pollinators and seed dispersers, and how these animals interact with plant traits and landscape or habitat features (Côrtes & Uriarte in press; Hadley & Betts 2011).

Deforestation and habitat fragmentation are proceeding at unprecedented rates in the tropics (FAO 2011; Whitmore 1997), with consequences for the ecology and genetic structure of natural populations (Aguilar *et al.* 2008; DiBattista 2008; Laurance *et al.* 2002). One of the main predictions of conservation genetics is that forest fragmentation disrupts gene flow by reducing dispersal distances and immigration of propagules from outside populations (Ouborg *et al.* 2006; Young *et al.* 1996). Fragmentation is also predicted to reduce effective population size, which can increase genetic drift and inbreeding, accelerating the loss of genetic variation (Aguilar *et al.* 2008). One way that effective population size can be reduced is by high reproductive dominance among plants. Reproduction dominance contributes to source-biased limitation, a type of dissemination limitation (García & Grivet 2011; Jordano & Godoy 2002) in which high variation among individuals in the quantity and quality of propagules results in fewer individuals successfully contributing genes to the new generations (Moran & Clark 2012b; Young & Pickup 2010).

In the long term, these detrimental effects are predicted to decrease population genetic diversity and increase genetic divergence among isolated patches (Aguilar *et al.* 2008; DiBattista 2008; Young *et al.* 1996). However, empirical evidence for this prediction is mixed, with many

studies failing to find strong population differentiation, decay of within-population genetic diversity, or an increase in inbreeding (Mimura *et al.* 2009; Moreira *et al.* 2009; Suárez-Montes *et al.* 2011; Winkler *et al.* 2011). This conflicting evidence is hardly surprising, given that neither plant nor disperser species exhibit uniform responses to habitat fragmentation (Hobbs & Yates 2003; Watling & Donnelly 2006).

A more effective approach to understanding the impacts of forest fragmentation on the genetic structure of plant populations is to emphasize the way in which changing landscapes alter the mechanisms that determine gene flow, namely seed dispersal and pollination. Direct assessment of these processes permits more realistic inference about the evolutionary consequences of fragmentation for natural plant populations (Bacles & Jump 2011; Sork & Smouse 2006) and provides insights into processes that drive contemporary gene flow, rather than relying on indirect comparisons of extant genetic variation, as a means of assessing historical gene flow (Meagher 2010; Oddou-Muratorio *et al.* 2010).

Highly polymorphic molecular markers (e.g., microsatellites) coupled with parentage analyses, can provide an accurate assessment of contemporary gene flow (Ashley 2010). Typically, researchers using highly polymorphic markers for parentage analysis exclude adults whose genotypes do not match those of seedlings. Alternatively, they use categorical approaches to assign the most likely single or pair of parents, based on log-likelihood ratios (Jones & Ardren 2003; Jones *et al.* 2010). These classic approaches, however, can provide poor results, if polymorphism is insufficient, and/or if the presence of null alleles or genotype mistyping errors are not accounted for (Chybicki & Burczyk 2010a; Jones *et al.* 2010). Moreover, researchers are often less interested in the parentage allocation *per se* than in population - level processes such as seed and pollen dispersal distances (Moran & Clark 2011; Moran & Clark 2012a; Oddou-

Muratorio & Klein 2008). In contrast, full probability models can jointly estimate population - level parameters and parentage, while incorporating both genetic and ecological data, such as spatial location of individual plants and reproductive status (Burczyk *et al.* 2006; Hadfield *et al.* 2006; Jones *et al.* 2010; Moran & Clark 2011).

Plant population - level characteristics that influence pollen and seed dispersal, such as adult density and flower and seed production, are often affected by landscape modification (Herrera *et al.* 2011; Kolb 2008; Leimu *et al.* 2006). Similarly, the abundance and behavior of animal pollinators and seed dispersers are likely to vary across heterogeneous landscapes (Aguirre *et al.* 2011; Hadley & Betts 2011; Magrach *et al.* 2011; Schleuning *et al.* 2011). Yet, studies of contemporary gene dispersal have rarely taken into account these ecological aspects of plant and animal populations. Few studies have conducted paternity or maternity analysis while considering the effects of plant and animal abundance or behavior across sites (Byrne *et al.* 2007; Dick *et al.* 2003; García *et al.* 2009a; Lander *et al.* 2010). Furthermore, studies of gene flow by both pollen and seeds of animal pollinated and dispersed plants across fragmented landscapes are very uncommon (but see Kamm *et al.* (2009)).

Here we use a hierarchical Bayesian approach to quantify the contribution of pollen and seed movement to gene flow for the Amazonian plant *Heliconia acuminata* L. C. Richard (Heliconiaceae), a common understory species of non-flooded forest of central Amazonia and the Guyanas (Berry & Kress 1991). *Heliconia acuminata* is pollinated by hummingbirds, and its seeds are dispersed by manakins and thrushes (Berry & Kress 1991; Uriarte *et al.* 2011). To conduct our study, we used mapped and genotyped individual plants in long-term demographic plots, established within an experimentally fragmented landscape. Specifically, we ask whether fragmentation and population density influence seed and pollen dispersal distance, immigration

of propagules from outside populations, and reproductive dominance among parents. We hypothesize that pollen and seed dispersal distances and immigration are lower in fragments than in continuous forest. In addition, we predict that reproductive dominance is higher in fragments than in continuous forest, due to these hypothesized reductions in pollen flow and seed dispersal distances and to the lower population sizes in these sites.

METHODS

STUDY SITE AND SYSTEM

The study was conducted in the Biological Dynamics of Forest Fragments Project (BDFFP) located 70 km north of Manaus, Brazil (2^{o} 30' S, 60' W, Fig. S1). The BDFFP is a 1000 km^2 landscape with several forest fragment reserves, ranging in size from 1 - 100 ha, as well as continuous forest. These fragments were isolated from 1980 - 1984 by clear - cutting the trees surrounding the patches and, in some cases, burning the felled trees (Gascon & Bierregaard 2001). Since isolation, fragments have undergone structural deterioration (Laurance *et al.* 2002), and secondary forests have colonized the surrounding matrix (Mesquita *et al.* 2001). Studies comparing bird capture rates before and after the isolation of the BDFFP's fragments suggest that spatial structure of the landscape is likely to affect the presence, abundance and movement of birds, including *Heliconia* pollinators and seed dispersers, and that these effects can be expected to vary, both spatially (Ferraz *et al.* 2003; Stouffer & Bierregaard 1995a, b) and temporally, as secondary forests develop in formerly cleared areas (Stouffer *et al.* 2006; Stouffer *et al.* 2009).

Heliconia acuminata has been the subject of a comprehensive demographic study since 1998 (Bruna 2003). Thirteen 5000 m^2 plots, in which all *H. acuminata* individuals have been

tagged and mapped, were established in continuous forest and fragments. We selected five plots for this study: two in continuous forest sites and three in 1 - ha fragments (Fig. S2.1). *Heliconia acuminata* is hermaphroditic, self-incompatible, and exhibits limited vegetative reproduction (E.M. Bruna and W.J. Kress, unpublished data). Differing from other *Heliconia* species, *H. acuminata* has a scattered distribution and is mainly found in the shaded understory (Bruna & Ribeiro 2005). In the study site, flowering usually begins in late January and continues through April (Bruna & Kress 2002). Flowering is correlated with plant size, with plants higher than 50 cm or with more than 3 shoots being more likely to flower (Bruna & Kress 2002; Gagnon *et al.* 2011). As is the case in other *Heliconia* species (Dobkin 1984, 1987), plants produce 20-25 flowers per inflorescence with each flower generally opening on a separate day and for only one day, preventing intra-inflorescence pollen transport by hummingbirds. Fruit maturation in the study site varies across the landscape and declines with increasing fragment size (Uriarte *et al.* 2011). In general, fruit maturation starts in March and continues through May (Bruna 2002). This species is one of the most abundant plants at the site (E. Bruna & W.J. Kress, personal observation). Each fruit produces two seeds (1.9 ± 0.02 seeds/fruit, mean ± SE, n=873 fruits, E. Bruna unpublished data). Plant fecundity (fruit and seed set) is independent of the density of nearby flowering conspecifics (Bruna *et al.* 2004).

Heliconia acuminata pollinators, the hermit hummingbirds *Phaethornis superciliosus* and *P. bourcieri*, are trapliners, can forage over large distances, move through a variety of habitats, and persist in primary and secondary forests (Stouffer & Bierregaard 1995a). Observational studies recorded low hummingbird visitation rates to *H. acuminata* flowers (median = 0.182 visits/hour) (Bruna *et al.* 2004). In our study site, the primary dispersers of *H. acuminata* seeds are the white-necked thrush (*Turdus albicollis*), the thrush-like-manakin (*Schiffornis turdinus*),

and several species of manakin (*Pipra erythrocephala*, *P. pipra*, *Lepidothrix serena*, *Corapipo gutturalis*). By modeling seed dispersal using radio-telemetry and feeding behavior data, Uriarte *et al.* (2011) estimated manakins in our sites to disperse seeds an average of 19 m from the maternal plant, while the thrush *Turdus albicollis* disperse 24 m away. At the individual plant level, removal of ripe fruits approximated 90% across the landscape and was not affected by forest fragmentation or neighborhood density (Uriarte *et al.* 2011).

For parentage analysis we collected samples of leaves from mapped *H. acuminata* seedlings instead of seeds because we were interested in "effective" pollen and seed dispersal, the ultimate result of both successful mating and seed deposition (Meagher & Thompson 1987). Plant tissue was collected in the five 0.5 ha plots in two years: 1999 and 2009. To assign seedlings to parents, we genotyped all plants that flowered between 1999 and 2009 (potential parents) and all seedlings established between 2000 and 2009 that were still alive in 2009, at ten nuclear microsatellite loci. In 2009, to increase the likelihood of determining the potential parents of seedlings inside the plot, we mapped and collected leaf tissue from all adults showing signs of current or past reproduction in a 20 m buffer around each plot. Old inflorescences can remain attached to the plant for more than a year, so it is relatively easy to identify potential reproductive individuals (P. Rubim, personal communication). This increased the sampling area for reproductive plants from 0.5 ha to 1.26 ha. Only plants that were alive in 1999 or 2009 could be sampled for genotyping. This means that some of the reproductive plants and seedlings that established after 1999 but died before 2009 could not be sampled (Table 2.1), while seedlings sampled in 2009 may not survive. If survival of plants is age and site dependent, sampling different age cohorts may bias the results. Proportion of dead seedlings did vary across populations (33-51% of all seedlings recruited between 1999 and 2008 died, $\chi^2 = 12.68$, $df = 4$, P

= 0.0129, *N* = 1046). We recognize that pollen and seed dispersal distances may be overestimated, due to the incomplete sampling of putative parents in the buffer zone and of seedlings that died between 1999 and 2009. Nevertheless, our main objective is to compare gene flow among populations, a task which should not be affected by this incomplete sampling since the same procedures were applied to all plots in the landscape.

Leaf tissue was either frozen in liquid nitrogen or dried in silica gel and then stored at -80°C. Total genomic DNA was manually extracted using a modified CTAB extraction method (Ferreira & Grattapaglia 1998) or by automatically using a AutoGenprep 965 robot (AutoGen Inc). Ten nuclear microsatellite markers previously developed for *H. acuminata* (Côrtes *et al.* 2009) were used to genotype *H. acuminata* individuals. The PCR protocols and genotyping procedures are described in Côrtes *et al.* (2009). Genotyping error rates resulting from mistyping and drop-out were calculated by regenotyping individuals for each locus (range 20 – 26% per loci). Across loci, 2.9% (range 1.4 – 5.1%) and 2.8% (range 0.9 – 6.6%) of the regenotyped individuals had been mistyped or had presented drop-out alleles.

GENE DISPERSAL MODEL

We modified the Bayesian approach developed by Moran & Clark (2011) to estimate pedigree and pollen and seed dispersal. The model permits the inclusion of prior information and multiple sources of uncertainty associated with genotyping and specific ecological processes, which results in more appropriate parameter estimates (Jones *et al.* 2010; Moran & Clark 2011). A second advantage of the model is that it incorporates the contribution of plants located outside the sampled area, so that immigration is also used to model dispersal kernel. Parentage analysis of hermaphroditic species usually assumes that the nearest assigned parent is the mother (Bacles

et al. 2006). Instead, in this approach, maternity and paternity are assigned with uncertainty, given the pollen and seed dispersal kernel (Moran & Clark 2011; Moran & Clark 2012a). The pedigree, pollen and seed dispersal parameter are jointly estimated based on offspring and adult genotypes, two types of genotyping error, distances between plants and plant phenology (Supplementary material S2.4), as follows (Moran & Clark 2011):

$$p\left(P, u_p, u_s \middle| \{G^O\}, \{d\}, e_1, e_2, \{f\}, \{c\}, \{r\}, \{s\}\right)$$

$$\propto \prod_k \left[\left(\frac{c_{i'} s_{i'i} p(d_{i'i}|u_p) f_i r_{ik} p(d_{ik}|u_s)}{\sum_{i,i'} c_{i'} s_{i'i} p(d_{i'i}|u_p) f_i r_{ik} p(d_{ik}|u_s)} \right) \text{x} \left(\frac{\prod_l p\left(G^O_{k,l} | G^O_{i',l'}, G^O_{i,l}, e_{1,l}, e_{2,l}\right)}{\sum_{i,i'} \prod_l p\left(G^O_{k,l} | G^O_{i',l'}, G^O_{i,l}, e_{1,l}, e_{2,l}\right)} \right) \right] p(u_s) p\left(u_p\right) \qquad \text{(Eqn. 1)}$$

where P is the pedigree; u_s and u_p are the seed and pollen dispersal parameters; G^O is the observed genotype of all individuals for locus l; d is the pairwise distance between individuals; f and c are the weight factors represented by the number of seeds (*i.e.*, fecundity of maternal plant i) and number of flowers (*i.e.*, pollen production of paternal plant i'), respectively; r is the plant - seedling temporal compatibility, indicating whether a seedling k recruited after mother i flowered (1 or 0); s is the flowering synchronization to assure that plants are able to mate by indicating whether flowering of plant $i'$and i occur in the same year (1 or 0); e_1 and e_2 are the mistyping and dropout errors of locus l; and $p(u_s)$ and $p(u_p)$ are the priors related to the dispersal parameters.

Flower production (c) was measured as the total number of flowers each individual plant produced over the study period, and is the product of the number of inflorescences and the average number of flowers per inflorescence. Fecundity (f) was calculated as the product of the number of seeds per flower (see Study site and system) and the maturation rate from flower to ripe fruits (from Uriarte *et al.* 2011). Maturation rate is higher for fragments, with a rate of 0.15 for CF1, 0.08 for CF2 and 0.5 for F1, F2 and F3 (Uriarte *et al.* 2011, M.T.B. da Silva,

unpublished data).

The distance kernel for both pollen and seed dispersal is given by the 2D - t function (Clark *et al.* 1999), which takes the form:

$$p(d) = \frac{1}{\pi u(1+\frac{d^2}{u})^2} \quad \text{(Eqn. 2)}$$

where parameters are as in Eqn. 1 and separate *u*'s are estimated for pollen and seeds. We chose the 2D - t function instead of other commonly used functional forms because it allows for both more events occurring at short and long distances relative to a normal distribution (Clark *et al.* 1999; Moran & Clark 2012a). To compare pollination and seed dispersal distances, we used the mode rather than the mean because we were interested in the most frequent dispersal events (Clark *et al.* 1999).

Pedigree and other parameters in Eqns. 1-2 were estimated using a Gibbs sampler, using parentage probabilities and ecological data. Implementation follows the code proposed by Moran & Clark (2011). Implementation and information on the effects of different priors and density of hypothetical parents on posteriors are provided in the Appendices (Supplementary material S2.2).

Reproductive dominance

Reproductive dominance was investigated using the pedigree recovered from the gene dispersal model and focusing only on the seedlings that had at least one parent identified within the plot. Reproductive dominance is a measure of the genetic contribution of reproductive plants to the seedlings in the population via either pollen or seeds. It was calculated using the probability of

parentage identity (PPaI) metric (Supplementary material S2.3). PPaI is analogous to the probability of paternal identity (PPI; Smouse & Robledo-Arnuncio 2005) and maternal identity (PMI; Grivet *et al.* 2005) and measures the probability that two offspring randomly sampled from a population share the genotype of either a father or mother. PPaI was estimated using a variation of the unbiased *r* - estimator R_0 (Eqn. S2.3), and ranges between 0 (seedlings do not share any parental genotype) to 1 (seedlings share genotypes of both parents).

RESULTS

DO SEED AND POLLEN DISPERSAL DISTANCES VARY BETWEEN FRAGMENTS AND CONTINUOUS FOREST?

Contrary to our expectation, pollen and seed dispersal distances were longer in fragments than in continuous forest (Fig. 2.1), which led to a more restricted dispersal in CF1 and CF2 relative to the fragment (Fig. 2.2). Modal distances were almost three and four fold larger in fragments for seed and pollen dispersal, respectively, relative to those for CF1 (Table 2.2).

Isolation was not the only factor influencing dispersal distances; dispersal values for CF2 were more similar to those in the fragments than to CF1 (Fig. 2.1). At the population level, absolute number and density of flowering plants was 5 to 14 times greater in CF1 than for the other areas, regardless of fragmentation status (Table 2.1), although there is inter - year variation (Supplementary material S2.4).

When considering gene dispersal from parental pairs located strictly within the plots, average effective pollination distance was always greater than average distance to the nearest reproductive plant (Fig. 2.3). Median distance between reproductive plants ranged from 3 m in CF1 to 15 m in CF2, whereas median of effective pollination ranged from 21 m in CF1 to 37 m

in F1. Effective pollination distance in CF2 exceeded this range (50 m), but only a single instance of inside pollination was recorded.

As for pollination, effective seed dispersal distances were generally longer than distance of seedlings to the nearest reproductive plant, except in CF2, where the median distance between seedlings to reproductive plants was greater (12 m) than effective seed dispersal (10 m) (Fig. 2.3). Median distance between seedlings and reproductive plants ranged from 3 m in CF1 to 12 m in CF2, whereas median of effective seed dispersal distance ranged from 10 m in CF2 to 24 m in F1.

DO PROPAGULE IMMIGRATION AND REPRODUCTIVE DOMINANCE VARY BETWEEN POPULATIONS?

Contrary to the predictions of conservation genetics, the probability of propagule immigration was not associated with fragmentation status, but rather was negatively related to plant density. Immigration of pollen and seeds was highest for CF2, with only one parental pair assignment within the sampled plot, and lowest in CF1, with only 2% of the seedlings generated from parent pairs located outside the plot (Table 2.2). Fragments, with intermediate plant densities, experienced intermediate rates of propagule immigration, with 13 - 23% of the seedlings with parent pairs located outside plots (Table 2.2). On average, 70% (range 62 - 91%) of the reproductive plants contributed any genes (via pollen or seed) to seedlings inside plot, with the exception of F1, in which more than 90% of the reproductive plants contributed genes (Table 2.2).

In general, probabilities that seedlings shared a parent (PPaI - values) were always smaller than 8%, indicating relatively even parental contributions to seedlings (R_0, Fig. 2.4). Fragmentation status was not associated with the degree of reproductive dominance. Rather,

plant density was a far more critical factor. CF2 and F3 exhibited the highest reproductive dominance, followed by F2 and F1 (R_0, Fig. 2.4). CF1 was the population with the most even genetic contribution of adults to seedlings (Fig. 2.4), with PPaI almost an order of magnitude smaller than CF2 and F3 (R_0, Fig. 2.4).

DISCUSSION

Our study examined the impacts of fragmentation on gene flow across plant populations using a novel and comprehensive approach that jointly exploits genetic and ecological data while incorporating multiple sources of uncertainty. By considering the contribution of outside parents via immigration of pollen and seeds, our approach provides a better description of the full dispersal kernel than traditional approaches.

Our results contradict the prediction of conservation genetics that fragmentation and small population sizes lead to genetic erosion. Instead, we found that gene flow was enhanced in fragmented and small populations relative to large populations in continuous forests. These results have important implications to what has so far constituted the paradigm of conservation genetics.

HOW DO FRAGMENTATION AND PLANT DENSITY INFLUENCE GENE FLOW?

The prevailing theory in conservation genetics predicts that small isolated populations suffer from reduced gene flow, high rates of inbreeding and genetic drift, eventually leading to reduced within - population genetic diversity, increased divergence across populations, and increased inbreeding (Aguilar *et al.* 2008; Ouborg *et al.* 2006; Young *et al.* 1996). Counter to this prediction, we found gene dispersal to be enhanced in small fragments with low density of

flowering plants, suggesting that some of the prevailing assumptions about the processes that lead to erosion of genetic diversity in fragmented populations may not be generally applicable. For instance, fragment boundaries do not always represent a barrier to animal and propagule movement (Hadley & Betts 2011; Kramer *et al.* 2008). At the study site, abundance of *H. acuminata* pollinators did not change before and after experimental landscape fragmentation (Stouffer & Bierregaard 1995a) and frugivorous birds used small fragments as the cleared matrix grew back into secondary forest (Stouffer & Bierregaard 2007).

High gene flow via pollen movement beyond boundaries of isolated forest fragments has been recorded elsewhere for animal-pollinated plants (Aldrich & Hamrick 1998; Dick *et al.* 2003; Kamm *et al.* 2009; Lander *et al.* 2010; Nason & Hamrick 1997; White *et al.* 2002). In contrast to pollen-mediated gene flow, gene flow via seed dispersal by animals between fragments and continuous forest has received scant attention and results to date are mixed. For instance, Hanson *et al.* (2007) assigned 14 out of 23 seed endocarps of *Dipteryx panamensis* to mothers outside fragments, demonstrating that bat-mediated dispersal can connect isolated patches. In contrast, parentage analysis of *Araucaria angustifolia* in Brazil showed that seed immigration into forest fragment was absent, possibly the result of autochory and limited dispersal by secondary dispersers (i.e., birds and rodents) (Bittencourt & Sebbenn 2007). These limited studies point to the key role that vector ability and willingness to move longer distances and cross inhospitable environments plays for the presence and magnitude of seed-mediated genetic connectivity.

Enhanced gene flow in fragmented populations, such as found in this study, can result from a variety of disperser responses to landscape fragmentation. Animals may move between fragments and continuous forests through preferential use of areas with high forest cover, leading

to greater dispersal distances for seeds consumed in fragments relative to those consumed in continuous forests. For instance, experimentally translocated hummingbirds (*Phaethornis guy*) in a mixed Costa Rica agricultural landscape successfully returned home, but often took tortuous paths to get there, following areas of high forest cover instead of crossing open agricultural matrix (Hadley & Betts 2009). A second mechanism leading to greater seed dispersal distances from fragments is a shift in the foraging behavior of the disperser. In a study in a fragmented landscape in South Africa, trumpeter hornbills (*Bycanistes bucinator*) generated a bimodal seed-dispersal distribution, with a first peak at 18 m associated with local foraging, and a second peak at 512 m, corresponding to the average distance between patches. In continuous forest, however, the distribution was unimodal with an average of 86 m (Lenz *et al.* 2011). The same two-component seed dispersal distribution has also been recorded for the European jay (*Garrulus glandarius*) in Mediterranean landscapes (Gómez 2003).

Beyond fragmentation, low density of reproductive plants also enhanced gene flow of *H. acuminata.* Average pollen and seed dispersal distances for the low-density continuous forest (CF2) were closer to fragments than to the high-density continuous forest site (CF1). Both theoretical and empirical studies predict that pollinators will spend more time visiting flowers within the same plant or forage on the nearest neighbor when plant density is low, ultimately reflecting in shorter pollination distances (Ghazoul (2005) and references therein). Lower reproductive success may occur if selfing produces less fit individuals or if nearest neighbor is related (which is expected given that fine-scale spatial genetic structure is common for plants (Vekemans & Hardy 2004)), and thus increasing biparental inbreeding (Ghazoul 2005). Recent paternity analyses across fragmented landscapes, however, have found extensive gene flow in populations with reduced plant density (Lander *et al.* 2010; Llorens *et al.* 2012). In line with

these studies, we found greater pollination distances and immigration of pollen in low density populations compared to the densest population. Our seed dispersal findings also corroborate the few seed dispersal studies on this matter. They report that increasing plant aggregation and abundance of fleshy fruits decrease seed dispersal distance as birds concentrate foraging in areas of higher fruit density (Herrera *et al.* 2011; Morales & Carlo 2006).

It is likely that in sites with sparsely distributed *H. acuminata*, birds have to travel longer distances and cover larger areas, searching for flowers and fruits, in order to meet their energetic requirements (Hadley & Betts 2011; Khamcha *et al.* 2012). This pattern may be particularly marked for specialist pollinators, such as fig wasps (Ahmed *et al.* 2009; Nason *et al.* 1996), and for frugivorous birds with narrow dietary preferences (Kinnaird *et al.* 1996; Kwit *et al.* 2004). Hummingbirds abundance in the study site is higher between January and April, period when *H. acuminata* is flowering (Stouffer & Bierregaard 1996), suggesting that these birds track these critical nutritional resources in time. Experiments in captivity and lab analyses have also shown that the high lipid content of *H. acuminata* fruits make them a preferred resource for manakins (S. Hashimoto unpublished data), also suggesting that certain frugivorous birds may track *H. acuminata* fruiting across the landscape.

It is important to note that pollination distances may have been underestimated in our study, given our incomplete understanding of the fine-scale temporal variation of *H. acuminata* flowering. Even though we were able to identify whether a plant flowered in a single year, we were not able to assess daily flower synchronization, which is required for successful pollination. The average number of open flowers per day in a Costa Rica population equaled 0.5/inflorescence with a density of 0.08 flowers/10 m^2 (Linhart 1973). Daily flowering of *H. acuminata* is potentially asynchronous given that plants produce few inflorescences and only a

few flowers open on a single day. Hummingbirds may need to travel much longer distances and search more actively for open flowers on a daily basis. The fact that the average inter-parent pair distance exceeded the distance to the nearest flowering plant reinforces the flowering asynchrony in *H. acuminata* given the widely-searching behavior of traplining hummingbirds.

Similarly, seed dispersal of *H. acuminata* did not occur near the maternal plant for the majority of the populations. Animal-dispersed seeds are typically deposited further from the maternal source plant, but in many cases, seeds are deposited below non-neighboring conspecifics (Godoy & Jordano 2001; Hardesty *et al.* 2006; Sezen *et al.* 2009). The chance that *H. acuminata* seeds are deposited below their own mothers is very low. Around 90% of the ripe fruits are removed from the plant (Uriarte *et al.* 2011), making it unlikely that fruits drop naturally to the forest floor. The high removal rates can be attributed to the overall low fruit production in the forest understory in the study site coupled with birds' preference for *H. acuminata* fruits (S. Hashimoto, unpublished data).

IMMIGRATION AND REPRODUCTIVE DOMINANCE

Our findings also contradict the theoretical expectation that reproductive dominance is higher in fragmented populations. The population with highest reproductive dominance was CF2, with 60% of all reproductive plants contributing to the genetic pool but only two individuals contributing more than 30% to the seedling genotypes. Immigration of pollen and seeds were also highest for this plot, with 62% of the seedlings originating from parents outside the plot. This high immigration rate is likely to increase effective population size of the recipient population and dilute reproductive dominance within the population. At the other extreme, the

population with the highest density of reproductive plants (CF1) had the most diverse array of parents contributing to the seedling genetic pool compared to other populations.

Despite these differences in reproductive dominance among populations, the values of PPaI were overall low, indicating that contributions of reproductive plants to seedlings genotypes were relatively even when compared to other systems. For example, adult *Symphonia globulifera* trees in pastures produced more than 68% of the seedlings in forest remnants of southern Costa Rica (Aldrich & Hamrick 1998). In a study of the palm *Iriartea deltoidea*, only two individuals contributed 56% of the genes of the second-growth founder population (Sezen *et al.* 2005).

These low values of reproductive dominance found in *H. acuminata* suggest that neither pollination nor dispersal are strongly limiting at the population level. Our results show that pollen from different plants is effectively dispersed but only few flowers of *H. acuminata* actually develop into fruits (Uriarte *et al.* 2011). In fact, green fruits are produced indicating that ovules are fertilized, but these often fail to develop into ripe fruits (E.B. personal observation). Seed disperser behavior is also likely to contribute to the low observed reproductive dominance. Given low fruit production, *Heliconia acuminata* seed dispersers seem to consume most of the produced fruits, regardless of the maternal attributes (plant size or density of flowering neighbors), and unlike in other systems, plants do not seem to compete for frugivores (Ghazoul 2005). The low reproductive dominance observed in our study may result from a large number of flowering plants (in CF1), flowering asynchrony, low fecundity and high seed removal rates of *H. acuminata* relatively to the highly fecund tree species that were the focus of previous studies. The relatively even reproduction and high immigration in fragments and low-density populations may increase the effective population size and diversity of contributing parents, which most likely will decrease biparental inbreeding and prevent signs of genetic erosion.

CAVEATS

We have largely ignored myriad ecological and genetic processes that occur between pollen deposition on floral stigmata and seedling establishment, concentrating instead on surviving offspring that will form the spatial structure of the gene pool that will produce the following generations (Cousens *et al.* 2008). The average seed dispersal distance in CF1 is congruent with the averages obtained by modeling seed dispersal using birds' movement and gut retention time (Uriarte *et al.* 2011), but the averages obtained in the other four populations were considerably longer than the modeled seed dispersal. It is possible that variance of pollen and fruit removal of *H. acuminata* is higher and that seed dispersal kernels are more restricted than actually observed among extant recruits. Post-dispersal processes may re-structure the spatial distribution of seedlings, so that mortality could reduce the number of offspring close to maternal plants (Choo *et al.* 2012; Isagi *et al.* 2007; Steinitz *et al.* 2011).

Moreover, the BDFFP landscape is surrounded by large extensions of primary forest and fragments are not that distant from other tracts of forest (minimum distance from fragments to forest is around 200 m). Under scenarios of true isolation it is likely that manakins and thrushes would not move to other forested patches, and, trapped within a fragment, would eventually disappear from the system, leading to genetic erosion. Nevertheless, our study shows that the secondary growth that grew back in cleared land enables the movement of dispersers and pollinators. Over 70% of global tropical forest cover consists of forest regrowth following logging, agricultural abandonment, or conversion to agroforests (FAO 2011), making our findings highly relevant to tropical forests elsewhere.

CONCLUSIONS

The conservation genetics paradigm predicts gene flow is reduced with habitat fragmentation and small population sizes. Our results exemplify the opposite of what have been anticipated. Given similar abundance of pollinators and seed dispersers, fragmentation seems to enhance gene flow at two levels. First it influences gene flow directly by promoting inter - patch movement. It also affects gene flow indirectly by reducing the abundance of plants within patches (Bruna 1999, 2002; Uriarte *et al.* 2010) and therefore, enhancing even more gene flow across fragments. As our study illustrates, continuous forest sites present striking variation of plant abundance, which in turn results in distinct gene dispersal outcomes. High abundance of reproductive plants on dense populations generates more equal reproductive contribution increasing genetic diversity of seedlings. It is possible that genetic diversity is maintained across the landscape by two exclusive processes: Immigration of propagules within fragments and low-density populations, and high diversity of parental contribution in high-density populations.

Plant dynamics and persistence in fragmented landscapes can be assessed using methods that allow contemporary and spatially explicit evaluation of ongoing genetic processes. As a step forward, we advocate that future studies evaluate contemporary gene flow by taking into consideration plant and dispersal vector features, as those vary across changing landscapes. Reformulating a new set of predictions within conservation genetics will require the contribution of additional studies to draw a more representative picture of how interactions between landscape configuration and organismal traits influence the processes of pollination and seed dispersal.

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Table 2.1- Plant characteristics of studied populations of *Heliconia acuminata* in BDFFP, including total number of reproductive plants (number of dead plants not sampled in parentheses), percentage (%) and density of flowering plants, and number of sampled seedlings (total number of seedlings ever recruited in parentheses).

Population name	Reserve number	Total No of flowering plants (1999-2009)	% Total flowering (1999-2009) (yearly range)	Total density of flowering plants (plants per m^2)	Average of total plant density (1999-2009) (yearly range)	No of seedlings (1999-2009)
CF1	1501	285 (3)	15 (0.4 - 12.3)	0.0226	732 (544 - 837)	374 (681)
CF2	None (Dimona)	21 (5)	5 (0 - 5.6)	0.0016	122 (110 - 132)	52 (80)
F1	3114	44 (2)	7 (0 - 3.4)	0.0035	227 (203 - 249)	118 (172)
F2	2107	50 (0)	9 (0 - 7.4)	0.0040	221 (208 - 232)	73 (112)
F3	2108	60 (3)	16 (2.4 - 15.0)	0.0047	185 (146 - 219)	83 (127)

Table 2.2- Modal pollen and seed dispersal distances (from the 2D-t function) with support intervals in parentheses, total number and percentage of seedlings with parents located inside and outside the plot and number and percentage of reproductive plants that were assigned as either pollen (fathers) or seed donors (mothers).

Population name	Pollination (m)	Dispersal (m)	Parent pair inside plot	Parent pair outside plot	Father outside	Mother outside	Contributing plants
CF1	16 (14-18)	14 (13-16)	276 (74%)	9 (2%)	8 (2%)	81 (22%)	212 (74%)
CF2	46 (36-57)	37 (25-44)	1 (2%)	32 (62%)	10 (19%)	9 (17%)	13 (62%)
F1	58 (47-64)	43 (39-45)	26 (22%)	38 (32%)	20 (17%)	34 (29%)	40 (91%)
F2	66 (55-77)	40 (30-43)	23 (31%)	18 (25%)	17 (23%)	15 (21%)	35 (70%)
F3	64 (54-71)	42 (39-45)	47 (57%)	11 (13%)	18 (22%)	7 (8%)	46 (76%)

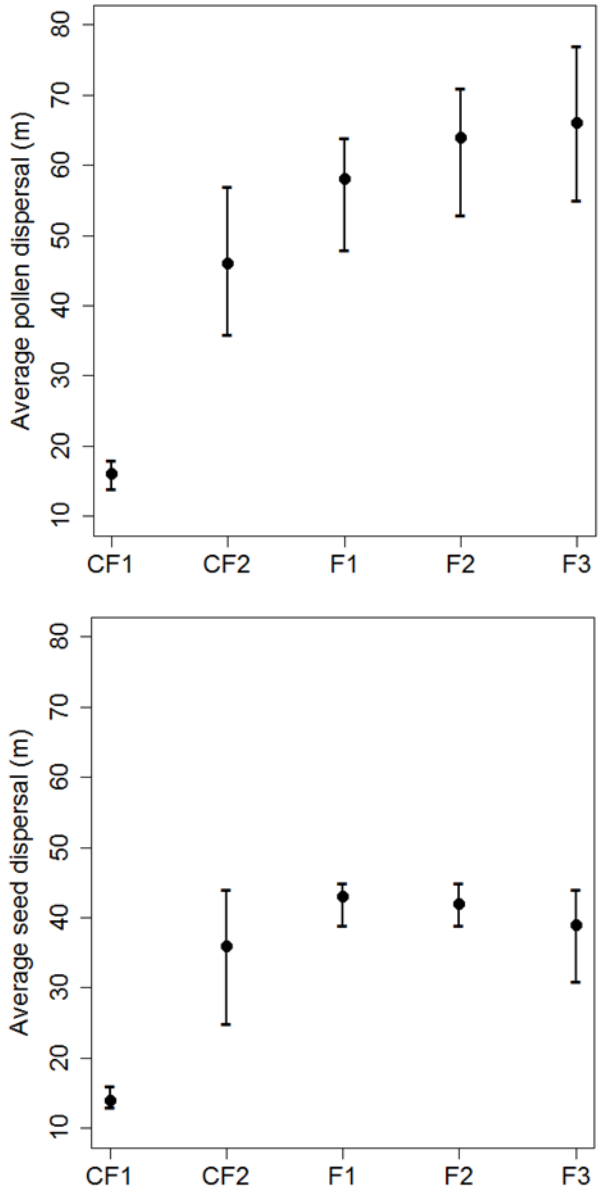

Figure 2.1- Average modal distance of pollen and seed dispersal and associated 95% support interval obtained from 50,000 simulations for each population of *Heliconia acuminata* in the BDFFP, Brazil.

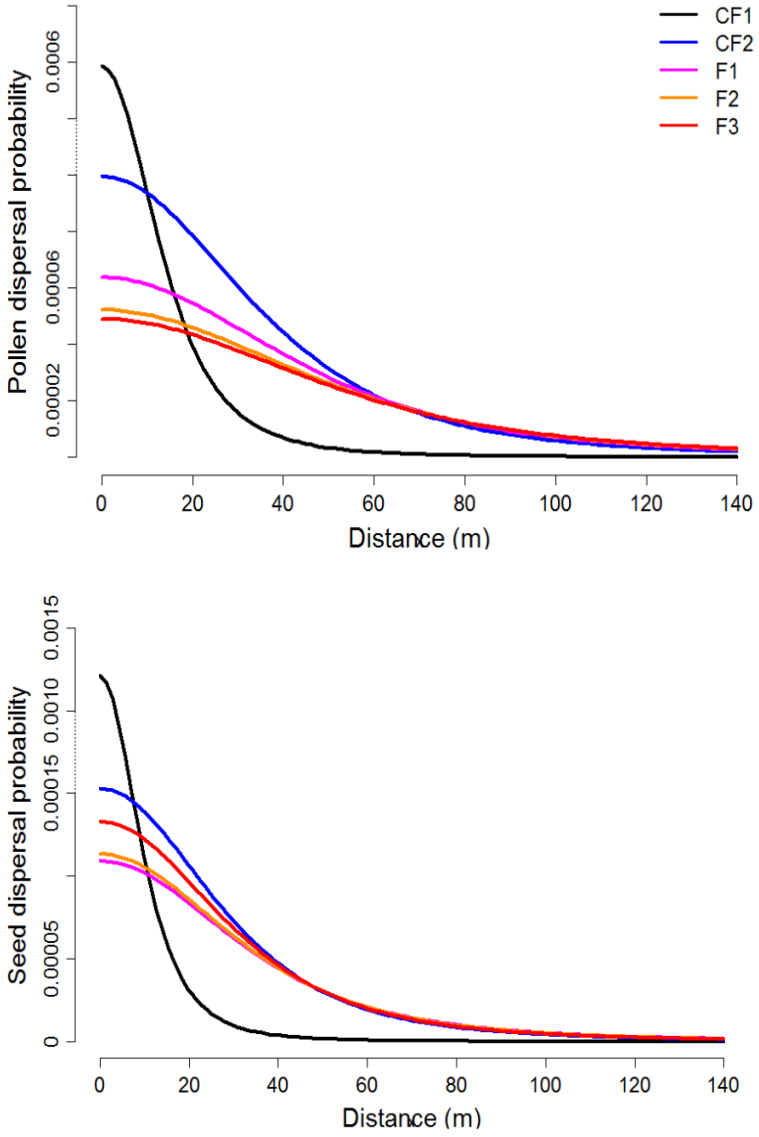

Figure 2.2- Modeled kernel using the 2Dt - function given the posterior mean of pollen dispersal (u_p) and seed dispersal (u_s) parameters. Scale of y-axis is different for pollen and seed dispersal, with seed dispersal reaching higher limits given it is more distance-restricted. Note that y-axis is broken to permit clear visualization of all kernels because probability of pollen and seed dispersal at short distances in CF1 was much higher than in other populations. X-axis was truncated at 140 m.

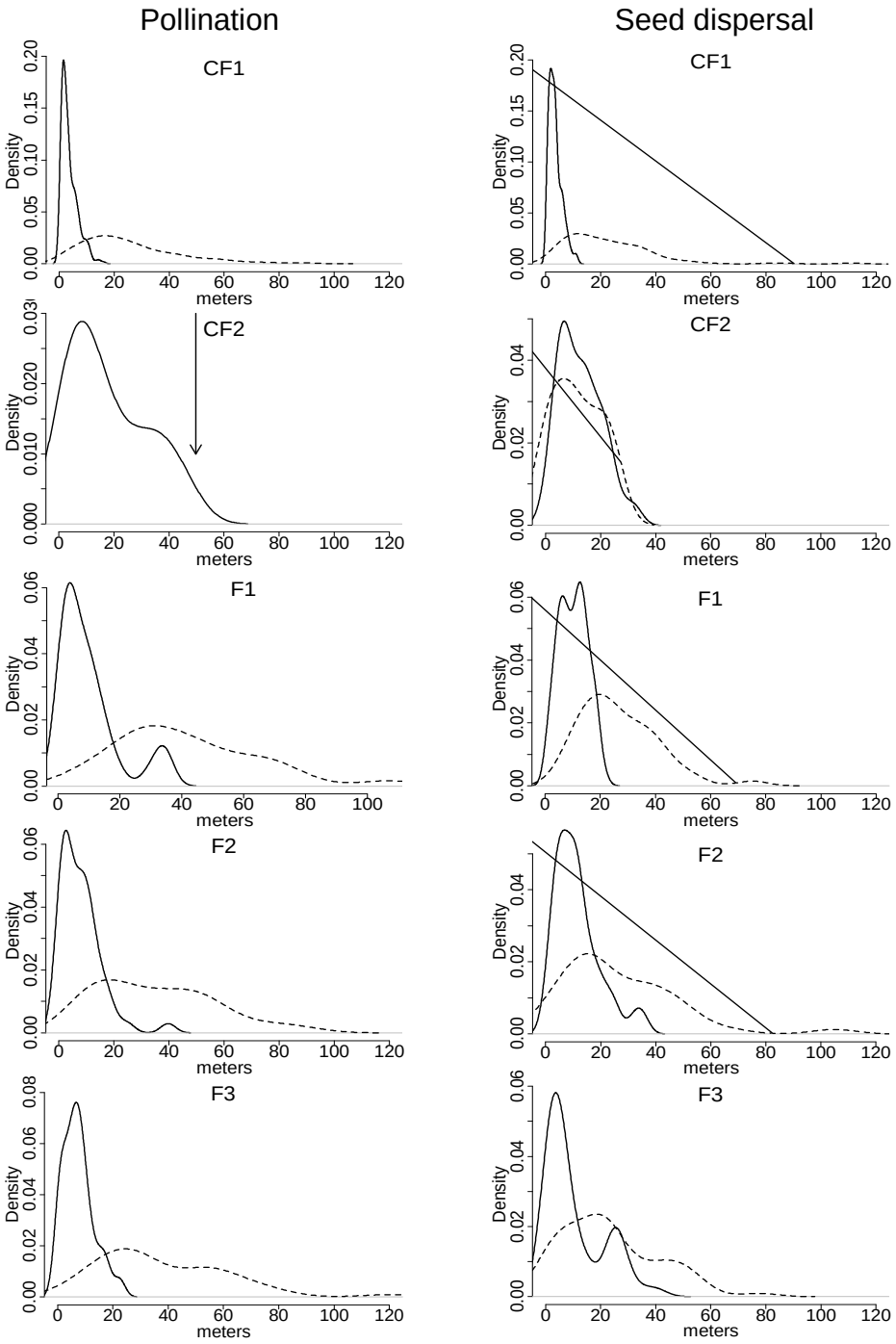

Figure 2.3- Frequency distribution of distances between sampled within - plots reproductive plants and seedlings. A.) Minimum distance between reproductive plants (potential pollination events) in black line and distance between actual parents (realized pollination events) in broken line. Pairwise distance between the single parental pair assigned in CF2 is noted by the arrow. B.) Minimum distance between seedlings and reproductive plants (potential seed dispersal events) in black line and distance between offspring and actual mother (realized seed dispersal events) in broken line.

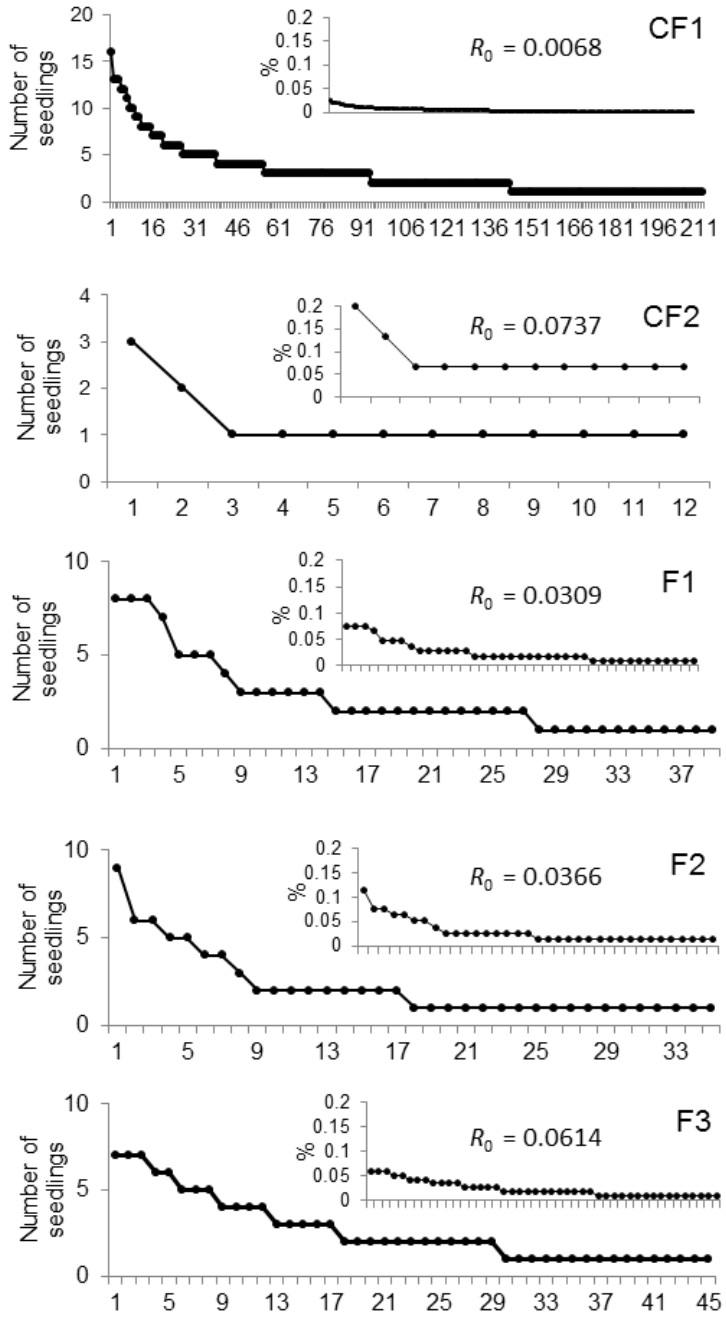

Figure 2.4- Number of seedlings each plant fathered or mothered (main graph); proportional genetic contribution of individual reproductive plants (graph inset) via either pollen or seeds to the next generations of seedlings and R_0 (PPaI - values) of *Heliconia acuminata* in each of the five populations in the BDFFP, Brazil. The y-axis represents the number of seedlings or proportional contribution (sum across reproductive plants is equal to one) and the x-axis represents each reproductive plant that contributed genes. The curve represents the decreasing ranking of the plants given their contribution. Flat curves indicate even contribution, whereas steep curves represent uneven genetic contribution.

CHAPTER 3 – FINE-SCALE SPATIAL PATTERN AND GENETIC STRUCTURE OF AN AMAZONIAN HERB ACROSS A HETEROGENEOUS LANDSCAPE

CITATION:

Côrtes, M.C., Uriarte, M., Bruna, E.M., Kress, W.J., Illian, J. in prep. "Fine-scale spatial pattern and genetic structure of an Amazonian herb across a heterogeneous landscape." For submission to Journal of Ecology.

ABSTRACT

Spatial pattern of plants and fine-scale genetic structure are intertwined and result from a multitude of ecological processes. Endogenous factors, such as dispersal limitation, can result in clustering of plants and genotypes, whereas habitat filtering can generate heterogeneous spatial distribution across environmental gradients. Disentangling the relative importance of endogenous and exogenous processes in generating observed spatial patterns is often difficult. Here we use a new statistical methodology to model marked point patterns to investigate the influence of endogenous factors (plant-plant interactions) and environmental covariates (light, slope and soil characteristics) on spatial pattern of the understory herb *Heliconia acuminata*. We also evaluate whether local spatial genetic structure is associated to spatial distribution of plants. We compared results for seedlings and adults to draw a more comprehensive picture of changes in the importance of different factors through time. We established five 50 x 100 m plots in two continuous forest sites and in three 1-ha fragments in the Biological Dynamics of Forest Fragments Project, Manaus, Brazil. Plants were mapped and genotyped using ten microsatellite markers. Environmental covariates were measured at 10 x 10 m scale. Seedlings are clustered within a 4 m – radius, but local spatial genetic structure is not associated with spatial pattern of seedlings or distance to nearest reproductive plant. These findings demonstrate that *H. acuminata* dispersal is contagious, but not distance - restricted or genetically structured (presence of highly related plants). The absence of an association between spatial pattern and local genetic structure for adults also suggest the absence of genetic structuring in seedlings over time. Light and zinc availability are positively associated with spatial patterns of seedlings and adults. The consistent effect of light and zinc on both cohorts may simply indicate carryover effects of seedlings on recruits over time. Additionally, these environmental factors may also favor survival and growth

of adults, reinforcing spatial structure of *H. acuminata*. Carbon is negatively associated with adults, which may be evidence of competition with large dominant trees. Modeling marked point patterns of natural plant populations can provide novel and comprehensive insights into the ecological processes that modulate spatial patterns and genetic structure of plant species. Integrative analyses that include both spatial location and plant characteristics across ontogenetic stages are likely to provide important insights into the factors that shape the spatial distribution of plants.

INTRODUCTION

The spatial distribution of individuals and genotypes in natural plant populations reflects a variety of ecological processes (Chung 2008; Jacquemyn *et al.* 2009; Oddou-Muratorio *et al.* 2004). Plant species often display strong spatial aggregation at both the population (Condit *et al.* 2000) and genotype levels (Choo *et al.* 2012; Degen *et al.* 2001), a pattern attributed to endogenous factors such as seed dispersal limitation (Hardy *et al.* 2006; Hubbell 2001; Schupp *et al.* 2002) and exogenous processes such as habitat filtering, driven by environmental heterogeneity (John *et al.* 2007; Palmiotto *et al.* 2004; Wang *et al.* 2011).

Seed dispersal generates the initial spatial and genetic template on which recruitment takes place, directly influencing density and spatial aggregation of conspecific plants (Fedriani *et al.* 2010; Sagnard *et al.* 2011; Zhou & Chen 2010). Dispersal-driven aggregation of fruiting plants may in turn attract and retain more frugivores, decreasing subsequent seed dispersal distances, which eventually may exacerbate patchiness of both plants and their genotypes (Carlo & Morales 2008; Fedriani *et al.* 2010). These population level processes influence spatial genetic structure (SGS), the non-random distribution of genotypes in the environment, typically measured at the population-level as the rate of decay of relatedness between pairs of individuals with increasing distance (Vekemans & Hardy 2004). Strong SGS may emerge if dispersal is distance - restricted, with progeny recruiting close to the mother, and if distance between fruiting plants is sufficiently high to prevent overlap of seed and gene shadows of different mothers (García & Grivet 2011). As plant density and/or seed dispersal distances increase, overlap of seed shadows from different mothers tends to homogenize the genetic composition of recruits, attenuating SGS (Hamrick *et al.* 1993; Hamrick & Trapnell 2011).

After seed deposition occurs, myriad ecological and environmental factors shape initial seed and gene shadows, modifying the SGS among surviving recruits. Density-dependent mortality, due to competition or pathogens, coupled with inbreeding depression, may result in the non-random removal of individuals and genotypes from the population (i.e., genetic thinning processes) (Bagchi *et al.* 2011; Collevatti & Hay 2011), whereas local factors favoring survival and growth of particular individuals or genotypes may facilitate recruitment of plants in microsites with favorable abiotic conditions (Comita *et al.* 2009; Santiago *et al.* 2012). These processes unfold over time, leaving their signature on the spatial patterns and SGS of different age cohorts within populations (Comita *et al.* 2007; Gomez-Aparicio 2008; Troupin *et al.* 2006). A decline in SGS from seedling to subsequent life history stages may be attributed to a number of density dependent factors, including competition between neighboring individuals and density-dependent predation (Choo *et al.* 2012; Chung *et al.* 2003; Steinitz *et al.* 2011; Zhou & Chen 2010). Conversely, an increase in SGS through a plant's life history stages may result from spatial overlap of recruits from multiple generations at favorable sites (Collevatti & Hay 2011; Jones & Hubbell 2006).

Disentangling the contribution of endogenous processes and exogenous environmental heterogeneity on spatial aggregation and SGS of plant populations is challenging, because similar spatial patterns can be generated by multiple combinations of distinct processes (Fortin & Dale 2005), and because standard analytical methods are compromised by the lack of independence among neighbors (Dale & Fortin 2002). Point process theory, however, offers a robust approach to analyze spatial point patterns (Illian *et al.* 2008). By relying on one or several snapshots of the spatial locations of all individuals located within a continuous pre-defined area, point processes analysis allows us to draw inferences about the ecological processes modulating

interactions between neighboring individuals (e.g. clustering), and plant responses to habitat heterogeneity (Law *et al.* 2009). Besides plant location and environmental heterogeneity, point process analyses can also incorporate the association between spatial location and specific plant characteristics, referred to as marks within a point pattern. Marks can be categorical (e.g., different plant species), or quantitative (e.g., individual tree diameter or genetic relatedness with neighbors) (Goulard *et al.* 1995; Law *et al.* 2009).

Despite these advantages, the complexity of mathematical concepts underlying the spatial point pattern analysis and computational difficulty of model fitting (Baddeley & Turner 2005; Illian *et al.* in press) have limited the application of point process analyses to tease apart the combined effect of dispersal limitation and environmental heterogeneity on spatial location of plants (Lin *et al.* 2011; Shen *et al.* 2009; Wang *et al.* 2011). Most of ecological studies dealing with point patterns of organisms have relied on elegant descriptive methods to characterize pattern properties, such as Ripley's K-function (Law *et al.* 2009; Wiegand & Moloney 2004). Only a few studies have coupled such descriptions of point patterns with traditional measures of SGS characterized at the population - level in an attempt evaluate the factors driving genetic and spatial structure of plant populations to more fully (Chung *et al.* 2009; Dounavi *et al.* 2010; Jacquemyn *et al.* 2009). To date, no studies exist that jointly model both spatial pattern and SGS through a marked point process to assess the importance of endogenous and exogenous processes on the spatial patterns and genetic structure of plant populations (Illian *et al.* 2008; Law *et al.* 2009). This approach is advantageous because allows for insights that go beyond description of separate patterns. It explicitly associates individuals' location in space, endogenous characteristics and habitat and, thus, permits a better understanding of how factors relate to

emerging spatial and genetic properties, which could be solely characterized using traditional measures of point patterns and SGS.

Here we take advantage of this new point process framework to disentangle the influence of endogenous factors (interaction with conspecifics via dispersal or competition) and environmental factors (soil characteristics, light availability, and slope as a proxy for soil moisture) on the fine-scale spatial pattern and local SGS of the understory herb *Heliconia acuminata* across five sites in an experimentally fragmented landscape in the Central Amazon, Brazil. For the marks, we rely on local SGS, rather than population - level depiction of SGS, which can be done by characterizing relatedness between each individual plant and its neighbors in a two-dimensional plane (Anselin 1995; Double *et al.* 2005; Zhou & Chen 2010). We also compare the results between seedlings and adult plants, to elucidate how the demographic processes driving spatial dynamics change throughout a plant's life cycle (Fuchs & Hamrick 2010; Jacquemyn *et al.* 2009). Specifically we ask:

(1) What endogenous and environmental factors influence the spatial distributions of seedlings and adult plants (Table 3.1)? The influence of endogenous factors will be evaluated by first assessing spatial interactions between individuals, which can be clustered, random or overdispersed. We predict that seedlings will be clustered, due to dispersal limitation or environmental preferences, and that adults will be clustered, due to carryover effects of seedlings recruitment or further association with specific habitat characteristics. The influence of environmental heterogeneity will be assessed by determining whether observed spatial patterns in plants are associated with environmental factors, namely soil quality, light and slope. We expect that the spatial distribution of seedlings will be influenced primarily by light availability, whereas adult plants will be associated with soil fertility.

(2) How does local SGS relate to the spatial pattern of seedlings and adult plants (Table 3.2)? The association between local SGS and spatial pattern of plants provides insights into seed dispersal patterns. We expect that local SGS is positively associated with spatial pattern of seedlings and/or to distance to nearest reproductive plant, indicating dispersal limitation. This limitation could result from directed dissemination of maternal progeny to specific microsites or from dispersal near maternal plants. In contrast, local SGS is expected to be more weakly associated with the spatial pattern of adults, due to overlap of seed and gene shadows of different mothers and habitat filtering processes acting over time.

METHODS

STUDY SITE

The study was conducted in the Biological Dynamics of Forest Fragments Project (BDFFP) located 70 km north of Manaus, Brazil (2^{o} 30' S, 60' W, see Fig. S3.1). The BDFFP is a 1000 km^2 landscape with several forest fragment reserves, ranging in size from 1-100 ha, as well as expansive stretches of continuous forest. The fragments were isolated between 1980 and 1984 by clear - cutting the trees surrounding the patches and, in some cases, burning the felled trees (Gascon & Bierregaard 2001). Since isolation, fragments have undergone structural deterioration (Laurance *et al.* 2011; Laurance *et al.* 2002), and secondary forests have colonized the surrounding matrix (Mesquita *et al.* 2001).

Heliconia acuminata is a hermaphroditic and self-incompatible understory plant (E.M. Bruna and W.J. Kress, unpublished data), and has been the subject of comprehensive demographic study in the BDFFP since 1998 (Bruna 2003). Thirteen 50 x 100 m plots, in which all *H. acuminata* have been tagged and mapped, were established in continuous forest (n = 6),

10-ha fragments (n = 3), and 1-ha fragments (n = 4). We selected five plots for this study: two in continuous forest sites and three in separate 1-ha fragments (Fig. S3.1).

Heliconia acuminata in this landscape represents an ideal system to disentangle the effects of environmental and biotic factors in shaping spatial aggregation and SGS of plant populations. Unlike other *Heliconia* species, *H. acuminata* has limited vegetative growth, exhibits a scattered distribution, and is mainly found in the shaded understory (Bruna & Ribeiro 2005). Density of *H. acuminata* adults is highly variable across the landscape (Bruna & Kress 2002). Environmental variation across the study plots is high, potentially influencing a number of demographic processes such as seed dispersal, seedling recruitment, growth and survival, and probability of flowering. The hermit hummingbird pollinators *Phaethornis superciliosus* and *P. bourcieri*, are trapliners. They can forage over large distances, move through a variety of habitats, and persist in both primary and secondary forests in the matrix (Stouffer & Bierregaard 1995a). In our study site, the primary dispersers of *H. acuminata* seeds are the white-necked thrush (*Turdus albicollis*), the thrush-like-manakin (*Schiffornis turdinus*), and several species of manakin (*Pipra erythrocephala, P. pipra, Lepidothrix serena, Corapipo gutturalis*). Frugivorous birds have been recorded using small fragments as the cleared matrix grew back into secondary forest (Stouffer & Bierregaard 2007). Seed production, seed dispersal limitation and density dependent interactions with conspecifics do not differ between fragments and continuous, but a more favorable light regime favors greater seedling establishment in continuous forests relative to fragments (Uriarte *et al.* 2010). Habitat quality, possibly mediated by soil characteristics, is also likely to be an important factor in limiting population size at the study site, where soils are extremely poor and acidic (Bruna *et al.* 2002; Laurance *et al.* 1999).

DATA COLLECTION

As part of the ongoing project (Bruna 2003), new seedlings have been marked and plants have been monitored annually since 1998, with height, the number of shoots, and the presence of an inflorescence being recorded. We used data from the 2009 census to measure the abundance and spatial distribution of all seedlings, adults, and reproductive plants in the five selected populations. For this study, seedlings are plants that are less than two years old (i.e., plants that recruited in 2007 or later), while all other plants are classified as adults. Adults flowering between 2006 and 2009, and potentially contributing seedlings to the study sites, are considered reproductive plants. Samples of leaf tissue from all individuals in the study plots were collected in 2009 and either frozen in liquid nitrogen or dried in silica gel, pending molecular analysis. A set of ten polymorphic microsatellite markers were used to genotype all plants alive in 2009 from each one of the five plots. DNA extractions, PCR protocols and fragment analysis methods are detailed in Côrtes *et al.* (2009).

To investigate the influence of abiotic characteristics on plant abundance and spatial genetic structure, we collected slope, light and soil quality data for all fifty 10 x 10 m subplots in each one of the five 0.5 ha plots. Slope was measured with a clinometer in each subplot corner, and the readings were averaged. Light was measured in two ways, historical and contemporary. Historical light levels are quantified as the number of gaps formed between 1999 and 2009 in each one of the subplots. This metric captures the changes in light incidence that might affect historical plant recruitment and establishment. Contemporary light was measured in 2007 with hemispherical photography (i.e., gap light index). Photographs were taken in the center of each of the subplots (see Uriarte *et al.* (2010) for more details). Contemporary light is expected to affect the recruitment and distribution of seedlings in this study. Soil quality was characterized

from soil cores collected from 0 - 10 cm (layer occupied by the roots of *H. acuminata*) at three randomly selected locations within each 10 x 10 m subplot. The three cores were homogenized and bulked into a single sample, yielding 50 samples for each 0.5 ha plot. Samples were analyzed for total N, P, K^{+}, Ca^{2+}, Na^{+}, Mg^{2+}, H^{++} Al^{3}+, total C, total organic material, Cu, Fe, Zn^{+}, Mn^{2+} and pH in water, total exchangeable bases, cation exchange capacity, base saturation and aluminum saturation index. Soil chemistry assays were conducted at the Soil Chemistry Laboratory of the Brazilian Agricultural Research Centre in Manaus (EMBRAPA) using standard protocols (Silva *et al.* 1998). Exploratory analyses uncovered high collinearity among some of these abiotic covariates. To avoid model overfitting, we excluded redundant covariates with correlation coefficients $|r| > 0.45$. The final list of covariates retained were slope, number of gaps (historical light), contemporary light (GLI), and soil pH, C, Na, Ca, Zn, Fe and C:N ratio. C was positively correlated with total organic material, the main macro nutrients (N, P, K^{+}), soil toxicity (H^{++} Al^{3+}, aluminum saturation index), Mg^{2+}, total exchangeable bases, and the micronutrients Cu and Mn^{2+}. The nutrient Ca was positively correlated with total exchangeable bases and pH was negatively correlated with H^{++} Al^{3}+ and aluminum saturation index (high levels indicating soil toxicity). Number of gaps, GLI, slope, C:N and the micronutrients Na, Fe, and Zn were not correlated to any environmental parameter. All the covariates were standardized with respect to the 0.5 plot population by subtracting the mean from each value and dividing by twice the standard deviation. Plots and maps of variation of all explanatory variables used in the analysis are provided in Figures S3.2a, S3.2b & S3.3.

The data set consists of spatial locations, recorded as x and y-coordinates, for all individuals within each of five 50 x 100 m plots (Figures S3.4 & S3.5). Along with location, a measure of local genetic relatedness is associated to each plant, which is an average value of relatedness between the focal plant and all neighbors within a 10 m radius (Figures S3.5 & S3.6). This threshold radius included all focal adults since it is below the minimum distance between adults (although two, seven, five, two and two seedlings were not included in CF1, CF2, F1, F2, and F3, respectively, because they were spatially isolated to have any near neighbor) and adult pairwise relatedness is, in general, not significant beyond 10 m. Pairwise relatedness was calculated between each focal plant and its neighbors using the Loiselle's kinship estimator (Loiselle *et al.* 1995; Vekemans & Hardy 2004), using the SPAGeDi software (Hardy & Vekemans 2002).

Local SGS may be dependent on the spatial distribution of plants, which in turn may be influenced by environmental factors and interactions with nearby conspecifics. To build this hierarchical model, we used a modern and flexible Bayesian framework that enables fitting complex data, including spatial information, quantitative marks and replicated point patterns. Integrated nested Laplace approximation (INLA) is a new tool that performs deterministic approximation (using Laplace approximations and algorithms for numerical integration) for Bayesian inference and allows for a much faster parameter recovery than typical Markov chain Monte Carlo (MCMC) methods (Rue *et al.* 2009). INLA may be used to analyze a general class of statistical models named latent Gaussian models, including, for instance, generalized linear, generalized additive, mixed and log-Gaussian Cox processes. Log-Gaussian Cox processes are used to model spatial point pattern data by expressing spatial variation at local and large scales and autocorrelation through a random structure, based on an underlying latent field (Illian *et al.*

in press). This latent field is the sum of known covariates and a random field. The latter is often referred to as a "spatially structured effect" as it is assumes that observations in neighboring locations are dependent. It describes spatial structure resulting from biotic or abiotic processes that are not explained by the covariates (Illian *et al.* in press).

Many complex and realistic models can be constructed due to the flexibility of the log-Gaussian Cox process (Illian *et al.* in press) and efficiency of INLA and efficiency of INLA (Rue *et al.* 2009). For instance, the model we use here contains two latent fields, one representing the spatial distribution of plants and the other representing local SGS, which share a component. Fitting a similar model without the use of INLA would have been computationally prohibitive. Statistical analyses were conducted using R version 2.13.1 and the R-INLA package (www.r-inla.org, Rue *et al.* 2009) and Spatstat (Baddeley & Turner 2005).

To fit the point pattern model with INLA, we subdivided each of the $k = 1, \ldots, 5$ plots in $N = n_{row}$ x n_{col} = 25 x 50 = 2500 grid cells $\{s_{ijk}\}$, each with an area of 4 m^2, with $i = 1, \ldots, n_{row}$, $j = 1, \ldots, n_{col}$. The response variable for plant location is measured as the number of individuals in each grid cell and the response variable for genetic relatedness is measured as the average relatedness of individuals in each grid cell. The number of points (plants) in the 2,500 grid cells of plot *k* is assumed to follow a Poisson distribution, and the average relatedness of plants in the grid cells of plot *k* is assumed to follow a normal distribution. Very fine grids provide very accurate estimations, but increase computation time, and that trade – off needs careful attention. An area of 314.2 m^2 around each individual was used to define neighboring individuals when calculating average genetic relatedness, but an area of 4 m^2 was used to define the grid cell area. The larger area for calculating individual average relatedness was chosen to allow for the inclusion of at least one neighbor for most of the individuals. Using an area of 4 m^2 would result

in missing data, which could compromise the analysis. The ideal is that different values of radius and grid cell area are used to assess differences in the results when changing scale resolution.

Our interest here is to model both the spatial pattern formed by the plants and the genetic relatedness with neighbors, or local SGS (i.e., the mark). We thus consider a joint model for these two response variables, both measured for each 4 m^2 grid cell. The joint model employs a shared spatially structured term between the different outcomes (spatial pattern and local SGS), to reflect dependence between the two response variables. Obviously, grid cells are not independent from each other with respect to average relatedness, because individuals are used multiple times to calculate genetic relatedness. We do, however, use a spatial effect to model local SGS, and this component assumes spatial dependency between observed values and also between local SGS and spatial pattern of individuals.

For seedlings, the full models for the latent field of the spatial pattern ($\eta_{ijk}^{(1)}$) and of the marks ($\eta_{ijk}^{(2)}$) take the form:

$$\eta_{ijk}^{(1)} = \beta_{01} + \sum_p \beta_p V_p + f\left(z_A(s_{ijk})\right) + f\left(z_D(s_{ijk})\right) + f_s^k(s_{ijk}),\ \ p = 1, \dots, 9 \qquad \text{(Eqn. 1)}$$

$$\eta_{ijk}^{(2)} = \beta_{02} + f\left(z_D(s_{ijk})\right) + \beta_s f_s^k(s_{ijk}) \qquad \text{(Eqn. 2)}$$

where β_{01} and β_{02} are the intercepts for the pattern and marks, respectively. V_p are nine light, slope and soil covariates measured at the s_{ijk} grid cell and $\beta_p$are parameters associated with these environmental covariates. The values of empirical covariates, originally measured at 10 x 10 m, have been interpolated to a grid of 25 x 50 m grid cells.

We construct two covariates: the first, z_A, is an aggregation index for seedlings and is based on the distance from the midpoint of grid cell $s_{ijk}$to the nearest seedling, and the second, z_D , is the potential mother-offspring distance and is based on the distance from the midpoint of grid cell s_{ijk} to the nearest reproductive adult. The function $f(z_A(s_{ijk}))$ is a smoothing function of the seedlings aggregation index, representing the local aggregation with conspecific seedlings, which, if clustered, primarily indicates contagious dispersal of seeds to the same microsite. The function $f(z_D(s_{ijk}))$ is a function of the potential mother-offspring distance and represents the spatial interaction between seedlings and reproductive plants, which, if clustered, indicates distance-restricted dispersal. The relationship between the response variable and the constructed covariate is often not linear, so a smoothing function is used here. The functional relationship between both constructed covariates z_A and z_D and response variables is modeled with a first-order conditional autoregressive process (or first-order random walk model, "rw1") (Illian *et al.* in press). It is known that there are plants outside the sampled plot and, thus, it is possible that distance to the nearest plant is actually smaller than reflected in the constructed covariate. To correct for potential edge effect we simply assumed that the constructed covariate is missing in cells in which the distance to the boundary is shorter than the distance to the nearest point.

Finally, $f_s^k(s_{ijk})$ is a spatially structured effect that accounts for large scale variation across the plot and for the spatial autocorrelation present in the data that is not accounted for by the empirical and constructed covariates. We assume a separate spatial effect for each plot *k.* This spatial structure is modeled using conditional autoregressive (CAR) model of second order (two-dimensional random walk, "rw2"). To jointly fit the model to the point pattern and the marks with equations (1) and (2), we use a common spatially structured term for both processes.

Thus, the spatial effects for the marks are proportional to the spatial effects for the patterns, a relationship which is defined by the parameter β_s.

Since we take a Bayesian approach, prior distributions need to be specified for the hyper - parameters of these models. Priors for estimating the functional relationship between the constructed covariate and outcomes (spatial pattern and local SGS) and the spatial structure are chosen from a log-gamma distribution. For the spatial effect, the choice of scale and shape parameters of the log-gamma distribution must trade off fitting an extremely smooth spatial effect with no explanatory power (no spatial large scale variation is included) to a fine scale spatial effect at the price of overfitting. The prior shape and scale parameters chosen for the estimation of the functional relationship of the constructed covariate were 15 and 0.009, and for the spatial structure were 15 and 0.1. These values were chosen because they allowed for an informative, but still smooth spatial structure.

For adults, the full models for the spatial pattern $\eta_{ijk}^{(3)}$ and for the marks $\eta_{ijk}^{(4)}$ are specified as:

$$\eta_{ijk}^{(3)} = \beta_{03} + \sum_p \beta_p V_p + f\left(z_{AA}\left(s_{ijk}\right)\right) + f_{sa}^{k}\left(s_{ijk}\right), \quad p = 1, \dots, 9 \qquad \text{(Eqn. 3)}$$

$$\eta_{ijk}^{(4)} = \beta_{04} + \beta_{sa} f_{sa}^{k}(s_{ijk}) \qquad \text{(Eqn. 4)}$$

Here, β_{03} and β_{04} are the intercepts; $\sum_p \beta_p V_p$ are the nine empirical covariates with respective β – parameters; $f(z_{AA}(s_{ijk}))$ is a function of the constructed covariate z_{AA}, which is an aggregation index of adults based on distance from midpoint of grid cell s_{ijk} to nearest adult conspecific, indicating habitat preference of facilitation among individuals; $f_{sa}^{k}(s_{ijk})$ and

$\beta_{sa} f_s^k(s_{ijk})$ are the spatially structured effect for adults and common spatially structured effect for the marks of adults, as described for equations (1) and (2). The prior shape and scale parameters used here are 60 and 10 for estimating the functional relationship of the constructed covariate on pattern and 60 and 0.1 for estimating the spatial effect.

We use the Deviance Information Criterion (DIC) to conduct model comparison and selection (Spiegelhalter *et al.* 2002). We first conduct a model selection of the environmental covariates, by running sub - models containing only the intercepts, without the constructed covariate and spatially structured effects. After selecting the empirical covariates, we tested different model combinations, including intercept, significant empirical covariates, constructed covariate and spatially structured effect. We report the DIC values of sub - models and the posterior estimates for the relevant parameters of the selected model.

RESULTS

SEEDLINGS

The joint model of pattern and marks of seedlings indicates that both clustering and environmental factors are associated with the spatial pattern. Relatedness with neighbors was variable among plants (mean = 0.0026 , range = -0.1888 - + 0.1975) and heterogeneous within plots (Fig. S6), but local SGS is neither associated with spatial pattern of seedlings (β_s = -0.0014, credible interval (CI) = -0.0198 - + 0.0185) nor to distance to reproductive plants (Fig. 3.1).

The final seedling model included light, soil nutrients, the seedlings' aggregation index, and the spatial effect common to individuals and SGS (Table 3.4). Although light, Zn, Fe and C:N were retained in the most parsimonious model when assessing just the effect of environmental covariates on spatial patterns of seedlings, Fe and C:N were dropped in the final

model as their β - parameters were not significantly different from zero anymore. In the final model, both light and Zn positively influenced the spatial distribution of seedlings of *H. acuminata* (Fig. 3.2). The functional relationship between seedlings' aggregation index and the pattern shows that seedlings are locally clustered within a 4 m - radius (Fig. 3.1), with 1.4 neighbors per cluster on average (range = 0 – 8). The flat relationship between the potential mother-offspring distance and the pattern (Fig. 3.1) suggests that seedlings' locations are random with respect to reproductive plants.

ADULTS

As for seedlings, the joint model of pattern and marks of adults indicates that both clustering and environmental factors are associated with spatial pattern, whereas local SGS was not associated with spatial pattern of adults (βsa = -0.01024, CI = -0.0184 - +0.0024). Despite the lack of relationship between local SGS and spatial pattern, relatedness with neighbors was variable among plants (mean = 0.012, range = -0.146 - +0.256) and heterogeneous within plots (Fig. S3.7)

The final model included historical light (measured as the number of gap openings since the plot was established), soil elements, the adults' aggregation index and the common spatial effect (Table 3.4). Light and other six soil covariates (C, Na, Ca, Zn and C:N) were significant, but only when analyzing the influence of environmental covariates on spatial pattern. After including the constructed covariate and the common spatial effect, the final model included only light, C and Zn (Table 3.4). Light and Zn were positively, whereas C was negatively associated with adults' spatial distribution (Fig. 3.2). The decaying functional relationship between number

of adults at the 4 m^2 grid cell scale and adults' aggregation index indicates clustering of adults up to 2 m (Fig. 3.1), with an average of 2.3 neighbors per cluster (range = 0 – 16).

DISCUSSION

By employing a very flexible and integrative approach, we were able to fit a complex model to evaluate the processes shaping both the spatial location of plants and their genetic relatedness with neighbors. We found that endogenous factors, most likely dispersal, result in clustering of seedlings, although genetic structuring was not related to spatial aggregation patterns or to distance to reproductive plants, as first hypothesized. Environmental heterogeneity played a crucial role in generating observed spatial patterns of *H. acuminata*. As predicted, light was important to seedlings, but also to adults. Adults were also associated with soil elements, but contrary to our predictions local density of adults was negatively related to C, which is highly correlated with main soil macro-nutrients. Adults also exhibited a clustered spatial distribution and, as expected, local SGS was not particularly dependent on spatial pattern *per se*. Our findings on the importance of local spatial interactions among individuals, genetic relatedness, and environmental factors in shaping the spatial distributions of seedlings and adults provide novel insights into the mechanisms that facilitate and restrict establishment and ultimately, spatial distribution of *H. acuminata*.

SEED DISPERSAL

The two dispersal mechanisms leading to identifiable spatial patterns in early stages of the plant cycle are distance-restricted and spatially contagious seed dispersal, *sensu* Schupp *et al.* (2002). The former refers to the leptokurtic shape of the seed dispersal curve, with the peak of seed

density occurring beneath or close to the maternal plant. The latter refers to the highly heterogeneous seed fall in space, largely driven by animal behavior (Côrtes & Uriarte in press; Schupp *et al.* 2002). The absence of a strong relationship between spatial pattern of seedlings and nearest reproductive plant indicates that dispersal of *H. acuminata* is not distance - restricted. This result is not surprising, given the relatively extensive seed dispersal of *H. acuminata* seeds. By conducting parentage analyses on seedlings, Côrtes *et al.* (in prep) recorded peaks of seed dispersal ranging from 14 m in one continuous forest plot (CF1) to around 40 m in the other populations, with most dispersal events exceeding the distance to the nearest reproductive plant.

The spatial clustering of seedlings suggests the likelihood of spatially contagious seed dispersal as a potential mechanism. Within the spatially contagious dispersal, genetic limitation arises if progeny from the same or related maternal sources are dispersed to the same patch, creating hotspots of kin propagules and thus intensifying local SGS (García & Grivet 2011). Even though we observed hotspots of high local SGS within plots (Fig S3.6), they were not related to the spatial pattern of seedlings, contrary to our prediction. That is, patches of high local density of seedlings do not necessarily present high local SGS. It is more likely that seeds from different maternal sources are being dispersed to determined patches, creating a homogenized genetic pool. Fruit removal of *H. acuminata* is close to 90% at the population level (Uriarte *et al.* 2011), leading to even parental contributions to the genetic pool of seedlings (Côrtes *et al.* in prep). Additionally, the main *H. acuminata* seed dispersers, manakins and thrushes, move actively throughout the landscape (Uriarte *et al.* 2011), probably bringing seeds from neighboring forest patches into sampled plots (Côrtes *et al.* in prep), elevating the genetic diversity of seedlings and attenuating strong local SGS in high density patches. As predicted, local SGS of adults failed to exhibit an association with adult spatial pattern. It is possible that

successive seed dispersal events from unrelated maternal plants over time are one of the mechanisms maintaining dense patches of not necessarily related plants.

Clustering of unrelated seedlings may indicate that directed dispersal is occurring. In this case, dispersers may spend more time in certain preferred areas of the forest where they end up disseminating seeds from different mother sources. This behavior has been recorded for males of the long-wattled umbrellabird *Cephalopterus penduliger* that, by disseminating genetically diverse pool of seeds into leks, creates a homogenized genetic structure of the Ecuadorian canopy palm tree *Oenocarpus bataua* (Karubian *et al.* 2010). However, although some of the *H. acuminata* dispersers maintain lekking sites, the fruiting season of *H. acuminata* does not overlap dispersers' mating season (M. Anciães, *personal communication*). It is also possible that seedlings are patchily distributed because they only establish under particular environmental conditions. In fact, seedlings distribution was associated with light and soil covariates. Without data on seed dispersal *per se* it is difficult to tease apart the direct effect of seed dispersal and environmental covariates, but it is most likely that they work in concert to shape the spatial distribution of seedlings.

Environmental heterogeneity

Spatial patterns of both seedlings and adults were associated with environmental heterogeneity. Light and zinc (Zn) were both important factors associated with spatial pattern of seedlings and adults, which can be the result of a legacy effect of seedlings manifesting on adults distribution or a positive effect of these factors on survival and growth of *H. acuminata*. Light was highly important to seedlings, with high local density associated with greater light availability. Using a spatially explicit modeling approach, Uriarte *et al.* (2010) found that light availability influenced

seedling establishment positively, especially in continuous forest sites, where light is more limited (Fig S3.2, light). Positive effects of light availability on seedlings was also found in *Heliconia metallica* in an Amazonian flood plain forest of Peru (Schleuning *et al.* 2009) and among recruits of many tropical tree species (Montgomery & Chazdon 2002; Ruger *et al.* 2009). Historical light availability (measured as the number of gap openings) was also an important factor influencing the spatial distribution of adults, possibly working in two non-exclusive ways. Spatial pattern may reflect cumulative seedling recruitment in microsites with high light incidence over time (e.g, gaps). Additionally, light may influence survival and growth of plants, favoring adult persistence in microsites with repeated or long-lived canopy opening events. In fact, increased growth of *H. acuminata* on fragment edges compared to forest interior was attributed to elevated light availability (Bruna & de Andrade 2011).

Zn was also positively associated with both seedlings and adults spatial patterns. Zn is an important micronutrient for protein synthesis in plants, and its deficiency can impair growth and cause root necrosis ("dieback") (Broadley *et al.* 2007). It is entirely possible that seedling mortality is elevated in microsites with low Zn concentration, so that both growth and survival are improved in microsites with elevated Zn availability. The association of plants with mycorrhizas generally improves mineral nutrition (Smith & Smith 2011), particularly enhancing assimilation of Zn in poor soils (Cavagnaro *et al.* 2010), such as those found in the BDFFP (Laurance *et al.* 1999). It is possible that in the absence of mycorrhizal infection, *H. acuminata* survival is compromised in patches of low Zn availability.

Cluster size (radius) decreased and number of neighbors within clusters increased from seedlings to adults. The intensification of clustering suggests that other factors or processes are increasing local spatial interaction of adults. The more aggregated distribution of adults with

respect to seedlings is, nonetheless, not common among tropical plants, where local negative density dependence results in spatial pattern thinning through ontogeny (Bagchi *et al.* 2011; Condit *et al.* 2000). A few non-exclusive explanations are possible. Interspecific competition with other plants in the community is one process that can intensify adults' aggregation, which may eventually constrain plants to small clusters, patchily distributed in the forest site. A second possibility is that adults may be associated with other environmental covariates that were not important during the seedling stage. This last explanation is based on the understanding that environmental conditions shape plant demography by reducing survival of young plants in unsuitable habitats and leading to close associations of adults with specific patches (Comita *et al.* 2007).

In fact, carbon was negatively associated with spatial distribution of adults, but not with seedlings. Herbaceous plants usually require less carbon per unit area than do woody plants, but they require more nutrients to replace losses of ephemeral tissues (Graves *et al.* 2006). On infertile soils, however, herbaceous growth is compromised, and light turns out to be an important promoter of growth (Graves *et al.* 2006). At BDFFP, carbon was positively and highly correlated with aluminum saturation, suggesting that *H. acuminata* may not persist in patches of high soil toxicity. Additionally, carbon was positively correlated with other nutrients, such as nitrogen, phosphorous and potassium, as well as with total exchangeable bases. It appears that *H. acuminata* establishment and persistence is higher in patches with low carbon and soil fertility, which counters our initial prediction that adult distribution is associated with high soil nutrient availability. Interestingly, stem density of trees at BDFFP is also negatively correlated with carbon, but tree biomass is positively correlated with carbon (Laurance *et al.* 1999). It is possible that richer soils (with higher carbon and soil fertility) support larger, more competitive dominant

trees, which may outcompete understory plants such as *H. acuminata*. Competitive pressure from *Quercus douglasii* on understory plant productivity in Californian grassland provides an example of this mechanism. The effect was present when the root system of *Q. douglassi* was superficial, occupying the upper soil horizons, directly interfering with roots of understory grass plants (Callaway *et al.* 1991). We suggest that the negative association of carbon is evidence of direct competition with large trees, and thus the decrease of cluster size for the adult cohort is due to competitive interspecific competition, rather than positive intraspecific facilitation or further habitat specialization. It is worth noting that since many of the soil parameters are correlated it is difficult to tease apart the relative importance of different environmental factors as determinants of plant spatial distribution. Experimental studies are necessary to investigate causal relationships and information on composition and spatial distribution of heterospecifics are needed to understand the role of intraspecific competition on *H. acuminata*.

Spatial scales

The large improvement of model fit when including the spatial effect in the marked point pattern analysis of adults indicates that there is spatial variation on aggregation of plants at larger scales (i.e., plot), which can reflect environmental gradients (John *et al.* 2007). Interestingly, the inclusion of a spatial effect in the seedlings' model did not improve the fit substantially. It is worth noting that different values of hyper - parameters were used to estimate the spatial effect, and none resulted in much lower DIC values for seedlings. It seems that variation in seedling distribution emerges at smaller, local scales, which is clearly demonstrated by the effect of plant clustering in the model. The results suggest that dispersal emerges as an important process that establishes seedlings patchiness, but that that light and soil play a role on shaping this initial

distribution. Adult spatial pattern, on the other hand, consists of a heterogeneous distribution of clusters across the plots, reflecting both successive seedling recruitment patterns and environmental conditions restricting the distribution of clusters, most likely interspecific competition with large trees.

Conclusion

We apply an emerging statistical methodology, INLA, to analyze the impact of environmental covariates and plant - plant interactions in driving the spatial distribution of *H. acuminata* seedlings and adults, while including genetic relatedness as marks. Our analysis shows that the spatial distribution of seedling is influenced by environmental factors, namely light and Zn, together with contagious, but not genetically structured dispersal. Clustering increases in adults and spatial variation at the plot level turns out to be more important for adults than for seedlings, indicating further effects of environmental factors. Light and Zn are also positively related to adults, whereas soil carbon seems to be negatively associated, suggesting that competition with large trees is associated with richer soils with more organic content. Adults local SGS is not related to spatial distribution, compatible with a lack of association between local SGS and spatial pattern for seedlings.

The methodology applied here offers a promising analytical approach that can be widely applied in ecological studies that involves spatial distributions of different species and cohorts, allowing for the inclusion of plant characteristics and simultaneous analysis of replicated point patterns in space and time. We also provide a different view of fine-scale spatial genetic structure analysis. Instead of summary descriptions of SGS at the population level, we characterize local SGS based on relatedness at the neighborhood scale, which can be modeled directly.

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Table 3.1- Potential influence of exogenous (environmental) and endogenous (interaction between seedlings, adults, and reproductive plants) factors on spatial pattern of seedlings and adults.

	Seedlings	Adults
Environment		
Soil	Importance of limiting factors, such as soil fertility and pH to seedling establishment	Importance of soil fertility and pH to plant survival and growth
Light	Light is positively associated with seedling recruitment	Historical light is positively associated with adults indicating long-term effect of light on successive recruitment of seedlings
Slope	Slope is an indication of soil moisture, with lower slopes being more favorable to seedlings	Slope is an indication of soil moisture, with lower slopes being more favorable to adults
Interactions between plants	Seedlings with	Adults with
random	seedlings: random seed dispersal reproductive: seed dispersal is random with respect to maternal plant	adults: legacy effect of random distribution of seedlings or thinning processes modifying adult spatial pattern

clustered	seedlings: seed dispersal is spatially-contagious or establishment is safe-site limited reproductive: seed dispersal is distance-restricted	adults: positive density dependence indicating legacy effect of clustered distribution of seedlings or association to environmental factors
overdispersed	seedlings: direct competition with other seedlings reproductive: direct competition with conspecifics in general	With other adults: negative density dependence indicating legacy effect of over-dispersed distribution of seedlings or competition among plants

Table 3.2 - Ecological interpretations of the resulting association between local SGS and spatial patterns of seedlings and adults.

Association between local SGS and spatial pattern	Seedlings	Adults
Not present	Seed dispersal is random	Legacy effect of local SGS of seedlings or overlap of seed/gene shadows of different mothers over time
Positive	Progeny is dispersed to the same microsite (spatially-contagious), regardless of distance to the mother, with high local density of seedlings presenting high relatedness	Legacy effect of local SGS of seedlings or overlap of seed/gene shadows of the same or related mothers over time
Negative	Progeny of different maternal plants are dispersed to the same microsite with favorable environmental conditions promoting high density of unrelated seedlings	Legacy effect of local SGS of seedlings or overlap of seed/gene shadows of different mothers over time associated with microsite suitability, increasing density in some patches

Table 3.3 - Number of plants of *Heliconia acuminata* in each of the sampled plot.

Plot	BDFFP reserve number	Size	Seedlings	Reproductive plants	Adults
CF1	1501	Continuous forest	144	128	762
CF2	None (Dimona)	Continuous forest	21	3	122
F1	3114	1-ha fragment	76	9	198
F2	2107	1-ha fragment	20	26	229
F3	2108	1-ha fragment	27	40	210
Total			288	206	1521

Table 3.4. Summary of DIC – values for joint models of the pattern and marks for seedlings of *Heliconia acuminata*. Multiple combinations of covariates were analyzed, but only the important results are reported here.

Plant cohort	Model	DIC
Seedlings	Only intercepts	1746.03
	Intercepts + significant covariates (Light, Zn, Fe, C:N)	1494.70
	Intercepts + Light, Zn, Fe, C:N + seedlings' aggregation	979.36
	Intercepts + Light, Zn, Fe, C:N + seedlings' aggregation + common spatial effect	976.47
	Intercepts + Light, Zn + seedlings' aggregation + common spatial effect	974.61
Adults	Only intercepts	3825.13
	Intercepts + significant covariates (Light, C, Na, Ca, Zn, C:N)	3662.63
	Intercepts + Light, C, Na, Ca, Zn, C:N + adults' aggregation	1662.14
	Intercepts + Light, C, Na, Ca, Zn, C:N + adults' aggregation + common spatial effect	1248.1
	Intercepts + Light, C, Zn + adults' aggregation + common spatial effect	1237.15

Fig 3.1 - Effect of the constructed covariate of: (a) seedlings (aggregation index) and (b) reproductive plants (potential mother-offspring distance) on the spatial pattern of seedlings; (c) reproductive plants on local SGS of seedlings; and (d) adult plants (aggregation index) and the spatial pattern of adults of *Heliconia acuminata*.

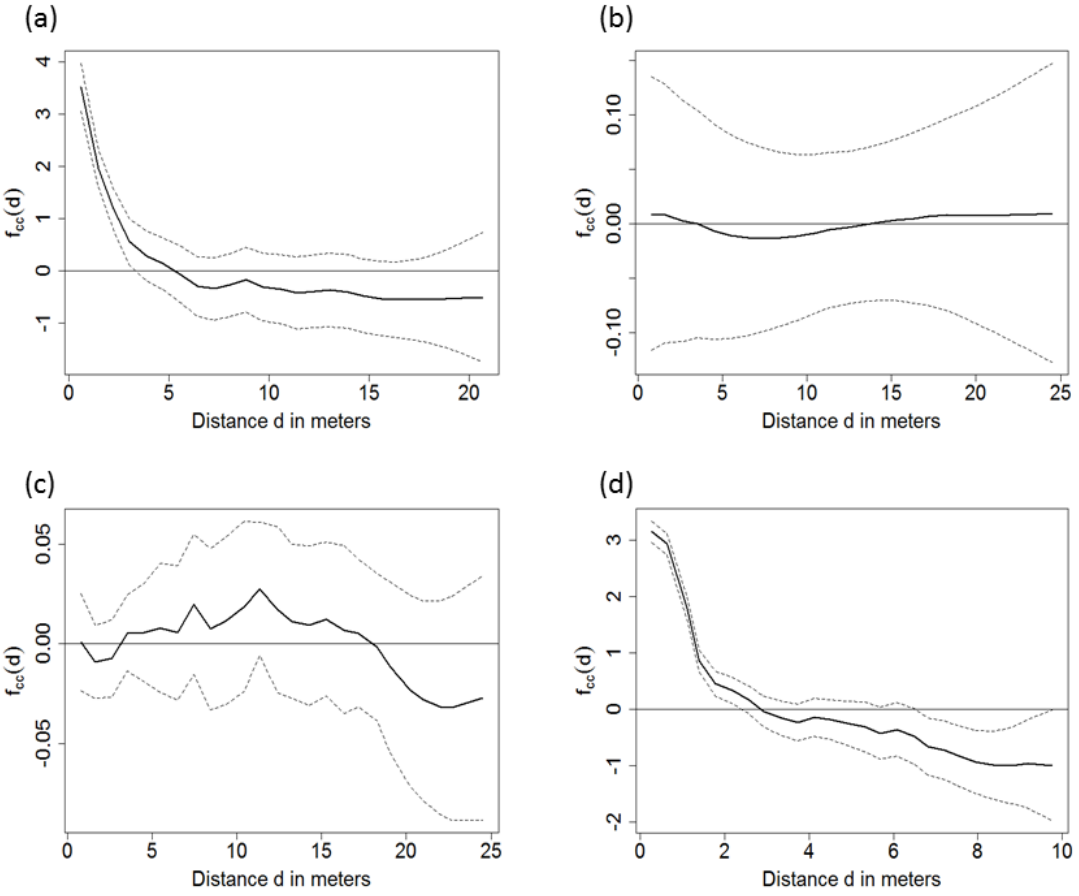


Fig 3.2 - Mean and 95% credible intervals of the posteriors of each environmental covariate included in the final marked point process model of *Heliconia acuminata*.

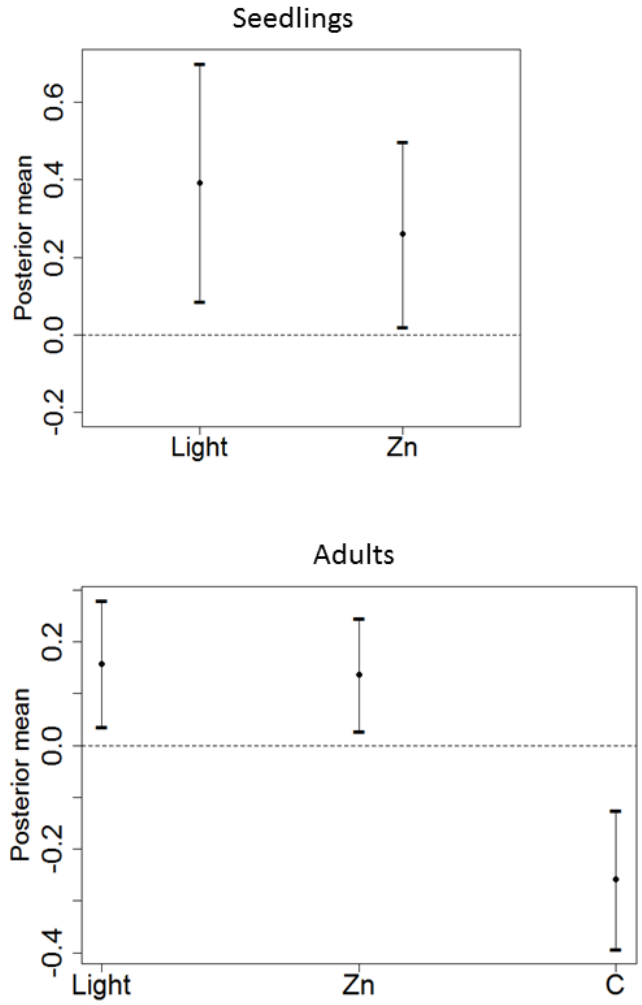
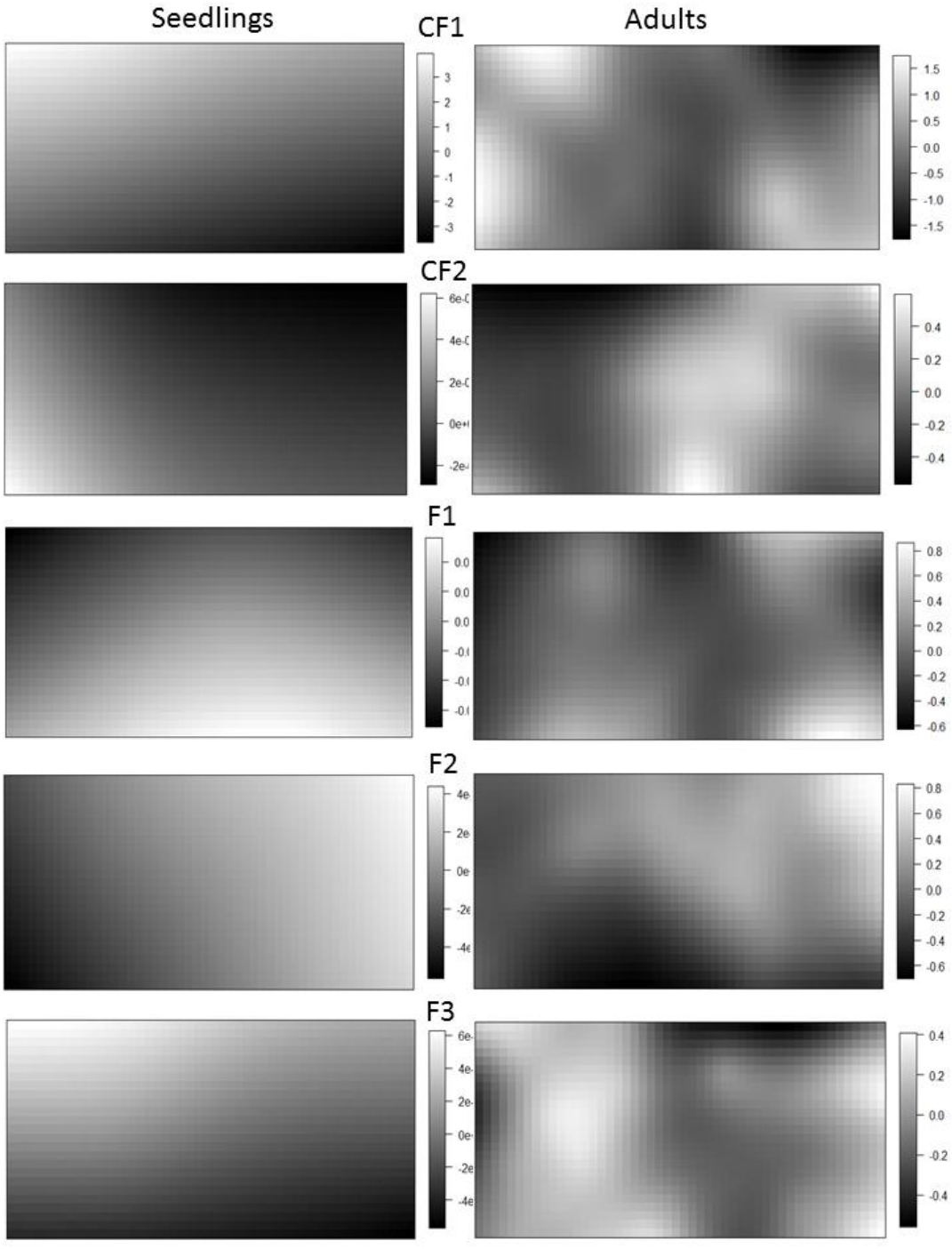


Fig 3.3 - Common spatial structure estimated for location and marks of seedlings and adults of *Heliconia acuminata* in the five sampled plots. The common spatial structure accounts for the large scale variation within the plot and for the spatial autocorrelation present in the data that is not accounted for by the empirical and constructed covariates.



CHAPTER 4 – INTEGRATING FRUGIVORY AND ANIMAL MOVEMENT: A REVIEW OF THE EVIDENCE AND IMPLICATIONS FOR SCALING SEED DISPERSAL

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ABSTRACT

General principles about the consequences of seed dispersal by animals for the structure and dynamics of plant populations and communities remain elusive. This is in part because seed deposition patterns emerge from interactions between frugivore behavior and the distribution of food resources, both of which can vary over space and time. Here we advocate for a frugivore - centered, process-based, synthetic approach to seed dispersal research that integrates seed dispersal ecology and animal movement across multiple spatio - temporal scales. To guide this synthesis, we survey existing literature using paradigms from seed dispersal and animal movement. Specifically, studies are discussed with respect to five criteria: selection of focal organisms (animal or plant); measurement of animal movement; characterization of seed shadow; animal, plant and environmental factors included in the study; and scales of the study. Most studies focused on either frugivores or plants and characterized seed shadows directly by combining gut retention time with animal movement data or indirectly by conducting maternity analysis of seeds. Although organismal traits and environmental factors were often measured, they were seldom used to characterize seed shadows. Multi - scale analyses were rare, with seed shadows mostly characterized at fine-spatial scales, over single fruiting seasons, and for individual dispersers. Novel animal and seed tracking technologies, remote environmental monitoring tools, and advances in analytical methods can enable effective implementation of a hierarchical mechanistic approach for the study of seed dispersal. This kind of mechanistic approach will provide novel insights regarding the complex interplay between the factors that modulate animal behavior and subsequently influence seed dispersal patterns across spatial and temporal scales.

INTRODUCTION

Seed dispersal is a crucial component of plant population dynamics with consequences to colonization of new habitats, spatial structure and maintenance of diversity (Bascompte & Jordano 2006; Schupp *et al.* 2002; Trakhtenbrot *et al.* 2005; Wang & Smith 2002). Twenty five to 80% of temperate plant species and 40 to 90% of tropical rainforest woody species depend on frugivores for seed dispersal (Howe & Smallwood 1982; Jordano 2000). Despite the critical role frugivores play in the organization of plant communities, a mechanistic understanding of the principles affecting animal dispersers and their consequences for the spatial and genetic organization of plant populations and communities remain elusive. Studies of seed dispersal have historically focused on individual components of the seed dispersal loop (Wang & Smith 2002), with numerous comparisons of seed dispersal patterns among units at the same scale (e.g., between habitats or dispersers). However, only a handful of these provide a comprehensive understanding of how seed dispersal "plays out" across spatial (Carlo & Morales 2008; García *et al.* 2005; García *et al.* 2011) or temporal (Prasad & Sukumar 2010) scales. Scaling seed dispersal is especially important given the rapid changes natural systems are currently facing. Deforestation and forest fragmentation, selective logging and defaunation modify plant-animal interactions with direct implications to the conservation and regeneration of natural habitats (Cordeiro *et al.* 2009; da Silva & Tabarelli 2000; Galetti *et al.* 2006).

Two key questions are relevant to all studies of seed dispersal by frugivores (Jordano (2007): (i) Which frugivore species (or individuals) contribute seeds, and to which locations? (ii) Which source plants contribute seeds, and to which locations? Together, these two processes generate the seed shadow, that is, the location where seeds from single plants are deposited (Nathan & Muller-Landau 2000). Our understanding of the processes generating seed shadows is

primarily limited by the difficulties involved in tracking seeds back to their source and in teasing apart the relative contribution of different dispersal agents to the spatial distribution of seeds. By using diverse methods, however, we can now begin to characterize some of the components that determine seed shadows, such as the distance and density from source tree, the density of dispersed seeds arriving at the target site, and less commonly, the number and extent of overlapping seed shadows (García & Grivet 2011; Jordano 2007).

The approaches used to characterize seed shadows can be divided into two broad classes: backward and forward tracking. Backward tracking, or source-based approaches (Jordano 2007), examine the spatial patterns of seed distribution with respect to distance from source plants (Muller-Landau *et al.* 2008) and then trace the movement of the seed back to its putative source. Backward approaches have commonly relied on inverse modeling, a statistical method in which the likelihood of obtaining the observed spatial patterns of seed dispersal or seedling establishment is calculated, based on a probability density function, linking the location of seed deposition with respect to the source (i.e., dispersal kernel) (Bullock *et al.* 2006; Clark *et al.* 1999; Nathan & Muller-Landau 2000; Ribbens *et al.* 1994). These approaches have some limitations. For instance, commonly used kernels generally capture dispersal of wind-dispersed species quite well, but fail to do so for animal-dispersed species (Hardesty *et al.* 2006; Holbrook & Loiselle 2007; Moran & Clark 2011; Russo *et al.* 2006). More recently, backward approaches have included the use of isotopes to track the seeds of ^{15}N enriched maternal plants and the application of molecular markers to more accurately assign the offspring to its maternal source (García & Grivet 2011; Godoy & Jordano 2001). Ultimately, molecular tools represent the only direct way to characterize the contribution of individual seed sources to a particular patch (e.g.,

seed trap, latrine, or roost), and to measure the number and extent of overlapping seed shadows (García & Grivet 2011; García *et al.* 2009a).

Forward, or target-tracking methods (Jordano 2007), follow the movement of seeds from the source plant to the deposition site. These methods include observing disperser foraging activity and tracking subsequent movements either remotely (i.e., radio - telemetry) or visually to infer seed displacement (Jordano & Schupp 2000; Westcott & Graham 2000), tagging fruits or seeds with threads (Forget 1990), attaching radio - tracking devices (Pons & Pausas 2007) or coded labels (Mack 1995) to seeds, and spraying fruits with fluorescent microspheres (Levey & Sargent 2000).

Forward - tracking mechanistic approaches offer an alternative to backward tracking techniques for modeling the seed shadow. In this case, plant and disperser traits and characteristics of the dispersal event itself can be directly incorporated into a predictive model of dispersal (Nathan & Muller-Landau 2000). Given the large number of variables that affect animal behavior and movement (Nathan & Muller-Landau 2000), these approaches are often ineffective. This is because animals do not move randomly in space. Rather, traits intrinsic to the dispersal vectors (e.g., physiology), together with extrinsic environmental factors, including landscape structure, food availability, competition and predation processes may ultimately determine how animals forage, move, and deposit seeds over space and time (Cousens *et al.* 2010; García *et al.* 2011; Nathan 2008). Unfortunately, forward tracking approaches rarely account for extrinsic factors.

Developing a mechanistic understanding of animal - mediated seed dispersal requires that we ask a third focal question in seed dispersal studies: why and how do frugivore species (or individuals) disperse seeds from an individual plant to a given deposition site? To answer this

question, we must comprehensively embrace the study of animal ecology (Giuggioli & Bartumeus 2010). These efforts should include studies of foraging behavior, the factors that shape the behavioral responses of animals to habitat and landscape structure, and the physiological traits that constrain foraging behavior. The nature of this third question demands the mechanistic study of the different intrinsic and extrinsic factors that modulate disperser behavior and seed deposition. Because the relative importance of these factors on animal-plant interactions is highly context - and scale - specific (Lehouck *et al.* 2009; Schupp *et al.* 2010), finding general patterns may prove challenging (Agrawal *et al.* 2007). Instead, future work needs to go beyond the study of a single temporal or spatial snapshot of a particular dispersal system (Burns 2004) to investigate how the relative importance of the intrinsic and extrinsic factors governing animal foraging behavior changes across spatio-temporal and taxonomic scales.

Our objective here is to motivate the adoption of a frugivore-centered seed dispersal research framework that goes beyond the pattern - based and snapshot view to a process - based and multi - scale examination of animal - mediated seed dispersal. To this end, we first combine elements from two existing frameworks, the seed dispersal effectiveness (Schupp 1993; Schupp *et al.* 2010) and movement ecology paradigm (Nathan *et al.* 2008), to examine the frugivore and plant traits, and environmental factors that influence the way animals interact with fruiting plants, shape seed shadows, and potentially influence plant recruitment. Second, we combine these frameworks to survey studies that explicitly link frugivory and seed deposition. Third, we survey promising technological developments and propose future directions for research aimed at advancing our understanding of the mechanisms influencing seed dispersal processes across spatial, temporal and taxonomic scales.

LINKING FRUGIVORES, FRUITING PLANTS, AND SEED SHADOWS

From a disperser perspective, seed dispersal effectiveness (SDE) can be described as the relative contribution of an individual disperser to a particular plant's seed shadow (Schupp 1993), although it can be more inclusive and also refer to the overall effectiveness of dispersal a plant receives from the complete suite of dispersers (Schupp *et al.* 2010). Ultimately, SDE is the product of the quantity (visitation frequency and rate of fruit consumption) and quality (treatment given to the seed and characteristics of the deposition site and pattern) components of seed dispersal (Schupp 1993; Schupp *et al.* 2010). Animal movement, one of the sub-components of seed dispersal quality, is the process that links frugivores, fruiting plants and seed shadows and thus deserves further attention. The movement ecology paradigm (Nathan *et al.* 2008) conceptualizes the drivers and components of animals movement and, in doing so, can add useful information to seed dispersal studies. In this paradigm, individual movement can be characterized by an internal state (why move?), motion capacity (how to move?), and a navigation capacity (when and where to move?), which are all modulated by biotic and abiotic stimuli (Nathan *et al.* 2008).

To help us integrate the elements of these two frameworks and organize our literature survey, we compartmentalize seed dispersal into two phases: frugivory and seed deposition. This two - phase compartmentalization aims to capture the effects that distinct plant and animal traits together with environmental characteristics have on the discrete sequence of disperser behaviors that lead to seed deposition (Fig.4.1). Hereafter we will use intrinsic factors to refer to inherent traits of the focal plant and animal, whereas extrinsic factors refer to abiotic or biotic environmental characteristics that influence either of these phases (Table 4.1).

FRUGIVORY PHASE: WHAT FRUITS ARE CONSUMED AND IN WHICH MANNER?

The frugivory phase is governed by the behaviors associated with fruit preference and selection, manipulation, and ingestion of fruits by animal dispersers (Fig. 4.1). The frugivory phase incorporates quantity and quality components of the SDE framework (Schupp 1993; Schupp *et al.* 2010). It is a cognitive process, with fruits as the intended targets that trigger the internal state of frugivores. The type and quantity of seeds taken and dispersed by frugivores are determined during this phase and are primarily influenced by morphological (e.g., size), physiological (e.g., nutritional requirements) and behavioral (e,g., reproductive status, social interactions) characteristics of the animals relative to those of the dispersed plants (Martin 1985). As a result, fruits of some species may be preferred over others, leading to disproportionally greater fruit removal rates for preferred species relative to other plant species in the community (Carlo *et al.* 2003; Wheelwright 1983), Animal morphology and physiology further determine whether a seed will be dispersed or predated, and also the speed of passage through the gut, which together with animal movement, determines where seeds are deposited (Cousens *et al.* 2010; Will & Tackenberg 2008). Moreover, disperser's life - history traits interact with environmental factors to generate large variation in fruit removal across plant population and communities (Table 5.1). For instance, males of manakins may establish leks on environmental hotspots, such as sites with high density of fruiting plants, to optimize foraging and attract females during the breeding season (Ryder *et al.* 2006), resulting in aggregated fruit removal and increased density of the local seed bank (Krijger *et al.* 1997). Extensive lists and description of the myriad intrinsic and extrinsic factors that can influence these patterns are provided elsewhere (Corlett 2011; Howe & Smallwood 1982; Jordano 2000; Schupp 1993).

From the plant's perspective, seed dispersers may affect the abundance of plants when dissemination is quantitatively limited (Schupp *et al.* 2002), meaning that independent of fruit production, dispersers remove a low proportion of fruits from the parents (Fig. 5.1). Reduced visitation rate and fruit removal may be due to low disperser abundance (Jordano & Schupp 2000), low consumption rates, or avoidance of plant species, possibly as the result of dispersers' diet preference or morphological constraints (Carlo *et al.* 2003; Jordano 2000). Another type of dissemination limitation during this phase is source-biased limitation (Jordano 2007) (Fig. 5.1), the result of unequal fruit removal among individual plants (Carlo & Morales 2008). In this case, few plants contribute seeds disproportionately to a given micro - site (García *et al.* 2009a) or to the whole population (Sezen *et al.* 2005), whereas other individuals fail to have their seeds dispersed. The most striking consequence of source - biased limitation is genetic. Dissemination limitation can be studied indirectly by observing animals' feeding behavior on individual plants (Fuentes *et al.* 2001), by marking individual plants and monitoring the number of fruits removed over time (Pizo & Almeida-Neto 2009), or by genotyping disseminated seeds and assessing the number of seed donors and the relative contributions of maternal plants to the population seed rain (García & Grivet 2011; Grivet *et al.* 2005).

SEED DEPOSITION PHASE: HOW AND WHERE ARE SEEDS DEPOSITED?

The seed deposition phase includes all behaviors that dictate where seeds are deposited, after consumption and digestion (Fig. 5.1), and largely incorporates SDE quality variables (Schupp 1993; Schupp *et al.* 2010). Animals' daily and seasonal activities will affect how and where seeds are deposited, and ultimately, disperser movement will be the most important post-frugivory factor affecting all three components of seed shadows (distance of seed from source

tree, density and distribution of dispersed seeds, and number and extent of seed shadows overlapping with conspecifics).

The study of animal movement has advanced steadily, by virtue of increases in temporal and spatial accuracy of GPS (global positioning system) tracking technology (Tomkiewicz *et al.* 2010), miniaturization of tracking devices (Wikelski *et al.* 2010), and the conceptualization of movement models borrowed from physics theory, such as random walk (or diffusion models if population-based), correlated and biased random walks, and Lévy statistics (Borger *et al.* 2008; Smouse *et al.* 2010).

Mechanistic models of animal's movement and home - range have emerged as an alternative to description of patterns of habitat use and selection. The models are based on stochastic rules of movement associated with probability distributions of movement lengths, orientations and turning angles (Moorcroft & Lewis 2006). Models of animal movement patterns have improved by considering the heterogeneous and complex nature of animal behavioral responses to intrinsic and extrinsic factors (Kie *et al.* 2010; Morales & Ellner 2002; Smouse *et al.* 2010). For instance, different speed or turning distributions can be assigned for distinct behavioral modes, or switches between modes can be modeled based on variations in internal states or environmental stimuli (Moorcroft & Lewis 2006). Navigation capacity accounts for the animal's ability to orient in space, determining the position and direction of each movement event. In order to predict seed dispersal events, we need to understand how external conditions affect animal internal states, in turn influencing the navigation status of the disperser (Cousens *et al.* 2010).

Social organization, territoriality and mating system are important aspects of animal behavior that play a role in determining the patterns of seed dispersal (Karubian & Durães 2009).

Many of these behaviors could explain the motivation (internal state) behind frugivore movement and navigation capacity. For example, one of the most important seed dispersers of the neotropical tree *Ocotea endresiana*, the three-wattled bellbird *Procnias tricarunculata*, disperses most of the seeds under song perches in canopy gaps (Wenny & Levey 1998). Here, the internal state is represented by the urge to attract females via display, and the navigation - dictated target is the song perch located in a gap. Such predictable seed deposition patterns favoring the recruitment of *O. endresiana* in gaps can be directly linked to disperser mating behavior.

Habitats are composed of a set of biotic and abiotic environmental variables that are heterogeneous in space and time (Beyer *et al.* 2010). How animals use habitat reflects a trade - off between their internal motivations and intended targets and the external conditions restricting their accessibility to those targets (Borger *et al.* 2008). An animal's movement is generally bounded by its home - range and by the territories of neighboring individuals or groups. Thus, home range determines the scale over which most of the animal seed dispersal occurs by any particular individual. Within these broad boundaries, various environmental characteristics can determine direction, length and speed of movement and frequency of use for specific habitats (Moorcroft & Barnet 2008) following a feeding event. Vegetation structure, water availability, topography, presence of competitors or predators, and abundance of a given food item are only a few of the many environmental factors that may exert an influence on the internal state of frugivores or modify their navigation capacities (Table 4.1).

In summary, understanding animal movement is essential to connect frugivores to seed shadows. The study of animal movement is still analytically challenging but the continuous application and subsequent refinement of analytical tools will help to advance the field of study

by providing the means to investigate the internal triggers and external conditions influencing how animals move and where they deposit ingested seeds.

A REVIEW OF STUDIES OF SEED SHADOWS FROM THE DISPERSER PERSPECTIVE

Numerous reviews have synthetized empirical work or discussed theoretical aspects of frugivore - mediated seed dispersal. These include a detailed description of morphological and physiological characteristics of frugivores and quality and distribution of food resources that ultimately affect plant-animal interactions (Corlett 2011; Jordano 2000; Schupp 1993), the importance of seed dispersal to the ecology, evolution (Howe & Smallwood 1982; Levin *et al.* 2003; Levine & Murrell 2003; Wang & Smith 2002), and genetics of plant populations and communities (Broquet & Petit 2009; García & Grivet 2011), and a survey of the scale at which seed dispersal processes are studied (Burns 2004; Kollmann 2000). In parallel, there have been a number of recent reviews in the field of animal movement (Borger *et al.* 2008; Cagnacci *et al.* 2010; Moorcroft & Barnet 2008; Nathan 2008; Schick *et al.* 2008). None of these reviews, however, has simultaneously considered seed dispersal, animal movement, and scaling issues. This is a critical need because integrating these processes will enable the development of a more mechanistic understanding of frugivore - mediated seed dispersal and open new avenues for researching the feedbacks between interacting organisms and the strength of biotic and abiotic context on modulating mutualisms (Agrawal *et al.* 2007).

To aid in this effort, we compiled studies that explicitly link fruiting plants, frugivores, and seed shadows. We did a literature search up to September 2011 and found 30 studies that met the following two criteria: (i) Individual plants, as fruit sources, were spatially linked to their seed shadow; (ii) Frugivore (individual or species) behavior was linked directly or indirectly to

both the fruiting plant and seed shadow. The studies cover a broad range of frugivores (birds, mammals and reptiles), plant life-forms (trees, shrubs and herbs), and biomes (tropical rainforests, deserts, temperate forests) (Table 4.2).

For each of the selected studies, we determined: (i) which and how many plants and animals were included and whether the study was centered on the plant' or animal' perspective; (ii); how animal movement was measured or inferred, (iii) how seed shadows were characterized; (iv) the number of intrinsic and extrinsic factors (see Table 4.1) included in the study; and (v) the spatial and temporal scale over which the studies were conducted. Below, we summarize and discuss our findings (Table 4.2).

SELECTION OF FOCAL PLANT AND ANIMAL DISPERSER SPECIES

Overall, studies of seed shadows evaluated either the seed dispersal role of chosen animals or seed dispersal pattern of specific plants and, thus, have primarily taken either the animal (N = 15 studies) or plant's perspective (N = 13 studies), with only two studies addressing dispersal from both perspectives (Table 4.2). There are good reasons behind this pattern. Reciprocal specialization in seed dispersal systems is very rare because frugivore diet is often diversified and most fleshy fruit bearing plants rely on a large assemblage of animals for their dispersal (Joppa *et al.* 2009). The research focus often reflects the interests of the investigators and the characteristics of the study system, while logistical restrictions limit the number of focal organisms and the taxonomic (e.g., species and genus) or organizational level (e.g., functional groups) considered in the study.

From an animal perspective, evaluating the outcomes of seed dispersal of all plant species consumed by a disperser species is often impractical. For instance, animal-focused studies (Table

4.2) took one of the following approaches to estimate gut retention time: seed shadows were generated using gut retention time obtained for a few plants species (Holbrook & Smith 2000), categories of plant species were created based on similar gut retention time (e.g., slow vs. fast seeds (Sun *et al.* 1997), gut retention time of different plant species were combined into a single average (Wehncke *et al.* 2003), or a model plant was used to understand seed dispersal patterns (Campos-Arceiz *et al.* 2008; Levey *et al.* 2005).

From a plant's perspective, tracking movement of all frugivores consuming a particular plant species is costly and time-demanding. Different approaches have been used to constrain the number of dispersers included in a study. The most straightforward method is to select the single most important seed disperser of the focal plant species. For instance, the spider monkey *Ateles paniscus* was selected for evaluating the seed shadow of the neotropical tree *Virola calophylla* (Russo *et al.* 2006) because it was responsible for dispersing 92% of removed seeds (Russo 2003). Seed dispersers, however, often have equivalent or similar roles in dispersing seeds from multiple, different focal plant species. Often, the few most important frugivores were studied individually (Holbrook & Loiselle 2007; Murray 1988), or subdivided into functional groups, based on their gut retention time (e.g., manakins vs. thrushes (Uriarte *et al.* 2011)), body - size or micro - habitat preference (small vs. medium - sized birds vs. mammals (Jordano *et al.* 2007)). Although measuring the individual contribution of each disperser is ideal, detailed information about individual dispersers may not always be necessary to understand or predict seed shadows.

MEASURING ANIMAL MOVEMENT

Frugivore movement was measured directly, through observation (N = 12) and/or remote tracking (N = 13), and indirectly by conducting maternity analysis on seeds (N = 6) (Table 4.2).

The most common method was to visually observe and record the time and location of animals throughout the day (e.g., Sun *et al.*, 1997; Wehncke *et al.*, 2003; McConkey & Chivers, 2007; Green, Ward & Griffiths, 2009). Depending on the home-range and patterns of habitat use, however, it may be difficult to sample longer movement bouts using these methods (Spiegel & Nathan 2007). Nevertheless, some of the selected studies successfully used radio and GPS telemetry to overcome this problem (e.g., Holbrook & Smith, 2000; Campos-Arceiz *et al.*, 2008; Lenz *et al.*, 2011).

Indirect measures of animal movement can be obtained by using genetic markers to assign seeds (i.e., offspring) to their source (i.e., maternal plant). The use of molecular tools can provide valuable insights into frugivores' social, reproductive and foraging behavior. For instance, Karubian *et al.* (2010) examined the effect of lekking behavior of the umbrellabird *Cephalopterus penduliger* on the directed-seed dispersal of the palm *Oenocarpus bataua* under leks. Directed-seed dispersal leads to the disproportionate dissemination of seeds to a particular micro-site, which has been hypothesized to result in the formation of pronounced genetic structure within the seed pool. The seed pools under leks were genetically characterized and they were highly heterogeneous, presenting weaker spatial genetic structure than seeds outside leks. These results have important implications for our understanding of how spatially-contagious dispersal interacts with source-biased dispersal (García & Grivet 2011).

In sum, different techniques used to measure movement elucidate different aspects of animal-plant interactions with some clear trade - offs. Observational methods allow detailed recording of animal behavior at limited spatial and temporal extents while remote tracking methods allow for accurate measurements of animal locations across larger areas, but fail to provide information on individual behavioral states. Expanding results from observational data to

larger scales through modeling has been successfully employed (Levey *et al.* 2005), however given our lack of understanding on how frugivore behavior transition across scales in most systems, extrapolating fine scale behavior may generate uncertainties and false predictions as we scale - up (see section below *Multi – scale analysis*). Molecular tools can accurately describe seed dispersal events, but cannot capture detailed information on disperser foraging behaviors, therefore limiting mechanistic generalization.

CHARACTERIZING THE SEED SHADOW

Seed shadows were characterized through observations of seed deposition in real-time (N = 3), predicted using movement and gut retention time data (N = 21), or through maternity analysis (N = 6) (Table 4.2). Visual tracking of animals' foraging and post-feeding movement until seeds are deposited requires that the researchers have an initial estimate of average or maximum gut retention time for the disperser. This method can capture isolated feeding (Yumoto *et al.* 1999). But, if an animal consumes fruits from conspecifics within the gut retention timeframe, identification of the exact maternal tree is impossible, unless genetic maternity analysis is also employed (Terakawa *et al.* 2009). Some of the selected studies, however, have bypassed this issue by assigning individual seeds to different plants when individual feces contained more than one seed (Bravo 2009; Russo *et al.* 2006).

Alternatively, some of the studies measured movement and gut retention time separately and later estimated seed dispersal curves by calculating the probability that a given seed consumed at time zero would be voided at a certain distance from the maternal plant, after a specific time of gut retention has elapsed. Four studies used fixed median, minimum and/or maximum values of gut retention time for calculating these probabilities (e.g., Westcott &

Graham, 2000; Levey *et al.*, 2005) and, in some cases, different curves were generated using temporal categories of gut retention time (e.g., fast, medium and long retention time (Weir & Corlett 2007)). However, gut retention times are highly variable, even within the same animal species and for the same plant species (Tewksbury *et al.* 2008; Traveset 1998), so dispersal curves generated using fixed values may be unrealistic. For this reason, several of the reviewed studies (N=13) sampled gut retention time values from empirical frequency distributions or probability distribution functions, which were then used to simulate seed dispersal events (e.g., Murray, 1988; Levey *et al.*, 2008; Uriarte *et al.*, 2011).

Studies often assume that seed dispersal decays with distance to putative maternal individuals and characterize seed movement by reporting maximum or mean distance to sources. These metrics are chosen because they are hypothesized to reflect the two main advantages of dispersal (Howe & Smallwood 1982): the escape hypothesis, which assumes that longer dispersal distances are beneficial because of high density-dependent mortality below maternal plants (Janzen 1970), and the colonization hypothesis, which assumes dispersal into a new environment is beneficial (e.g., tree-fall gaps) (Cain *et al.* 2000). Furthermore, distance metrics are useful to evaluate whether the seed dispersal of a particular plant population is distance-restricted (i.e., dissemination limitation) (Fig. 4.1), with implications for the fine-scale recruitment, distribution of plants and long-distance dispersal (Cain *et al.* 2000; Clark *et al.* 1999; Schupp *et al.* 2002).

However, the idea that seed dispersal obeys a decay function with respect to distance from the source plant has recently come into question. For instance, parentage analysis has shown that seeds dispersed beneath a conspecific canopy need not be from that specific mother tree (Godoy & Jordano 2001) and that the adult plant nearest to the seedling is rarely the parent

(Hardesty *et al.* 2006; Sezen *et al.* 2009). Thus, basing models of seed dispersal on distance alone may yield unrealistic predictions about the spatial distribution and gene flow of many plant species (Ashley 2010). Although some of the reviewed studies went beyond reporting distance metrics and acknowledged the fact that seed movement is anisotropic and non-random (Santamaría *et al.* 2007; Scofield *et al.* 2010), few evaluated the factors that underlie spatial variance in seed dispersal (Russo *et al.* 2006). Often, seeds may be disproportionately deposited in certain sites, leading to clumped patterns of seed dispersal and spatially-contagious type of dissemination limitation (Fig. 4.1) (Schupp *et al.* 2002). For instance, Russo *et al.* (2006) demonstrated that the distribution of *Virola calophylla* seeds dispersed by the spider monkey *Ateles paniscus* was strongly leptokurtic (many seeds were dropped beneath the sleeping trees), with a long fat tail (due to longer seed dispersal events during in-transit movements) and multimodality (due to the clumped deposition under sleeping sites at various distances from the parent tree).

In summary, seed shadows are more accurately measured by using molecular tools, but can be mechanistically described by combining animal movement and gut retention time. As gut retention time is highly variable, probability distribution of retention times should be used over single values (e.g., average or maximum). Moreover, components of seed dispersal other than distance from source plants should be considered. Mapping seed shadows allows for a two-dimensional examination of seed deposition and additional components, such as the density of seeds at different deposition microsites, can be uncovered.

INCORPORATING INTRINSIC AND EXTRINSIC FACTORS INTO SEED DISPERSAL STUDIES

Many of the selected studies evaluated other extrinsic and intrinsic factors that may influence dispersal (Table 4.1). These factors included plant traits (N = 12 studies), animal traits (N = 19) and environmental (N = 17) factors (Table 4.1 & 4.2). Commonly, however, the variation in these factors were not incorporated in the characterization of the seed shadow (N=8). Plant traits included crop size (Carlo & Morales 2008), plant aggregation patterns (Carlo & Morales 2008; Morales & Carlo 2006), and distance to distinct microhabitats (García *et al.* 2009a). Animal traits included abundance of dispersers (Uriarte *et al.* 2011), digestive physiology (e.g., effect of ingestion on germination (Santamaría *et al.* 2007)), behavioral states, (e.g., foraging, sleeping, defecating (Russo *et al.* 2006)) and movement states (e.g., perching time, move length and move direction (Levey *et al.* 2005; Sun *et al.* 1997)). A few studies also characterized quality of the deposition site in function of presence and size of gaps (Murray 1988), vegetation height (García *et al.* 2009a), and availability of sleeping trees (Russo *et al.* 2006) (Table 4.2).

Animals move purposefully, their internal states and navigation capacities change over time according to the influence and interplay of intrinsic and extrinsic factors. In general, however, seed dispersal models integrating displacement and gut retention time probabilities make no assumptions about the reasons animals move (Cousens *et al.* 2010). Some studies have added realism to seed dispersal models by making displacement probabilities a function of intrinsic and extrinsic factors. For example, in order to understand the effect of forest fragmentation on seed dispersal of the understory herb *Heliconia acuminata*, Uriarte *et al.* (2011) parameterized a mechanistic simulation model which incorporated the effects of landscape structure on animal movement decisions and resulting seed shadows. Other studies examined effects of corridors (Levey *et al.* 2005), patch shape and landscape heterogeneity (Levey *et al.* 2008), and daily activity of frugivores (Russo *et al.* 2006).

We advocate for the explicit inclusion of a greater number of intrinsic and extrinsic factors in studies that aim to link frugivory to seed deposition. The use of predictive models driven by a set of foraging and dispersal rules may help us test hypotheses about the relevance of such factors for seed dispersal under different environmental conditions and along natural and human - modified gradients.

SCALE OF ANALYSES

The relative importance of individual factors on determining frugivory and seed deposition processes and patterns differed with the choice of spatial, temporal, or taxonomic scales (García *et al.* 2011; Kollmann 2000; Schupp *et al.* 2010) (Fig. 4.2). Among the reviewed studies, seed shadows were mostly studied at fine - spatial scales (0.1 - 50 ha) and over single fruiting seasons (days to months) (Table 4.3, third and fourth level in Fig. 4.2). Furthermore, most studies were conducted at the individual disperser level, and multiple seed shadows were summed or averaged to generate composite population-level seed shadows (Holbrook & Loiselle 2007; Santamaría *et al.* 2007).

At the population level, factors measured at fine scales are considered to influence the observed pattern of dispersal (Fig. 4.2). However, factors occurring at larger scales, such as phylogenetic constraints of frugivores or biome structural characteristics, may influence fine scale processes (Fig. 4.2). This is a critical issue if we are to scale-up from individual studies to a general understanding of the factors that influence variation in seed dispersal patterns across years and regions. Despite the important effect that the choice of scale may have on our understanding of seed shadows, very little has been done to actually disentangle the influence of factors acting at different scales on seed shadows. Among the reviewed studies, Carlo & Morales

(2008) examined the effect of plant aggregation at the neighborhood (fine - scale) and landscape (5000 x 5000m plot) scales on fruit removal rates and seed dispersal of individual plants. Westcott & Graham (2000) analyzed the fractal geometry of the trajectory of an understory bird *Mionectes oleagineus* to find that its movement complexity was not scale invariant.

Although the majority of studies included in this survey did not attempt to perform cross - scale analysis, many of them conducted comparisons within spatial, temporal and/or taxonomic categories (Table 4.3). Spatially, seed deposition was compared among different micro - habitat, habitats, landscapes or geographical regions. Despite the well - known striking temporal variation in fecundity (e.g., mast seeding) for many tree species (Kelly & Sork 2002), annual and inter-decadal comparisons remain rare in studies of seed dispersal (but see Jordano, 1994; Herrera, 1998). Finally, taxonomic comparisons were conducted in the selected literature between congeneric species (e.g., Holbrook & Smith, 2000), or functional groups (Jordano *et al.* 2007).

TECHNICAL PROSPECTS FOR STUDIES OF DISPERSAL

The study of seed dispersal in the wild confronts a series of logistical and technical limitations. Researchers have creatively adapted existing tools to collect data relevant to understanding seed shadows (Bullock *et al.* 2006). As we make use of the newly available tools, we expect advances in our understanding of the components of the frugivore-plant system that remain largely unexplored (e.g., the effect of odor and sound on fruit-animal interactions (Corlett 2011)). In this section, we discuss the potential of new technical tools to help unveil the factors that determine disperser activities.

Animal-specific contribution to seed shadows

One technical difficulty that often impedes linking individual species of frugivores to the disseminated seed is to identify and quantify the contribution of different animal dispersers to a plant seed shadow. Seed dispersal interactions are highly complex and often involve multiple frugivore species shaping the combined seed shadow of one focal plant species. With a few exceptions, identifying which frugivore dispersed which seed is a daunting task. In some cases, system particularities, such as noticeable signatures of seed deposition by different frugivores (e.g., regurgitation vs. defecation) or microhabitat use by a single frugivore, might make it possible to track a seed back to its disperser and then back to its seed source (Fedriani *et al.* 2010; Jordano *et al.* 2007). Many dispersers, however, do not have distinguishable deposition patterns and the definition of discrete, biologically meaningful microsites can be difficult. One way to overcome this limitation is to identify the dispersers using genetic tools. DNA barcoding has been used by ecologists to identify animal species based on samples of hair, feces or urine (Valentini *et al.* 2009). Defecated or regurgitated seeds can provide enough animal material to isolate DNA and run the analysis to assign unknown specimens to known species using publically available databases (GenBank, Barcode of Life Database). This approach can provide useful information on the contributions of different frugivores to seed shadows (Marrero *et al.* 2009).

Tools for monitoring intrinsic and extrinsic factors

Understanding the environmental and physiological variables that trigger different internal states on animals can be challenging primarily because obtaining appropriate data on animal behavioral states and environmental characteristics is not always easy. At fine scales, ground surveys can be

conducted to gather information on certain intrinsic and extrinsic factors, such as abundance and distribution of fruit sources, which can be appropriate for animals that forage within restricted areas. Often, however, frugivores rely on patchy resources scattered across large areas or highly heterogeneous and inaccessible habitats (Lehouck *et al.* 2009). Satellite and aerial imagery is a valuable tool to help map points of interest (e.g., fruiting trees (Caillaud *et al.* 2010)) and characterize habitats at broader scales. Remote monitoring data (e.g., land use, climate) collected at different temporal and spatial resolutions has become increasingly available allowing detailed characterization of association between biota and environment (Kearney & Porter 2009), including disperser-plant interactions (Marquez *et al.* 2004). Light detection and ranging (LiDAR) provides information on vertical habitat structure (e.g., presence of gaps, vegetation stratification) arising as another tool to model animal-habitat relationships (Vierling *et al.* 2008). Biotelemetry, on the other hand, enables the characterization of the intrinsic factors that modulate animal foraging and movement. This promising advance involves remote monitoring of animal activities, physiological states and environmental conditions, such as temperature, frequency of wing beats or heart rate, using specialized sensors (Cooke *et al.* 2004). Combining biotelemetry and movement tracking technology can provide information on frugivore activities and their specific location in space and time. For instance, to better understand the role of the nocturnal frugivorous oilbird *Steatornis caripensis* on seed dispersal, Holland *et al.* (2009) used GPS - telemetry and accelerometers to monitor behavioral changes remotely. They were able to identify when birds were inactive in roosts or foraging in trees, and could distinguish flights to roost or foraging sites, based on the frequency of wing beats. Their results indicated that oilbirds are effective seed dispersers, due to their extensive foraging activities outside caves and non-

overlap of foraging and roosting sites. Valuable information about animal behaviors, such as resting, feeding, walking or flying, can be retrieved remotely using these tools.

Although many of these tools are new, costly, and logistically challenging, their increasing application in a broad range of studies will expand our understanding of seed dispersal across a broad range of scales. When combined with ground surveys, they may allow a more accurate and detailed understanding of links between environment and animal behavior, providing us with the material to explore the mechanisms behind frugivore - mediated seed dispersal at different scales.

A PROPOSAL FOR INTEGRATED ANALYSES

As ecologists we are faced with the challenge of linking processes that are highly space-time dependent and identifying general patterns that we can seldom extrapolate to other systems. In this section we first propose a flexible mechanistic approach as a way to integrate multiple processes within a comprehensive framework in order to shed some light on the effects of different assumptions on specific patterns, in our case, seed dispersal. Second, we focus on scaling issues as a way to pinpoint generalities in complex systems. Instead of looking for pattern similarities across systems, we propose concentrating on the mechanisms that lead to differences across temporal, spatial and taxonomic scales.

BUILDING A SPATIALLY-EXPLICIT MECHANISTIC MODEL: A HYPOTHETICAL EXAMPLE

To understand how biotic and abiotic factors affect seed dispersal one useful approach is to apply a spatially - explicit mechanistic model in which sub-models are tested and simulations are constructed using a series of nested routines. This approach allows for a comprehensive analysis

of the processes taking place and predicting the relative effects of factors on seed dispersal according to alternative scenarios. Inspired by many existent studies (Carlo & Morales 2008; Levey *et al.* 2008; Russo *et al.* 2006; Uriarte *et al.* 2011) we describe a hypothetical example to demonstrate how one can test for the relevance of different factors on the generation of seed shadow.

In our example, we are interested in the seed shadow of a canopy tree *T* generated by a medium - sized canopy bird *B* in a dense forest. Specifically, we ask what is the relative importance of abiotic and biotic factors on shaping the seed shadow? The first step is to identify underlying models describing aspects of the frugivory and seed deposition phases, and thus select a series of traits and factors believed to influence each one of these aspects. Appropriate data on animal and plant natural history and ecology should be collected, and for that a myriad of traditional and modern techniques are available (see section above *Tools for monitoring intrinsic and extrinsic factors*). Alternatively, data from similar systems can be used to parameterize some of the sub-models. For instance, gut retention time of a related disperser species can be used in the absence of specific information for the disperser under study (Levey *et al.* 2008).

Here, we have data on location of every tree *T* and all heterospecific trees that also produce bird - eaten fruits during tree *T* fruiting period within a pre - defined plot of 300 x 300m. Available data for intrinsic plant traits for plant *T* include crop size for a sample of trees, mean fruit size per plant, and fruit removal (based on observations of bird visitation) for a sample of trees. For intrinsic traits of bird *B* we have: gape size and body size for males and females (from a sample of captured birds), gut retention time for males and females from experiments in captivity, and movement behavior (from radio-telemetry); as extrinsic bird factors:

abundance/activity of other frugivorous birds (from bird counts and mistnet captures established in a grid system within the plot); as abiotic factors: location of gaps.

The second step is to build statistical models given our scientific hypotheses and select the best one. Given a set of nested alternative models we drop the covariates that do not improve the likelihood of a particular hypothesis given the dataset and select the most parsimonious one based on AIC (or AICc) values. The components included and the respective covariates considered in each of our models are (Fig. 4.3):

(1) Probability that a tree *T* is visited by bird *B*: hypothesized to be affected by crop size, number of heterospecifics in the neighborhood, number of conspecifics in the neighborhood, distance to nearest gap, and number of other frugivores in the neighborhood. This model represents the attractiveness of each tree *T* to birds *B*. Crop size and number of plants in the neighborhood are predicted to attract frugivores (Carlo 2005; Saracco *et al.* 2005; Sargent 1990). Plants located in gaps or close to them may be less attractive, as open environments make birds more vulnerable to predators (Howe 1979; Martin 1985). It is worth noting that high fruit production in gaps, however, can concentrate activity of understory birds (Levey 1988; Levey 1990), which may outweigh the predation risk. Also, the activity of other frugivores in the area represents the level of competition in the neighborhood (Carlo 2005), which may repel birds *B* to approach certain trees (Fadini *et al.* 2009; Martin 1985; Pratt 1984).

(2) Fruit removal rates (number of fruits removed per visit): hypothesized to be affected by the probability that a bird can swallow the fruit, body size, distance to gaps, and activity of other frugivores in the area. Larger birds are able to consume more fruits per bout (Jordano & Schupp 2000), so if there is sexual dimorphism in body size, we expect that males, on average, will remove different amounts of fruits compared to females. Fruit removal rates are strongly affected

by visit duration (Pratt & Stiles 1983), which in turn can be influenced by distance to gaps and activity of other frugivores. Birds visiting trees that are close to gap openings or located in neighborhoods with higher frugivore activity are expected to spend less time in the tree due to predation risk (Howe 1979) and competition pressure (Martin 1985; Pratt 1984), respectively.

(3) Bird's movement: analyzed through four components. a) Direction of movement: hypothesized to be affected by previous move direction and location of gaps, under the prediction that birds avoid flying into gaps; b) move length; c) speed; and d) perch time: hypothesized to be affected by distance to gaps (i.e., predation risk) and frugivore activity in the neighborhood (i.e., competition pressure).

(4) Gut retention time: bird's gender and body size (which may be correlated).

The second step is to use the parameters estimated from the most parsimonious models for each of the four components to define probability distributions to be sampled in a series of simulations. The simulation routines start from scenarios created from our empirical data. We create a plot with the same size and distribute plant *T* and heterospecifics in the same numbers as the observed. Plants and gap openings are randomly distributed (but location can follow a specific spatial distribution model). Crop size and average fruit size are assigned to each tree *T* from probability distributions fit to data. Each simulation starts from a bird randomly located in the map. We assume that bird's internal state is motivated by eating fruit *T* and thus move in the landscape searching for tree *T* given a constant speed, until gut is satiated (the maximum number of eaten fruits can be measured from captivity experiments and may vary according to size and gender). Movement rules are dictated by model 1. After satiated, bird's internal state changes and rules governing behavior are then based on model 3. Steps go as follows (Fig. 4.3):

(1) Bird is randomly located in the map. Assign gender to the bird (given natural proportions from mistnet data, although basing sex-ratio on mistnet data can be biased given that capture rates for males and females may differ because of specific movement patterns, (Remsen & Good 1996). If sexual dimorphism in size was detected (see component 2), each gender is associated to a different body and gape size average and GRT. If there is no dimorphism, a combined body and gape size and GRT are used.

(2) The bird visits the most attractive tree *T* within a 20 m radius (expected visual field). Level of attractiveness is based on component 1 and distance to each plant. Attractiveness decreases with distance from bird *B* to plant *T*.

(3) The bird stays and consumes fruits if gape size is wider than average fruit size of tree *T*, meaning that the bird will more likely swallow the fruits from that tree. If there is gape size dimorphism between males and females, than the probability of removal is affected by gender. If the bird consumes fruits, we go to step 4, otherwise the bird drops pecked fruit under the crown and moves to a different tree (step 2).

(4) Number of fruits removed is defined by model 2, sampled from a distribution bounded between 1 and maximum number given gut size limits (that may vary according to gender). At this point, plant identity and time are recorded. Perch time is conditional on the number of fruits removed. If bird consumes less than the maximum gut size, we repeat step 2 - 4 (movement dictated by the urge to eat fruit *T*) until bird is satiated. Seeds may be voided during perching if time elapsed since first feeding bout exceeds gut retention time. If bird is satiated, we go to step 5.

(5) Movement is now dictated by an unknown internal state. Direction, move length and speed are chosen based on each sub-model 3a, 3b, and 3c. Bird lands in a point in the map and

remain perched given sub - model 3d. Seeds may be voided during perching if time elapsed since first feeding bout exceeds gut retention time. The bird regurgitates as many seeds as eaten in the respective feeding bout and specific location where seeds are deposited is recorded. If the bird does not regurgitate all seeds, repeat step 5. If all seeds are regurgitated, go to step 6.

(6) Sum total of dispersed seeds by all simulated birds. If sum corresponds to total expected fruit removal by birds *B* during fruiting season of tree *T*, stop the simulation. If not, start with a new bird in step 1.

At the end of each of the many simulations, seed shadows can be evaluated individually for each tree or summed across individuals to characterize the population seed shadow. Probability distribution functions can be fit to the simulated data and two - dimensional depictions of the resulting seed shadows can be done to assess the variance in the spatial distribution of seeds. The assessment of the relative importance of different factors included in the model can be done by conducting sensitivity analyses by changing the input values of different factors one at a time and studying the outcomes (Calviño-Cancela & Martín-Herrero 2009; Uriarte *et al.* 2011). For instance, in the hypothetical example, we can remove variability in fruit removal per visit or change proportion of males and females in the population, in case gender differences were found.

Stochastic mechanistic simulation models are also useful for hypothesis testing, so that, for example, one may test the effect of bird extinction or reduced plant abundance on seed shadow. Likewise, genetic data can be incorporated, and differential gene shadows can also be simulated and modeled given a set of assumptions and factors.

Building such comprehensive model is not a trivial task. The challenges are to gather a complete data set on animal, plant and environmental characteristics and to develop adequate

algorithms for the simulation models. Clearly, the field of seed dispersal would benefit immensely from interdisciplinary collaborations between plant, animal and quantitative ecologists and, particularly, by developing such initiatives within long-term studies in permanent plots for which environmental data and mapped plant data are already available.

MULTI-SCALE ANALYSIS

Our compilation of the existing literature identified only a few studies that examined seed dispersal across multiple spatial, temporal, or taxonomic scales. This finding is in line with Kollmann (2000), who reviewed 136 studies to examine the spatial scale (e.g., microhabitats, habitats, regions) in which the intensity of particular components of seed dispersal (e.g., frugivore abundance, fruit removal, seed rain) were more strongly determined. Kollman (2000) and Burns (2004) argue that the lack of studies investigating seed dispersal at different scales may have so far precluded the emergence of general principles in the field. We believe that uncovering general principles and patterns will occur as frugivore-mediated seed dispersal is treated as a complex and integrated process that varies within and among scales rather than as a series of components examined individually at arbitrary scales.

Although literature on scaling issues in frugivory and seed deposition is scant, studies of animal movement and habitat selection undertaken at multiple scales can provide some insights on animal-mediated seed dispersal research. In animal ecology, four hierarchical spatio-temporal levels of resource selection have been identified (Gaillard *et al.* 2010; Johnson 1980): first order level refers to the geographical distribution range of the animal species; second order, selection of home-range; third order, selection of a patch or habitat; and fourth order, selection of a micro-site (e.g., nest) or item (e.g., prey). These levels are tied to spatio-temporal scales with relevant

resource selection and habitat performance parameters chosen according to the scale of study (Gaillard *et al.* 2010). For example, a proxy for studying resource selection and habitat performance at the species level (first order) is the probability of extinction, a process that should be investigated at the scale at which the pattern emerges, that is, across biomes and over millions of years. In contrast, at the individual level (fourth order), a useful proxy for measuring performance is energy gain from a food item (Fig. 4.2, Gaillard *et al.* 2010). At fine spatial and temporal scales animals tend to select resources and habitats that increase their immediate performance. As scales increase, behavioral decisions influence demographic parameters and population performance. And finally, environmental niches emerge at broad scales, over evolutionary time (Gaillard *et al.* 2010).

From fine to intermediate temporal scales, some effort has focused on understanding how animals' daily (e.g., sleeping, foraging, moving) and seasonal (e.g., mating, migrating) activities are tied to the spatio-temporal extent of analysis (Amano & Katayama 2009; Fryxell *et al.* 2008; Johnson *et al.* 2002; Mayor *et al.* 2009; Morales & Ellner 2002). Aside from searching for invariant scaling properties, multi-scale analysis provides a means to investigate the correlations between specific behavioral states and landscape features and how these are conditional to specific scales (Schick *et al.* 2008). For instance, Fryxell *et al.* (2008), used datasets from different sources (radio and GPS tracking devices) to test how the movement modes of elks (*Cervus elaphus*) change across spatio-temporal scales, ranging from minutes to years and meters to 100 km. At coarser scales, elks shifted from dispersive, exploratory movement to home-ranging behavior through time. At finer scales, however, elks responded to patchiness in local resources, displaying shorter moves and greater turning frequency when foraging than when exploring for food. Fryxell *et al.* (2008) concluded that multiphasic movement patterns

were present at all spatio - temporal scales, as a result of a combination of internal state, resource abundance, distribution of other individual elks, and navigational capability.

Here we propose a similar hierarchical approach for seed dispersal studies, in which a set of intrinsic and extrinsic factors are selected based on research questions and study system and then located along spatial and temporal gradients (Fig. 4.3). For instance, in our hypothetical example described above (section *Building a spatially – explicit mechanistic model*), we can evaluate how rates of fruit removal vary with the abundance of plants in the fruiting community at the regional level, which in turn may be determined by underlying gradients of soil fertility and rainfall (Gentry & Emmons 1987). The ultimate goal is to link causal relationships to dispersal outcomes from fine to large-scales, using factors related to different organization levels (Fig. 4.2) as the covariates. Although not explicitly linking frugivores activity to seed deposition, a handful of studies have investigated the exact scale in which seed dispersal operates (Aukema 2004; García & Chacoff 2007; García *et al.* 2009b; García *et al.* 2011). García *et al.* (2011), for example, assessed the relative importance of food availability and habitat structure on explaining scale-dependent variability on seed-frugivore interactions and looked for generalities by comparing patterns across three distinct ecosystems.

Scaling frugivory and seed deposition to higher levels in the temporal and spatial continuum over evolutionary time (right upper corner of Fig. 4.2) requires a more powerful conceptual approach and may bring another level of uncertainty into the models. The same four hierarchical levels identified in animal ecology studies can be translated to animal - mediated seed dispersal and pertinent proxies for studying these interactions and outcomes should be chosen according to the scale (Fig. 4.2). Seed shadows are predominantly viewed as the deterministic outcome of local processes at finer scales, with no considerations of the larger

spatio - temporal context in which they are embedded. Historical, systematic, and biogeographic information are seldom incorporated in investigations of seed dispersal (but see Garrido *et al.*, 2002; Almeida-Neto *et al.*, 2008; Kissling *et al.*, 2009). Biotic interactions are pervasive in all environments and have been recorded in the geologic past (Jablonski 2008; Tiffney 2004). Because of their relative transient nature, however, biotic interactions have been mostly dismissed as an important force molding species and clade level dynamics, largely because we lack understanding of how local processes cascade upwards to clade dynamics, and vice-versa (Jablonski 2008). At fine scales (fourth and third order), seed shadows are the first template over which plant recruitment takes place (Wang & Smith 2002), and thus are the means by which plants expand their range, exchange genes (or conversely lead to genetic differentiation and speciation), and colonize new habitats. Ultimately, population-level processes mold community composition and reflect on biogeographic and phylogeographic patterns over evolutionary time scales (first order) (Givnish 2010) (Fig. 4.2A). Community-wide and multi-trophic interactions are particularly important given that multiple animal dispersers are shared among plant species. Networks of mutualistic interactions are often affected by other trophic levels through cascading effects, with consequences for evolution and coevolution of species and implication for conservation of system's stability and robustness (Carlo & Yang 2011; Guimarães *et al.* 2011; Pocock *et al.* 2012). Conversely, seed shadows are also contingent on large-scale patterns (Fig. 4.2B). To address these feedbacks between scales more explicitly (Agrawal *et al.* 2007), we will have to assemble large data sets, spanning environmental gradients over time and space, using for instance molecular analysis (see below) or paleoecological data (Tiffney 2004).

By examining contemporary and historic gene flow across environmental gradients, molecular markers can help us elucidate some of these feedbacks (Oddou-Muratorio *et al.* 2010).

Using a hierarchical approach, one can scale from individual gene shadows to analyses of variance in allele frequency among populations and regions to measure levels of genetic differentiation and connectivity, with implications for incipient speciation (Broquet & Petit 2009). For instance, Voigt *et al.* (2009) studied the spatial genetic structure of two congeneric *Commiphora* plant species at the local and regional level. At the local scale (i.e., within forest sites), the Malagasy species with few dispersers and shorter seed dispersal distances, exhibited greater genetic structure than the South African species, with a diverse assemblage of frugivores and longer seed dispersal distances. At the regional scale (i.e., among forest sites), however, this pattern was reversed. This unexpected result was associated with the historical habitat distribution of *Commiphora* in both sites: longer persistence of ecosystems in Madagascar allowed for some level of gene flow across the island, until recent human-induced forest fragmentation. In contrast, naturally isolated patches of scarp forests since the Last Glacial Maximum in South Africa may have precluded high levels of gene flow across the region. Estimating the relative contribution of different factors acting at local, regional and historical levels on seed shadows (Fig. 4.4) would be extremely helpful, not only for understanding processes of seed dispersal, but to better evaluate its role in maintaining natural populations, communities and shaping the spatial distribution of plants over large geographic ranges and evolutionary time.

CONCLUSIONS

1- A mechanistic understanding of frugivore-mediated seed dispersal requires that we embrace animal ecology and characterize the environment more fully and comprehensively within an integrated framework. The number of variables that modulate seed dispersal outcomes,

however, is very large; they can relate to characteristics of the dispersed plants, animal dispersers, or environmental factors. Building the bridge between frugivory and seed deposition will require that we take full advantage of the new tools available for studying animal and seed movement, monitoring environmental and physiological factors, and analyzing large plant and animal community datasets.

2- Which variables to include in our studies will depend on the study system, the questions we aim to address, and the availability and cost of techniques. Studying all pertinent variables may sound impractical, but simulation models based on empirical data or theoretical concepts can help to evaluate particular hypotheses and predict resulting outcomes across relevant natural or anthropogenic gradients.

3- Seed dispersal processes and outcomes are highly context dependent, and results will mostly differ according to the scale. Advances in seed dispersal research are likely to emerge as we move from describing patterns to actually exploring the reasons why processes differ as we shift scales. To forecast seed dispersal and its outcomes (e.g., spatial distribution of plants), we first need to be able to identify relevant correlations between specific biotic and abiotic factors and pinpoint the scales at which these relationships emerge. Instead of predicting patterns, we should start predicting the magnitude of the effects certain factors and their interactions have on processes of interest.

Assessing how the relative importance of the factors that modulate frugivory and seed deposition scales-up over time and space and across taxonomic levels will require a hierarchical multi-scale approach. Such an approach is likely to foster the development of general principles in the study of frugivory and seed dispersal.

4- Building spatially-explicit mechanistic models that incorporate several plant, animal and environmental factors and investigating such processes at multiple spatial, temporal and taxonomic scales are challenging tasks. Much can be gained, however, from building collaborative working groups, which bring together plant, behavioral and physiological ecology with those studying movement ecology and mathematical modeling.

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Table 4.1- Intrinsic and extrinsic factors related to individual and populations traits of frugivores and plants, and abiotic characteristics of the environment that may affect the frugivory and seed deposition phases.

Factors	Frugivore	Plant	Abiotic
Intrinsic	Body size	Fruit size	NA
	Nutrient requirement	Crop size	
	Age / sex	Nutrient content	
	Digestive system	Color	
	Territory or home range size	Accessibility	
	Reproductive status		
	Spatial memory		
Extrinsic	Abundance	Abundance	Light incidence
	Competition	Plant aggregation	Temperature
	Predation	Community phenology	Topography
		Vegetation structure	Climate
			Soil

Table 4.2. Characteristics of the reviewed studies. Plant traits are related to the maternal plant and thus affect the frugivory phase. Animal traits affect seed deposition (GRT and Mov) and frugivory (Other). Deposition site characteristics (DSC) affect the seed deposition phase. Gut retention time (GRT) was estimated observing animals in the field (F) or in captivity (C), or extracted from theoretical probability distributions (T). Movement (Mov) of animals was directly observed in the field (O), measured using GPS-loggers (GPS), radio-telemetry (RT), spool-and-line (SL), inferred by conducting maternity analysis on dispersed seeds (G), or characterized theoretically (T). Seed shadow (SS) was characterized by direct observation (O), predicted using movement and GRT (P) or measured through maternity analysis on dispersed seeds (G).

Focus	Plant life form (No species)	Disperser (No species)	Habitat (Location)	Spatial extent	Period of study	Plant traits	Animal traits			DSC	SS	Reference
							GRT	Mov	Other			
Animal	Diverse	Monkey (1)	Atlantic forest and chaco (Argentina)	4 ha	2 yrs	0	F	O	5	Yes	O	Bravo (2009)
	Tree (1)	Elephant (1)	Deciduous forest (Sri Lanka/Myanmar)	NA	1 yr	0	C	GPS	2	No	P	Campos-Arceiz *et al.* (2008)
	Diverse	Tortoise (1)	Amazonian forest (Peru)	1,420 ha*	1 yr	0	F	RT	4	Yes	O	Guzman & Stevenson

												(2008)
	Diverse (8)	Hornbill (2)	Forest (Cameroon)	526,000 ha*	1 yr	1	C	RT	0	No	P	Holbrook & Smith (2000)
	Diverse	Tortoise (1)	Amazonian forest (Brazil)	8,000 ha*	3 yrs	0	F	RT, SL	1	No	P	Jerozolimski *et al.* (2009)
	Diverse (5)	Hornbill (1)	Fragmented sub-tropical forest (South Africa)	NA	2 yrs	0	C	GPS	0	Yes	P	Lenz *et al.* (2011)
	Bush (1)	Bird (1)	Open temperate forest (USA)	8 landscapes (50 ha ea)	2 yrs	0	C	O	3	Yes	P	Levey *et al.* (2008)
	Bush (1)	Bird (1)	Open temperate forest (USA)	8 landscapes (50 ha ea)	2 yrs	0	C	O	3	Yes	P	Levey *et al.* (2005)

	Diverse	Gibbon (1)	Dipterocarp forest (Indonesia)	<50 ha†	1 yr	1	F	O	0	Yes	P	McConkey & Chivers (2007)
	Diverse (6)	Turacos (3)	Tropical forest (Rwanda)	350 ha	14 mo	1	C	O	3	No	P	Sun *et al.* (1997)
	Diverse (9)	Monkey (1)	Tropical forest (Panama)	50 ha	4 mo	0	C	O	1	Yes	P	Wehncke *et al.* (2003)
	Diverse	Bird (3)	Vegetation mosaic (Hong Kong)	NA	2 yrs	0	C	RT, O	0	No	P	Weir & Corlett (2007)
	Shrub/treelet (6)	Bird (1)	Tropical forest (Costa Rica)	115 ha	1 yr	0	C	RT	3	No	P	Westcott & Graham (2000)
	Diverse	Cassowary (1)	Tropical forest (Australia)	NA	2 / 7yr	0	C	RT	0	No	P	Westcott *et al.* (2005)

	Diverse	Monkey (2)	Tropical forest (Colombia)	17-70 ha†	21 days	0	F	O	1	Yes	O	Yumoto *et al.* (1999)
Plant	Tree (1)	Mice (1)	Evergreen forest (Japan)	0.1 ha	< 5 mo	0	-	G	0	Yes	G	Abe *et al.* (2006)
	Shrub (1)	Bird (2)	Cattle ranch (Puerto Rico)	18 ha	3 mo	3	T	T, O	1	No	P	Carlo & Morales (2008)
	Tree (1)	Bird (3 groups)	Mediterranean (Spain)	25 ha	< 1.5 mo	8	-	G	0	Yes	G	García *et al.* (2009a)
	Mistletoe (1)	Bulbul (1)	Desert (Israel)	7 wadis (area NA)	14 mo	0	C	O	1	Yes	P	Green *et al.*(2009)
	Tree (1)	Toucans (2 groups)	Tropical forest (Ecuador)	84 ha	≈ 2 yrs	1	C	RT	1	No	P	Holbrook & Loiselle (2007)
	Tree (1)	Bird (3	Mediterranean	26 ha	4 yrs	0	-	G	1	Yes	G	Jordano *et*

		groups)	(Spain)									*al.* (2007)
	Theoretical	Bird	Theoretical	2,500 ha	30 days	2	T	T	2	No	P	Morales & Carlo (2006)
	Herb/shrubs (3)	Bird (3)	Tropical forest (Costa Rica)	14 plots (0.013 - 0.24 ha)	2 yrs	2	C	RT	0	Yes	P	Murray (1988)
	Tree (1)	Monkey (1)	Amazonian forest (Peru)	≈ 300 ha	2 yrs	1	F	O	4	Yes	P	Russo *et al.* (2006)
	Shrub (1)	Lizard (1)	Insular mediterranean (Spain)	2.91 ha	7 days	1	C, F	RT	4	Yes	P	Santamaría *et al.* (2007)
	Tree (1)	Woodpecker (1)	Oak savanna (USA)	2380 ha*	1 yr	0	-	G	0	Yes	G	Scofield *et al.* (2010)
	Shrub (1)	Bird (2)	Desert (Israel)	wadis (50 - 800 m	4 mo	1	C	RT, O	2	Yes	P	Spiegel & Nathan

				wide)								(2007)
	Herb (1)	Bird (2 groups)	Fragmented Amazonian forest (Brazil)	13 plots (0.5 ha ea)	1 yr	2	C	RT	4	Yes	P	Uriarte *et al.* (2011)
Plant/Animal	Palm (1)	Umbrellabird (1)	Tropical forest (Ecuador)	30 ha	NA	0	-	G	0	Yes	G	Karubian *et al.* (2010)
	Tree (1)	Macaque (1)	Evergreen forest (Japan)	70 ha	1 mo	0	-	G	0	No	G	Terakawa *et al.* (2009)

* Spatial extent based on area reported for reserve, not for study

† Spatial extent based on area reported for animal(s) home range

Table 4.3- Spatial, temporal and taxonomic scales considered by some of the reviewed studies.

	Scale aspects	Examples	Reference
Spatial	Fine vs. broad scale	Neighborhood vs. landscape	Westcott & Graham (2000), Jordano *et al.* (2007), Spiegel & Nathan (2007), Carlo & Morales (2008)
	Micro-habitat	Conspecific plants, sleeping trees	Yumoto *et al.* (1999), Wehncke *et al.* (2003), Russo *et al.* (2006), McConkey & Chivers (2007), Spiegel & Nathan (2007), Bravo (2009), Green *et al.* (2009)
	Habitat	Gaps, rock out-crop, shrub-dominated, grassland	Murray (1988), Yumoto *et al.* (1999), Westcott & Graham (2000), Abe *et al.* (2006), Jordano *et al.* (2007), Santamaría *et al.* (2007), Guzman & Stevenson (2008), García *et al.* (2009a), Karubian *et al.* (2010)
	Landscape	Fragmented forest	Levey *et al.* (2005, 2008), Lenz *et al.* (2011), Uriarte *et al.* (2011)
	Geographical	Biomes	Campos-Arceiz *et al.* (2008)
Temporal	Daily	Morning vs. afternoon	Westcott *et al.* (2005), Russo *et al.* (2006), McConkey & Chivers

			(2007), Santamaría *et al.* (2007)
	Monthly	Across months	McConkey & Chivers (2007)
	Seasonal	Dry vs. Wet	Weir & Corlett (2007), Campos-Arceiz *et al.* (2008), Guzman & Stevenson (2008), Jerozolimski *et al.* (2009)
	Year	Between years	Levey *et al.* (2005)
Taxonomic	Plants	Among plants	Murray (1988), Holbrook & Smith (2000), Westcott & Graham (2000)
	Animals	Between small vs. large birds	Murray (1988), Sun *et al.* (1997), Yumoto *et al.* (1999), Holbrook & Smith (2000), Holbrook & Loiselle (2007), Jordano *et al.* (2007) (2007), Spiegel & Nathan (2007), Weir & Corlett (2007), Uriarte *et al.* (2011)
	Within animals	Groups of monkeys	McConkey & Chivers (2007)

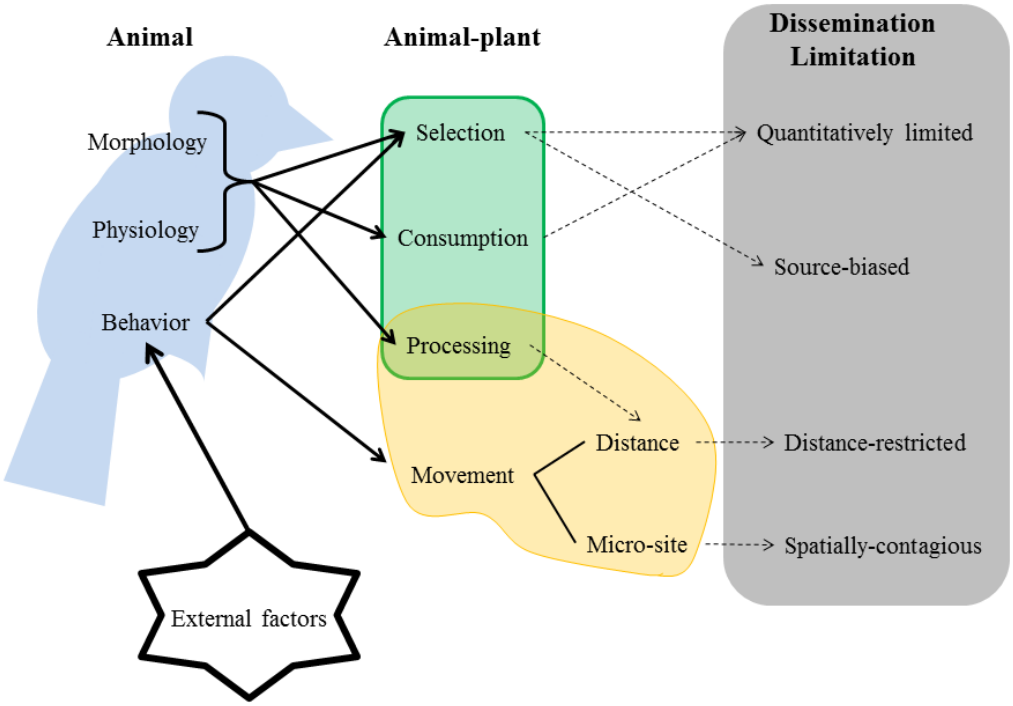
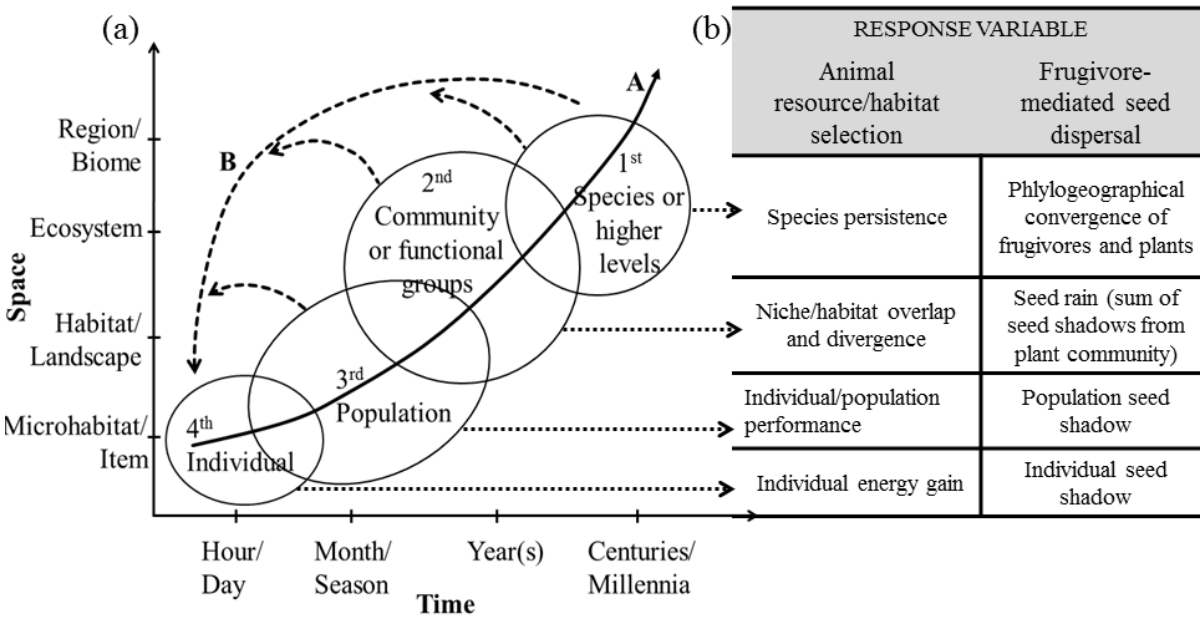


Figure 4.1- Schematic of the effects of animal characteristics during the frugivory phase (green box) and the seed deposition phase (yellow box). From the plant's perspective (gray box), different aspects of animal-plant interactions may result in distinct kinds of seed dispersal and dissemination limitation.



(b)

RESPONSE VARIABLE	
Animal resource/habitat selection	Frugivore-mediated seed dispersal
Species persistence	Phlylogeographical convergence of frugivores and plants
Niche/habitat overlap and divergence	Seed rain (sum of seed shadows from plant community)
Individual/population performance	Population seed shadow
Individual energy gain	Individual seed shadow

Figure 4.2- (a) Cross-scale variation in the processes that determine the organizational level of frugivory and seed deposition along a spatial and temporal gradient. Plant-frugivore interactions can be examined from different perspectives, as follows: A) Seed shadows are summed across individuals from single plants (fourth order) to higher organization levels (third and second orders) and matched to appropriate factors in each level (e.g., abundance of conspecifics or other fruiting plants). B) Factors included in the first order (e.g., regional species pool, rainfall) can influence individual seed shadows at finer scales. (b) Proxies for measuring the response variables in seed dispersal studies at each scale, adopting the framework for animal studies of habitat and resource selection (see text for details).

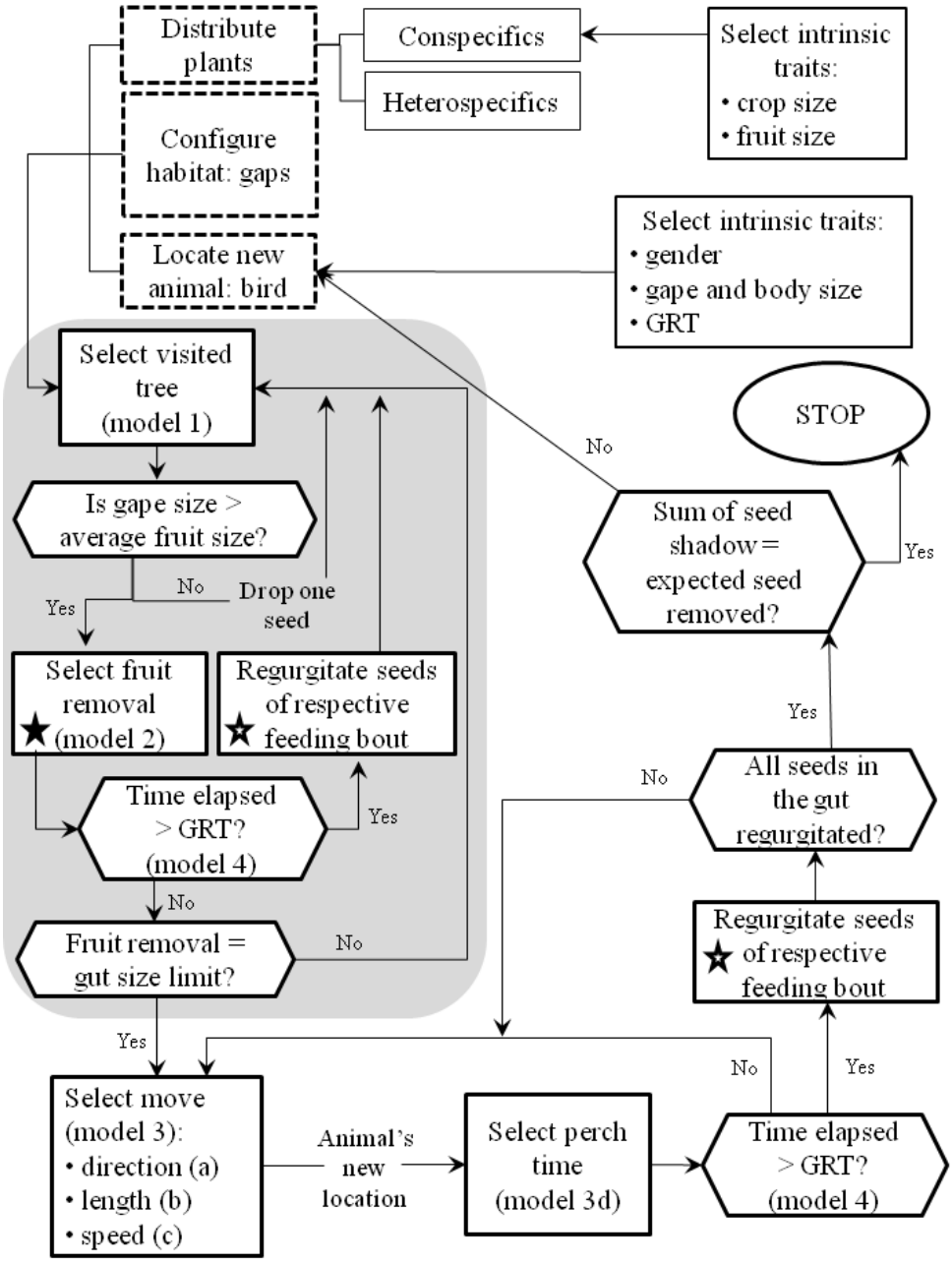


Figure 4.3- Flow diagram representing the steps within the seed dispersal simulation. The gray box encompasses the animal's internal state in which the feeding behaviors are governed by the urge to eat fruits from tree *T* (frugivory phase). It is assumed that after animal is satiated, animal's internal state changes to an unknown state and navigation is mediated by environment and motion capacity (taking most of the seed deposition phase). Black filled star indicates that identity of the maternal plant, location and time of feeding bout are registered. White filled star indicates the moment that time, location and number of deposited seeds are registered. See text for more details.

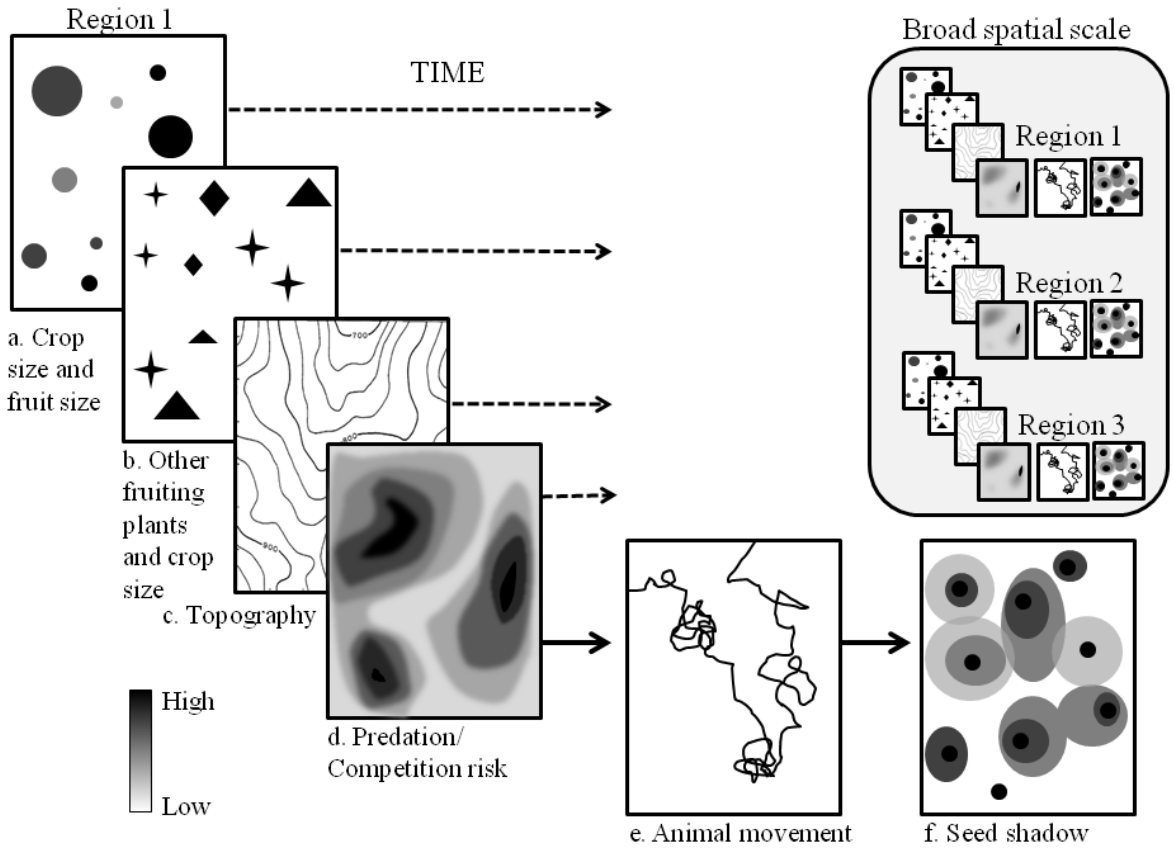


Figure 4.4- Schematic representation of a hierarchical multi-scale analysis of the factors that determine animal movement (e.) and seed shadow (f.) across multiple temporal and spatial dimensions. Different factors are spatially mapped and layers are overlaid to search for relationships among plant traits (a.), biotic (b.) and abiotic (c.) environmental characteristics, and animals traits (d.). Each map can be replicated over time at distinct resolutions generating, for example, a plant phenology map for the focal species (a. over TIME) and plant community (b. over TIME). The relationship among factors can be additionally studied at different spatial scales, from fine (individuals within maps) to regional scale (comparison of different regions at broad-spatial scale).

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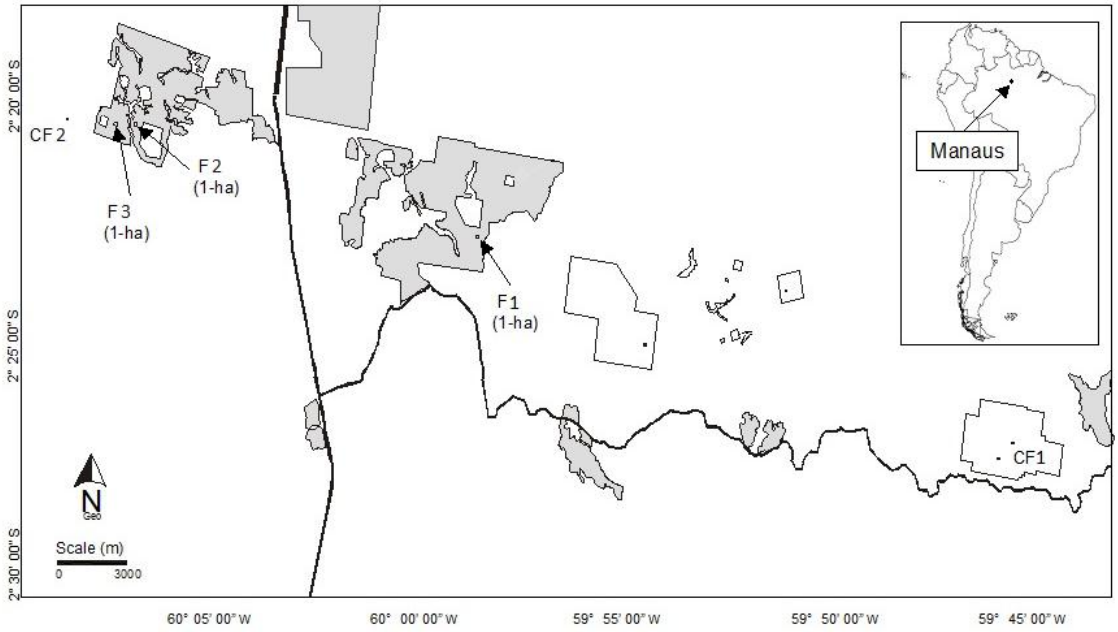
APPENDICES

SUPPLEMENTARY MATERIAL: CHAPTER 2

1- STUDY SITE



Supplementary Figure S2.1- Map of the study area with location of continuous forest sites (CF1 and CF2) and three 1-ha fragments (F1, F2 and F3).

2- IMPLEMENTATION OF THE BAYESIAN MODEL

a) Implementation:

In the genetic component of the model, the probability of observing the seedling's genotype, given its parental genotypes, $P(G_k^O|G_i^O,G_{i'}^O)$ is calculated by producing an (n_a+1) x (n_a+1) matrix of parentage probabilities for each seedling, with rows representing the identified potential mothers within the plot, plus a hypothetical mother from outside the plot, and columns representing the identified potential fathers within the plot, plus a hypothetical father from outside the plot. The matrix represents the probability that a seedling *k* is produced by mother *i'* and father *i*, relative to all potential parental combinations, given genotyping errors and

Mendelian inheritance. This step is conducted separately and is independent of ecological variables and dispersal parameters.

Priors were defined based on previous information about the pollen and seed vector movements. A prior of u_s = 500 (standard deviation 1000) was used for seed dispersal kernel, representing a conservative mode dispersal distance of 18 m, which is the average seed dispersal distance found for the main frugivores of *Heliconia* in the study area (Uriarte *et al.* 2011). A prior of u_p = 2000 (standard deviation 1500) was used for pollen dispersal kernel, corresponding to a conservative modal pollination distance of 36 m. Although there is no data on hummingbird movement in the area, these pollinators can move across the heterogeneous landscape at the study site and elsewhere, covering distances of 1000 m in less than four hours (Hadley & Betts 2009; Stouffer & Bierregaard 1995a). Parameters u_p and u_s were drawn from a normal truncated distribution, with lower and upper bound of 10 and 15,000 for u_p and 10 and 3,000 for u_s. We used different priors to evaluate the effect of changing initial conditions on the recovery of the dispersal kernel parameters (Supplementary material S2.2b).

The contributions of adult plants from outside the plot could be incorporated in the model by assigning hypothetical plants at 10 interval distances of 10 m, going outwards from the sampled plot. Pollen and seed production outside the plots was calculated as the mean production within plots, multiplied by the plant density within each of the rectangular rings delimited by the hypothetical plants. For populations in continuous forest sites (CF1 and CF2), density of flowering plants outside plots was set as the density of flowering plants within plots, under the assumption that density, although variable in space, is homogeneous within a continuous forest of similar environmental conditions. By contrast, areas outside fragment plots encompass secondary growth vegetation where density of *Heliconia acuminata* is, in general, lower than in

forested areas (Bruna & Ribeiro 2005). We set the density of flowering plants to 0.0008 plants/m^2 outside the Dimona plots (F2 and F3) and 0.0012 plants/m^2 outside F1, based on the results of Bruna & Ribeiro (2005). Genotypes of hypothetical plants, as well as of plants with missing genotypes at locus *l*, were set as the product of the allele frequencies of the respective population (Moran & Clark 2011). Results may be sensitive to the density of hypothetical parents outside the sampled plot. It is likely that density of flowering *H. acuminata* will increase as secondary forest regenerates in the matrix, which may lead to changes in animal space use and movement. Thus we repeated the analysis by setting the density of flowering plants outside plots equal to density of plots inside the fragments (Supplementary material S2.2.c).

Implementation of Gibbs sampler:

1. Chain is initialized by generating for each seedling a random pedigree $P_k=(m_k,f_k)$ following a multinomial distribution of the parentage probabilities matrix. For each step of the Gibbs sampler,
2. Parameters u_s and u_p are sampled from a normal truncated distribution given P_k. The conditional distribution is:

$$p(u_p, u_s|P)$$

$$= \prod_k \left[\left(\frac{c_{i\prime}\, s_{i\prime i} p(d_{i\prime i}|u_p) f_i r_{ik} p(d_{ik}|u_s)}{\sum_{i,i\prime} c_{i\prime} s_{i\prime i} p(d_{i\prime i}|u_p) f_i r_{ik} p(d_{ik}|u_s)}\right)\right] p(u_p|m_p, s_p) p(u_s|m_s, s_s) \qquad \text{(Eqn. S2.2.a1)}$$

Where *i* and *i'* are the inputted father and mother, m_p and m_s are the prior mean values and s_p and s_s are the prior standard deviations of the normal truncated distribution. If the conditional

probability based on the new values (p_{new}) is greater than the conditional probability of the current values (p_{now}), p_{new} is accepted. If $p_{new} < p_{now}$ then the new values are accepted with probability $a = p_{new} / p_{now}$.

1- Pedigree P_k is sampled conditioned on u_s and u_p parameters. New mothers and fathers are proposed for each seedling by sampling from the parentage probability matrix. Now, parental pair which parentage probability for seedling k is 0 is excluded from pool of combinations. This step is to prevent sampling parent combination with incompatible genotypes. The conditional probability of the proposed pedigree given proposed parameters is:

$$p(P_k = (i,i')|u_s, u_p) = p(d_{ii'}, d_{i'k}|u_s, u_p, P_k)p(G_k^O|G_i^O, G_{i'}^O)$$

$$= \frac{c_{i'}\, s_{i'i} p(d_{i'i}|u_p) f_i r_{ik} p(d_{ik}|u_s)}{\sum_{i,i'} c_{i'} s_{i'i} p(d_{i'i}|u_p) f_i r_{ik} p(d_{ik}|u_s)} \frac{p(G_k^O|G_i^O, G_{i'}^O)}{\sum_{i,i'} p(G_k^O|G_i^O, G_{i'}^O)} \quad \text{(Eqn. S2.2.a2)}$$

The new pedigree is accepted or rejected using the same approach as described in the previous step.

4. Steps 2-3 are repeated until chain reaches the defined total number of iterations. For each chain we run 100,000 simulations with a burn in of 50,000.

b) Effect of varying priors on posteriors

Results may be sensitive to the chosen priors, thus, to evaluate the effect of different initial conditions on the recovery of the parameters we tested different prior means of pollen (800, 2000, 8000) and seed dispersal (100, 500, 2000) parameters and standard

deviations for pollen (500, 1500, 4000) and seed dispersal (500, 1000, 1500). We selected only one population (F2) to run different priors.

In general, mean posteriors were more sensitive to standard deviations than to mean priors and variation was higher for pollination parameters, most likely because range of values were wider than for seed dispersal. Mean posteriors of pollination varied from 2,400 to 14,000 and seed dispersal varied from 1,800 to 2,800. Lower mean posteriors of seed dispersal (almost half of the current value) were found at low mean priors (100 and 500) and standard deviation (500). In all other conditions, mean posteriors did not change considerably (Table S2.2.b). Mean posteriors of pollen dispersal also showed a higher increase at low standard deviation values, increasing 2.5 times at standard deviations of 500 and only 1.4 times at standard deviation of 4000 (Table S2.2.b). Two-folding the standard deviation increased the mean posterior of seed dispersal parameter by less than 50% at low priors and no differences were found among the posteriors by increasing standard deviations at high mean prior (Table S2.2.b). For pollen dispersal parameters, tripling the standard deviations doubled the mean posterior on average, with decreased difference as the mean prior increased (Table S2.2.b).

The results were not highly sensitive to changes in prior means but more sensitive to standard deviations. Influence of wider standard deviations to parameters and pedigree recovery have been associated to low number of zero genetic mismatches inside the sampled plot, which is pronounced under high genotyping errors and few parents within plots (Moran & Clark 2011). Also, higher uncertainty around the parameter values were found for populations with lower sample size (CF2), which was further worsen by the

high immigration rate and thus reduced number of parent pairs within sampled plot (Chybicki & Burczyk 2010b).

Supplementary Table S2.2.b- Summary of posterior means (and support interval) obtained for pollen and seed dispersal based on different priors (mean and standard deviations) for population F2. Underlined numbers highlighted in bold face indicate the parameters used in the present study.

Pollen dispersal parameters			Seed dispersal parameters		
Prior mean	SD	Posterior mean and Credible interval	Prior mean	SD	Posterior mean and Credible interval
800	500	2400 [1700-3100]	100	500	1800 [1200-2500]
800	1500	4800 [2100-7600]	100	1000	2700 [2300-3000]
800	4000	11000 [8900-14000]	100	1500	2800 [2400-3000]
2000	**500**	3100 [2400-3900]	**500**	**500**	2100 [1500-5800]
2000	1500	6100 [4300-7600]	500	1000	2700 [2300-3000]
2000	4000	10000 [7300-12000]	500	1500	2800 [2500-3000]
8000	500	8200 [7400-9200]	2000	500	2700 [2200-3000]
8000	1500		2000	1000	2700 [2200-3000]
8000	4000	14000 [11000-15000]	2000	1500	2700 [2200-3000]

c) Effect of density of hypothetical parents on posteriors

Fragments may be less isolated in terms of quantity of resources in the surroundings. To test the effect of higher density of plants in the matrix we increased the density of hypothetical parents outside the plots in the gene dispersal model. Setting the flowering density equal to flowering density inside forest fragments reduced distances of pollination and seed dispersal in all three fragments (Table S2.2.c). Posterior mean of pollen dispersal decreased around 3 times in F1 and F2 and less than 2 times in F3. Decrease in posterior mean of seed dispersal was more subtle in F1 and F2, decreasing by around 15% (Table S2.2.c). Seed dispersal parameter of population F3, however, was 4 times lower comparing to the original scenario (Table S2.2.c).

Changing the initial conditions in the gene dispersal model altered the pollen and seed dispersal distances of *H. acuminata*. Assuming a greater number of flowering plants outside sampled plots in fragmented sites decreased gene dispersal distances in all three fragments. This is expected given the potential role that density of flowering plants have on behavior and patterns of space use by birds. That is, we created a scenario of low patch isolation so animals have access to a continuum of food resources throughout the environment. Given the availability of resources in the neighborhood, animals do not need to move further distances to find food, and therefore, do not disperse propagules at longer distances.

Supplementary Table S2.2.c- Posterior means (and support intervals within brackets) of the pollination parameter (u_p) and seed dispersal parameter (u_s) for the current scenario in which density of flowering plants outside plots is lower than within fragment and new scenario in which density of flowering plants outside plots is equal inside fragments. Values highlighted in bold face indicate non-overlapping support interval between current and new scenarios.

Fragment	Modal pollination distance		Modal seed dispersal distance	
	Current	New	Current	New
F1	58 [47-64]	36 [27-44]	43 [39-45]	39 [27-44]
F2	66 [55-77]	34 [20-46]	40 [30-43]	39 [32-44]
F3	64 [54-71]	50 [41-63]	42 [39-45]	19 [12-28]

3- PROBABILITY OF PARENTAGE IDENTITY (PPAI)

Probability of parentage identity was calculated for each *Heliconia acuminata* population, using the *r* - estimator, as follows:

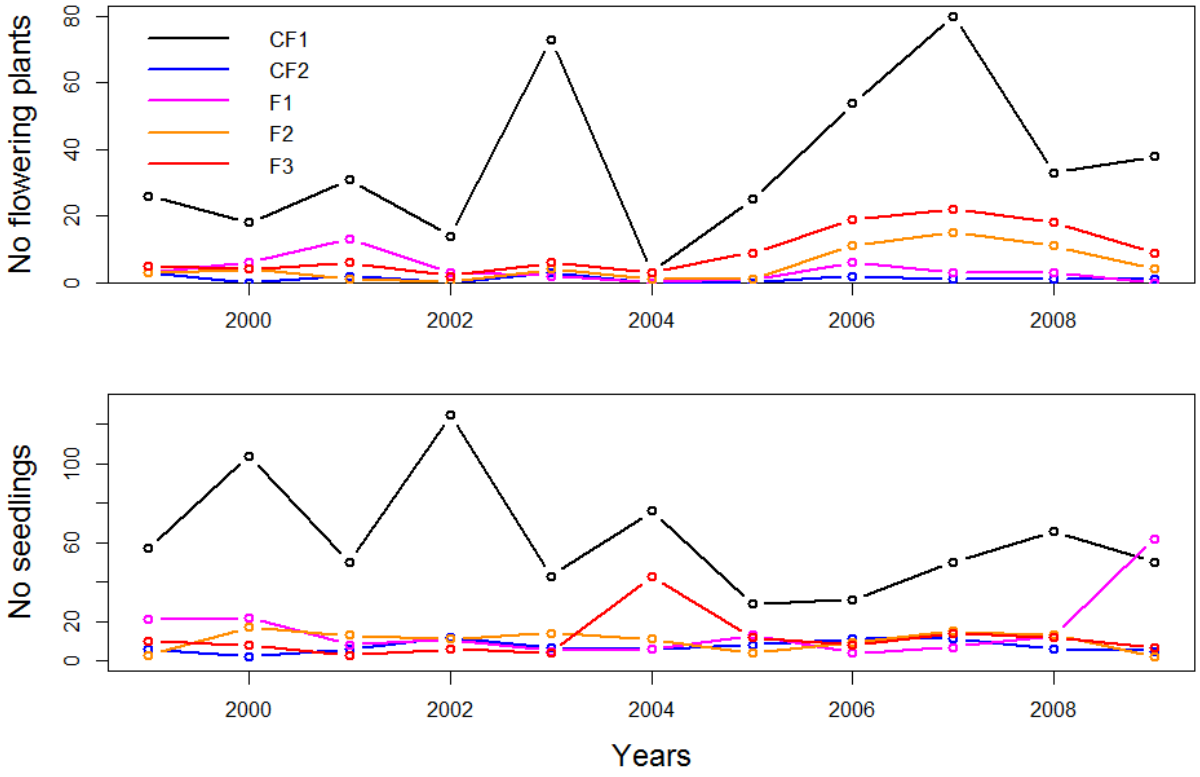
$$R_0 = \sum_{k=1}^{K} \left(\frac{X_k(X_k-1)}{2N(2N-1)} \right) \quad \text{(Eqn. S2.3)}$$

Where, X_k is the number of seedlings that plant *k* either fathered or mothered, and *N* is the total number of offspring recruited for which either father or mother are inside the sampled plot. A total of two sets of chromosome, one coming from each parent, are multiplied by the number of offspring. The total number of chromosome sets is used instead of number of offspring because we are interested in the contribution of both fathers and mothers to the seedlings genotypes, meaning that one seedling can have up to two inside plants contributing to its whole genotype.

4- FLOWERING AND SEEDLING PHENOLOGY

The proportion of flowering plants was very low in all populations but was higher for CF1 and F3, with around 15% of all recruited plants flowering at least once during the 11 year-study

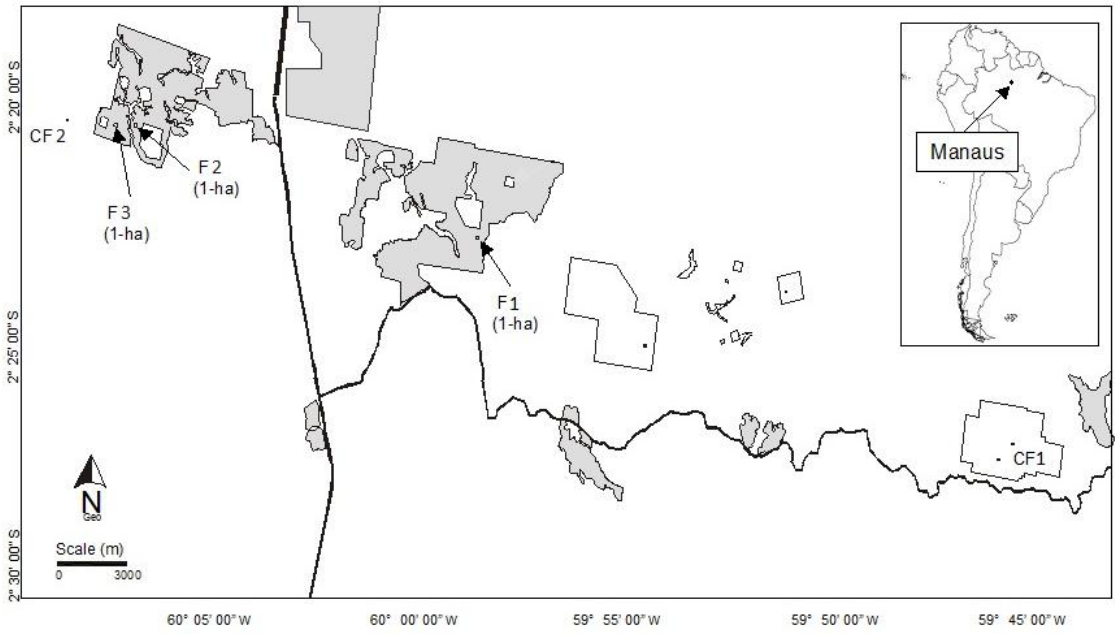
period. In contrast, only about 7% of the plants flowered in CF2, F1 and F2, with some years lacking any flowering events (Table 2.1, Fig. S2.4).



Supplementary Figure S2.4- *Heliconia acuminata* phenology at the study site: A. Number of flowering plants, and B. Number of seedlings recruited along the 11-yr study period. B. depicts the total number of seedlings ever recruited, including the ones that were not included in the genetic analysis.

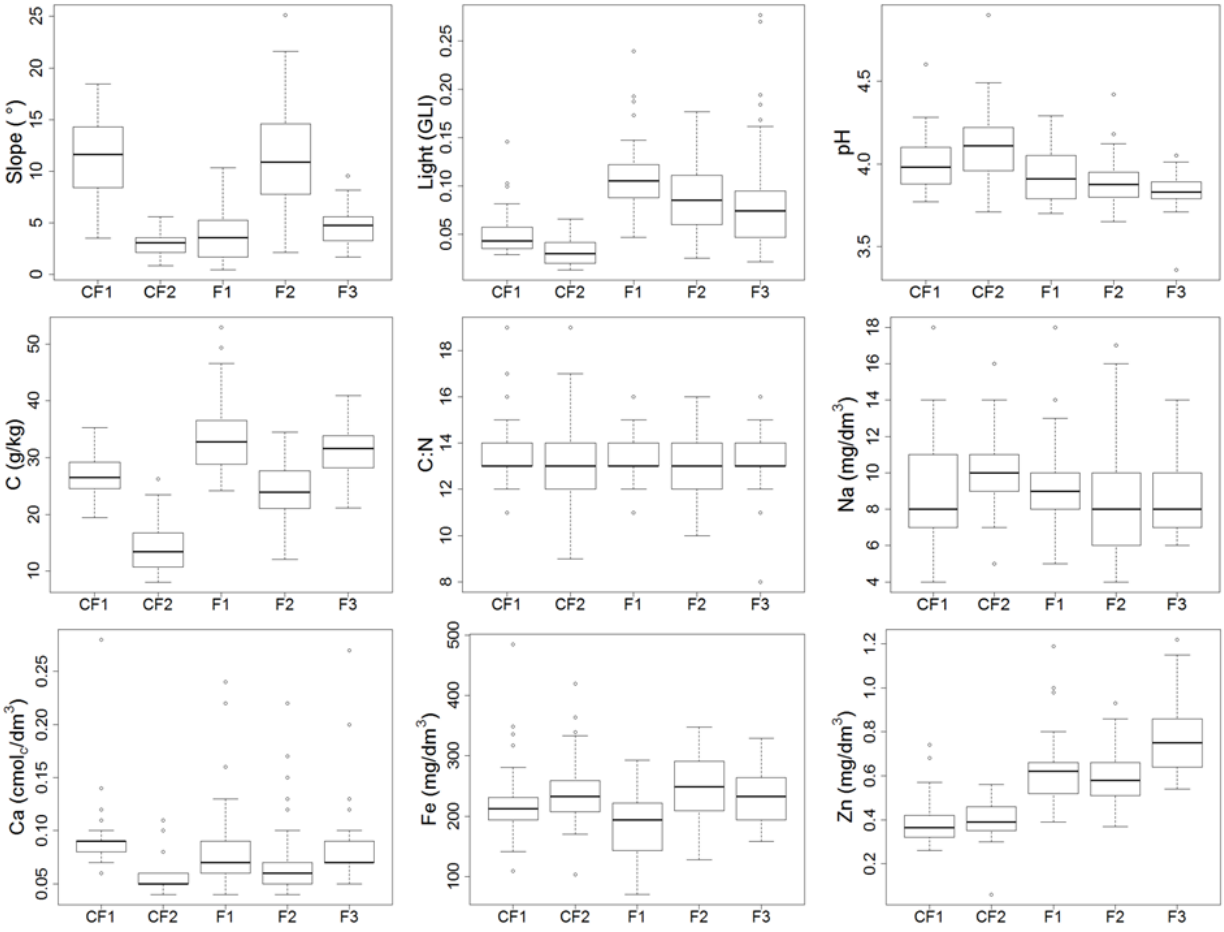
SUPPLEMENTARY MATERIAL: CHAPTER 3

1- STUDY SITE

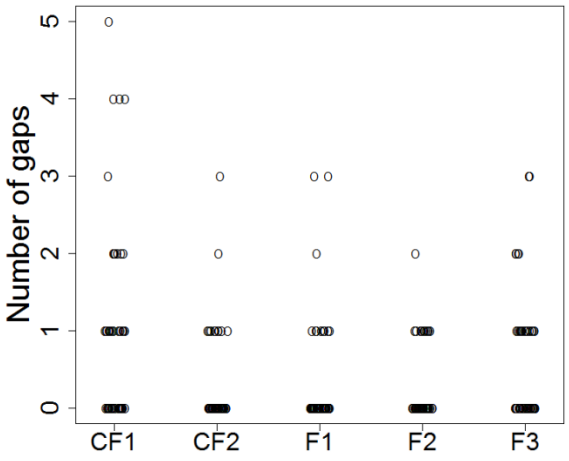


Supplementary Figure S3.1- Map of the study area with location of continuous forest sites (CF1 and CF2) and three 1-ha fragments (F1, F2 and F3).

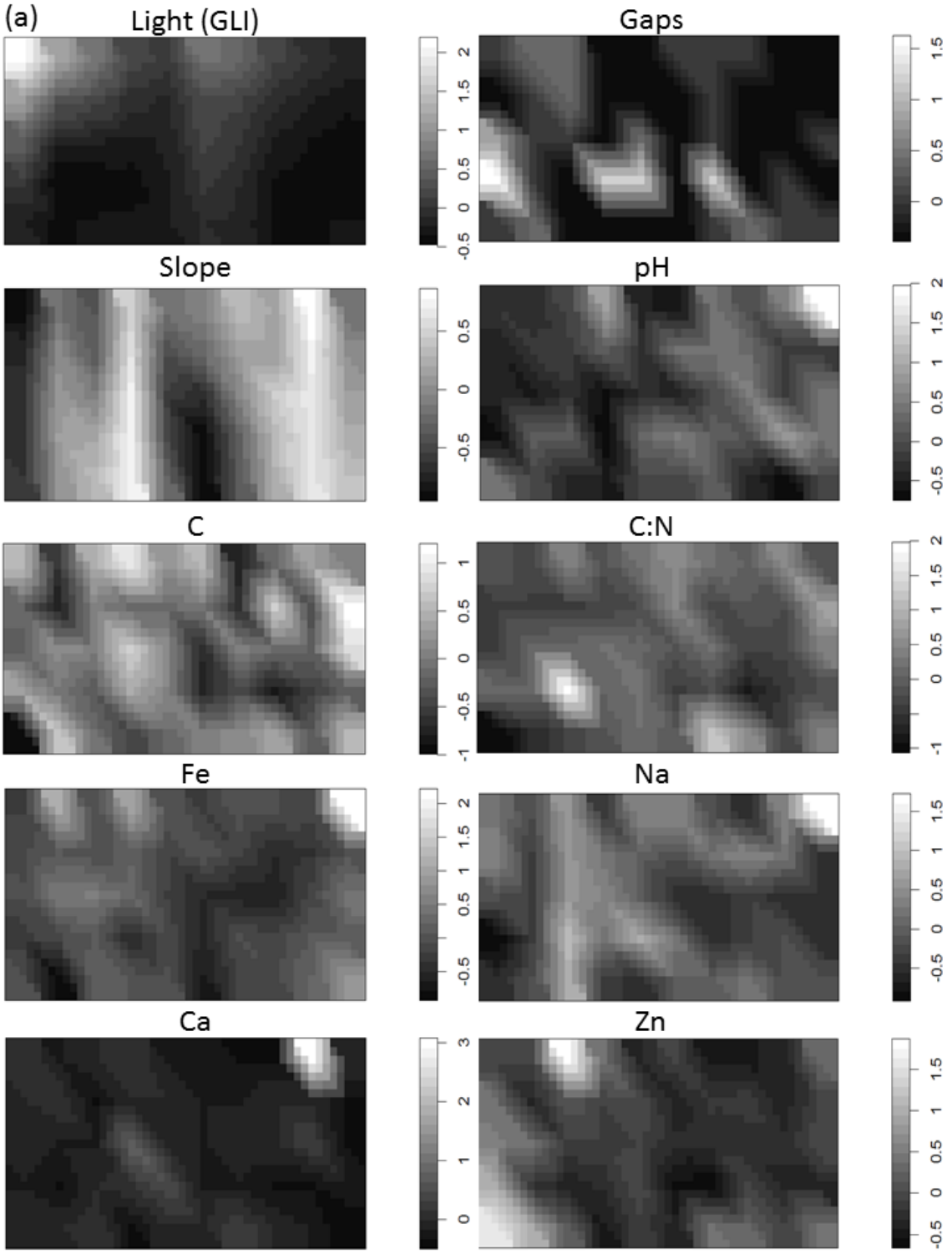
2- VARIATION OF ENVIRONMENTAL COVARIATES

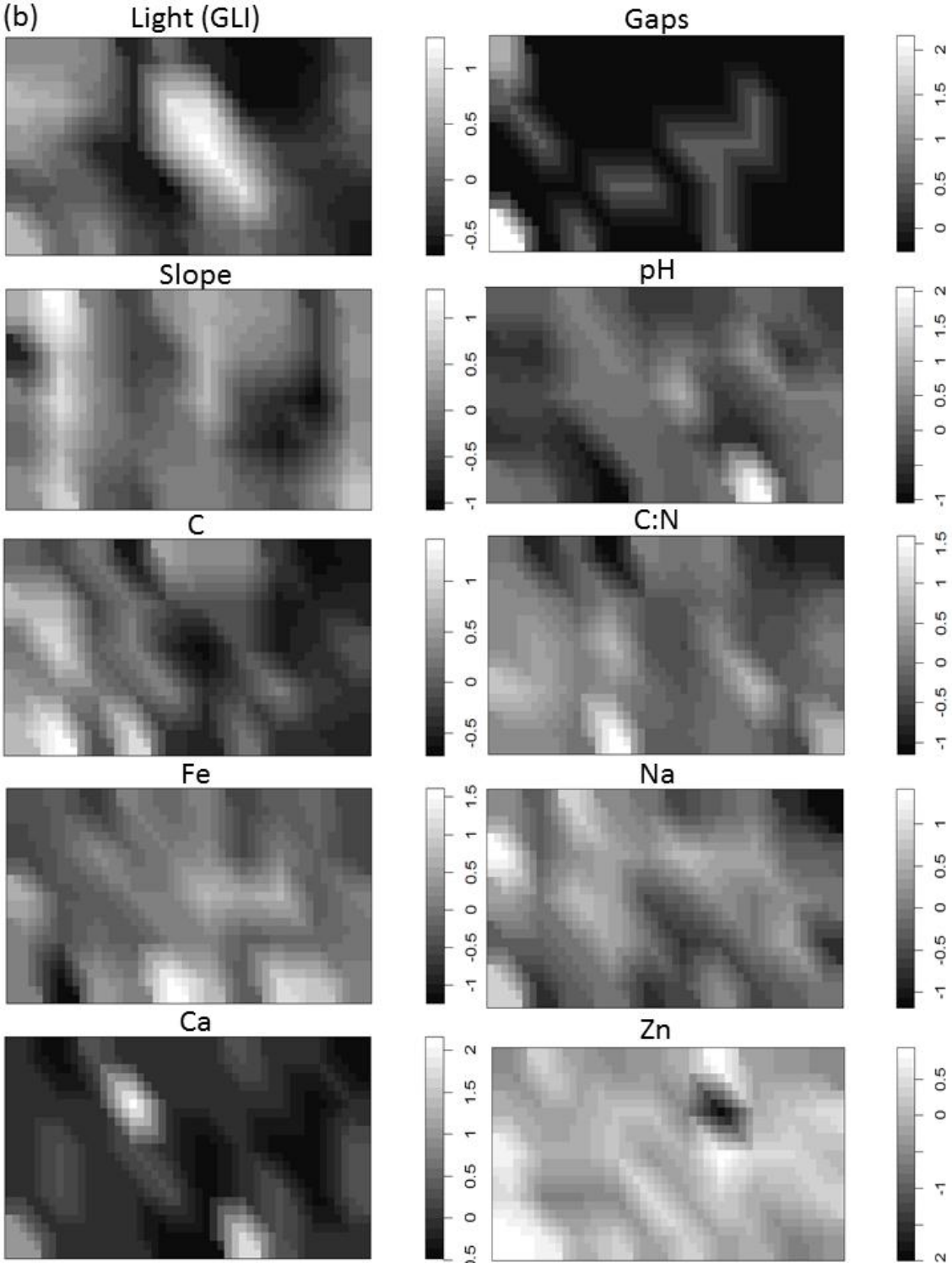


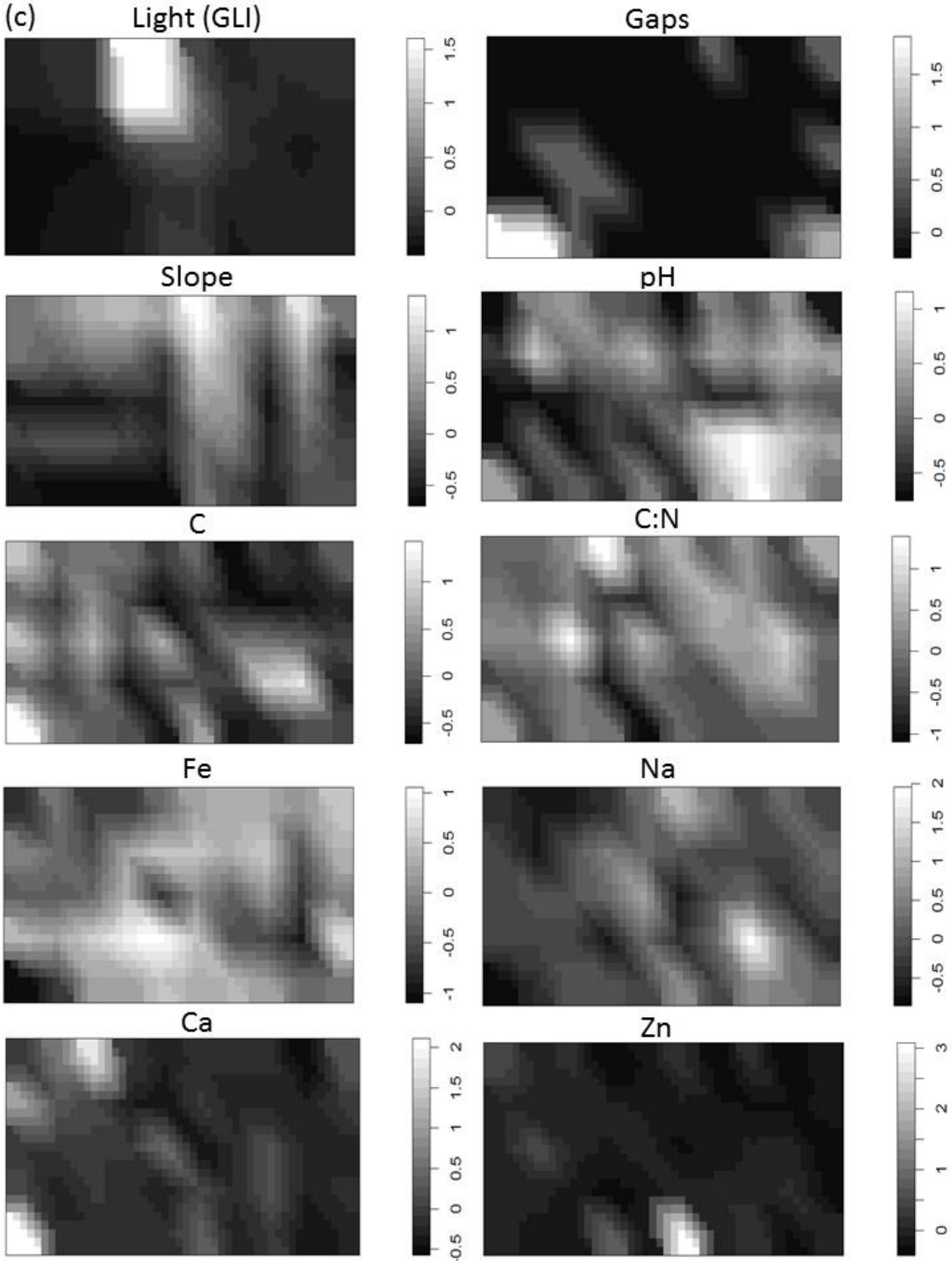
Supplementary Figure S3.2a - Boxplots of the environmental covariates used in the analyses to characterize environmental heterogeneity in each plot. Variation is shown for raw values across the original scale of 10 x 10 m (N = 50 subplots).

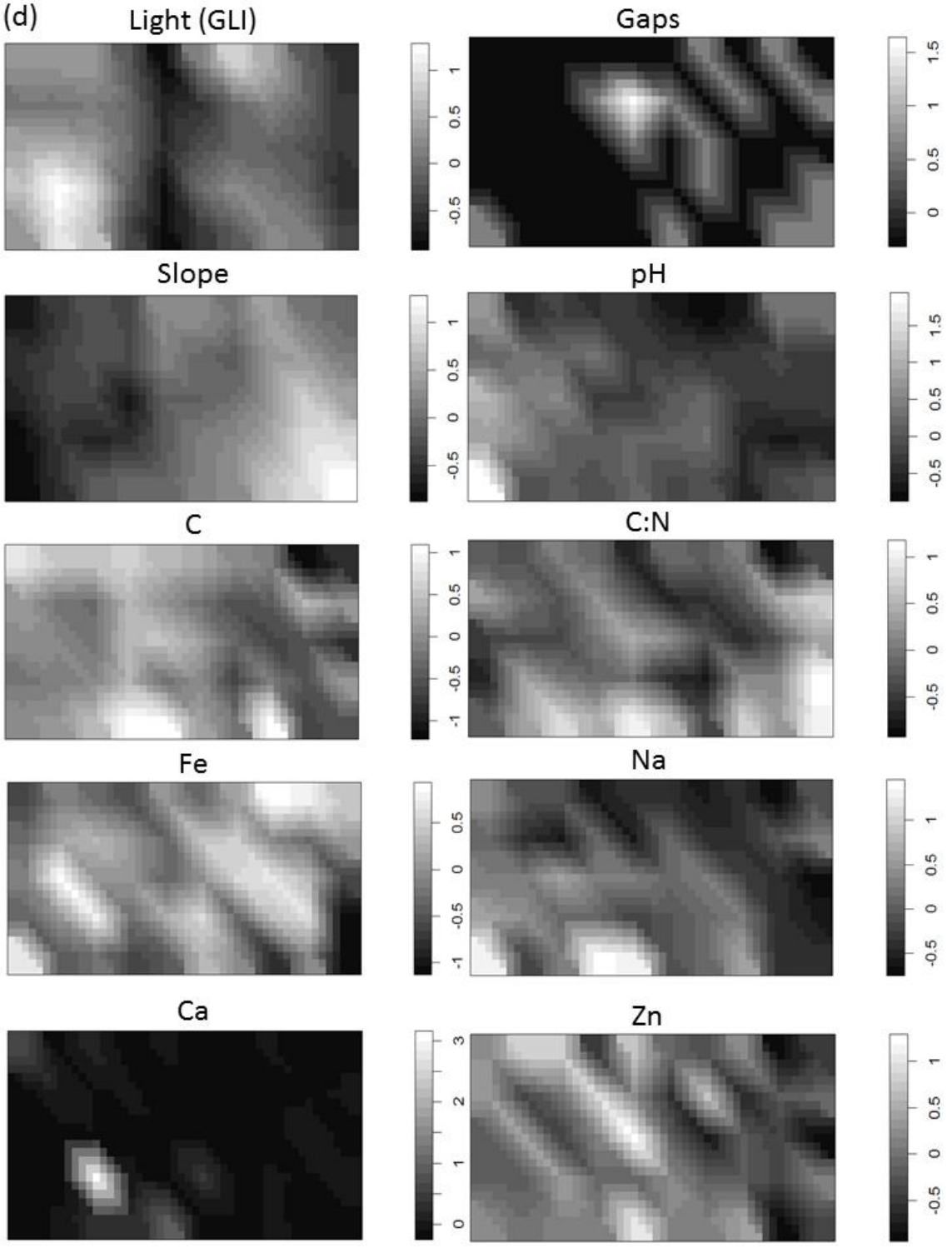


Supplementary Figure S3.2b - Number of gaps formed in each plot between 1999 and 2009. Values are jittered to allow visualization of frequency of occurrence of gaps measured at the 10 x 10 m scale (N=50 subplots).


(a)
Light (GLI)
Gaps
Slope
pH
C
C:N
Fe
Na
Ca
Zn


(b)
Light (GLI)
Gaps
Slope
pH
C
C:N
Fe
Na
Ca
Zn


(c)
Light (GLI)
Gaps
Slope
pH
C
C:N
Fe
Na
Ca
Zn


(d)
Light (GLI)
Gaps
Slope
pH
C
C:N
Fe
Na
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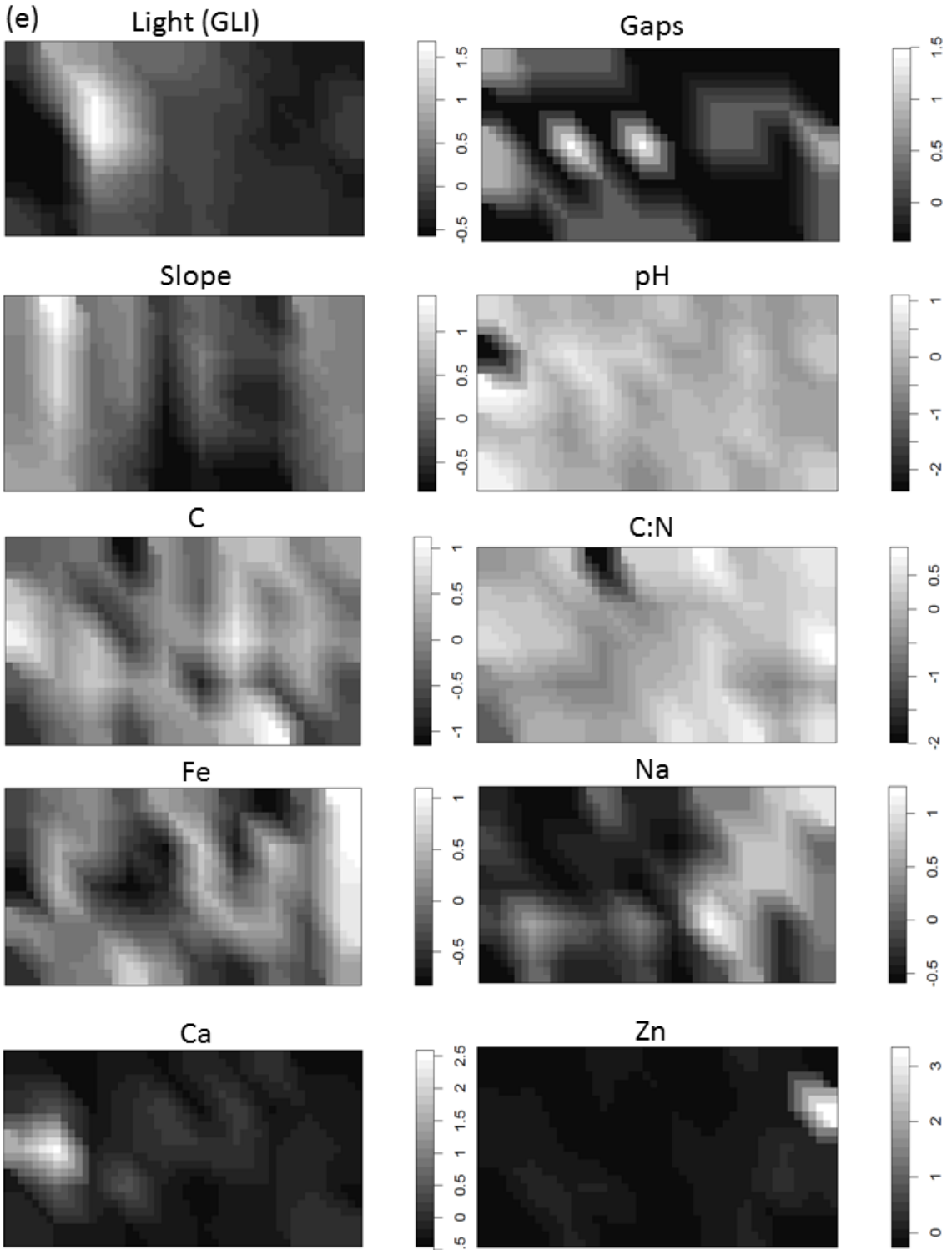
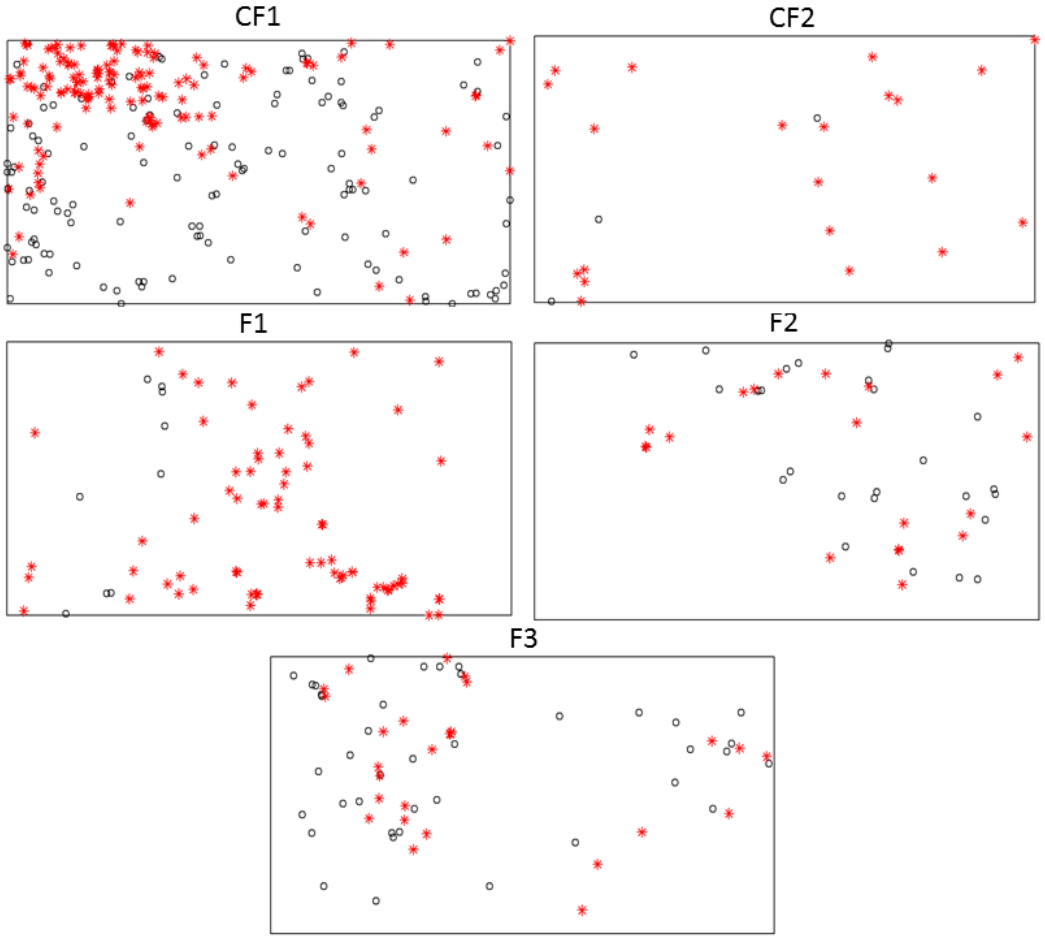
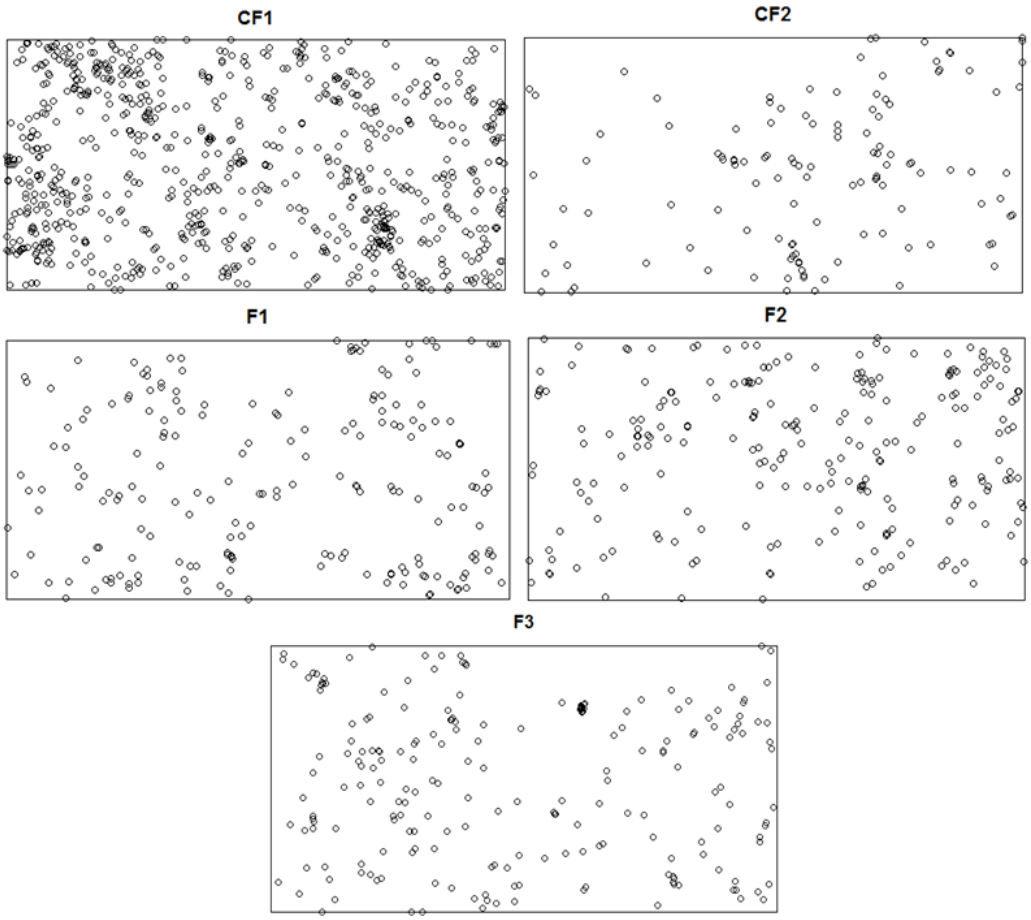


Supplementary Figure S3.3 - Map of the standardized and interpolated environmental covariates represented at the 2 x 2 m scale in each plot: (a) CF1, (b) CF2, (c) F1, (d) F2, and (e) F3.

3- POINT PATTERNS OF SEEDLINGS, REPRODUCTIVE PLANTS AND ADULTS

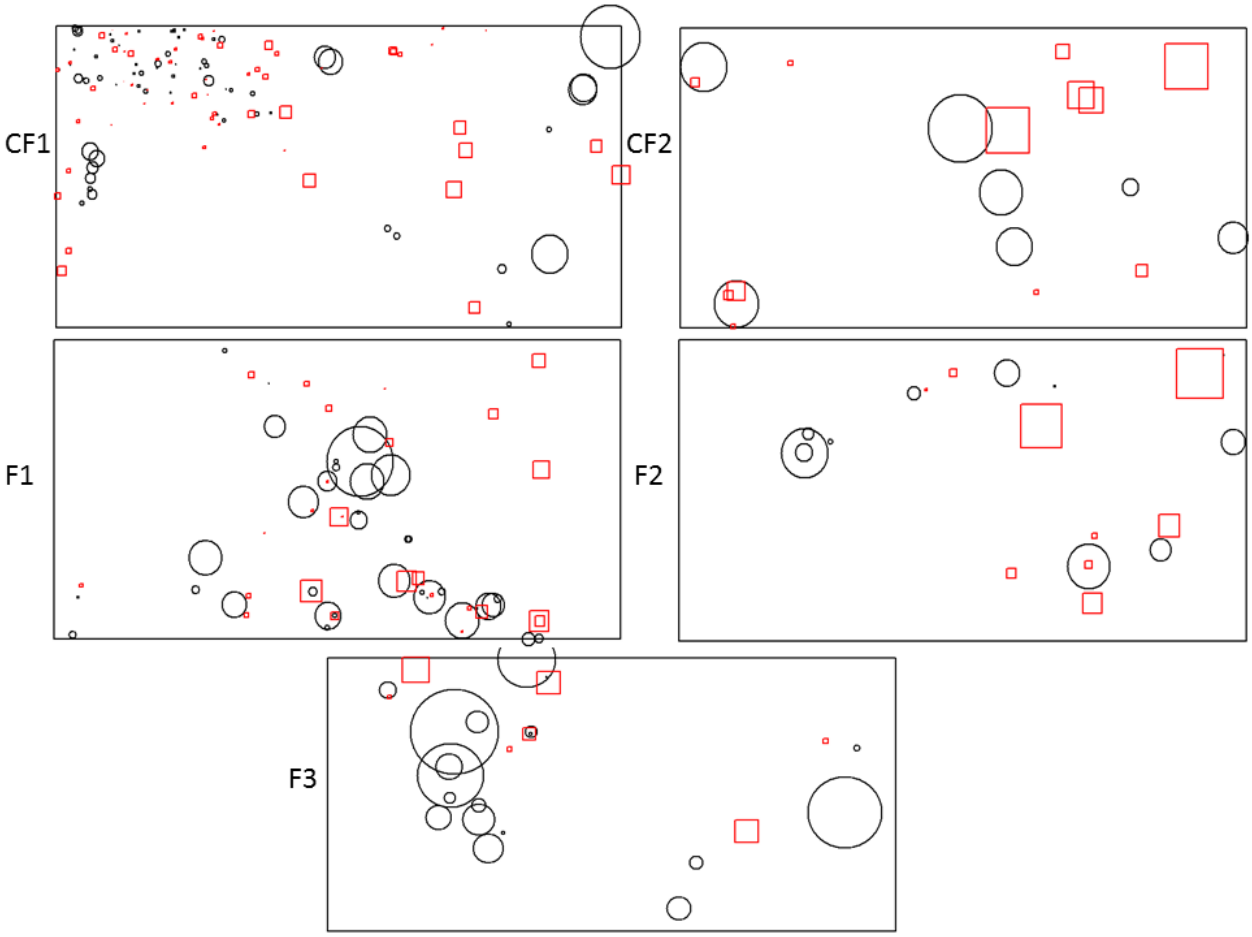


Supplementary Figure S3.4 - Point pattern of seedlings (red stars) and reproductive plants (black circle) of *Heliconia acuminata* in each plot.

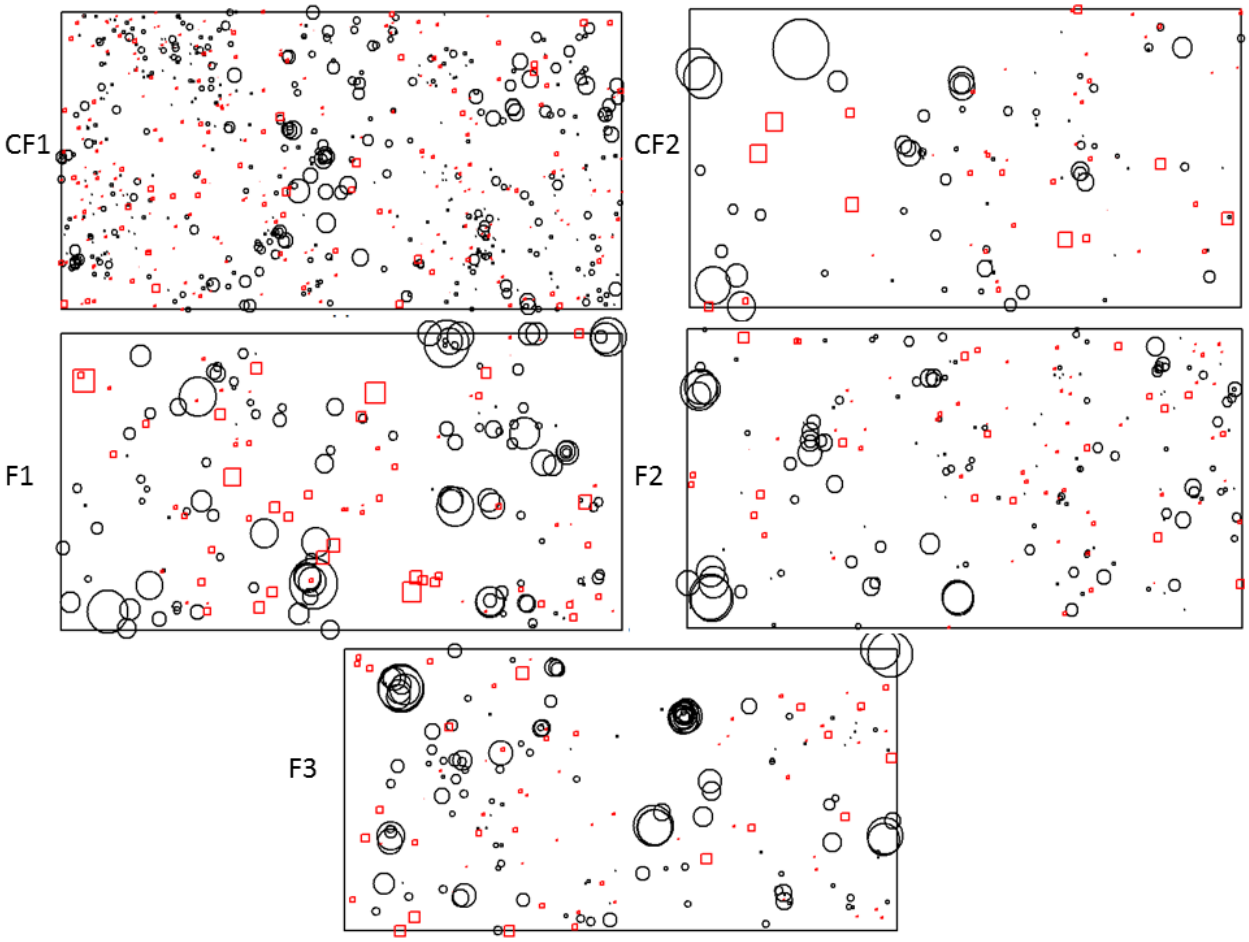


Supplementary Figure S3.5 - Point pattern of adult plants of *Heliconia acuminata* in each plot.

4- MARKED POINT PATTERN OF SEEDLINGS AND ADULTS



Supplementary Figure S3.6 - Marked point pattern of seedlings of *Heliconia acuminata* in each plot. Size of symbol is proportional to relatedness with neighbors. Positive and negative relatedness values are represented by black circles and red squares, respectively.



Supplementary Figure S3.7 - Marked point pattern of adult plants of *Heliconia acuminata* in each plot. Size of symbol is proportional to relatedness with neighbors. Positive and negative relatedness values are represented by black circles and red squares, respectively.