REGENERATION OF ANIMAL-DISPERSED TREE SPECIES IN TROPICAL PREMONTANE WET FOREST FRAGMENTS

by

ANNA SUGIYAMA

(Under the Direction of Chris J. Peterson)

ABSTRACT

Tropical wet forests support one of the most diverse terrestrial ecosystems, yet are currently subject to forest fragmentation globally. Despite such prevalence, studies in the tropics come predominantly from lowland forests. Forest fragmentation effects may differ regionally and applying insights from a few well-studied sites elsewhere may be misleading. Inequality also exists in fragmentation effects on different functional groups. Regeneration of animal-dispersed, shade-tolerant tree species are considered most vulnerable to forest fragmentation but light requirements may change ontogenetically, and our understanding of how forest fragmentation may affect different growth stages is limited. I studied various stages of regeneration of five animal-dispersed tree species in tropical premontane wet forest fragments in Costa Rica. First, I assessed forest fragmentation effects on various early regeneration stages and demonstrated that not all stages are equally affected, but rather effects are limited to particular times. I also compared germination of seeds from small and medium-sized fragments and showed consistently higher germination from small fragments, in contrast to results from most previous studies. Then, I ranked relative light requirements of species whose life-history traits were unknown and tested whether species with lower light requirement are more negatively affected by forest fragmentation for all size classes. There was evidence for ontogenetic effects of forest fragmentation. The general prediction that species with low light requirement are more vulnerable to forest fragmentation did not apply at an individual-species level. Finally, I assessed the biological aspect of species regeneration by testing the Janzen-Connell hypothesis in forest fragments. The Janzen-Connell hypothesis was supported for all non-pioneer species but only for the smallest size class, and the underlying mechanisms proposed by the Janzen-Connell hypothesis were largely rejected. While mixtures of species with different life histories may result in complex spatiotemporal plant dynamics, the spatiotemporal dynamics of some species may be predictable based on positive distance-dependent survivorship. To conclude, my results emphasize the importance of studying various regeneration stages and individual sizes in underrepresented study systems. Such studies should provide better understanding of the species and underlying mechanisms for species regeneration in fragmented landscapes, allowing significant interface between biological understanding and conservational applications.

INDEX WORDS: Abundance, animal-dispersed tree, biotic infection, coefficient of skewness (g_1), conservation, Costa Rica, distance-dependence, *Drypetes brownii* (Putranjivaceae), edge effect, establishment distance, *Ficus tonduzii* (Moraceae), fruit size, germination, growth, invertebrate herbivory, Janzen-Connell hypothesis, *Lacistema aggregatum* (Lacistemataceae), Las Cruces Biological Station, light environment, light requirement, maternal effect, medium-sized fragment, ontogenetic shift, pioneer species, pre-dispersal predation, population recruitment curve, post-dispersal predation, *Quararibea aurantiocalyx* (Malvaceae), realized dispersal, regeneration, restoration, secondary dispersal, seed dispersal, seedling establishment, seed source, size class, size distribution, spatiotemporal dynamics, shade-tolerant species, stage-specificity, survivorship, *Tapirira mexicana* (Anacardiaceae), tropical premontane wet forest

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DEDICATION

To my parents, Yukiko and Yoshikuni Sugiyama.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1. Prevalence of forest fragmentation and bias in existing studies

Tropical forests are among the most diverse terrestrial ecosystems, and such biodiversity supports a variety of ecosystem functioning and services (Balvanera *et al.*, 2006; Isbell *et al.*, 2011). They are irreplaceable in supporting high biodiversity (Gibson *et al.*, 2011) but forest cover is decreasing globally at an alarming rate. While reduction rate in forest area has decreased by about 3 million ha per year compared to that 10 years ago, forests are still disappearing by 5.2 million ha per year, which is an area about the size of Costa Rica (FAO, 2011). Most deforestation is caused by land conversion for agriculture and urbanization (FAO, 2011). Much of the slowdown in deforestation is primarily caused by reforestation in temperate and boreal zones, and most forest loss still takes place in tropical regions (FAO, 2011). Furthermore, owing to detectability of different types of disturbances in tropical forests using remote-sensing, apparently intact forests may actually be far more disturbed than what we realize (Peres *et al.*, 2006). Along with such worldwide deforestation, areas that are forested are subject to fragmentation (Skole and Tucker, 1993). Forest fragmentation is so pervasive in tropical regions (Gascon *et al.*, 2000; Riitters *et al.*, 2000) that most historically forested tropical landscapes now exist as small archipelagoes of forest islands, and such small patchy forest fragments are becoming the norm in once continuously forested landscapes.

Although forest fragmentation is a global phenomenon, there is a major bias in existing studies. In Latin America and the Caribbean, with almost half of the land covered by forest (FAO, 2011), the great majority of plant ecology studies come from lowland forests (Turner and Corlett, 1996b; Carson *et al.*, 2008). The two best-studied sites in Latin America, Barro Colorado Island (BCI) of the Smithsonian Tropical Research Institute (STRI) in Panama and La Selva Biological Station of the Organization for Tropical Studies (OTS) in Costa Rica, are both tropical lowland wet forests. Insights on forest fragmentation are best represented by studies conducted in Biological Dynamics of Forest Fragments Project (BDFFP), which is in lowland tropical forest of Brazil. However, considering that forest fragmentation is ubiquitous (Gascon *et al.*, 2000; Riitters *et al.*, 2000), applying insights from a few well-studied sites to other fragmented forests may be misleading because fragmentation effects can differ regionally (Bouroncle and Finegan, 2011) as a result of difference in various factors, such as topography and microclimate. There may also exist altitudinal bias in forest cover and fragment size (Mendoza *et al.*, 2005; Tabarelli *et al.*, 2010b) and different processes may act at higher elevations. One of the primary reasons for such bias owes to the difficulty in having true replicates of forest fragments and conducting experimentally controlled studies (Debinski and Holt, 2000), particularly at a landscape scale. Outside BDFFP, there is also a bias towards small forest fragments (< 10 ha) with few studies on medium-sized forest fragments (Turner and Corlett, 1996b; Zuidema *et al.*, 1996; Debinski and Holt, 2000). Thus, while insights gained from the long-term studies conducted at BDFFP (Laurance *et al.*, 2002; Laurance *et al.*, 2011) are invaluable in advancing both biological and conservational understanding of the impacts of forest fragmentation in lowland forest of northwestern South America, more studies from underrepresented sites are critically needed for a better understanding of such a global phenomenon.

1.2. Effects of forest fragmentation on plant species regeneration

Ever since forest fragmentation has been recognized as another major threat along with deforestation, various consequences of forest fragmentation have been revealed (Saunders *et al.*, 1991; Andren, 1994; Collinge, 1996; Young *et al.*, 1996; Laurance *et al.*, 2002; Ries *et al.*, 2004; Aguilar *et al.*, 2006; Ewers and Didham, 2006; Laurance *et al.*, 2011). Forest fragmentation is a complex phenomenon because it physically alters landscape characteristics as well as various aspects of the forest environment concurrently. Such alteration includes reduction in forest area, habitat loss, isolation from the larger expanses of surrounding forest, conversion of surrounding matrices, and increase in forest edges. Consequences of forest fragmentation are also affected by the shape of the forest fragment (Ranta *et al.*, 1998), and such confounding factors often have been tested individually to isolate each factor (Debinski and Holt, 2000; Fahrig, 2003; Lawes *et al.*, 2005; Ewers and Didham, 2006). One serious consequence of deforestation is loss of species diversity (Wilcox and Murphy, 1985; Turner, 1996; Zuidema *et al.*, 1996; Fahrig, 2003). Negative effects of forest fragmentation on animal communities, well-represented by birds and mammals (Andren, 1994; Turner, 1996), may also cause the local extinction of such species (Fahrig, 2002; Pacheco *et al.*, 2006).

Regeneration of plant species is negatively affected by such local extinction of animal species because over 80% of the plant species in tropical forests are animal-dispersed (Howe and Smallwood, 1982). Such fragmentation impacts on animal species lead to unequal consequences on plant species with different life-history traits. Seed dispersal is reduced in fragmented forests or forests with poachers for various reasons. In such forests, dispersers may be locally extinct, their abundance and species richness may be reduced, or their movements may be inhibited between isolated forest fragments when compared to intact, continuous, or large forests

(Cordeiro and Howe, 2003; Cramer *et al.*, 2007a; Herrera and Garcia, 2010). Such reduced seed dispersal leads to declines in relative abundance of animal-dispersed species compared to species with abiotic dispersal modes, such as wind- or gravity-dispersed species (Tabarelli *et al.*, 1999; Metzger, 2000; Terborgh *et al.*, 2008). Among animal-dispersed species, negative effects on seed dispersal is particularly severe for species with large seeds (de Melo *et al.*, 2006; Laurance *et al.*, 2006a; Cramer *et al.*, 2007b; Terborgh *et al.*, 2008; Melo *et al.*, 2010) because there are potentially fewer dispersers that can disperse large than small seeds (Hughes *et al.*, 1994; Corlett, 1998; Kitamura *et al.*, 2002; Alcántara and Rey, 2003; Galetti *et al.*, 2003; Gosper *et al.*, 2005). Additionally, large-bodied species require larger expanses of forest habitat and are usually the first to disappear from fragments (Dirzo *et al.*, 2007; Michalski and Peres, 2007). Similarly, among different pollination modes, insect-pollinated species are considered more vulnerable (Steffan-Dewenter and Tscharntke, 1999; Kolb and Diekmann, 2005) than wind-pollinated or self-pollinated species (but see Jump and Penuelas, 2006) due to habitat loss and inhibited movements of pollinators between isolated fragments (but see Nason and Hamrick, 1997). Therefore, regeneration of species that is more dependent on animals for pollen and seed dispersal is expected to be more negatively affected by forest fragmentation.

A major consequence, which almost unavoidably occurs as a result of forest fragmentation, is edge effects (Murcia, 1995; Laurance, 2000), especially when forests are fragmented by clearance for cultivation or pasture (Kapos *et al.*, 1997). Edge effects are any phenomenon, either abiotic or biotic, that results from two adjacent ecosystems being separated by an abrupt transition (Murcia, 1995), such as forest-pasture or forest-road transitions. When forest is fragmented, the forest perimeter to interior ratio increases. Thus, even when the total forest area may be equal, an increase in number of fragments will likely lead to more extensive edge effects, which is also used as a justification for SLOSS (single large or several small) arguments (Fahrig, 2003) along with justifications based on the island biogeography theory (MacArthur and Wilson, 1967). Abiotic edge effects include increased air temperature, light intensity, and vapor pressure deficit, and lower soil moisture content at forest edges compared to forest interior (Kapos, 1989; Didham and Lawton, 1999; Gehlhausen *et al.*, 2000). The distances over which edge effects are detected varies by the species or metrics assessed, but most abiotic consequences of edge effects are detected < 100-200 m (Didham and Lawton, 1999; Laurance, 2000; Laurance *et al.*, 2006a). On some occassions, edge effects can penetrate up to 300 m (Laurance *et al.*, 1998) or even 500 m (Laurance, 1991). As a result of such abiotic alteration in the environment near forest edges, plant community structure of plant is expected to shift toward a more early-successional suite of species in small forest fragments. While pioneer (early-successional, shade-intolerant) species and lianas proliferate on the edges of forest fragments (Sizer and Tanner, 1999; Laurance and Williamson, 2001; Laurance *et al.*, 2006b; Tabarelli *et al.*, 2008; Tabarelli *et al.*, 2010a), shade-tolerant (late-successional) species, which may also be dependent on fewer dispersers (Hamann and Curio, 1999), decline in relative abundance (Benitez-Malvido, 1998; Laurance *et al.*, 1998; Tabarelli *et al.*, 1999; Laurance *et al.*, 2006a; Püetz *et al.*, 2011; but see Bouroncle and Finegan, 2011). This exacerbates the biodiversity consequences of fragmentation because the majority of species- and structural-diversity in tropical wet forests is provided by non-pioneer species, and pioneer species constitute a small proportion of the entire plant community (Hubbell *et al.*, 1999; Laurance *et al.*, 2006a; Tabarelli *et al.*, 2008). Among different life forms, tree recruitment (Benitez-Malvido and Martinez-Ramos, 2003), especially of species with large individual sizes (Laurance *et al.*, 2006a; Santos *et al.*, 2008), is reduced in fragmented forests. Therefore, insect-pollinated, animal-dispersed, non-pioneer tree species are one of the groups particularly at risk of extinction as more forests become fragmented.

1.3. Importance of considering various stages of regeneration processes

The cycle of species regeneration will not be complete if any of the stages fail. In that regard, early stages are of special concern if they are negatively affected by forest fragmentation because they can act as bottlenecks for species regeneration. Early stages of species regeneration include pre-dispersal seed predation, primary seed dispersal, post-dispersal seed predation, secondary seed dispersal, seed longevity, germination, and seedling establishment, all of which can be affected by forest fragmentation. Yet, studies have shown that the effects of forest fragmentation on early stages are not consistent. Such inconsistent effects may also vary by how each stage is evaluated across different studies. For example, some studies report significantly higher pre-dispersal predation in continuous forest (Cascante *et al.*, 2002; Nabe-Nielsen *et al.*, 2009), while others find no difference among fragment sizes (Burgos *et al.*, 2008). It may also be highly species- (Chacoff *et al.*, 2004) or year-dependent (Rabasa *et al.*, 2009). Post-dispersal predation may also be affected differently by edge effects as a result of forest fragmentation. Edge effects may lead to higher post-dispersal predation close to forest edges than in forest interiors (Lopez-Barrera *et al.*, 2005), higher in the interior than at edges (Restrepo and Vargas, 1999; Chauvet and Forget, 2005), or no difference (Holl and Lulow, 1997), depending on the adjacent vegetation or habitat type and species. In contrast to the aforementioned stages, most studies of primary dispersal (Cordeiro and Howe, 2003; Cramer *et al.*, 2007a; Herrera and Garcia, 2010; but see Bach and Kelly 2004), seed viability (Nason and Hamrick, 1997; Rocha and Aguilar, 2001; Cascante *et al.*, 2002; Henriquez, 2004; Valdivia and Simonetti, 2007; but see

Mathiasen *et al.*, 2007; Burgos *et al.*, 2008; Ashworth and Marti, 2011), germination environment (Bruna, 1999, 2002), and seedling establishment (Santos and Telleria, 1994; Benitez-Malvido, 1998; Rodriguez-Cabal *et al.*, 2007; Cordeiro *et al.*, 2009) are negatively affected by forest fragmentation. Such negative effects may be caused by a combination of both dispersal limitation and edge effects. Compared to the aforementioned stages, only a handful of studies have assessed pre-dispersal fungal infection of seeds (Myster, 1997; Kuprewicz and Garcia-Robledo, 2010), secondary dispersal in the context of forest fragmentation (Xiao *et al.*, 2004; Cole, 2009) or edge effects (Galetti *et al.*, 2003), and seed longevity (Devlaeminck *et al.*, 2005; Kolb and Diekmann, 2005). Thus, there is a bias in stages assessed in the context of forest fragmentation.

Use of appropriate methods is also an important consideration (Baraloto *et al.*, 2010). Differences in methods used across studies make simple comparisons even difficult among studies. Ideally each study should consider different stages for a broader understanding of the phenomenon. Even for a given species, edge effects may act on both early (Benitez-Malvido, 1998; Herrera and Garcia, 2010; Uriarte *et al.*, 2010) and later stages (Laurance *et al.*, 2000; Nascimento and Laurance, 2004; Oliveira *et al.*, 2008). Furthermore, some tropical tree species are known to undergo a change in light requirements over their lifespan, a phenomenon called ontogenetic shift (Clark and Clark, 1992; Dalling *et al.*, 2001; Wright *et al.*, 2003; Niinemets, 2006). Although simple functional grouping of species based on the classical view of pioneer and shade-tolerant species dichotomy (Swaine and Whitmore, 1988; Whitmore, 1989) is still common (e.g., Schnitzer and Carson, 2001; Uriarte *et al.*, 2004; van Gelder *et al.*, 2006), increasing evidence suggests that most tropical tree species are not extremely light-demanding or shade-tolerant (Wright *et al.*, 2003). Rather, species that have the same light requirements throughout their entire life may be exceptions (Poorter *et al.*, 2005). Thus, shade tolerance of species should be viewed as a continuum (Condit *et al.*, 1996; Wright *et al.*, 2003), which had partly been recognized earlier (e.g., Whitmore, 1989). Habitat associations may also differ across various stages (Comita *et al.*, 2007; Kanagaraj *et al.*, 2011). However, individuals that are considered in forest ecology studies are generally restricted to certain size classes (but see Nascimento and Laurance, 2004). Thus, how fragmentation may differentially affect various ontogenetic stages is largely unknown. A conclusion based on assessing the effects of forest fragmentation effects on just one life-history stage may be misleading because fragmentation effects may be limited to a certain time frame and a fragmentation effect may be missed depending on the growth stage and process assessed. Particularly when restoration strategy is considered, use of one stage (e.g., seed, seedling) may not be fruitful in restoration if potential ontogenetic shifts or habitat associations are not recognized.

1.4. Overview of the dissertation

The overarching goal for my dissertation was to assess the effects of forest fragmentation on various aspects of regeneration in animal-dispersed tropical trees. Are animal-dispersed tree species regenerating in small forest fragments that are becoming the norm in forested landscapes? Are insights obtained from the few well-established sites directly applicable to other sites? With strong concerns for the future of tropical forests, I initiated my dissertation as a conservation-oriented project. My ultimate goal was to seek a realistic and practical way to conserve the tropical forests of Latin America and elsewhere, with their unique ecosystems and high species diversity. As a practical dissertation project, I considered evaluating the effects of forest fragmentation on regeneration of five animal-dispersed tree species to seek practical methods for their conservation and restoration, and to obtain insights which could be applied in a broader context or to a broader array of species. However, as my projects progressed and I learned about basic ecological studies, I came to realize the importance of a basic understanding of biology of study species for any applied ecological research. Insights or ecological theories from basic studies cannot be set aside and considered separately when ecological applications are considered. Ecological processes proposed in basic studies may be altered in fragmented forests, which have important implications for both basic and applied ecology. Therefore, another major goal for my dissertation was to integrate biological understanding and conservation applications.

To address some of the issues mentioned so far, I studied different aspects of forest fragmentation impacts on regeneration of five animal-dispersed tree species in Costa Rica. The dissertation research was based at Las Cruces Biological Station (LCBS; 8° 47' N, 82° 58' W), a field station owned and operated by the Organization for Tropical Studies (OTS) in Coto Brus county, southern Costa Rica (Fig. 1.1.). The natural vegetation of the area is classified as tropical premontane wet forest (Holdridge *et al.*, 1971) with elevation ranges between 1,000 and 1,400 m (1,170 ± 58 m). This elevation range is currently underrepresented in existing studies of tropical plant ecology. Temperature ranges between 14 and 29°C with mean of 20°C. Dry season is from December through March. Both El Niño and La Niña years were included during the study years between 2008 and 2011 (Fig. 1.2.) and the severe drought during the wet season of 2009 in a El Niño year affected fruit production of many tree species at the community level in 2009, and even affected lianas in addition to trees in 2010. While Costa Rica is the only country in Central America and one of only five countries in Latin America and the Caribbean that increased in forest area in the past 10 years (FAO, 2011), the area where this study was conducted is situated in a highly deforested part of Costa Rica where overall forest cover within a 15 km radius of the station is estimated at 27% (Daily *et al.*, 2001). Thus, the medium-sized forest fragment at LCBS (209 ha) is the largest remaining primary forest in the immediate area. Evidence from aerial photos suggests that the forest reserve at LCBS was finally isolated as a fragment around 1978 to 1979, with earlier forest cutting in the 1960s (R. Zahawi, *personal communication*). In addition to the forest reserve at LCBS, trees in four smaller primary forest fragments (AGZ: 33 ha, CED: 27 ha, LL: 19 ha, PC: 4 ha) were used (Fig. 1.1.). All five fragments are within Coto Brus county with similar elevations (1,175 ± 27 m) and climate, and have not had recent major disturbances such as logging.

Five animal-dispersed tree species from different families were selected as focal species, based on the abundance of reproductive trees in the fragments during May to August 2009. The five focal species included: *Drypetes brownii* Standley (Putranjivaceae), *Ficus tonduzii* Standley (Moraceae), *Lacistema aggregatum* (P.J. Bergius) Rusby (Lacistemataceae), *Quararibea aurantiocalyx* W. S. Alverson (Malvaceae), and *Tapirira mexicana* Marchand (Anacardiaceae). All five species produce fleshy fruits that are dispersed by animals during the early wet season. The limited information available on mammal species in this area report that large herbivorous mammals such as howler monkeys (*Alouatta palliata*), spider monkeys (*Ateles geoffroyi*), Baird's tapirs (*Tapirus bairdii*), and white-lipped peccaries (*Tayassu pecari*) are locally extinct in Coto Brus (Pacheco *et al.*, 2006), although these species still exist in other regions of Costa Rica. Time since local extinction of these large mammals in LCBS forest is estimated to be 20 to 50 years (R. Zahawi, *personal communication*). Dispersal limitation is one of the major concerns in degraded landscapes (Holl *et al.*, 2000; Zartman and Nascimento, 2006), and local extinction of these animals may have negative consequences on regeneration of the focal species. While none of the focal species are known as typical pioneer species (e.g., *Cecropia*, *Heliocarpus*, *Vismia*), there was no *a priori* knowledge of where these species fit in the pioneer-to-shade-tolerant continuum (Condit *et al.*, 1996; Wright *et al.*, 2003), and few descriptions of these species were available.

In this dissertation, I present results from five different projects. In Chapter 2, I assessed impacts of edge effects on multiple early stages of regeneration using one of the focal species, *T. mexicana*. While previous studies have assessed various stages of regeneration independently on individual species (e.g., Bruna, 2002; Cramer *et al.*, 2007a; Kuprewicz and Garcia-Robledo, 2010), few studies have assessed multiple stages in a single study, especially those specifically assessing edge effects. Thus, I studied whether edge effects differentially affect different early stages of regeneration processes for a given species. Edge effects were evaluated as a function of distance from edges where focal trees occurred. To evaluate how edge effects may act differentially on different stages of regeneration processes, I assessed all of the following: pre-dispersal seed predation, primary seed dispersal, post-dispersal seed predation, secondary seed dispersal, *ex situ* germination, *in situ* seed longevity, 1st-yr and 2nd-yr seedling abundance, 2nd-yr seedling survivorship, and relative growth.

In Chapter 3, I assessed the potential of small to medium-sized fragments to serve as seed sources for three of the focal species (*F. tonduzii*, *L. aggregatum*, and *Q. aurantiocalyx*). Seed source is an important consideration for conservation and restoration but many studies show reduced rates in germination for seeds collected from small fragments (Nason and Hamrick, 1997; Rocha and Aguilar, 2001; Cascante *et al.*, 2002; Henriquez, 2004; Valdivia and Simonetti, 2007). However, large forests are becoming increasingly rare such that, in practice, collecting seeds from trees in large forests is often difficult. I conducted germination tests in a screen house at LCBS to isolate the effect of the environment where seeds germinate (Bruna, 1999, 2002) from the quality of seeds collected from different forest fragments (Nason and Hamrick, 1997; Cascante *et al.*, 2002). I also followed seeds from different maternal trees to evaluate variation among maternal trees. Additionally, to assess whether seedlings obtained from certain sources have a better chance of establishment when transplanted elsewhere, I also measured seedling survivorship and growth up to two years. Two years later in 2011, when larger fruit crop was available for one of the species *L. aggregatum*, I conducted more rigorous tests with larger sample sizes.

In Chapter 4, I aimed to rank relative light requirement for the five focal species and tested whether species with lower light requirement are more negatively affected by forest fragmentation as generally predicted by studies based on species functional groups (e.g., Laurance *et al.*, 2006a; Tabarelli *et al.*, 2008). In a highly diverse ecosystem of tropical wet forests, life-history traits are largely unknown for many species (Wright *et al.*, 2003), which is particularly so at underrepresented sites. While classification of species into different functional groups has been based on various methods (e.g., Kiama and Kiyiapi, 2001; Chazdon *et al.*, 2010), measuring different traits solely to classify species into different functional groups will be labor intensive and difficult for canopy trees. In fact, classifying species into different functional groups is often an unlikely objective of a study. Phenotypic plasticity is also common (Rijkers *et al.*, 2000), and species may also undergo ontogenetic shifts (Clark and Clark, 1992; Dalling *et al.*, 2001; Wright *et al.*, 2003; Niinemets, 2006). Thus, if a single census of size distributions serves as a reliable predictor for life-history traits (Wright *et al.*, 2003) and could be used to predict conservation status, it would be useful for many poorly described species. I assessed light requirements of each species based on size distributions of regenerating individuals. Considering all individual sizes, I assessed effects of forest fragmentation based on the following metrics:

size distributions between different-sized fragments for a given species, species abundance, edge effects on number of regenerating individuals, survivorship, growth, realized dispersal, herbivory and biotic infection damage, and light availability.

In Chapter 5, I studied spatiotemporal dynamics of the five focal species to elucidate some of the underlying mechanisms for species regeneration in forest fragments. Originally inspired by the paper published in 1983 by Augspurger in *Oikos*, I empirically tested whether median distance of individuals from the closest reproductive conspecific (hereafter referred to as 'establishment distance') shifts outward over time by comparing individuals of different sizes, as a substitute for time. Establishment distance is the center of spatial distribution in relation to the closest reproductive conspecific independent of other coexisting species. Augspurger (1983a) demonstrated that establishment distance of seedlings shifted outward with time. She also demonstrated that shifts in establishment distance were determined by location of the light gaps where seedling survivorship was enhanced as a result of reduced fungal infection. While many papers have been published on spatial aggregation or autocorrelation of plants, surprisingly few papers had been published on directional shifts in relation to the parent or the closest reproductive conspecific. Even for studies that assessed spatial distributions of different-sized individuals, the major interest has predominantly been to determine whether individuals are aggregated (clustered) or not (e.g., Condit *et al.*, 2000; Picard *et al.*, 2009). Independent of whether species are aggregated or not, I empirically tested whether establishment distances are greater with increasing size class, and whether such outward shifts are caused by positive distance-dependent survivorship, as expected theoretically. Thus, I empirically tested the Janzen-Connell (J-C) hypothesis (Janzen, 1970; Connell, 1971) in a temporal context, which has rarely been conducted (Carson *et al.*, 2008). The J-C hypothesis states that mortality will be higher close to the maternal tree where seed and seedling densities are high due to higher seed predation, herbivory, or pathogen infection. It was originally proposed to explain high species coexistence in tropical forests because such positive distance-dependent or negative density-dependent survivorship will not allow conspecific offspring to occupy the space near the reproductive tree, which allows entry of other species. To explain potential underlying mechanisms for such distance-dependent survivorship, I also assessed degree of invertebrate herbivory and biotic infection, and light availability and heterogeneity along the distance gradient.

In Chapter 6, I report experimental tests of the J-C hypothesis by transplanting seedlings of known origins using one of the focal species *F. tonduzii*. While forest fragmentation effects were assessed in the study in Chapter 4, independent of forest fragmentation effects, survival of individuals is affected by various factors, such as maternal origin, environment, initial plant size, and distance from the maternal tree, as formulated by the J-C hypothesis. While the J-C hypothesis has been tested many times both in the tropics and temperate forests, most tests have been conducted in lowland tropical forests (Carson *et al.*, 2008) and it has rarely been tested in the context of forest fragmentation (but see Wright and Duber, 2001). Thus, whether the J-C effects are altered by forest fragmentation is largely unknown. I assessed relative importance of these factors on seedling survivorship and growth in forest fragments by transplanting the seedlings obtained from the study in Chapter 3 to two distance classes from either maternal (home) or non-maternal reproductive tree (away) in different-sized forest fragments.

Finally, in the final chapter, I summarize the major findings from the previous chapters and conclude with some discussion of the biological and conservation implications of my findings.

1.5. Figures

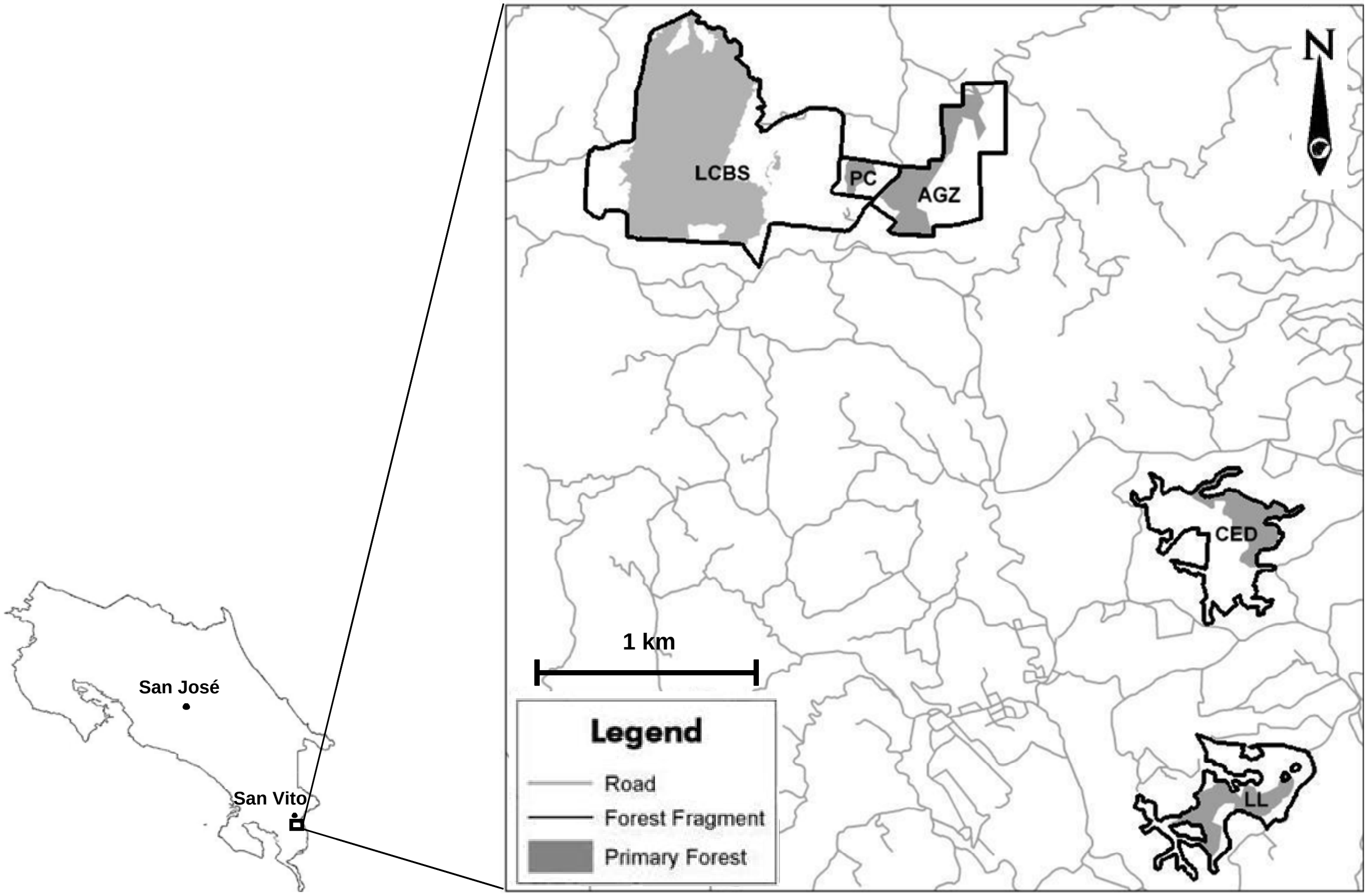

Figure 1.1
Map and configuration of the five forest fragment sites in Coto Brus county, Costa Rica. The closest town to Las Cruces Biological Station (LCBS) is San Vito. Abbreviations and primary forest areas are; LCBS (209 ha), AGZ (33 ha), CED (27 ha), LL (19 ha), and PC (4 ha). Contours and configuration of primary forest covers where focal trees were located are shown. Forest areas between fragments were distinctly isolated by roads, pastures, and garden areas. Primary forest covers for the study sites are; LCBS (64.2%), AGZ (37.9%), CED (31.3%), LL (29.2%), and PC (23.5%).

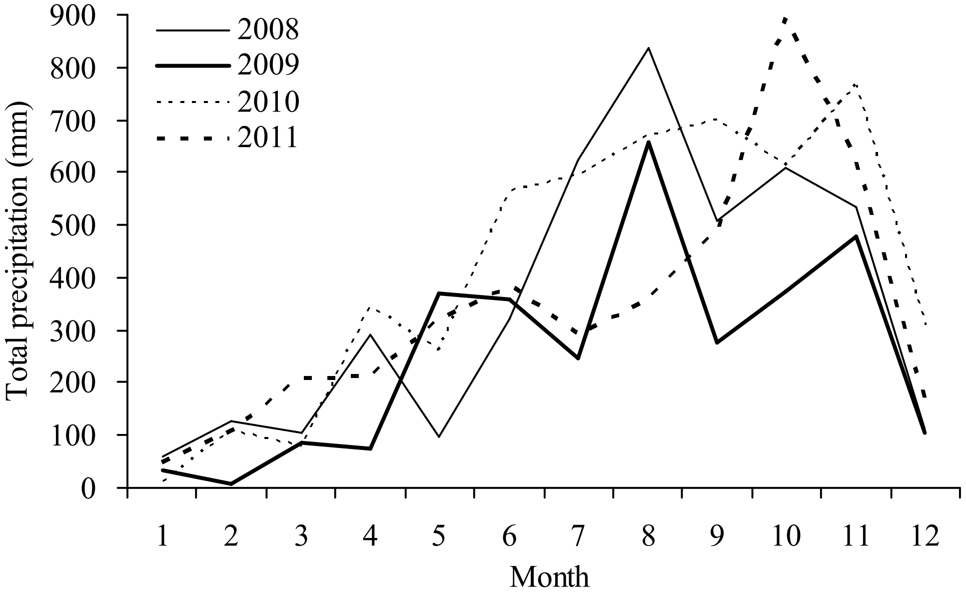

Figure 1.2
Monthly precipitation for the study years between 2008 and 2011. Annual precipitation was 4,223 mm in 2008, 3,063 mm in 2009 (El Niño year), 5,011 mm in 2010, and 4,110 mm in 2011 (La Niña year). Bold solid line and bold dotted lines show El Niño and La Niña years, respectively.

CHAPTER 2

EDGE EFFECTS ACT DIFFERENTIALLY ON MULTIPLE EARLY REGENERATION STAGES OF A SHADE-TOLERANT TREE *TAPIRIRA MEXICANA*[1]

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[1] Sugiyama, A. and Peterson, C. J. Accepted by *Biotropica*. Reprinted here with permission of publisher.

2.1. Abstract

Forest fragmentation is pervasive in tropical landscapes, and one pathway by which fragmentation may negatively impact populations is via edge effects. Early life-stages are particularly important for species regeneration since they act as bottlenecks, but how edge effects may act differentially on different life-stages is unknown. This study evaluated edge effects on multiple early life-stages of a currently common animal-dispersed, shade-tolerant tree Tapirira mexicana (Anacardiaceae). The study was conducted in tropical premontane wet forest fragments in a highly deforested region of Costa Rica. The stages assessed were: pre-dispersal predation, primary dispersal, post-dispersal predation, secondary dispersal, ex situ germination, in situ seed longevity, 1st-yr and 2nd-yr seedling abundance, 2nd-yr seedling survivorship, and basal diameter growth. Results showed that impacts of edge effects were not equal across stages, but were limited to specific stages and times. One stage which may act as a bottleneck for species regeneration was pre-dispersal predation. Over 60% of the seeds were predated by larvae, and predation was higher near the edge than interior habitat. Seeds lost viability within 10 days in the forest. Germination to 1st-yr seedling stage was also lower near edges, but such effect was eliminated within a year after that. Primary dispersal, seedling survivorship, and growth were not affected by proximity to edges, and both secondary dispersal and post-dispersal predation were rare. This study demonstrates that current population abundance may not guarantee future species persistence and the importance of considering multiple life-stages for a comprehensive assessment of forest fragmentation effects on species regeneration.

2.2. Resumen (Spanish abstract)

La fragmentación de los bosques es un fenómeno generalizado en los paisajes tropicales; una forma en que la fragmentación puede influir negativamente las poblaciones de plantas es a través de los efectos de borde. Las etapas de la vida tempranas son particularmente importantes para la regeneración de las especies ya que actúan como cuellos de botella, pero se desconoce cómo los efectos de borde pueden actuar diferencialmente sobre las distintas etapas de la vida. El presente estudio evaluó los efectos de borde en varias etapas de la vida temprana de Tapirira mexicana (Anacardiaceae), una especie de árbol común que es tolerante a la sombra, dispersada por animales. El estudio se realizó en fragmentos de bosque tropical premontano húmedo en una región altamente fragmentada de Costa Rica. Las etapas de evaluación fueron: la depredación previa a la dispersión, dispersión primaria, la depredación post-dispersión, la dispersión secundaria, la germinación ex situ, la longevidad de las semillas in situ, la abundancia de plántulas de primer y segundo años, supervivencia de plántulas de segundo año, y crecimiento en el diámetro basal. Los resultados muestran que el impacto del efecto de borde no fue igual entre todas las etapas, pero se limita a etapas y épocas específicas. Una etapa que puede actuar como un cuello de botella para la regeneración de la especie fue la depredación antes de la dispersión. Más del 60 por ciento de las semillas fueron depredadas por larvas, y la depredación fue mayor cerca de los bordes que en el hábitat interior. Las semillas perdieron su viabilidad dentro del bosque en los primeros 10 días. La etapa desde la germinación a plántula de 1 año también se vio reducida cerca de los bordes, pero este efecto desapareció en el plazo de un año después de eso. La dispersión primaria, la supervivencia de las plántulas, y el crecimiento no se vieron afectados por la proximidad a los bordes, y tanto la dispersión secundaria como la depredación post-dispersión fueron raros. Este estudio demuestra que la abundancia de la población actual no

puede garantizar la persistencia de las especies futuras y la importancia de considerar varias etapas de la vida para una evaluación integral de los efectos de la fragmentación del bosque sobre la regeneración de las especies.

2.3. Introduction

Tropical forests support the most diverse terrestrial ecosystems, yet are currently subject to numerous threats including extensive clearing and forest fragmentation. Various consequences of forest fragmentation have been reported (Laurance *et al.*, 2002; Fahrig, 2003), but edge effects are one of the most important (Laurance, 2000), especially when forests are fragmented by clearance for cultivation or pasture, as is the case for many tropical forests (Kapos *et al.*, 1997). However, species with different life histories and regeneration modes are not equally affected by fragmentation or edge effects. Among different pollination modes, insect-pollinated species are more vulnerable (Kolb and Diekmann, 2005) than species with wind- or self-pollinated species (but see Jump and Penuelas, 2006). Likewise, due to local extinction of animal dispersers in fragmented forests, animal-dispersed species are more likely to be negatively impacted while species with abiotic dispersal modes increase in abundance (Metzger, 2000; Terborgh *et al.*, 2008). Among different life histories, pioneer species and lianas increase in abundance with creation of edges (Laurance *et al.*, 2001; Tabarelli *et al.*, 2008), while relative abundance of late-successional shade-tolerant species declines (Benitez-Malvido, 1998; Tabarelli *et al.*, 1999). Among different growth forms, recruitment of trees, particularly large-sized species, is reduced (Benitez-Malvido and Martinez-Ramos, 2003). Therefore, insect-pollinated, animal-dispersed, shade-tolerant tree species are one of the groups particularly at risk of extinction as forests become more fragmented.

Different stages of regeneration processes are also affected differentially by edge effects. Edge effects act on both early (Benitez-Malvido, 1998) and later life-stages (Oliveira *et al.*, 2008) but early life-stages are of a special concern since they act as bottlenecks for species regeneration. Early regeneration characteristics/processes include: seed longevity (Devlaeminck *et al.*, 2005; Kolb and Diekmann, 2005), germination (Bruna, 2002; Cascante *et al.*, 2002), and seedling abundance (Santos and Telleria, 1994; Rodriguez-Cabal *et al.*, 2007). Similarly, plant regeneration is strongly affected by interactions with other organisms, such as: pre-dispersal seed predation (Cascante *et al.*, 2002; Chacoff *et al.*, 2004; Burgos *et al.*, 2008) and fungal infection (Myster, 1997; Kuprewicz and Garcia-Robledo, 2010), post-dispersal predation (Restrepo and Vargas, 1999; Chauvet and Forget, 2005; Lopez-Barrera *et al.*, 2005), and primary (Bach and Kelly, 2004; Herrera and Garcia, 2010) and secondary dispersal (Galetti *et al.*, 2003; Xiao *et al.*, 2004; Cole, 2009). However, not all studies have specifically assessed edge effects, and impacts of edge effects on pre-dispersal predation, pre-dispersal fungal infection, and germination are unknown. A handful of studies that have specifically assessed edge effects report mixed results. For example, post-dispersal predation may be higher near edges than in forest interior (Lopez-Barrera *et al.*, 2005), higher in the interior than at edges (Restrepo and Vargas, 1999; Chauvet and Forget, 2005), or may not differ (Holl and Lulow, 1997), depending on the adjacent vegetation or habitat type and species. Other stages negatively impacted by edge effects include secondary dispersal (Galetti *et al.*, 2003) and seedling abundance (Benitez-Malvido, 1998), while primary dispersal (Bach and Kelly, 2004) or seed longevity (Devlaeminck *et al.*, 2005) may not be affected by edge effects.

Although edge effects are one of the key mechanisms causing the impacts of forest fragmentation (Murcia, 1995; Laurance, 2000), a surprisingly limited number of studies have directly assessed edge effects on different stages of regeneration processes. Instead, many studies on forest fragmentation presume edge effects as a cause for any observed differences on different stages by comparing continuous forest and fragmented forest. Additionally, most existing studies have focused on only one or a few of those stages, which makes evaluation of cumulative effects or comprehensive evaluation of species regeneration difficult. Thus, whether all stages are equally important or one or multiple stages act as a bottleneck for species regeneration is unknown. Furthermore, the great majority of studies on forest fragmentation in the tropics come from lowland tropical forest (Turner, 1996), which may not be directly applicable to fragmented forests in higher elevation ranges. There is also a bias towards small fragments (< 10 ha) and more studies on under-represented medium-sized fragments are needed (Zuidema *et al.*, 1996).

To address some of these issues, this study was conducted in underrepresented small to medium-sized, tropical premontane wet forest fragments. Edge effects on multiple early stages of regeneration processes were evaluated using a currently common animal-dispersed, shade-tolerant canopy tree *Tapirira mexicana*. Currently common species are typically not of conservation interest, but current abundance may not predict future persistence of the species in forest fragments, particularly for species with characteristics expected to be vulnerable to fragmentation. Here, edge effects were specifically assessed for the following stages of regeneration processes: (1) pre-dispersal seed predation; (2) primary dispersal; (3) *ex situ* germination; (4) post-dispersal seed predation; (5) secondary dispersal; (6) *in situ* seed longevity; (7) 1st-yr seedling abundance; (8) 2nd-yr seedling abundance; (9) 2nd-yr seedling survivorship; and (10) relative growth in basal diameter.

2.4. Methods

Study sites

This study was carried out near Las Cruces Biological Station (LCBS; 8° 47′ N, 82° 58′ W), of the Organization for Tropical Studies (OTS) in Coto Brus county, Costa Rica (Fig. 1.1). The forest reserve at LCBS (209 ha) is the largest remaining fragment of primary forest in the immediate area; overall forest cover within a 15 km radius of the station is estimated at 27% (Daily *et al.*, 2001). Natural vegetation of the area is classified as tropical premontane wet forest (Holdridge *et al.*, 1971), across an elevation range of 1,000-1,400 m. There is a distinct dry season from December through March. Annual precipitation was 4,223 mm (2008) and 5,011 mm (2010), while 2009 (El Niño year) was an unusually dry year with only 3,063 mm of rainfall; this affected fruit production of many tree species during the early wet season of 2009 and 2010 (A. Sugiyama, *personal observation*).

In addition to the forest reserve at LCBS, four smaller primary forest fragments (Fig. 1.1; 4-33 ha) were used. All five fragments are within Coto Brus county with similar elevations and climate, have not had recent major disturbances, and none have newly created forest edges.

Study species

Tapirira mexicana Marchand (Anacardiaceae), local common name “cirrí”, is an animal-dispersed, shade-tolerant canopy tree (Table 2.1). Although it is considered to be dioecious and insect-pollinated (F. Oviedo, *personal communication*) based on the pollination system of a closely related species *Tapirira guianensis* (Lenza and Oliveira, 2005), information on this species is scarce. *T. mexicana* was chosen as the study species for several reasons. First, *T. mexicana* is an animal-dispersed, shade-tolerant tree, all of which are characteristics that are expected to be negatively affected by fragmentation (Benitez-Malvido, 1998; Kolb and Diekmann, 2005; Terborgh *et al.*, 2008). Second, despite the above characteristics, *T. mexicana* is currently common (local density *ca.* 13 individuals of > 1m tall/ha) with a wide distribution through Latin America and wide elevation ranges (500-1,800 m), which facilitated locating the focal trees. Finally, it produces copious fruits during the early wet season with a long fruiting period (peak: May to July), which was critical for studying multiple regeneration stages in a single season. Fruits are green at maturity, with white sap in the carp, and contain one seed, the seed coat of which is tightly attached to the carp. Since the carp is *ca.* 1.5 mm, when a fruit is predated by terrestrial vertebrates, its seed is also preyed upon, usually destroying the embryo. On occasion, two to three fruits are fused, and thus one diaspore may have two to three embryos with separate partitions (A. Sugiyama, *personal observation*). Potential mammal and avian frugivores are Central American agouti (*Dasyprocta punctata*), blue-crowned motmot (*Momotus momota*), crested guan (*Penelope purpurascens*), clay-colored thrush (*Turdus grayi*), fiery-billed aracari (*Pteroglossus frantzii*), and Swainson's thrush (*Catharus ustulatus*), all of which are known to occur in the forest fragments studies here, although their relative abundances are unknown (F. Oviedo, *personal communication*).

All focal trees used in this study occurred in the primary forest area of each fragment at different distances from the forest edge (Table 2.1), and were producing fruits in 2009. None of the *T. mexicana* trees in the region produced fruits in 2008, 2010, or 2011 (A. Sugiyama, *personal observation*). Focal trees were at least 50 m from each other to minimize overlapping seed shadows. Due to an unusually dry wet season when the study was conducted, the number of focal trees or fruits varied depending on the availability of fruits when each regeneration stage was studied. In assessing edge effects on different stages of regeneration processes, focal trees were classified into forest "interior" (≥ 200 m) and "edge" (< 200 m) habitats, since studies show that edge effects are typically detected up to *ca.* 100-200 m (e.g., Didham and Lawton, 1999).

Pre-dispersal seed predation

In order to assess pre-dispersal predation, fruits without visible damage (total n = 1,341) were collected between the end of June and late July 2009 from nine focal trees (interior, n = 4; edge, n = 5; LCBS, n = 4; LL, n = 4; PC, n = 1). Collected fruits were kept moist until examination, which was conducted within 5 days. The fruits were opened and the seeds were inspected for embryo and cotyledon color, presence of insect larva and apparent fungal hyphae. Seeds were classified as "intact" (white embryo and cotyledon), "white embryo and black cotyledon", "black embryo and cotyledon", "larva", and "fungus". The presence of larva (*ca.* 4-5 mm long, *ca.* 1.5 mm wide) was considered a pre-dispersal event because collected fruits had no visible damage.

Ex situ *germination test*

Germination tests were conducted in a common screen house environment at LCBS to control for germination environment while testing the quality of seeds collected from trees at different distances from the edge (34-525 m). Fruits were collected between the end of May and late June 2009 (total n = 796) from 13 focal trees (interior, n = 5; edge, n = 8; LCBS, n = 8; AGZ, n = 1, LL, n = 3; and PC, n = 1). Collected fruits were kept moist and sown the day of collection on sterilized soil without extracting the seed since seed coat is tightly attached to the carp. The screen house allowed natural irradiation and day length, and fruits were watered daily with ample water. Germination was monitored until the end of the field season (46.1 ± 2.8 days), unless seeds were obviously dead prior to that.

Secondary dispersal, post-dispersal predation, and in situ *seed longevity*

Fruits were placed around 15 focal trees (interior, n = 6; edge, n = 9; LCBS, n = 7; AGZ, n = 1; CED, n = 1; LL, n = 5; and PC, n = 1) to track post-dispersal fate of seeds. Experiments were initiated on three different dates (10 July 2009, LCBS, AGZ, and PC; 12 July 2009, all LCBS; 17 July 2009, CED and LL). Since pre-dispersal predation may vary greatly by source tree and affect seed viability, the fruits used for this experiment were a composite from several sources. Fruits were used within 3 days of collection from at least three non-focal trees. Seed movement was tracked by sewing a 30 cm section of polyester thread (40/2) to the carp close to the peduncle (far end from where the embryo exists in the seed) since simply using seed removal as a proxy for seed predation may not be valid (Vander Wall *et al.*, 2005). Since the fruit size was small, the thread used here was much finer than thread or flagging tape used elsewhere (e.g., Russo, 2005; Cole, 2009). Bonding glue was not used, to avoid any effects on fruits or disperser behaviors. Many studies suggest no effect on behavior of dispersers with this technique (e.g., Forget and Wenny, 2005; Xiao *et al.*, 2006), and when the effect of threads on disperser behavior or seed longevity was tested using both threaded and unthreaded fruits prior to this study, there was no effect (t-test, n = 62).

Since post-dispersal predation may be higher close to the mother tree where seed input is high, as proposed by the Janzen-Connell (J-C) hypothesis (Janzen, 1970; Connell, 1971), the experiment was conducted in a fashion that tested the proposed distance-dependent seed mortality. Threaded fruits were placed at four distances (5 m, 10 m, 20 m, and 40 m) in two transects (5 × 40 m) radiating in random directions from the base of each focal tree. To keep track of dispersal beyond the distance classes, different-colored thread was used for each distance. At each distance, five fruits were placed on the ground surface and threads were hidden by litter (n = 5 fruits/distance × 4 distances/transect × 2 transects/focal tree × 15 focal trees). To relocate the fruits, plastic straws were placed into the ground *ca.* 50 cm away from the threaded fruits. The status of fruits was checked after 1 day, 4 days, 10 days, 2 week, and 20 days, and any litter covering fruits was removed each time. Because rotten fruits may be ignored by animals, when more than half of the fruits had blackened within 4 days of the initial fruit placement, fruits were replaced so that more than half of the fruits were green.

In situ seed longevity was assessed by recording the number of days until the fruit blackened. Although the black fruits were rotten, the existence of green fruits does not guarantee viability until germination. Larval predation should be fatal for *T. mexicana* seeds but blackening could occur after 20 days, when this experiment terminated.

1st-yr seedling abundance

In situ germination and 1st-yr seedling survivorship were inferred by recording the number of *T. mexicana* seedlings naturally occurring in the same transects 1 year after fruiting in 2009 (27 April 2010 to 30 May 2010). A total of six trees (interior, n = 2; edge, n = 4; LCBS, n = 3; AGZ, n = 1; LL, n = 1; and PC, n = 1), at least 220 m away from the closest reproductive conspecific, were used as focal trees. All the *T. mexicana* seedlings (< 50 cm tall) within the transects were tagged, and basal diameter, number of leaves, and distance class (0-40 m, 1 m interval) were recorded. None of the focal trees produced fruits in 2008 or 2010, and very few seedlings were present in 2009 when focal trees were fruiting (A. Sugiyama, *personal* *observation*). From these observations, the number and developmental stage of leaves (mature *T. mexicana* individuals have compound leaves), and the growth data, it was clear that the majority of seedlings were from the 2009 cohort.

Inferred primary seed dispersal

For all the transects used for assessing 1st-yr seedling abundance, no fruits were removed in 2009 to avoid modification of the seed shadow. Thus, primary dispersal was not directly studied. Instead, it was inferred indirectly based on the seedling distances from the focal tree. Considering only 1st-yr seedlings, seedlings that occurred beyond the crown extent of focal tree + 3 m were classified as "dispersed". Since crown extent differed for each transect even for the same tree, the crown extent was measured for each transect.

2nd-yr seedling survivorship and growth rate

Tagged *T. mexicana* seedlings (n = 2,001) in transects were re-censused after a year (24 April 2011 to 3 June 2011). Survival, basal diameter, and number of leaves were recorded. Relative growth in basal diameter was calculated as $(d_2 - d_1)/(d_1) \times 100$, where d_1 and d_2 are basal diameters in 2010 and in 2011, respectively.

Statistical analyses

For analysis of seed integrity and pre-dispersal predation, G-tests were used to test for differences between forest interior and edge habitats in the proportion of seeds in the five categories (*e.g.*, intact, larva), since each seed was mutually exclusively assigned to one category. For the other stages, each focal tree was treated as an experimental unit (two transects were pooled to represent each tree) and generalized linear model tests were performed to test for differences between forest interior and edge habitats. Distributions and links used were: beta distribution and logit link (proportion of fruits affected by animals, proportion of seeds “dispersed”, and 2nd-yr seedling survivorship), Poisson distribution and log link (1st-yr and 2nd-yr abundance), and Gaussian distribution and identity link (*in situ* seed longevity and relative growth). SAS 9.2 software (SAS Institute Inc., 2009) was used to perform all analyses, and R^2 and errors reported here are adjusted R^2 and SE, respectively, unless otherwise indicated.

2.5. Results

Pre-dispersal seed predation

Pre-dispersal predation was substantial for *T. mexicana* (62.5 ± 6.7%). In contrast, the proportions of intact seeds (white embryo and cotyledon; 17.6 ± 4.9%), seeds with fungal infection (2.5 ± 1.0%), dead seeds without larva or hyphae (black embryo and cotyledon; 6.1 ± 1.6%) were generally low. Non-intact seeds without damage by larvae or hyphae (white embryo and black cotyledon; 11.3 ± 2.7%), which could potentially lead to death, were also present. Between habitats, the proportions of seeds with larva and dead seeds without larva or hyphae were 1.5 and 2.6 times higher in forest edge, respectively (Fig. 2.1), a significant difference between forest interior and edge habitats ($G = 28.2$, df = 4, $P < 0.0001$).

Ex situ *germination*

No *ex situ* germination was obtained for *T. mexicana* in the screen house environment. Since pre-dispersal predation was substantial (*ca.* 90% larval predation for some trees) and seeds were not extracted, additional seeds, confirmed to be intact by removing a small portion of the carp, were sown in July 2009 (n = 150). However, none of these seeds germinated either.

Secondary dispersal and post-dispersal predation

Secondary dispersal and post-dispersal predation were both very low for *T. mexicana*. Out of 634 fruits, only 15 fruits were ever affected by animals (all the fruits were relocated); three fruits were eaten (only the thread was left behind), nine fruits were bitten, and there were rare events of burial (0.5%; thread was sticking out from the ground). Such animal activity was not associated with dates of initial fruit placement, days after initial fruit placement, or distance from the focal tree. Whenever the fruits were affected by animals, the activities above happened within *ca.* 30 cm (2.9 ± 2.0 cm) of where fruits were initially placed. As animal activity was overall very low, there was no significant difference between forest interior and edge habitats ($F_{1,15} = 2.8$, $P = 0.12$).

In situ *seed longevity*

Overall, most *T. mexicana* seeds lost their viability within 10 days in the forest (7.8 ± 0.2 days) and less than 5% of the seeds were alive after 20 days (Fig. 2.2). However, mean *in situ* seed longevity was not significantly different between forest interior and edge habitats (interior, 7.7 ± 0.3 days; edge, 7.8 ± 0.2 days; $F_{1,13} = 0.06$, $P = 0.81$).

1st-yr and 2nd-yr seedling abundance

For both 1st-yr and 2nd-yr seedlings, numbers of seedlings occurring around the focal trees were greater in forest interior than in edge habitats (1st-yr: $F_{1,4} = 816.4$, $P < 0.0001$, 2nd-yr:

$F_{1,3} = 161.9$, $P = 0.0010$). The difference in seedling abundance was greater for 1st-yr than for 2nd-yr seedling abundance (Fig. 2.3).

Inferred primary dispersal

Based on 1st-yr seedling distances from the focal trees, 11.2 ± 15.0% of *T. mexicana* seedlings occurred beyond crown + 3 m and were classified as individuals that germinated from "dispersed" seeds. However, the proportion of "dispersed" seeds did not differ between forest interior and edge habitats (interior, 8.7 ± 2.1%; edge, 15.9 ± 21.9%; $F_{1,3} = 1.3$, $P = 0.34$).

2nd-yr seedling survivorship and growth rate

Between the first and second years after germination, 55.0 ± 7.4% of *T. mexicana* seedlings survived, and had a mean relative growth of 55.1 ± 1.0% in basal diameter. However, neither survivorship ($F_{1,3} = 0.13$, $P = 0.75$) nor mean relative growth ($F_{1,3} = 0.73$, $P = 0.45$) differed between forest interior and edge habitats.

2.6. Discussion

This study demonstrates that not all stages are equally affected by edge effects, and that there is a critical time frame when edge effects may act. One major process that may become a bottleneck for regeneration of *T. mexicana* was pre-dispersal predation. On average, *ca.* 60% and up to *ca.* 90% of seeds were killed before dispersal by larvae of *Anastrepha* (Tephritidae) flies (Lopez *et al.*, 1999). Such level of pre-dispersal predation is high since *ca.* 80% of the species from various taxa have pre-dispersal predation of less than 60% (Kolb *et al.*, 2007). Such high pre-dispersal predation led to typically less than 20% of seeds remaining intact. Observed edge effects on pre-dispersal predation may result from higher insect abundance (Fowler *et al.*, 1993) and larger fruit or floral sizes near forest edges (Brody and Mitchell, 1997; Fenner *et al.*, 2002). Indeed, when fruit size produced by trees in forest interior and edge habitats was compared, fruits near edges were significantly larger (Table 2.1). Although inter-annual variation is possible (Rabasa *et al.*, 2009) fruiting was not annual or even biennial for *T. mexicana*, which might result from a severe drought in 2009 when fruiting occurred (Wright *et al.*, 1999). As fruiting interval elongates with more frequent drought events (Malhi and Wright, 2004), impacts on species regeneration from one fruiting event become more severe. Note that since only fruits that were apparently intact were used, the number reported here is a best-case scenario.

Germination environment was also important for regeneration of *T. mexicana*. *Ex situ* germination was not obtained under full sun, even after excluding the possibility of failure due to substantial pre-dispersal predation. Yet, germination does not appear to be particularly difficult for *T. mexicana* in forest environments since numerous seedlings are observed (*e.g.*, 17.5 seedlings/m^2 under the crown), despite high pre-dispersal predation. Since fruits were watered daily, high temperature and/or light intensity and not humidity seem to be responsible for germination failure in the screen house, although reduced humidity near the edge may further exacerbate such consequences. Major abiotic consequences of edge effects include increases in air temperature, which can change 2-4°C (Didham and Lawton, 1999) or in some cases, up to *ca.* 8°C (Kapos, 1989) within 200-250 m from the edge. Similarly, humidity drops, and light availability also increases with proximity to forest edges (Kapos, 1989; Gehlhausen *et al.*, 2000). Reduced germination via such abiotic environmental changes can indeed reduce seedling abundance (Benitez-Malvido, 1998; Bruna, 2002). In this study, the number of 1st-yr seedlings per focal tree also increased linearly with distance from the edge although such a spatial trend was no longer evident in the second year (Chapter 4). While pre-dispersal predation was generally higher near edges (Fig. 2.1), since it was not linearly related with distance from the edge, pre-dispersal predation cannot, by itself, explain the linear trends for 1st-yr seedling abundance. Thus, edge effects on both abiotic conditions and pre-dispersal predation likely led to reduced seedling abundance in edge habitats (Fig. 2.3).

Such reduction of germination under a hot/dry environment suggests that seeds of *T. mexicana* are likely recalcitrant (Benitez-Malvido, 1998; Ellis, 2003). Recalcitrant seeds, which possess no dormancy and lose their viability quickly when desiccated, are fairly common among non-pioneer species in tropical wet forests (Tweddle *et al.*, 2003). Indeed, *in situ* longevity of *T. mexicana* seeds were very short (typically they died in 8 days), so was presumably much shorter in the screen house environment. Note that *in situ* seed longevity reported here is an underestimate because the status of seeds was only checked up to 20 days. Since none of the seeds that stayed green until 20th day germinated, germination of *T. mexicana* should occur after 20 days and it is possible that most seeds that died within 20 days had pre-dispersal predation. Thus, while edge effects on *in situ* seed longevity were not detected, edge effects may become apparent after 20 days, perhaps most clearly at the germination stage. Studies on impacts of edge effects or forest fragmentation on seed longevity are still scarce and most have focused on seed banks (Devlaeminck *et al.*, 2005; Kolb and Diekmann, 2005), although recalcitrant seeds do not form seed banks. Results here suggest that storing seeds or using seeds in open areas for restoration will likely prove difficult for *T. mexicana*.

While abundances were significantly lower in forest edge habitats for both 1st-yr and 2nd-yr seedlings (Fig. 2.1), edge effects on 2nd-yr seedling survivorship were not detected. Note that 2nd-yr seedling abundance and survivorship are not identical. While 2nd-yr seedling abundance was defined as the number of individuals that existed in 2011, survivorship was the probability that individuals survived between 2010 and 2011. Similarly, 1st-yr seedling abundance reflects germination and survivorship of seedlings between 2009 and 2010. Thus, edge effects on 2nd-yr seedling abundance but not for 2nd-yr seedling survivorship suggest that the difference between forest interior and edge habitats for 2nd-yr seedling abundance is a carryover of a pattern generated in the previous year. While reduction in seedling abundance can occur via both reduced germination and increased mortality of seedlings (Benitez-Malvido, 1998), results here suggest that edge effects on *T. mexicana* are restricted only up to 1 year after germination, which was also true for later stages (A. Sugiyama, *unpublished data*). A given species may ontogenetically change its physiological traits such as light requirement (Clark and Clark, 1992; Dalling *et al.*, 2001; Wright *et al.*, 2003) and since edge effects were detected only until 1 year after germination, *T. mexicana* presumably possess tolerance of hot environments after 1 year. This is also supported by success in transplanting 5-11 month-old *T. mexicana* seedlings, obtained from nursery, to pasture (Loik and Holl, 2001). Thus, if *T. mexicana* seedlings are germinated in shade environment or those older than a year are used as transplants, restoration efforts may be fruitful.

Another stage not directly affected by proximity to edges was inferred primary dispersal. While one might expect increased dispersal in forest interior, which is more likely in larger forest (Herrera and Garcia, 2010), or near edge habitats if bird dispersers prefer larger fruits (Pizo and Almeida-Neto, 2009), bird abundance in edge habitat can be highly variable among species and between seasons (Bolger *et al.*, 1997; Restrepo and Vargas, 1999). One caveat in this study is that the existence of a seedling is a combined effect of dispersal and survivorship (*i.e.*, realized dispersal). Therefore, if there is a disproportionally higher survivorship for dispersed individuals (as expected by the J-C hypothesis) among seedlings only in one habitat type, it may obscure the actual dispersal patterns. Indeed, “dispersed” individuals survived significantly greater in the forest interior here ($G = 84.7$, df = 3, $P < 0.0001$), although true disperser abundances were unknown. Thus, if trees in the forest interior are visited less by dispersers, realized dispersal in forest interior might have been an overestimate. However, one of the few studies on the J-C effect with different animal levels showed that, in sites with low disperser and predator levels, the increase in seedling density was greater for non-dispersed than dispersed seeds (Wright and Duber, 2001).

Similar to primary dispersal, secondary dispersal and post-dispersal predation were not affected by proximity to edges. Results from food preference trials with agoutis (considered the main seed predator) suggest that fruits of *T. mexicana* are not preferred when other fruits are available (A. Sugiyama, *unpublished data*). Due to such overall low animal activities on fruits, there was no evidence for the J-C pattern, and long secondary dispersal was not observed. Although burial was rare, studies report improved germination for buried seeds (Wenny, 1999; Cole, 2009) and burial should provide insulation from heat and dehydration for *T. mexicana*.

Currently common species are typically not considered for conservation practices. However, current conservation status does not guarantee population persistence in the future, particularly for animal-dispersed shade-tolerant species which provide much of the species- and structural diversity in tropical wet forests (Hubbell *et al.*, 1999; Tabarelli *et al.*, 2008). *T. mexicana* is indeed one such species with multiple early stages acting as regeneration bottlenecks. Edge effects can be detected up to *ca.* 100-200 m from the forest edge (Didham and Lawton, 1999; Laurance *et al.*, 2006a) and since many focal trees in this study were within 200 m from the edge (Table 2.1), all the stages could potentially be affected by edge effects. However, not all stages equally experienced edge effects and the stages that were affected were rather limited, both in stage and time. Specifically, edge effects were only detected between pre-dispersal and a year after germination, which might have been missed if only seedlings of later stages were assessed. Since forest fragmentation is a relatively recent and rapid phenomenon compared to the long life span and regeneration cycle of shade-tolerant trees, studies on its long term effects still require further evaluation. The current population abundance of *T. mexicana* is presumably maintained by high fecundity that counteracts high pre-dispersal predation and low germination and seedling abundance near the edge, but such diagnosis can only be made when multiple stages are assessed. Thus, consideration of multiple life-stages will become increasingly important for efficient and successful conservation practices as forests become increasingly fragmented in many landscapes.

2.7. Tables

Table 2.1
Tree size, focal tree location, and fruit size of *Tapirira mexicana*. *T. mexicana* is a canopy tree that reaches 30 m in height with maximum crown radius of 10 m at maturity. Mean (± SD) tree size (dbh, height, and mean crown extent), location of focal trees (elevation and distance from the forest edge), and fruit size (length, width, and fresh weight) are given for each fragment. N indicates the sample size for the measurements of focal tree and fruits. * $P < 0.01$, ** $P < 0.001$, *** $P < 0.0001$.

Habitat	Tree size			Tree location		N	Fruit size			N
	DBH (cm)	Height (m)	Mean crown size (m)	Elevation (m)	Distance from forest edge (m)		Length (cm)	Width (cm)	Weight (g)	
Interior	44.0 ± 9.9	28.1 ± 3.7	4.6 ± 0.8	1204 ± 53	345 ± 111***	7	1.73 ± 0.17	1.13 ± 0.12	0.93 ± 0.18	180
Edge	44.5 ± 21.3	265.3 ± 4.9	5.3 ± 2.0	1174 ± 68	109 ± 43	9	1.95 ± 0.16*	1.30 ± 0.10**	1.23 ± 0.25*	210
Total	44.3 ± 16.8	26.5 ± 4.5	5.0 ± 1.6	1187 ± 62	212 ± 143	16	1.85 ± 0.20	1.22 ± 0.14	1.09 ± 0.27	390

2.8. Figures

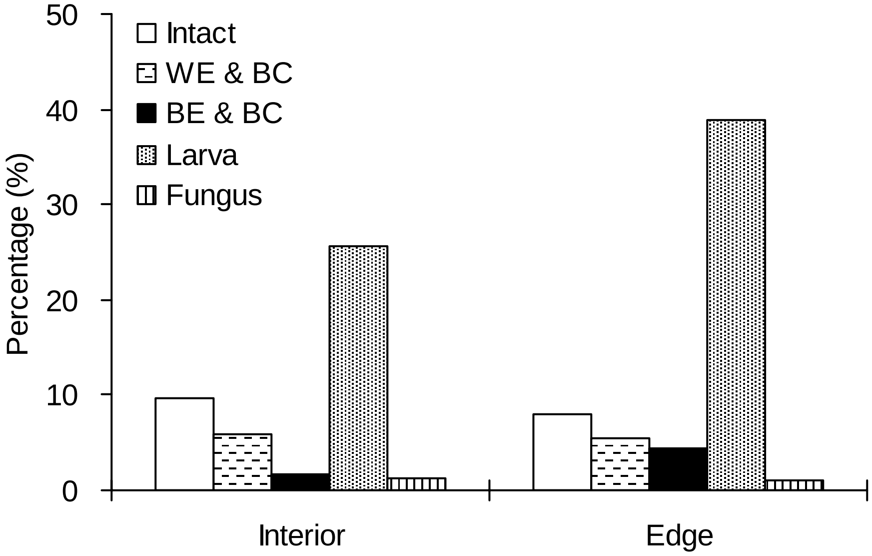

Figure 2.1
Proportional difference in five seed integrity categories between forest interior and forest edge habitats. Percentage for each category totals up to 100 percent and results from G-tests were significant. Five seed integrity categories are: intact, white embryo and black cotyledon (WE & BC), black embryo and black cotyledon (BE & BC), larva, and fungus.

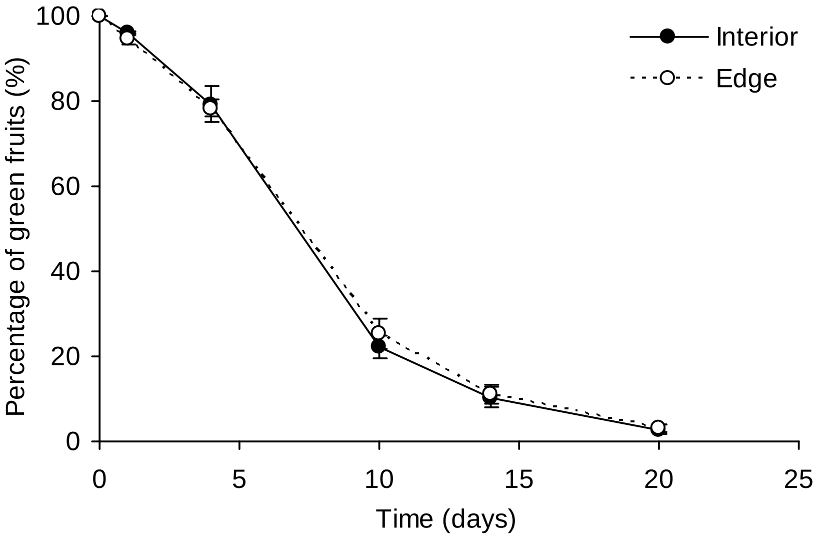

Figure 2.2
Mean proportion of green fruits (*in situ* seed longevity) after placement in the forest interior and edge habitats for *T. mexicana*. Mean longevity was 7.8 days. Error bars represent SE.

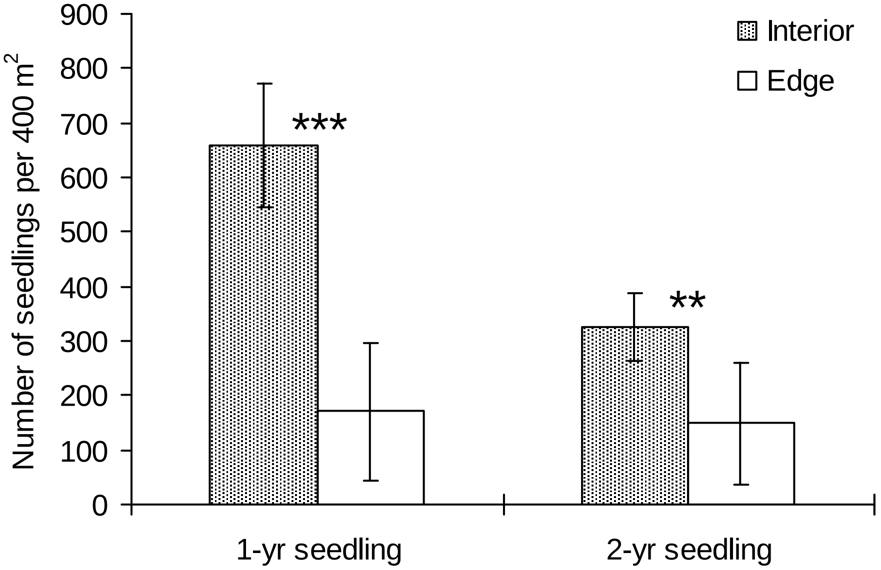

Figure 2.3
Number of 1st-yr and 2nd-yr seedlings occurring in transects (400 m^2) around the focal trees in forest interior and edge habitats for *T. mexicana*. ** $P < 0.001$, *** $P < 0.0001$. Error bars represent SE.

CHAPTER 3

HIGHER SEED GERMINATION FROM SMALLER THAN MEDIUM-SIZED PREMONTANE FOREST FRAGMENTS FOR THREE ANIMAL-DISPERSED TREE SPECIES IN COSTA RICA [2]

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[2] Sugiyama, A. and Peterson, C. J. Submitted to *Plant Biology*, 01/30/2012.

3.1. Abstract

Forest fragmentation is pervasive in tropical landscapes, and animal-dispersed tree species are among the most threatened. Seed source is an important factor for active conservation efforts for such species, but many studies show lower germination levels for seeds collected from small fragments compared to larger forests. However, the increasing rarity of large forests makes them difficult to be used as seed sources. We assessed the potential of small to medium-sized fragments (4-209 ha) to serve as seed sources for the conservation and restoration of three such species (*Ficus tonduzii*, *Lacistema aggregatum*, and *Quararibea aurantiocalyx*) in Costa Rican premontane wet forests. Germination, seedling survivorship, and growth for two years were quantified in a screen house environment. Two years later, more rigorous germination tests were conducted using *L. aggregatum*. Within a species, germination levels differed substantially among source trees. Seeds from some individuals had no germination, suggesting that fecundity alone may be an incomplete indicator of individual fitness. Furthermore, in contrast to many previous studies, germination and seedling survivorship were better for seeds from smaller fragments for all three species, and for both study years. Subsequent seedling survivorship did not counteract the trends generated by germination, and the better performance of seeds from smaller fragments was retained for two years as *ex situ* seedlings. Higher seed quality in smaller fragments was associated with larger seed size and potential outcrossing in trees near forest edges. Our results suggest a previously unrecognized potential of small fragments as seed sources.

3.2. Introduction

Tropical wet forests are one of the most diverse terrestrial ecosystems, yet they are becoming increasingly fragmented in historically forested landscapes (Gascon *et al.*, 2000). Various negative impacts of forest fragmentation have been reported (Fahrig, 2002; Laurance *et al.*, 2002) but such impacts are not equivalent across species. For example, animal-dispersed plant species are more strongly impacted than species with abiotic dispersal modes (Metzger, 2000; Terborgh *et al.*, 2008) due to the local extinction of animal dispersers in fragmented forests (Cordeiro and Howe, 2001). Among different life forms, tree recruitment is especially reduced in forest fragments (Benitez-Malvido and Martinez-Ramos, 2003). Therefore, animal-dispersed tree species are one group particularly threatened by forest fragmentation.

To maintain populations in fragments, a species must successfully regenerate, and a critical first stage for species regeneration is germination. Fragmentation can affect germination either abiotically, in which environments of fragmented forests are unsuitable for germination (Bruna, 1999, 2002), or biotically, by impairing seeds (Chacoff *et al.*, 2004). Regarding the abiotic pathway, germination can be lower in fragments than in continuous forest when seeds from the same source are sown in both sites (Bruna, 1999, 2002). Similarly, regarding a biotic pathway, germination can be reduced for seeds collected from isolated trees or fragments compared to those from continuous forest when germinated in a common environment (Nason and Hamrick, 1997; Rocha and Aguilar, 2001; Cascante *et al.*, 2002; Henriquez, 2004; Valdivia and Simonetti, 2007).

In conservation, if species regeneration is hindered by dispersal limitation or low seed quality, seeds from sources that produce higher-quality seeds could be used to supplement existing seed rain. Similarly, transplantation of seedlings requires high-quality seeds with high establishment success. Thus, either *in situ* or *ex situ*, where to collect seed is an important factor to be considered in conservation and restoration (Vander Mijnsbrugge *et al.*, 2010). If seed quality is lower for trees in fragments or on isolated trees, as studies suggest (e.g., Cascante *et al.*, 2002; Valdivia and Simonetti, 2007), seeds should be collected from large undisturbed forests. However, as forests become increasingly fragmented in many landscapes (Gascon *et al.*, 2000), small forest fragments are becoming the norm, and thus, often provide the only practical source of seeds. Fragments used for comparison with continuous forests are also biased towards very small fragments (< 10 ha) and more studies on medium-sized fragments are needed (Zuidema *et al.*, 1996). Moreover, a general perception is that the negative impacts of fragmentation increase with reduction in fragment size, and thus, there is much discussion of whether small fragments have much conservation value (Ghazoul, 1996; Turner and Corlett, 1996a; Arroyo-Rodriguez *et al.*, 2009).

Our main goal was to assess the potential of small to medium-sized fragments as seed sources for conservation or restoration practices. To address issues above, we conducted *ex situ* germination tests for three animal-dispersed tree species with seeds collected from one medium-sized (209 ha) and several smaller premontane wet forest fragments (4-33 ha). We hypothesized that seeds collected from trees in the medium-sized fragment would be of higher quality than those from the smaller fragments. In germination tests, we prioritized practicality in actual conservation or restoration practices and tracked survivorship and growth for two years to assess their utility as transplants elsewhere. We also aimed to assess whether predicting good source trees for collecting fruits was possible for our focal species. Two years later in 2011, we conducted more rigorous tests and assessed inter-annual variation for one species that had large fruit crop.

3.3. Methods

Study sites

This study was conducted at Las Cruces Biological Station (LCBS; 8° 47' N, 82° 58' W), a field station of the Organization for Tropical Studies (OTS) in Coto Brus county, southern Costa Rica (Fig. 1.1; Table 3.1). The area is situated in a highly deforested landscape with overall forest cover within a 15 km radius of the station is estimated at 27% (Daily *et al.*, 2001). The forest reserve at LCBS is the largest remaining primary forest fragment (209 ha) in the immediate area. Evidence from aerial photographs show that LCBS forest was finally isolated as a fragment around 1978 to 1979, with partial isolation beginning in the 1960s (R. A. Zahawi, *personal communication*). Natural vegetation in the area is classified as tropical premontane wet forest (Holdridge *et al.*, 1971), and elevations range from 1,000 to 1,400 m. Temperature ranges between 14 and 29°C (mean: *ca*. 20°C) with a distinct dry season from December through March. Annual precipitation was 4,223 mm and 5,011 mm in 2008 and 2010, respectively, while 2009 (El Niño year) was an unusually dry year with only 3,063 mm of annual precipitation, which affected fruit production of many tree species during the early wet season of 2009 and 2010 (A. Sugiyama, *personal observation*). In contrast, 2011 was a La Niña year with wet dry season and dry wet season. In addition to the forest reserve at LCBS, fruits were collected in four smaller primary forest fragments (Fig. 1.1; AGZ: 33 ha, CED: 27 ha, LL: 19 ha, PC: 4 ha). All five fragments are within 8 km of LCBS with similar elevations and climate, and have not had recent major disturbances.

Focal species

Based on the abundance of reproductive trees in the study sites and fruit availability during early wet season of 2009, we selected three animal-dispersed tree species (Table 3.2); *Ficus tonduzii* Standley (Moraceae), *Lacistema aggregatum* (P.J. Bergius) Rusby (Lacistemataceae), and *Quararibea aurantiocalyx* W. S. Alverson (Malvaceae). The number of seeds per fruit varies among species. *F. tonduzii* has many small seeds per fruit, *L. aggregatum* has one seed per fruit, and *Q. aurantiocalyx* has an average of two seeds per fruit. All three species produce fleshy fruits dispersed by birds, rodents, white-faced capuchin monkeys (*Cebus capucinus*), and bats (*F. tonduzii*), all of which are known to occur in all the fragments (relative abundance unknown; F. Oviedo, *personal communication*). None of the seeds possess dormancy under screen house conditions (A. Sugiyama, *personal observation*). Fruiting phenology varies among the species. *F. tonduzii* produces fruits all year round as a species but the same individual trees do not produce fruits annually. *L. aggregatum* and *Q. aurantiocalyx* have a more distinct peak in fruiting phenology, between April and June (peak: April-May) and May and August (peak: June-July), respectively. In contrast to the fruiting phenology, flowering phenology/mode is largely unknown. *F. tonduzii* and *L. aggregatum* are monoecious while it is unknown for *Q. aurantiocalyx* (F. Oviedo, *personal communication*). Similarly, pollination mode is unknown but for *F. tonduzii*, which is pollinated by small fig wasps (Agaonidae). Among the three species, *Q. aurantiocalyx* is listed as “Endangered C2a” in version 2.3 of the ICUN red list.

Germination tests

We conducted germination tests in a screen house with natural insolation and day length. To minimize seed-shadow overlap, focal trees of at least 120 m (2009) and 30 m (2011) apart from other reproductive conspecifics were randomly chosen in the primary forest area of each fragment. When sample size was very small in the initial collection and fruits were available later, we collected additional fruits on different days within a month but there was no consistent date effect. We collected fruits without visible damage under each focal tree, which is a common practice (Cascante *et al.*, 2002). The number of focal trees varied depending on fruit availability (Table 3.2). We kept fruits in plastic bags with wet paper towels to prevent moisture loss until measurements and germination tests. Fruits were sown intact the day of collection in sterilized soil to avoid fungal infection from the soil or potential microbial effects on germination or early mortality of germinants (e.g., Bever *et al.*, 2010). The soil was forest floor-decomposed organic material (e.g., litter) that was boiled for further decomposition, followed by sterilization at 105°C for a minimum of 16 hours prior to use. We watered fruits daily and germination was followed until the end of the field season when no new germinants were observed for more than a week, unless the seed was obviously dead prior to that.

Unlike some previous germination tests, we did not extract seeds from each fruit, for two reasons. First, our main goal was to assess the potential of small fragments as seed sources for conservation or restoration practices. Therefore, feasibility and practicality were given the highest priority. Some seeds of tropical tree species are hard to extract, and extraction can possibly damage the seeds. If we can obtain reasonably high germination levels without extracting the seeds from the fruit, it would be useful for actual conservation practices. Ideally, germination tests should be conducted on both conditions but low overall sample size did not allow such comparisons in 2009. Second, maturity level of seeds within a fruit was unknown for *F. tonduzii*. When fruits were cut open, seed color varied from white to brown, presumably reflecting differences in seed maturity. Not only would extracting small seeds be time consuming but it would also introduce variation in maturity levels within a fruit, which was not of interest here. Thus, we recorded germination per fruit (fruit germination treatment). This method does not account for the effect of digestion or handling by animals, but our goal was to assess maternal effects on seed quality produced by trees in different-sized fragments *ex situ*.

To assess whether evaluation of germination per fruit had biased the results, we evaluated germination on a per-seed basis for *Q. aurantiocalyx* retrospectively. This attempt was not conducted for *F. tonduzii* due to the difficulty in estimating the exact number of seeds per fruit. We assumed an average of two seeds per fruit and used the actual number of seedlings emerging instead of evaluating fruits binomially (i.e., germinated vs. failure).

Seedling survivorship and growth

At the end of the field season in August 2009, we transplanted all germinated seedlings to a larger planting space within the screen house using the same sterilized soil as for the germination tests. In January 2010, we individually tagged and transplanted the seedlings to individual plastic transplant bags with drainage holes to allow further growth. At the end of April 2010, we recorded basal diameter, seedling height, and survivorship since August 2009 to assess early seedling development. After the first seedling measurements in April 2010, a total of 288 *F. tonduzii* seedlings of all sizes (i.e., no size bias in seedlings left) and one *Q. aurantiocalyx* seedling were excluded for a different study. In June 2011, we recorded 2-yr survivorship and growth for the rest of the seedlings. We calculated growth rate as $(g_t - g_{t-1})/(d_t - d_{t-1})$ where g_t is growth (basal diameter or height) at time t and d_t is growing days at time t when the measurement took place.

Since initially high germination levels may be counteracted by low subsequent survivorship, we also evaluated individual persistence up to two years by cumulative probabilities. With the initial stage of seed as 100%, we calculated cumulative probabilities for subsequent stages (germination, 1-yr seedling, and 2-yr seedling) as $n_{t-1} \times p_t$ where n_t is number of individuals at time t (stage) and p_t is probability of being at a given stage.

Inter-annual variation

To assess inter-annual variation and to increase sample sizes, we attempted to collect fruits again in 2010. However, none of the focal species produced fruits during the wet season of 2010, and overall fruit production in the forest was even lower than in 2009 (A. Sugiyama, *personal observation*). In early wet season 2011 we used *L. aggregatum*, which was the only species with adequate seed crop, for further germination tests to assess inter-annual variation. We re-visited all the focal trees used in 2009 and added trees in the primary forest area that were fruiting in late April to mid May of 2011. In 2011, we used a total of 36 focal trees (LCBS: 20, AGZ: 4, CED: 5, LL: 7) and a total of 11,374 fruits.

We conducted germination tests in the same manner as in 2009, but this time we carefully extracted seeds from half of the collected fruits for each tree (seed germination treatment) to assess the effect of seed extraction on germination. Before seeds were extracted and measured for size (length, width, and fresh weight), we measured fruit size. Although only visibly intact fruits were collected, when seeds were extracted some fruits proved to be predated before dispersal by larvae or nematodes. Since such damage could not be assessed in the fruit germination treatment, such dead seeds were also included in the sample size for seed germination treatments when fruits were visibly intact. Therefore, there was no bias in the denominator when germination percentages were calculated for fruit and seed germination.

In 2011, we also recorded days until germination for individual fruit and seed. Since there was some mortality between germination and the end of the field season in late June 2011 (duration 53 days ± 1.4 standard error (SE) per tree), we also calculated early mortality percentage for both germination treatments. We calculated early mortality as $(s_{max} – s_{fin})/(d_2 - d_1) \times 100$, where s_{max} and s_{fin} are number of maximum germinants and number of final germinants, and d_2 and d_1 are final day of the field season when recording took place and day when germination test was initiated, respectively.

Statistical analyses

We conducted stepwise model selection to evaluate factors that potentially affect seed quality (entry and stay levels: 0.06). We treated each focal tree as an experimental unit, and germination, 1-yr and 2-year seedling survivorship, and 1-yr and 2-year growth rates were the response variables. Predictor variables were fragment size, distance of the focal tree from the nearest forest edge, elevation, dbh, and mean fruit size for each focal tree. Additionally, we included germination percentage and seedling growth or survivorship in predicting survivorship or seedling growth, respectively. When there were multiple significant variables left in the best model, we also conducted simple linear regressions or correlation analyses to evaluate the direction of its effect.

In assessing whether seed quality or seedling performance from small fragments are low, we compared focal trees in different sites: i.e., between the medium-sized LCBS fragment and the four smaller fragments pooled, collectively referred to as smaller fragments (SF). Hereafter, we call these categories “site groups” (i.e., LCBS vs. SF). Comparisons between continuous or large forest and combinations of small fragments are common in forest fragmentation studies (e.g., Bruna, 1999; Cascante *et al.*, 2002). Here, we compare site groups due to the distinct division in fragment sizes and to balance sample sizes. For each species, we evaluated differences in germination and seedling survivorship among maternal trees by G-tests (Fisher’s exact tests for small sample size). We assessed differences in germination and survivorship between site groups (fixed effect) by generalized linear mixed models (GLMM) with binomial distribution and logit link, where trees were nested within site groups as a random effect. When there was a quasi-complete separation problem (e.g., no germination for trees from one site group), logistic regression was used with Firth’s penalized maximum likelihood estimation, with trees nested within site groups. We used GLMMs with a Gaussian distribution and identity link to test for differences between site groups in growth rates and in cumulative probabilities for each stage.

For germination tests of *L. aggregatum* in 2011, we added seed germination and early mortality percentages in the models as response variables, and mean seed size and days until first germination as predictor variables for model selections. We analyzed the rest of the tests for the seed germination treatment in the same manner as the 2009 data. We used GLMMs (binomial distribution and logit link) for differences in final germination and early mortality percentage between site groups and germination treatments (fixed effect). Additionally, we compared days until first germination between site groups and germination treatments with GLMM (Poisson distribution and log link). As seed size was not measured in 2009, we used simple linear regressions to assess relationships between fruit and seed size for *L. aggregatum* in 2011.

Finally, we assessed inter-annual variation in germination (fruit germination treatment) and fruit sizes for trees of *L. aggregatum* that produced fruits in both years. We conducted GLMMs (Gaussian distribution and identity link) for differences in fruit size for each tree, with the Tukey-Kramer adjustment for multiple comparisons for year and site group interactions. For differences in fruit size between years, we conducted t-tests. For all analyses, we used SAS 9.2 software (version 9.2, SAS Institute Inc., Cary, NC, US). Data that did not meet the normality assumption were log-transformed, or angular-transformed for proportion data, prior to analyses for regressions, correlations, and Gaussian distribution in GLMMs. All R^2 values reported here are adjusted R^2, correlation coefficients are Pearson correlation coefficients, errors are standard deviation (SD) except for SE in percent data, and germination percentage is the final germination percentage, unless otherwise indicated.

3.4. Results

Germination tests in 2009

Germination percentages varied among the focal species (Table 3.3), and within species germination varied substantially among maternal trees for all three species (Fig. 3.1; *F. tonduzii*: $G = 79.8$, df = 4, $P < 0.0001$; *L. aggregatum*: Fisher's exact test, $P < 0.0001$; *Q. aurantiocalyx*: Fisher's exact test, $P < 0.0001$). Furthermore, for all the species, seeds from some trees had no germination. Germination was affected by several factors. Fruit size was important for all three species (*F. tonduzii*: width, $P = 0.053$; *L. aggregatum*: length, $P = 0.057$; *Q. aurantiocalyx*: weight, $P < 0.0001$). Additionally, while fragment size was important for *F. tonduzii* ($P = 0.0079$; overall: $F = 117.9$, $P = 0.0084$) and *L. aggregatum* ($P = 0.0037$; overall: $F = 10.2$, $P = 0.0049$), distance from the edge ($P = 0.027$) was important for *Q. aurantiocalyx* (overall: $F =$

111.0, $P < 0.0001$). As opposed to the hypothesis, germination percentages were higher for SF than for LCBS for all three species (Fig 3.1; *F. tonduzii*: $\chi^2 = 11.0$, $P = 0.0009$; *L. aggregatum*: $F = 6.2$, $P = 0.032$; *Q. aurantiocalyx*: $\chi^2 = 23.3$, $P = 0.003$). When focal trees within each site group were individually inspected, germination percentage and distance from the edge were negatively related (Fig. 3.2).

Fruit size was an important predictor for germination in all three species. When fruit size was assessed separately, percent germination was positively correlated with mean fruit size for each species but it was significant for only one species (*F. tonduzii*: $r = 0.68$, $P = 0.20$; *L. aggregatum*: $r = 0.43$, $P = 0.17$; *Q. aurantiocalyx*: $r = 0.93$, $P = 0.0003$). One might also expect that larger trees produce larger fruits with seeds that show higher germination rates but tree size had no effect on germination (data not shown). When fruit size was assessed between site groups, fruit size was significantly larger for SF than for LCBS for all three species (*F. tonduzii*: $F_{4,113} = 29.1$, $P < 0.001$; *L. aggregatum*: $F_{11,467} = 39.5$, $P < 0.001$; *Q. aurantiocalyx*: $F_{8,144} = 7.3$, $P < 0.001$).

When germination was evaluated per seed for *Q. aurantiocalyx*, fruit size (width, $F = 12.7$, $P = 0.012$) was the only significant predictor for germination. However, significant differences among focal trees (Fisher's exact test, $P < 0.0001$) and between site groups ($\chi^2 = 6.1$, $P = 0.017$) were retained.

Seedling survivorship and growth

Seedling survivorship and growth rates also varied among species during the first year after germination (Table 3.3). As for germination, 1-yr seedling survivorship varied among maternal trees for two of the species (Fig. 3.1; *F. tonduzii*: $G = 115.6$, df = 2, $P < 0.0001$; *L.*

aggregatum: Fisher's exact test, $P = 0.31$; *Q. aurantiocalyx*: Fisher's exact test, $P < 0.0001$). In contrast, none of the predictors were significant for 1-yr and 2-yr seedling survivorship and neither differed between site groups for any of the species (data not shown).

For 1-yr basal diameter growth, no predictors were significant for *F. tonduzii*, but distance from the edge was significant for *L. aggregatum* ($F = 20.5$, $P = 0.045$) and germination percentage was for *Q. aurantiocalyx* ($F = 19.5$, $P = 0.011$). In predicting 1-yr height growth, germination percentage (*F. tonduzii*: $F = 132.4$, $P = 0.055$), fragment size (*L. aggregatum*: $F = 596.7$, $P = 0.0017$), and fruit size (*Q. aurantiocalyx*: length, $F = 11.5$, $P = 0.027$) were significant. There was no difference in 1-yr seedling growth rates (both basal diameter and height) between site groups (data not shown) except for 1-yr basal diameter growth for *Q. aurantiocalyx* ($F = 6.5$, $P = 0.052$), with higher growth rate for seedlings from SF than those from LCBS (Fig. 3.3). In predicting 2-yr seedling basal diameter or height growth, no variable was significant, and neither differed between site groups for any species (data not shown).

The relationship between predictors for 1-yr basal diameter growth that proved to be significant from model selections were positively correlated for both *L. aggregatum* (distance from edge: $r = 0.95$, $P = 0.045$) and *Q. aurantiocalyx* (germination percentage: $r = 0.81$, $P = 0.027$). Similarly, significant predictors for 1-yr height growth were positively correlated for *F. tonduzii* (germination percentage: $r = 1.00$, $P = 0.055$) and *Q. aurantiocalyx* (fruit length: $r = 0.86$, $P = 0.027$), but not for *L. aggregatum* (fragment size: $r = -0.041$, $P = 0.95$).

When 2-year individual persistence was assessed, there were some interchanges among the trees for the three stages (germination, 1-yr seedlings, and 2-yr seedlings; Fig. 3.4). However, subsequent survivorship did not counteract the low germination from LCBS for all three species.

Thus, trends in 2-year persistence were generated by germination, and for all the species and stages, differences between site groups were significant.

Germination tests for L. aggregatum *in 2011*

In 2011, different variables influenced germination of *L. aggregatum*. Distance from the edge, instead of fragment size, was important in predicting both fruit ($P = 0.0001$; overall: $F = 15.7$, $P < 0.0001$) and seed ($P < 0.0001$; overall: $F = 27.2$, $P < 0.0001$) germination treatments, and fruit size was no longer important. Days until first germination were also significant (fruit: $P = 0.021$, seed: $P = 0.017$). Germination percentage was negatively correlated with mean days until first germination for both treatments (fruit: -0.55, $P = 0.0027$; seed: -0.51, $P = 0.0030$), which were significantly longer for LCBS (23.8 ± 7.3 days) than for SF (19.9 ± 5.9 days) in seed ($F = 7.6$, $P = 0.014$) but not for fruit germination treatment (LCBS: 23.6 ± 5.2 days, SF: 24.4 ± 7.0 days, $F = 0$, $P = 0.97$). Distance from the edge was negatively related to fruit germination percentage as in 2009 (Fig 3.2b), which was also true for seed germination treatment ($R^2 = 0.33$, $P = 0.0001$). As a result, germination percentage was significantly higher for SF than for LCBS again in 2011 (Fig. 3.5a) for both treatments (fruit: $F = 10.4$, $P = 0.0028$; seed: $F = 16.0$, $P = 0.0003$).

Distance from the edge ($P = 0.020$) was again an important variable along with fruit size (width, $P = 0.0039$) in predicting early mortality for fruit germination (overall: $F = 4.9$, $P = 0.017$). However, only days until first germination ($P = 0.021$) was significant for seed germination treatment (overall: $F = 7.2$, $P = 0.017$). Early mortality was positively related to distance from the edge (fruit: $F = 5.5$, $P = 0.027$, $R^2 = 0.15$; seed: $F = 6.3$, $P = 0.018$, $R^2 = 0.15$) but neither fruit size nor days until first germination was correlated with early mortality (data not shown). Similarly, early mortality did not differ between site groups (Fig. 3.5b; fruit: $F = 0.02$, $P = 0.89$; seed: $F = 3.8$, $P = 0.06$).

Fruit and seed size were positively correlated (Fig. 3.6) and significantly higher germination percentage was achieved for the seed (11.0 ± 3.3%; $F = 357.5$, $P < 0.0001$) than for the fruit germination treatment (2.9 ± 0.6%). In addition to improved germination, seeds germinated faster when they were extracted from the fruit (Fig. 3.7; $F = 73.4$, $P < 0.0001$; fruit: 24.3 ± 6.7 days, seed: 20.3 ± 6.1 days).

Inter-annual variation in germination levels of L. aggregatum

Seven out of 12 *L. aggregatum* trees used in 2009 produced fruits again in 2011 (Fig. 3.8). Of those trees, seeds from two trees in LCBS did not germinate both years and seeds from only one non-LCBS tree had relatively high germination both years. The rest of the trees only fruited in one year. Fruits were generally larger in 2011 than in 2009 (Table 3.4), and trees that produced large fruits in 2009 generally produced large fruits in 2011 as well. However, there was no obvious relationship between fruit size and germination for these trees.

3.5. Discussion

Germination levels can vary greatly by source tree even within species (Fig. 3.1; Fig 3.4a). While fecundity itself may vary greatly among maternal individuals (Herrera, 1991), our results indicate that the sole use of fecundity (e.g., fruit or seed number) as a proxy for individual fitness must be viewed with caution especially if fecundity is based on a single growing season. One consequence of low or zero germination from particular mother trees is that even fecund individuals may contribute little or no genes to future cohorts via seeds. Such patterns may lead to low genetic diversity in the long term, although for long-lived species they may be counterbalanced by multiple reproductive episodes. Due to the drought in 2009, fruit production was very low at the community level (A. Sugiyama, *personal observation*). Obtaining sufficient number of seeds is one of the difficulties in restoration practice (Vander Mijnsbrugge *et al.*, 2010) but such drought events may increase with global climate change (Beniston and Stephenson, 2004), which has already been observed in other tropical regions (Malhi and Wright, 2004). Low fecundity will exacerbate deleterious demographic consequences of low or zero germination. Since germination was recorded per fruit in this study, germination reported here is a best-case scenario for species with multiple seeds per fruit (Nason and Hamrick, 1997). For species whose fruiting interval is not annual, impacts from one fruiting event become more severe as fruiting interval increases. Our findings suggest that future cohorts may derive from a smaller proportion of the adult population than is apparent from simple surveys of adult fecundity.

Small fragments may potentially be used as seed sources. Seeds from smaller fragments had higher germination for all the focal species (Fig. 3.1), and this pattern was consistent across two reproductive events for *L. aggregatum* (Fig. 3.5a). This result was unexpected, since most previous studies report the opposite trend (e.g., Cascante *et al.*, 2002). Two important variables affecting germination were fragment size or distance from the edge and fruit size for all three species. Thus, there was a positive edge effect on germination. Reduced outcrossing or inbreeding depression is considered the primary cause for reduced germination in smaller forest fragments (Menges, 1991; Gonzalez-Varo and Traveset, 2010). Similarly, reduced population size may negatively affect outcrossing (Yates *et al.*, 2007; Aguilar *et al.*, 2008) and consequently reduce germination rates (Menges, 1991; Mathiasen *et al.*, 2007; Gonzalez-Varo *et al.*, 2010).

While we did not assess genetic consequences, large fruit/seed size likely led to higher germination for our focal species. Such a relationship between seed (Khera *et al.*, 2004) or fruit size (Khan *et al.*, 1999) and germination is well documented. Similarly, larger fruits may have more seeds per fruit (Wolfe and Denton, 2001) for species with multiple seeds. Larger fruit/seed production in smaller fragments may have been affected by both genetic and environmental causes. Outcrossed pollen and large pollen load may increase seed size (Jordano, 1993; Wolfe, 1995; Hufford and Hamrick, 2003) and affect seed vigor (Schlichting *et al.*, 1990). While outcrossing rates in small fragments are often reduced (Yates *et al.*, 2007; Aguilar *et al.*, 2008), isolated trees or trees in small fragments may have comparable (Rocha and Aguilar, 2001) or even higher outcrossing rates when compared to those in medium-sized fragments (Smith-Ramirez and Armesto, 2003; Mathiasen *et al.*, 2007). Pollen dispersal can exceed 10 km by active pollinator movements (Nason and Hamrick, 1997), especially when remnant trees serve as stepping-stones (Cascante *et al.*, 2002; Fuchs and Hamrick, 2011) or when fragments are connected (Gonzalez-Varo *et al.*, 2010). Mating systems or pollination modes for our focal species are unknown, except for *F. tonduzii* where extensive pollen flow has been reported for a congener (Nason and Hamrick, 1997). Potentially comparable or even higher outcrossing rates in SF than for the medium-sized LCBS may result in larger fruit/seed size in SF. Another non-mutually exclusive possibility is of differing environmental effects on maternal trees in small fragments (Roach and Wulff, 1987). Air temperature can change 2-4°C (Didham and Lawton, 1999; Sizer and Tanner, 1999) or in some cases, up to *ca.* 8°C (Kapos, 1989) within 200-250 m from the edge. High temperatures and intense light can increase fruit size (Roach and Wulff, 1987; Mazer and Gorchov, 1996) and such increases in temperature near the edge may physiologically increase the productivity of trees in smaller fragments. Increase in temperature may also increase pollination success (Goldwin, 1992), and competitive release of parent trees may lead to more vigorous progenies (Wright and Vanschaik, 1994; Mulkey *et al.*, 1996), resulting in higher productivity in small fragments (Neal *et al.*, 2010). Finally, fruits produced in smaller fragments may have less pre-dispersal predation (Cascante *et al.*, 2002; Nabe-Nielsen *et al.*, 2009).

Apparently contradictory results from previous studies of seed germination from different-sized fragments may be caused in part by how fragment sizes are defined. Sizes considered to be “small”, “medium”, and “large” vary among studies. The few studies with at least three sizes suggest similar trends when fragment sizes are re-classified as in the present study, and when small vs. medium-sized fragments are compared (Mathiasen *et al.*, 2007; Gonzalez-Varo *et al.*, 2010; but see Nason and Hamrick 1997). A similar trend of reduced mean fruit set for medium population sizes is also reported (Yates *et al.*, 2007). Previous studies reporting higher germination levels for large or continuous forests (Cascante *et al.*, 2002; Valdivia and Simonetti, 2007) utilized large forests of > 500 ha. Lower germination for medium-sized fragments than for small or large forests may result from two contrasting effects. With increase in fragment/population size, outcrossing may typically increase while fruit/seed size decreases. In medium-sized fragments, neither higher outcrossing rates, which also affects seed size (Hufford and Hamrick, 2003), nor large fruit/seed size may be able to compensate for these contrasting effects. Fragment/population sizes considered to be “medium” vary depending on factors such as species, connectivity of fragments, amount of pollen flow, mating system, and pollination mode, and further studies specifically testing this idea may reveal potentially complex effects of fragment/population size and seed germination.

Maternal effects on seedling survivorship and growth were not as obvious as those on germination. Thus, patterns of 2-yr individual persistence were determined by patterns generated by germination (Fig. 3.4), which is consistent with results elsewhere (Cascante *et al.*, 2002). Inconsistent effects of fragmentation on seedling survivorship and growth are common (Roach and Wulff, 1987; Kery *et al.*, 2000; Verdu and Traveset, 2005). With many correlated variables and maternal effects acting on different stages, fragmentation impacts on early post-germination stages are still complex and difficult to explain (Khera *et al.*, 2004; Broadhurst *et al.*, 2008). Distance from the edge was positively related with early mortality of *L. aggregatum* in 2011 for both treatments, which may represent inbreeding depression (Hufford and Hamrick, 2003) at an early stage (Gonzalez-Varo and Traveset, 2010). Number of days until first germination was also important in predicting the germination success of *L. aggregatum* in 2011. Mean days until germination were shorter for SF, again suggesting better seed quality. Number of days until germination is evolutionarily important since early emergence often affects seedling growth and survival (Verdu and Traveset, 2005). Correlation between seed size and days until germination is also known (Rocha and Aguilar, 2001; Sletvold, 2002; Norden *et al.*, 2009). However, it has rarely been addressed in the context of forest fragmentation.

There are some possible caveats due to our methods. First, we recorded germination on a per-fruit basis in 2009. Two of the species have multiple seeds per fruit, and if germination was measured on a per-seed basis, it could have been lower than the results reported here. This may partly explain the observed difference in germination among species (Table 3.3). However, when effect of such evaluation method was assessed for *Q. aurantiocalyx*, both inter-individual variation and difference between site groups still held. The existence of a carp had an effect on germination rate (Fig. 3.5a) and days until germination (Fig. 3.7) for *L. aggregatum*, which may also apply for the two other species. However, difference in germination levels between site groups (Fig. 3.5a) and a negative relationship against distance from the edge (Fig. 3.2b) still held for the seed germination treatment. Therefore, our main findings do not seem to have been biased by the evaluation method. Second, fruits collected under the mother tree might have been biased because frugivores may prefer larger fruits (Foster, 2008; Pizo and Almeida-Neto, 2009), and some birds may select for large fruits with small seeds (Sobral *et al.*, 2010). If larger fruits with high-quality seeds were more selectively removed from the trees in LCBS with potentially higher animal abundance and species richness, only smaller seeds might have been left for germination tests in LCBS. However, Jordano (1995) showed the opposite. When he compared the seed mass at the beginning of the fruiting season and the seed mass removed by the frugivores, the latter was significantly smaller and such pattern was consistent across his two-year study period. Studies have also shown that larger fruits are dropped more frequently due to greater difficulty of handling regardless of their preferences (Levey, 1987; Sallabanks, 1993). Finally, as all three focal species in this study were selected on the basis of fruit production when it was an unprecedentedly dry wet season, the fact that these three species were able to produce fruits in such a drought might have had a “filtering” effect.

Two conservation implications arise from our results. Small fragments may have substantial, previously unrecognized, potential as seed/germplasm sources for restoration. Small fragments may still maintain high genetic diversity due to high levels of gene flow between small fragments or isolated trees (Cascante *et al.*, 2002; Fuchs and Hamrick, 2011) or species diversity (Obbens *et al.*, 2001; Yates *et al.*, 2007; Sanchez-Gallen *et al.*, 2010), and retain considerable resilience to disturbances (Higuchi *et al.*, 2008). As more forests are reduced to small fragments (Gascon *et al.*, 2000), maintaining small fragments in landscapes is important, not only as vegetation cover but also for genetic connectivity (Gonzalez-Varo *et al.*, 2010). Another important implication is the potential for predicting a good source tree. Three implications of our results arise regarding conservation practices for our focal species. First, seeds are more likely to germinate if collected from trees near the forest edge, which is more likely to be in small fragments with easy access, if no large expanses of forests are available. Second, the better performance of seeds from smaller fragments was retained for two years as *ex situ* seedlings (Fig. 3.4) when mortality is expected to be high. Finally, simplicity and feasibility play an important role in actual conservation practices. Some local rules may already exist (Khan *et al.*, 1999) but for our focal species, seed quality was estimated by fruit size, which is visible and easy to determine on site. Such fruits were more likely to be collected from trees near the edge with easier access and fruits could be collected from the ground without reaching the high canopy. Although removing fruit tissue improved germination for *L. aggregatum*, the whole fruit could be sown and seedlings obtained. Depending on the difficulty of seed extraction, availability of the fruits, number of seedlings needed, and people involved in the conservation practice (e.g., small children), whether seed extraction is worth the effort should be determined. In integrating academic scientists and restoration practitioners (Young *et al.*, 2005), such simple rules can have great value in the actual conservation practices.

3.6. Tables

Table 3.1
Mean distance from the edge for focal trees used as seed sources in each forest fragment. Primary forest areas and mean distance from the edge are given. Errors represent SE. N indicates number of focal trees whose fruits were collected (all species pooled) for germination tests in 2009 and 2011 for each site.

Site	Primary forest size (ha)	Distance from edge (m)	N
PC	4	10 ± 0	1
LL	19	156 ± 25	9
CED	27	105 ± 41	7
AGZ	33	86 ± 21	7
LCBS	209	322 ± 25	31

Table 3.2
Mean tree and fruit size of focal trees of the study species *F. tonduzii*, *L. aggregatum*, and *Q. aurantiocalyx* that were used as seed sources in 2009. Errors are standard deviation and n indicates the number of focal trees whose fruits were collected and used for germination tests in 2009. The number of focal trees in each fragment was as follows; *F. tonduzii* (LCBS: 2, AGZ: 1, CED: 1, PC: 1), *L. aggregatum* (LCBS: 6, AGZ: 5, CED: 1), and *Q. aurantiocalyx* (LCBS: 7, LL: 2).

Species	Family	Tree size					Fruit size				
		DBH (cm)	Height (m)	Mean crown (m)	Max crown (m)	N	Length (cm)	Width (cm)	L/W ratio	Weight (g)	N
Ficus tonduzii	Moraceae	42.7 ± 19.8	18.0 ± 4.0	4.9 ± 1.3	7.1 ± 2.1	5	3.35 ± 0.60	2.91 ± 0.54	0.87 ± 0.06	13.91 ± 6.97	191
Lacistema aggregatum	Lacistemataceae	10.6 ± 3.0	9.2 ± 3.5	2.4 ± 0.6	3.0 ± 0.9	12	0.65 ± 0.11	0.96 ± 0.19	1.53 ± 0.25	0.24 ± 0.09	994
Quararibea aurantiocalyx	Malvaceae	20.4 ± 3.9	18.6 ± 2.7	3.3 ± 0.8	3.9 ± 0.8	9	2.45 ± 0.42	3.05 ± 0.36	1.27 ± 0.19	8.37 ± 2.74	202

Table 3.3
Mean final fruit germination, 1-yr growth rate, and survivorship of the seedlings for germination tests conducted in 2009. Errors represent standard deviation except for percent data in which standard errors are given. N indicates the number of seedlings that survived and were used in the measurements.

Species	Germination (%)	Duration (days)	Basal diameter growth rate (mm day^{-1})	Height growth rate (cm day^{-1})	Survivorship (%)	Growth (days)	N
Ficus tonduzii	29.8 ± 5.5	53.8 ± 10.4	0.013 ± 0.004	0.043 ± 0.016	68.1 ± 20.0	324.0 ± 3.9	313
Lacistema aggregatum	3.1 ± 1.0	45.0 ± 14.2	0.011 ± 0.004	0.054 ± 0.021	40.6 ± 13.2	316.8 ± 10.4	13
Quararibea aurantiocalyx	19.3 ± 1.6	48.7 ± 10.0	0.015 ± 0.004	0.057 ± 0.013	71.1 ± 14.5	316.9 ± 12.0	42

Table 3.4
Inter-annual comparison of mean fruit size and fruit shape (L/W ratio: length/width) for focal trees of *L. aggregatum* that fruited in both study years. Errors represent standard deviation. Different superscript letters indicate significant difference between the two years for each fruit size dimension or shape at α = 0.05. N indicates the number of fruits used for fruit germination treatment.

Site	Tree	2009					2011				
		Length (cm)	Width (cm)	L/W ratio	Weight (g)	N	Length (cm)	Width (cm)	L/W ratio	Weight (g)	N
LCBS	LA1	1.03^{b} ± 0.15	0.67^{b} ± 0.10	1.53^{a} ± 0.18	0.28^{a} ± 0.10	70	1.20^{a} ± 0.14	0.77^{a} ± 0.08	1.56^{a} ± 0.14	0.27^{a} ± 0.06	180
LCBS	LA2	0.96^{b} ± 0.13	0.67^{b} ± 0.10	1.44^{a} ± 0.17	0.27^{a} ± 0.10	60	1.06^{a} ± 0.11	0.80^{a} ± 0.09	1.33^{b} ± 0.13	0.30^{a} ± 0.07	180
LCBS	LA5	1.06^{a} ± 0.13	0.66^{b} ± 0.06	1.62^{a} ± 0.15	0.28^{a} ± 0.07	83	1.03^{a} ± 0.12	0.76^{a} ± 0.10	1.37^{b} ± 0.13	0.26^{b} ± 0.09	220
AGZ	LA8	0.84^{b} ± 0.08	0.62^{b} ± 0.06	1.38^{a} ± 0.18	0.18^{a} ± 0.05	120	0.93^{a} ± 0.09	0.71^{a} ± 0.07	1.31^{b} ± 0.15	0.20^{a} ± 0.04	130
AGZ	LA10	0.98^{b} ± 0.11	0.72^{b} ± 0.11	1.38^{a} ± 0.16	0.27^{b} ± 0.09	104	1.03^{a} ± 0.11	0.82^{a} ± 0.10	1.26^{b} ± 0.13	0.30^{a} ± 0.08	70
AGZ	LA11	0.81^{b} ± 0.11	0.57^{b} ± 0.08	1.43^{a} ± 0.15	0.16^{a} ± 0.05	100	0.87^{a} ± 0.12	0.64^{a} ± 0.10	1.37^{a} ± 0.12	0.16^{a} ± 0.06	28
CED	LA12	1.33^{a} ± 0.18	0.74^{a} ± 0.09	1.81^{a} ± 0.29	0.31^{a} ± 0.08	78	1.25^{b} ± 0.13	0.73^{a} ± 0.07	1.72^{a} ± 0.19	0.27^{b} ± 0.06	195

3.7. Figures

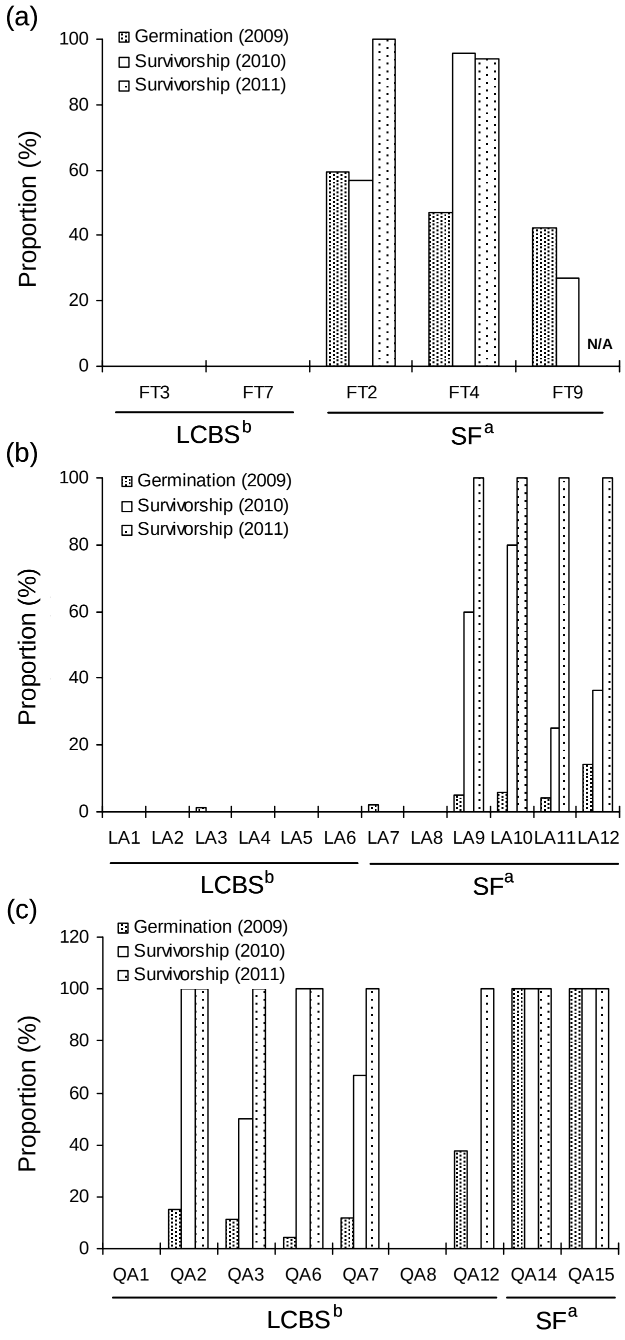

Figure 3.1
Germination and 1-yr and 2-yr seedling survivorship for the germinants among focal trees from germination tests conducted in 2009. Three panels correspond to (a) *F. tonduzii*, (b) *L. aggregatum*, and (c) *Q. aurantiocalyx*. Horizontal bars below focal trees show trees from each site group, and different superscript letters show significant difference at α = 0.05 for germination percentage. Data for 2-yr seedling survivorship was not available for individual FT9 since all the seedlings were used for a different study.

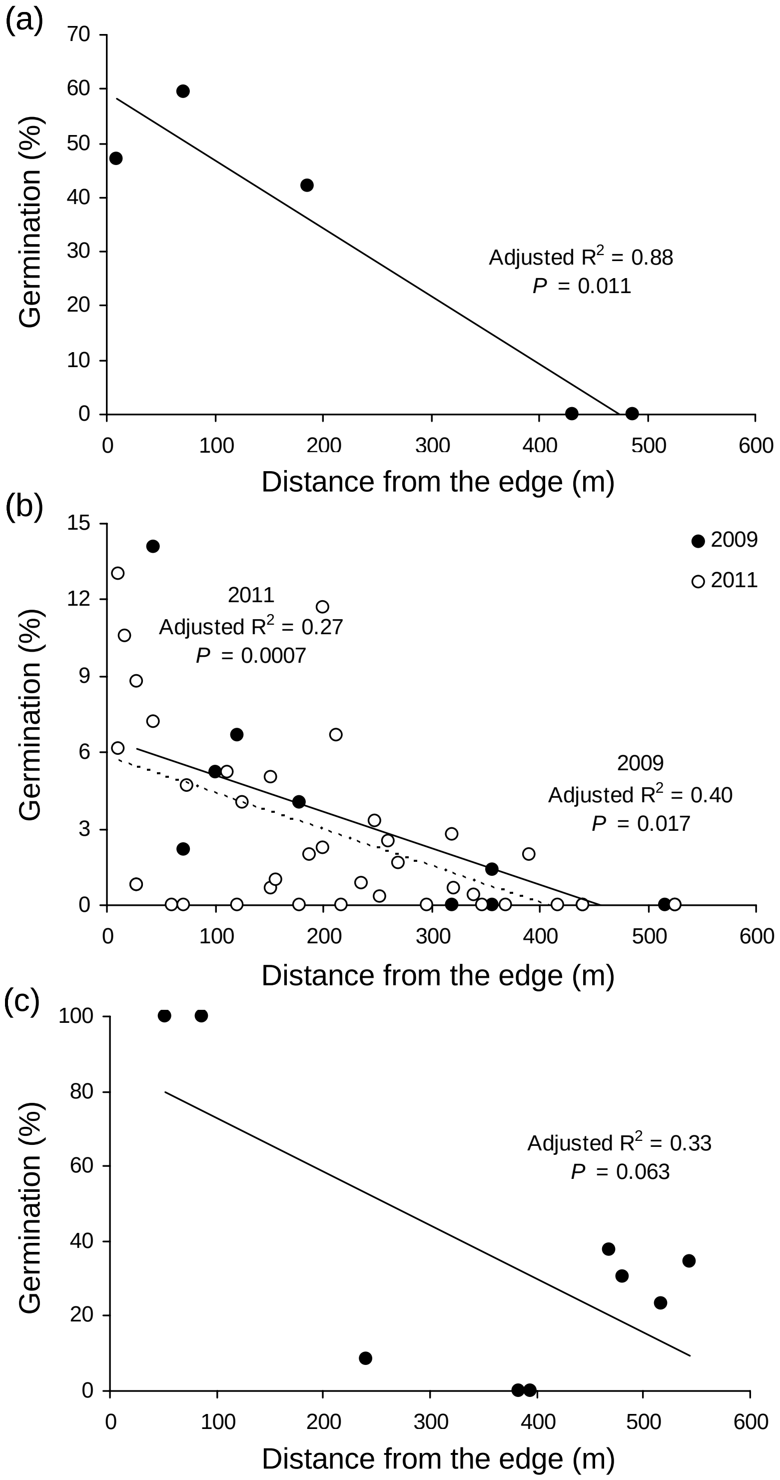

Figure 3.2
Relationship between germination percentage and distance from the edge for the fruit germination treatment. Solid and dotted line show results from 2009 and 2011 (*L. aggregatum* only), respectively. Three panels correspond to (a) *F. tonduzii*, (b) *L. aggregatum*, and (c) *Q. aurantiocalyx*. *P* values are from simple linear regressions and actual statistical tests were conducted using transformed data when the raw data were not normal.

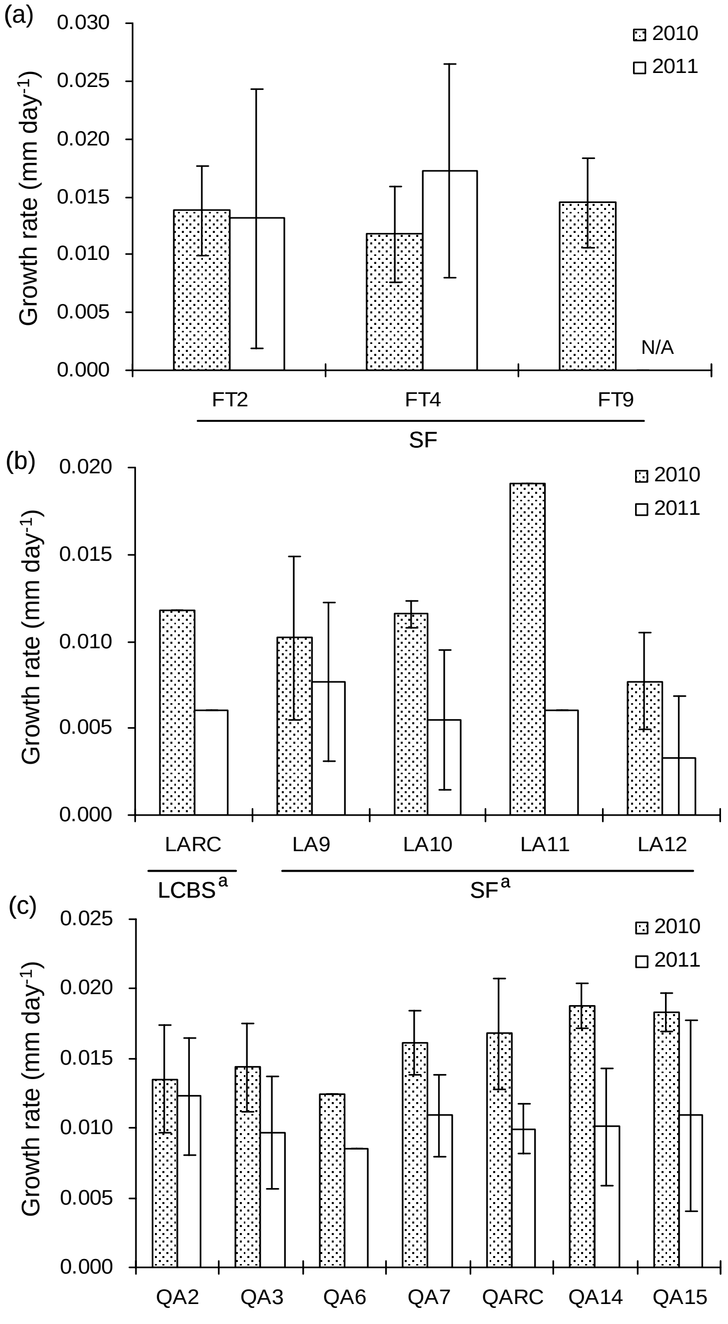

Figure 3.3
Variation in basal growth rate of 1-yr and 2-yr seedlings among focal trees. Three panels correspond to (a) *F. tonduzii*, (b) *L. aggregatum*, and (c) *Q. aurantiocalyx*. Horizontal bars below each focal tree show trees from each site group. Different superscript letters show significant difference at $\alpha = 0.06$ for 1-yr growth rate (2010). While basal growth rate of 1-yr seedlings for *Q. aurantiocalyx* was higher for SF than for LCBS (LCBS: 0.015 ± 0.003 mm day^{-1}, SF: 0.019 ± 0.001 mm day^{-1}), there was no significant difference for 2-yr growth for all the species. Data for 2-yr seedling growth rate was not available for individual FT9 since all the seedlings were used for a different study. Error bars represent standard deviation.

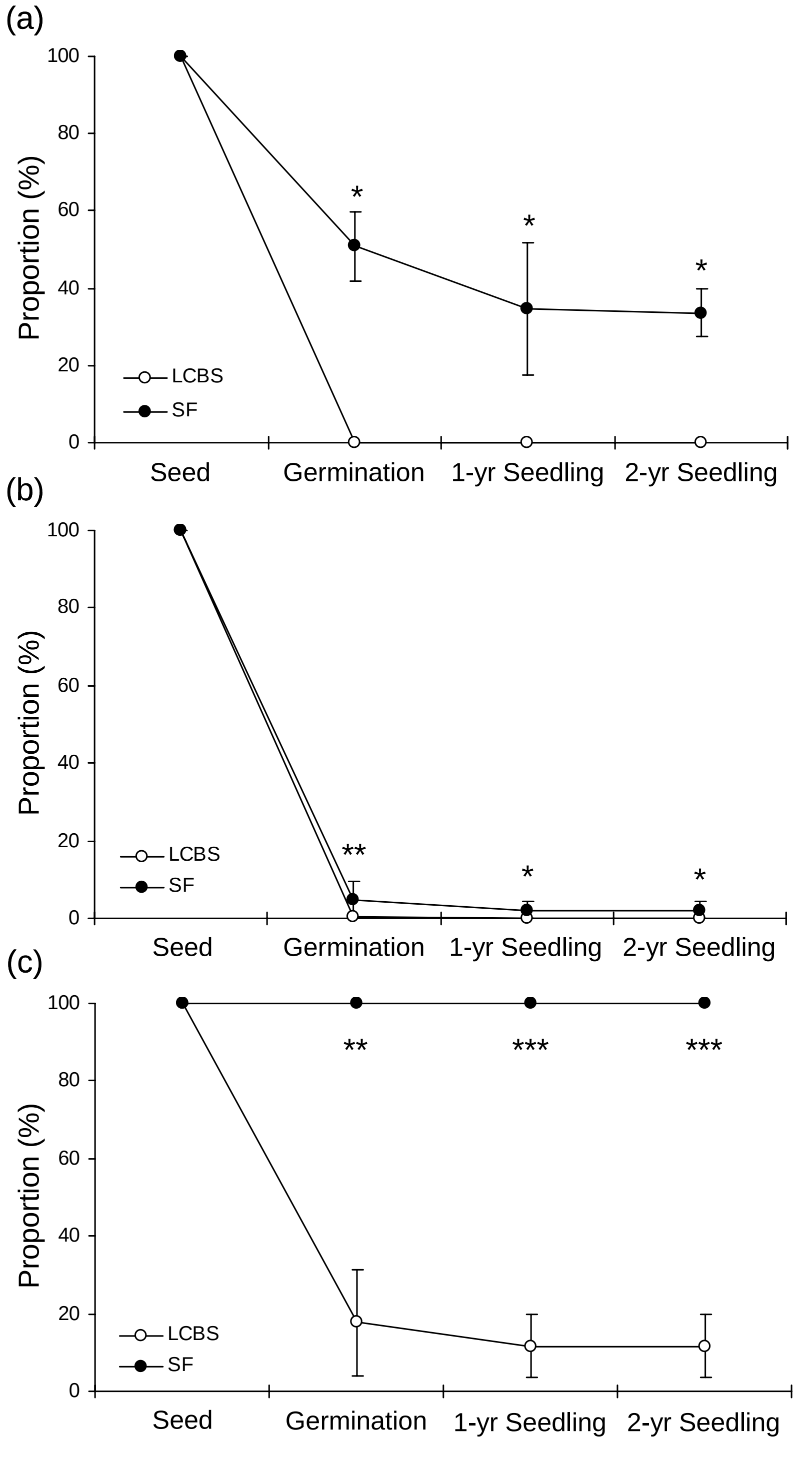

Figure 3.4
Accumulative probabilities of individual persistence for germination, 1-yr seedling, and 2-yr seedling stages. Three panels correspond to (a) *F. tonduzii*, (b) *L. aggregatum*, and (c) *Q. aurantiocalyx*. For all three species and for all three stages, proportions of individuals that persisted at each stage were higher for SF than for LCBS and the trend generated at germination stage was not counteracted by subsequent stages up to two years in the screen house. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0001$.

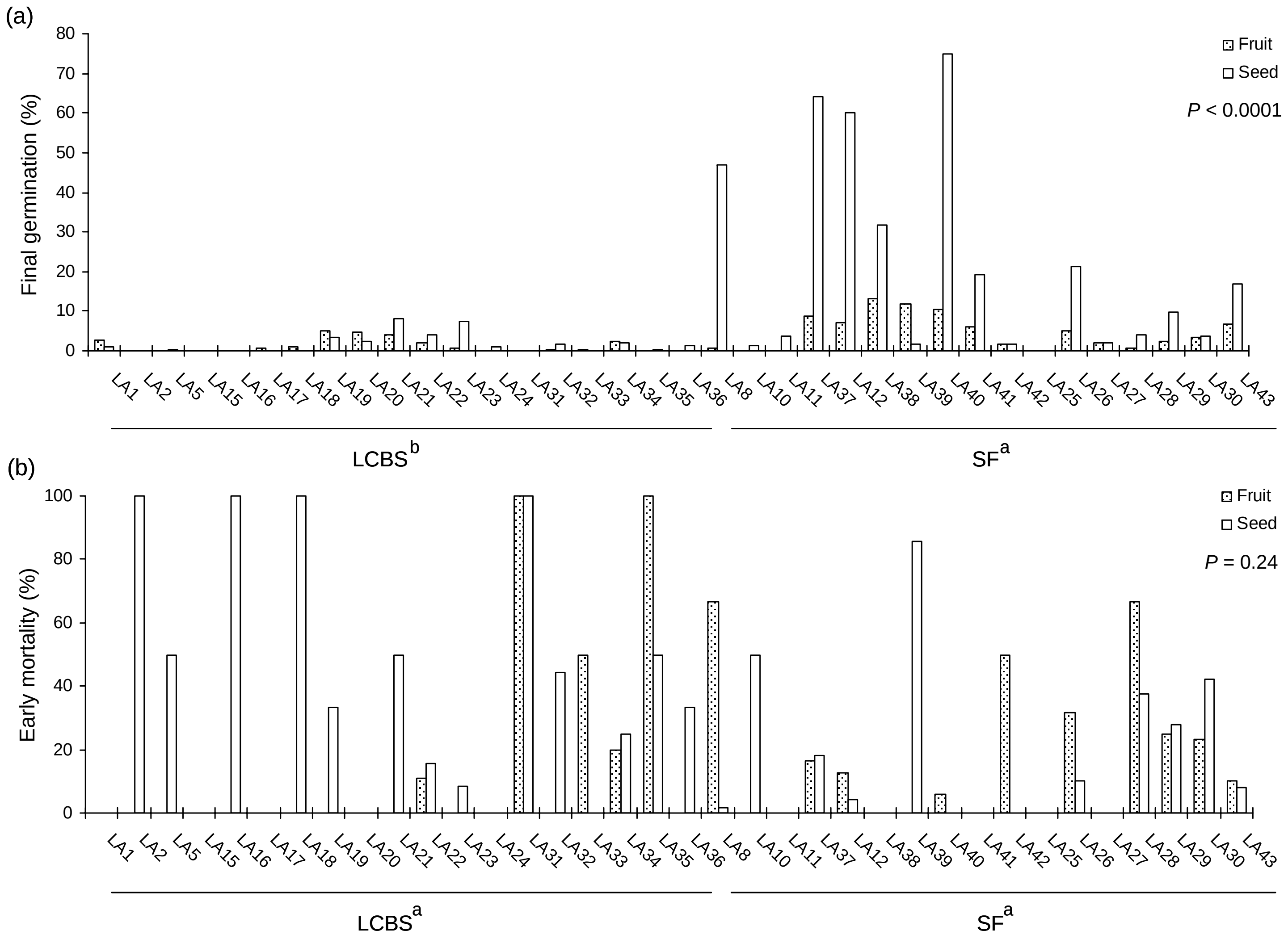

Figure 3.5
Germination and early mortality from fruit and seed germination treatments conducted in 2011 for *L. aggregatum*. Two panels show results for (a) final germination percentage and (b) early mortality. Horizontal bars below focal trees show trees from each site group. Different superscript letters show significant differences between site groups at $\alpha = 0.05$. *P*-values indicate difference between the germination treatments.

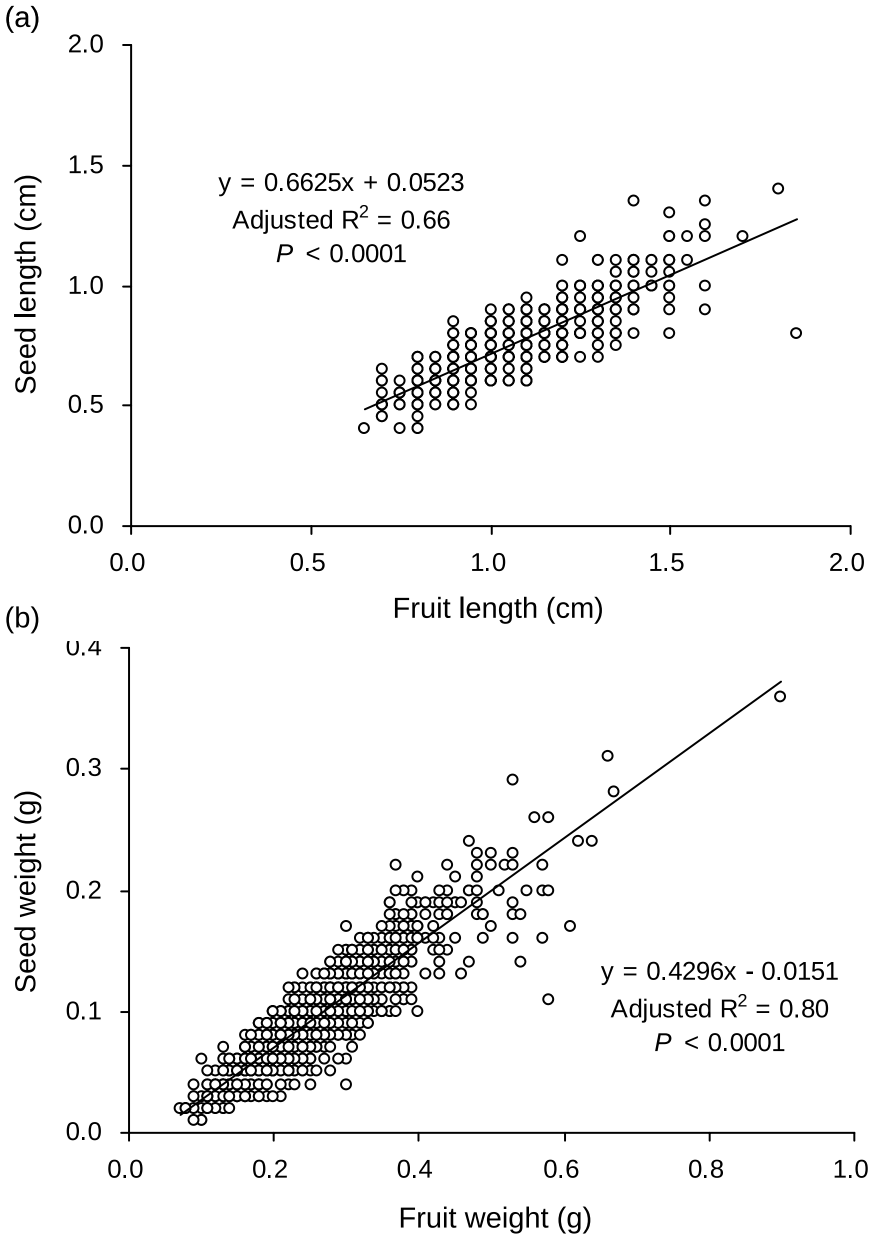

Figure 3.6
Relationship between fruit and seed size of *L. aggregatum* in 2011. Regressions for (a) length and (b) fresh weight show that fruit size was a good indicator for seed size (n = 1,198). Actual statistical tests were conducted on transformed data. All fruit dimensions were positively correlated (length: r = 0.81, $P < 0.0001$, width: r = 0.76, $P < 0.0001$, weight: r = 0.90, $P < 0.0001$).

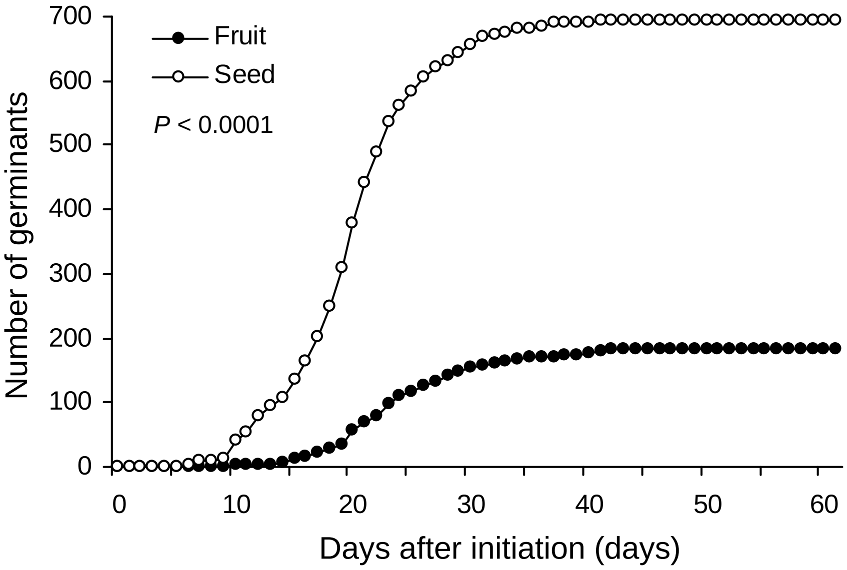

Figure 3.7
Accumulative germination of *L. aggregatum* in 2011 for fruit and seed germination treatments. *P*-value indicates difference in mean number of days until germination between fruit and seed germination treatments. Actual statistical test was conducted on transformed data and sample size for both germination treatments were equal (n = 5,687 each).

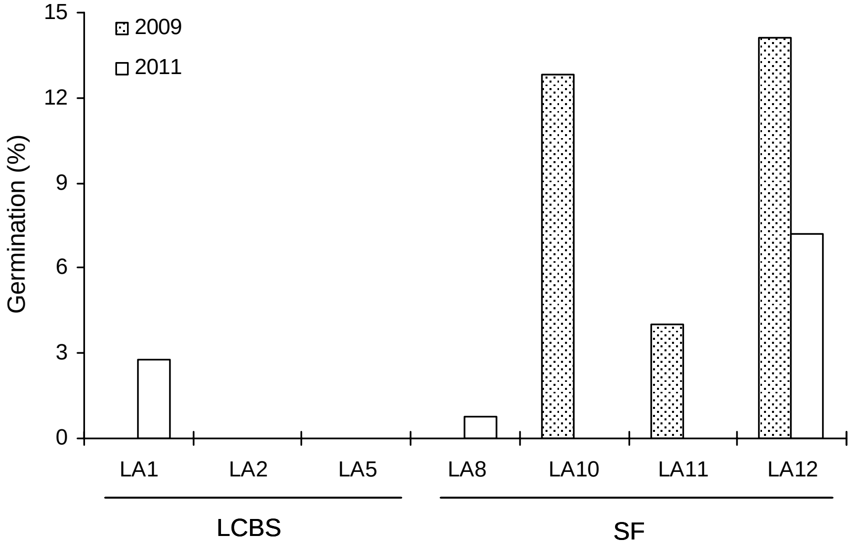

Figure 3.8
Inter-annual variation in germination levels of *L. aggregatum* for fruit germination treatment. There was significant year and site group interaction effect on germination percentage (overall: *F* = 4.5, *P* = 0.0037). When comparison was restricted to these seven individuals that produced fruits in both 2009 and 2011, difference between site groups was significant only for 2011 (2009: t = 0.03, adjusted *P* = 1.00; 2011: t = 2.8, adjusted *P* = 0.029).

CHAPTER 4

MULTI-STAGE EVALUATION REVEALS ONTOGENETIC EFFECTS OF FOREST FRAGMENTATION ON TREE REGENERATION IN TROPICAL PREMONTANE WET FORESTS[3]

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[3] Sugiyama, A. Submitted to *Journal of Ecology*, 04/04/2012.

4.1. Abstract

1. Forest fragmentation is pervasive in the tropics, and shade-tolerant species are considered more threatened than pioneer species. However, a priori knowledge of species life history is largely lacking for many tropical tree species, and it would be useful if a single census of size distributions serves as a reliable predictor for species life history. Conversely, species that are typical pioneer or shade-tolerant species throughout their lifespans are rather rare, with some species undergoing ontogenetic shifts in their light requirements. However, how fragmentation may act on different life-history stages is unknown.

2. In premontane wet forest fragments of southern Costa Rica, I assessed the effects of forest fragmentation with various metrics for five animal-dispersed tree species of all sizes for which there was little a priori knowledge of life histories. The goal of this study was to rank the relative light requirements for species with little a priori knowledge, and to test whether species with lower light requirements are more negatively affected by forest fragmentation.

3. The general prediction that species with low light requirement are more vulnerable to forest fragmentation did not apply at an individual-species level. Even for animal-dispersed tree species that are expected to be threatened by forest fragmentation, effects of forest fragmentation were rather restricted to certain metrics and stages assessed. Negative impacts of edge effects on the number of regenerating individuals were detected only during very early stages for one species, and vice versa for another species. Thus, there was evidence for non-unidirectional ontogenetic effects of forest fragmentation.

4. *Synthesis*. While dichotomous classification based on species light requirements at a certain growth stage is still common, results from this study suggest that ontogenetic shifts of species in their light requirements may be more common than had been previously recognized,

rather than an exception. Recognition of such potential ontogenetic shifts is particularly critical in the context of forest fragmentation to properly assess its effects and to determine the growth stage to be used for restoration. Results here emphasize the importance of considering a wide range of individual sizes and multiple metrics for assessments of forest fragmentation.

4.2. Introduction

Land conversion and urbanization are causing deforestation and fragmentation of tropical forests globally (Gascon *et al.*, 2000; FAO, 2011). Since tropical forests support one of the most diverse terrestrial ecosystems, consequences of forest fragmentation are immense (Laurance *et al.*, 2002) but such impacts are not equivalent across species. Due to local extinction of animal dispersers in fragments (Cordeiro and Howe, 2001), animal-dispersed species are more impacted compared to species with abiotic dispersal modes (Terborgh *et al.*, 2008). Likewise, while pioneer species proliferate on forest edges (Tabarelli *et al.*, 2010a), shade-tolerant species decline in relative abundance (Laurance *et al.*, 2006a). Such shifts in species composition exacerbate the biodiversity consequences of fragmentation because the majority of species- and structural-diversity in tropical wet forests is provided by shade-tolerant species (Tabarelli *et al.*, 2008). Among different life forms, tree recruitment is reduced in fragmented forests (Benitez-Malvido and Martinez-Ramos, 2003). Therefore, animal-dispersed, shade-tolerant tree species are considered to be one of the groups most threatened by forest fragmentation.

While results from studies on plant functional groups reveal such trends, life-history traits are largely unknown for many tropical tree species (Wright *et al.*, 2003). Dispersal syndromes may be fairly easily distinguishable but identifying light requirements for individual species can be challenging because a given genus can include species with a wide spectrum of light requirements, from pioneer to shade-tolerant species (e.g., Davies *et al.*, 1998; Uriarte *et al.*, 2004; Slik, 2005). Classification of species into different functional groups has been based on various methods, such as size distributions (Condit *et al.*, 1998; Wright *et al.*, 2003), presence in different forest types or gaps (Kiama and Kiyiapi, 2001), canopy stratum and growth rates (Chazdon *et al.*, 2010) and ecophysiological traits (Davies, 1998). However, measuring various traits solely to classify species into different functional groups, which is typically not the goal of a study, is labor-intensive. Thus, if a single census of size distributions serves as a reliable metric for functional groups, it will be useful particularly in a highly diverse system like tropical forest (Wright *et al.*, 2003).

Species for which *a priori* knowledge is known, functional grouping of species based on the classical pioneer-shade tolerant species dichotomy (Swaine and Whitmore, 1988) is common (e.g., Uriarte *et al.*, 2004). Germination and recruitment of typical pioneer species depend on gap creation and the seed banks they form (Swaine and Whitmore, 1988; Clark and Clark, 1992). However, increasing evidence suggests that most tropical trees are not extremely light-demanding or shade-tolerant (Wright *et al.*, 2003) and species' light requirements should be viewed as a continuum (Condit *et al.*, 1998) instead of a dichotomy. Moreover, some tropical tree species may undergo a change in light requirement over their lifespan, a phenomenon called ontogenetic shift (Clark and Clark, 1992; Dalling *et al.*, 2001; Wright *et al.*, 2003; Niinemets, 2006). Response to light intensity may change with growth, and species with the same light requirements throughout their life may be rather exceptional (Poorter *et al.*, 2005). In addition to such potential ontogenetic shifts, including a wide range of individual sizes is important in considering potentially different habitat associations across various stages (Kanagaraj *et al.*, 2011). Effects of forest fragmentation have been assessed by various types of impacts on tree populations such as size distribution of individuals (de Souza *et al.*, 2010), density of seedlings (Benitez-Malvido, 1998) or larger individuals (Michalski *et al.*, 2007), survivorship and growth (Alvarez-Aquino *et al.*, 2004), seed dispersal (Cordeiro and Howe, 2001) and degree of herbivory or biotic infection (Ruiz-Guerra *et al.*, 2010). However, individuals considered in such studies are generally restricted to certain sizes (but see Nascimento and Laurance, 2004) and how fragmentation may act on different ontogenetic stages is unknown. Considering such ontogenetic shifts in the context of forest fragmentation is important because restoration success may be dependent on which life-stage is used (Chapter 2).

The goal of this study was to rank light requirements of species with little *a priori* knowledge, and to test whether species with lower light requirement are more negatively affected by forest fragmentation as predicted from general trends of different functional groups. Instead of classic pioneer and shade-tolerant dichotomy classification, I ranked the relative light requirements of five animal-dispersed species, with no *a priori* knowledge of their life histories, based on size distributions of individuals (Condit *et al.*, 1998; Wright *et al.*, 2003). To assess whether light requirement is associated with vulnerability to forest fragmentation, I assessed species fragmentation effects based on size distributions between different-sized fragments for a given species, species abundance, edge effects on number of regenerating individuals, survivorship, growth, realized dispersal, herbivory and biotic infection damage and light availability. Similar approaches of ranking light requirements of species and associating them with different disturbance levels have been conducted (Davies *et al.*, 1998; Slik, 2005; reviewed in Chazdon *et al.*, 2010). If light requirements of the species based on a single census of size distribution serves as a proxy for vulnerability to forest fragmentation, it will be useful in identifying species that require active conservation efforts.

4.3. Methods

Study site

This study was based at Las Cruces Biological Station (LCBS; 8° 47' N, 82° 58' W), a field station of the Organization for Tropical Studies (OTS) in Coto Brus county, southern Costa Rica (Fig. 1.1). The area is situated in a highly deforested part of southern Costa Rica where overall forest cover within a 15 km radius of the station is estimated at 27% (Daily *et al.*, 2001) and the forest reserve at LCBS (209 ha) is the largest remaining primary forest fragment in the immediate area. Evidence from aerial photos suggests that LCBS forest was finally isolated as a fragment around 1978 to 1979, with earlier forest cutting in the 1960s (R. Zahawi, *personal communication*). Limited information available on the mammal species in this area report that large herbivorous mammals such as howler monkeys (*Alouatta palliata*), spider monkeys (*Ateles geoffroyi*), Baird's tapirs (*Tapirus bairdii*), and white-lipped peccaries (*Tayassu pecari*) have become locally extinct in the vicinity of LCBS (Pacheco *et al.*, 2006), while viable populations of these species still exist in other regions of Costa Rica. Time since local extinction of these large mammals in LCBS forest is estimated to be 20 to 50 years (R. Zahawi, *personal communication*). The natural vegetation of the area is classified as tropical premontane wet forest (Holdridge et al., 1971) with elevation ranges between 1,000 and 1,400 m. Such elevation ranges and fragment size are still underrepresented in existing studies (Turner, 1996; Zuidema *et al.*, 1996). Temperature ranges between 14 and 29°C (mean: 20°C), and there is typical dry season from December through March. Annual precipitation was 4,223 mm in 2008 and 5,011 mm in 2010, but 2009 (El Niño year) was an unusually dry year (annual precipitation: 3,063 mm), which affected fruit production of many tree species during the early wet season (A. Sugiyama, *personal observation*). In contrast, 2011 was a La Niña year with a wet dry season and a dry wet season (annual precipitation: 4,110 mm).

In addition to the forest reserve at LCBS, four smaller primary forest fragments (AGZ: 33 ha, CED: 27 ha, LL: 19 ha, and PC: 4 ha) were used (Fig. 1.1). All five fragments are within 8 km of each other, with similar elevations and climate, and have had no recent major disturbances or newly created forest edges. Comparisons between continuous or large forest and small fragments is common (e.g., Bruna, 1999) and here, I compared the largest fragment (LCBS) and combination of the four smaller fragments (SF), due to the distinct division in fragment sizes and to balance the sample size of the trees used in different fragments. I will call these categories "site groups" (i.e., LCBS vs. SF) hereafter.

Focal species

Based on the abundance of fruiting trees in the forest fragments during May to August 2009, I selected five animal-dispersed tree species, *Drypetes brownii* (Putranjivaceae), *Ficus tonduzii* (Moraceae), *Lacistema aggregatum* (Lacistemataceae), *Quararibea aurantiocalyx* (Malvaceae), and *Tapirira mexicana* (Anacardiaceae), as focal species (Table 4.1). All five species produce fleshy fruits dispersed by animals (Appendix A), and the seeds do not appear to possess dormancy under full sun except for *D. brownii*, which germinate the following wet season (A. Sugiyama, *personal observation*). Although none of these species are known as typical pioneers in the region (e.g., *Cecropia obtusifolia*, *Heliocarpus appendiculatus*), their relative light requirement on the pioneer-to-shade-tolerant spectrum is unknown. I randomly selected trees that produced fruits in 2009 as focal trees in the primary forest of each fragment (n = 3 trees × 2 site groups × 5 species). Focal trees were at least 100 m away from each other to minimize overlapping seed shadows, occurred at varying distances from the forest edge, and did not fruit in 2008, 2010, or 2011.

Size distribution

In mid June 2010, I assessed the size distribution of existing individuals in 20 × 20 m plots randomly established in each fragment (Table 4.2; LCBS: 10, AGZ: 4, CED: 4, LL: 3, PC: 2). Within each plot, I recorded basal diameter and dbh (when height > 3 m) for all individuals of the five focal species. In addition, I classified individuals into six size classes: SS (small seedlings: < 50 cm tall), SD (seedlings: 0.5-1 m tall), SP (small saplings: 1-3 m tall), LP (large saplings: > 3 m tall, < 5 cm dbh), UN (understory: 5-10 cm dbh), and CP (canopy: > 10 cm dbh). Identification of small individuals was realized by germination trials from the seeds. Due to proportional bias in number of individuals toward SS individuals, I classified size classes other than SS as non-SS. Hereafter, I will call these two categories as "size groups" (i.e., SS vs. non-SS).

To rank the relative light requirement of the species, I calculated the coefficient of skewness (g_1), which has been employed for tropical tree species (Wright *et al.*, 2003). The statistic g_1 summarizes the evenness or skewness of truncated distributions (Bendel *et al.*, 1989), which is calculated as

$$g_1 = \frac{n \sum_i (x_i - \bar{x})^3}{(n-1)(n-2)\, s^3}$$

where n is the number of individuals, x_i is the logarithm of basal diameter for individual *i*, $\bar{x}$ is the mean of x_i, and s is the standard deviation of x_i. Here, I used basal diameter in place of dbh (Wright *et al.*, 2003) to include small individuals not included in the previous study (i.e., SS, SD, and SP). When the distribution is skewed to the right (i.e., many small and few large individuals),

g_1 yields large values and *vice versa* for the distribution skewed to the left, which may take negative values. Note that g_1 parameterizes shape and does not depend directly on the mean or variance of the distribution (Bendel *et al.*, 1989).

Plant-size distributions are known to reflect species life histories (Condit *et al.*, 1998; Wright *et al.*, 2003). Typical pioneer species are represented by few small individuals (small, usually negative g_1) because their regeneration is dependent on creation of gaps, and small individuals either die quickly or grow rapidly into larger size class if the light levels remain high (Wright *et al.*, 2003). In addition to making inferences on species life history, size distribution statistics have also been used in an attempt to predict future population trends from current size distributions (Condit *et al.*, 1998 and references therein; Feeley *et al.*, 2007) or to compare the conservation status between different habitat types (de Souza *et al.*, 2010). Such an attempt has proved successful at a local scale (Feeley *et al.*, 2007). The logic behind such prediction of future trends is analogous to the population pyramid of humans; the population is likely to grow or maintain its current size if there are relatively more small (young) individuals (i.e., large g_1). In this study, I compared size distributions across species and for a given species between site groups to infer the life history of the focal species (i.e., light requirement) and the future population trend at a local scale (i.e., forest fragmentation effects), respectively. I expected that species with large g_1 (i.e., species with low-light requirements) would have smaller g_1 in SF than LCBS, suggesting negative effects of forest fragmentation.

Species abundance

When the size distribution was assessed in plots, I assessed effects of fragmentation on individual density. Based on general predictions from different functional groups (Laurance *et al.*, 2006a; Tabarelli *et al.*, 2010a), I expected that species with high-light requirements would be less affected by fragmentation and would be more abundant in SF or have comparable abundances between site groups. Since density is greatly affected when a reproductive tree is present in a given plot, I also calculated commonness by the proportion of plots that included at least one individual of any size.

Edge effects on number of regenerating individuals

Edge effect is considered one of the primary consequences that negatively affect species regeneration as a result of forest fragmentation (Benitez-Malvido, 1998). With the expectation that species with high-light requirements would be more common or be less affected near edges, I established two 5 × 40 m transects in random directions from each focal tree to assess whether edge effects affect the number of naturally occurring individuals. Since the existence of a reproductive tree can have a greater influence on number of individuals over the distance from the edge, I used transects instead of plots because the highest number of individuals is expected around focal trees, which were all fruiting in 2009. While there was no substantial difference in apparent fecundity among the focal trees (A. Sugiyama, *personal observation*), focal trees may differ in fecundity or quality of seeds they produce as a result of edge effects (Chapter 3), which then may affect the number of individuals that germinate and establish in the transects. Thus, I evaluated edge effects on realized establishment; the collective effects of fecundity, seed quality, germination, and offspring establishment. Between the end of April and early June 2010, I tagged individuals of all sizes conspecific to the focal tree and recorded basal diameter, dbh (when > 3 m tall), distance class from the focal tree (in 1 m intervals), size class (same categories as plots above), and number of leaves (SS individuals only) in each transect. The great majority of the SS individuals in this study were from the 2009 cohort based on the number and developmental stage of leaves, the growth data (see below), and the fact that there were very few SS individuals in 2009 when focal trees were fruiting (A. Sugiyama, *personal observation*). Thus, initial recording of SS individuals in 2010 largely reflected 1-yr seedling establishment except for *D. brownii*.

Individual survivorship and growth

To assess effects of forest fragmentation on individual survival and growth (Alvarez-Aquino *et al.*, 2004), I conducted a recensus between late April and early June 2011. I expected that species with high light requirements would have similar survivorship and growth between site groups. I calculated relative growth as $(d_2 - d_1)/(d_1) \times 100$, where d_1 and d_2 are basal diameters in 2010 and in 2011, respectively. In addition, I considered increase in leaf number for SS individuals.

Realized dispersal

Reduced seed dispersal in small fragments may lead to low seedling recruitments (Cordeiro and Howe, 2001). Since I did not modify the seed shadow, I assessed seed dispersal indirectly as realized dispersal (Chapter 2) to test whether seed dispersal was lower in SF compared to LCBS, particularly for large-seeded species, which is a trait typically associated with shade-tolerant species (Poorter *et al.*, 2008). I classified SS individuals that occurred beyond the crown periphery plus 3 m in 2010 as seedlings from dispersed seeds. Since crown shapes were irregular and the crown extent often differed by its directions, I measured crown size above each transect. Because the presence of a seedling is a result of both dispersal and survival/establishment, I only considered SS individuals to best reflect dispersal.

Invertebrate herbivory and biotic infection

Forest fragmentation may enhance or suppress invertebrate herbivory (Ruiz-Guerra *et al.*, 2010). To assess effects of forest fragmentation on levels of invertebrate herbivory and biotic infection, I recorded invertebrate herbivory and evidence for biotic infections (e.g., galls, fungal infection) non-destructively for all SS individuals, which is the stage expected to be most critically affected by these events (Coley, 1983a). Since herbivory frequency may affect seedling performance via plants' induced response (Kessler and Baldwin, 2002), I assessed both invertebrate herbivory by frequency (number of holes or bites) and the leaf area consumed. I evaluated herbivory frequency by categorizing each individual into three categories: intact (no herbivory), severe frequency (≥ 10 herbivory events), and some frequency for the rest. Similarly, to assess area affected by herbivory, I categorized each individual as follows: intact (no herbivory), severe herbivory area (≥ 50% of all existing leaf area lost), and some herbivory area for the rest. Because of the evaluation scheme, seedlings classified as intact for frequency were also intact for area. Finally, independent of herbivory status, I classified individuals binomially by the presence of biotic infection. I conducted initial recording between the end of April and early June 2010. Thus, I did not monitor individuals periodically for about a year after germination, except for *D. brownii*. To assess impacts of herbivory and biotic infection on seedling survivorship, I recensused SS individuals in 2011.

Light environment

Light environment is one of the most important factors that affects plant performance of all sizes (Montgomery and Chazdon, 2002) and is known to be affected by fragmentation (Kapos, 1989). To test whether light availability is greater in SF than LCBS and as a linear function of distance from the edge, I took hemispherical photos with a leveled fisheye converter (Nikon FC-E8 0.21×) mounted on a Nikon Coolpix 885 camera (Nikon Corporation, Tokyo, Japan). Between the end of April and early June 2011, I took photographs under overcast conditions at 1m height every 5 m along the two transects up to 40 m for each focal tree (n = 8 distances × 2 transects × 3 trees × 2 site groups × 5 species). I calculated percent canopy openness using Gap Light Analyzer (GLA) 2.0 (Frazer *et al.*, 1999). Since GLA does not allow automatic thresholding of pixels, I used automatic thresholding function on SideLook 1.1 (Nobis, 2005) for binarization of photographs prior to image analyses in GLA.

Statistical analyses

To rank species by light requirements, I pooled data across sites for interspecific comparisons. I used generalized linear mixed models (GLIMMIX) to assess fragmentation effects by comparing the site groups (fixed effect) with either plots or focal trees (experimental unit) nested within site groups as a random effect. For both size groups, I used GLIMMIX to examine differences in site groups in their size distributions (Gaussian distribution and identity link), individual density, increase in leaf number (Poisson distribution and log link), commonness, survivorship, herbivory and biotic infection, realized dispersal (binomial distribution and logit link), and relative growth (beta distribution and logit link). In evaluating whether herbivory or biotic infection in 2010 affected overall survivorship recorded in 2011, I used logistic regression. To test whether edge effects explained the patterns observed between site groups, I conducted simple linear regressions between number of individuals in transects (data from two transects were combined to represent each tree) and distance from the edge for each size group. I compared canopy openness between site groups to assess difference in light environment using ANOVA with sites nested within site groups. Linear regression was conducted between canopy openness (averaged across to represent each tree) and distance from the edge to assess edge effects on forest light environment. I conducted all statistical procedures with SAS 9.2 software (SAS Institute Inc., 2009), and log-transformed (angular-transformed for proportion data) all the data that did not meet the normality assumption before the analyses for regressions, correlations, and Gaussian distribution in GLIMMIX. I report adjusted R^2 values here and errors are SE, unless otherwise indicated.

4.4. Results

Species light requirement rank based on size distributions

Suggesting highest and lowest light requirement, g_1 ranged between 0.01 (*F. tonduzii*) and 5.85 (*T. mexicana*), respectively. Since SS individuals constituted a large part of the individuals in plots and resulted in large values, I also calculated g_1 only for non-SS individuals (Table 4.3). Based on g_1 without SS individuals, the ranks of species from those with the highest light requirements were: *F. tonduzii* (-0.82) > *L. aggregatum* (0.19) > *Q. aurantiocalyx* (1.27) > *D. brownii* (1.72) > *T. mexicana* (1.87). Thus, species with highest and lowest light requirement were consistent regardless of inclusion of SS individuals.

For a given species, there was no significant difference in g_1 between site groups for any of the species, although g_1 was generally higher for LCBS than for SF, except for *T. mexicana* (Fig. 4.1). Such a pattern was largely retained when SS individuals were excluded (Table 4.3).

Species abundance

For both size groups, individual density was not consistently higher in either site group, and there were few significant differences between site groups (Table 4.3). Density ranged between the lowest *F. tonduzii* (33 ± 24/ha) and the highest *L. aggregatum* (655 ± 460/ha) for SS individuals, and the lowest *T. mexicana* (41 ± 16/ha) and the highest *Q. aurantiocalyx* (178 ± 119/ha) for non-SS individuals. As expected, when a reproductive tree was present in a given plot, there were copious SS individuals, which led to large variation among plots. The only significant differences were observed for *Q. aurantiocalyx*, with higher density in LCBS than in SF for both size groups, and *D. brownii*, with higher density in SF than in LCBS but only for non-SS individuals. Similar results were observed for commonness, which ranged between 17.4% (*F. tonduzii*) and 82.6% (*D. brownii*) for SS, and 34.8% (*T. mexicana*) and 87.0% (*D. brownii*) for non-SS individuals (Table 4.3). However, differences between site groups were significant only for *Q. aurantiocalyx*, which was higher in LCBS than in SF, only for non-SS individuals.

Edge effects on number of regenerating individuals

Evaluation of multiple stages revealed evidence for ontogenetic effects of edge effects for some species (Fig. 4.2). For *T. mexicana*, the number of SS individuals increased with distance from the edge in 2010 (Fig. 4.2e). However, such a positive relationship disappeared after a year.

Further supporting such a trend, there was no such edge effect for non-SS individuals (all > 2-yr) for both years (Fig. 4.2j). In contrast, edge effects were detectable for non-SS (Fig. 4.2i) but not for SS individuals (Fig. 4.2d) for *Q. aurantiocalyx*, which persisted between 2010 and 2011. Such patterns were observed for *F. tonduzii* also, with apparent edge effects on non-SS in 2010 (Fig. 4.2g) but not on SS individuals (Fig. 4.2b). However, number of individuals near reproductive trees was very low for *F. tonduzii*.

Individual survivorship and growth

Survivorship ranged between 33.9 ± 8.2% (*L. aggregatum*) and 69.4 ± 4.7% (*Q. aurantiocalyx*) for SS individuals and 50.0 ± 16.8% (*F. tonduzii*) and 96.1 ± 1.4% (*Q. aurantiocalyx*) for non-SS individuals. With an exception of *F. tonduzii*, survivorship was consistently higher for non-SS than SS (Table 4.3). However, there was no significant difference between site groups for either size group. In contrast to survivorship, relative growth was consistently higher for SS than non-SS individuals (Table 4.3), ranging between 9.0 ± 5.3% (*F. tonduzii*) and 55.1 ± 1.0% (*T. mexicana*) for SS individuals and 5.7 ± 2.7% (*L. aggregatum*) and 14.0 ± 3.4% (*T. mexicana*) for non-SS individuals. However, there was no significant difference between site groups for relative growth of both size groups and increase in leaf number for SS individuals.

Realized dispersal

Realized dispersal was apparently not affected by forest fragmentation, which ranged from 6.2 ± 9.8% (*Q. aurantiocalyx*) to 77.8 ± 12.0% (*F. tonduzii*). Although mean percent dispersal was higher in LCBS than in SF except for *D. brownii*, there was no significant difference between site groups for any of the species (Table 4.3).

Invertebrate herbivory and biotic infection

Invertebrate herbivory was very common. More than half of the individuals of all species had some level of herbivory a year after germination. Proportion of intact individuals ranged from 10.2 ± 3.4% (*T. mexicana*) to 46.1 ± 4.7% (*D. brownii*) in 2010, although seedlings of *D. brownii* in 2010 germinated in 2010. For all species, proportion of intact individuals was reduced in 2011 (Table 4.4). In contrast, the proportion of severe herbivory frequency increased from 2010 to 2011 except for *F. tonduzii* with a low number of seedlings, ranging from 0 ± 0% (*F. tonduzii*) to 46.2 ± 3.3% (*T. mexicana*). Compared to severe herbivory frequency, severe herbivory area was low, typically less than 2% except for *T. mexicana*. Similarly, biotic infection was less than 8% in 2011 even with increased levels from 2010. Such levels of biotic damages were not different between site groups for any of the species and for both years except for significantly high severe herbivory frequency in SF than LCBS for *D. brownii* in 2010 and an opposite pattern for *Q. aurantiocalyx* in 2011 (Table 4.4). For all but *F. tonduzii* (frequency: $F_{1,7} = 0.0$, $P = 0.97$, area: not available), either severe herbivory frequency (*L. aggregatum*: $F_{1,1087} = 5.7$, $P = 0.018$) or area (*D. brownii*: $F_{1,2585} = 5.1$, $P = 0.024$; *Q. aurantiocalyx*: $F_{1,957} = 12.4$, $P = 0.0004$; *T. mexicana*: $F_{1,2001} = 108.5$, $P < 0.0001$) in 2010 significantly affected survivorship.

Light environment

Light environment in the forest was affected by forest fragmentation. Canopy openness was significantly greater in SF (8.6 ± 0.1%) than in LCBS (8.1 ± 0.1%; $F_{4,479} = 5.8$, $P = 0.0001$)

as expected. Edge effects were responsible for this difference and canopy openness decreased linearly with distance from the edge ($R^2 = 0.10$, $F_{1,28} = 4.3$, $P = 0.048$).

4.5. Discussion

In highly diverse tropical forests where little is known about most species, it will be useful if simple measurements reveal life histories reliably. Additionally, if such life histories are associated with vulnerability to forest fragmentation, it will be useful in identifying species that are threatened. Based on size distributions, I ranked the light requirement of five animal-dispersed tree species and tested whether species with lower light requirement are more negatively affected by forest fragmentation. Three major findings from this study are the following: 1) effects of forest fragmentation may only be detectable for certain metrics and stages assessed, 2) the general prediction that species with low light requirement are more vulnerable to forest fragmentation does not apply at an individual-species level, and 3) effects of forest fragmentation can change ontogenetically.

Based on g_1, light requirement was highest for *F. tonduzii* and lowest was *T. mexicana* (Fig. 4.1; Table 4.3). Density of SS individuals for *F. tonduzii* was very low even around reproductive trees where the highest density is expected (Fig. 4.2), and this led to low g_1 value, which suggests that *F. tonduzii* is a pioneer species. Such SS individuals, not considered in previous studies, led to a larger values of g_1 (0.01 to 5.85) than those previously reported. For 73 canopy tree species, Wright *et al*. (2003) reported g_1 between -1.14 and 1.86 for species with highest and lowest light requirement, respectively. While I did not intend to compare actual g_1 values across studies, g_1 calculated without SS individuals led to values closer to those previously reported (-0.82 to 1.87). The rank based on g_1 calculated without SS individuals

(Table 4.3) also matched the light requirements of the five focal species based on other observations and traits (Appendix B). Thus, while g_1 without SS individuals still includes size classes smaller than those in previous studies (Wright *et al.*, 2003), g_1 appears to be a good predictor of species life histories for a wide range of individual sizes. When g_1 was compared between site groups for a given species, g_1 was generally greater in LCBS than in SF (Fig. 4.1), suggesting potential decay in future populations by forest fragmentation. The few exceptions in such trends likely resulted from large errors for small sample size for some species and methods employed here (Appendix B).

Abiotic forest fragmentation effects were detected in light environment where light availability was greater in SF than in LCBS and declined with distance from the edge, as shown previously (Kapos, 1989). However, even for animal-dispersed tree species, which are considered one of the groups negatively affected by forest fragmentation (Benitez-Malvido and Martinez-Ramos, 2003; Terborgh *et al.*, 2008), negative effects of forest fragmentation were rather limited in both stage and metrics assessed. One of the few negative fragmentation effects was detected in abundance of *Q. aurantiocalyx*, where density and commonness were substantially reduced in SF compared to LCBS for both size groups (Table 4.3). *Q. aurantiocalyx* is listed as “Endangered C2a” in version 2.3 of the IUCN red list, and forest fragmentation may further exacerbate such conservation status. In contrast, density of non-SS individuals was higher in SF compared to LCBS for *D. brownii*. One might conclude from this that while SS individuals are abundant, non-SS individuals in LCBS may not survive to reach larger size classes. However, there was no difference in large-sized individuals (LP-CP) between site groups and the observed difference was due to the higher abundance of middle-sized individuals (SD-SP) in SF. As expected, survivorship of SS individuals was generally higher in

LCBS than SF but this pattern was reversed for non-SS individuals. However, this difference was not significant and no consistent trends existed for growth (Table 4.3). While difference in survivorship for SS individuals reflects contemporary effects of fragmentation, one year of data may not be representative for non-SS individuals which included different-aged individuals. As for growth, relative growth was consistently higher for SS than non-SS individuals (Table 4.3), which was consistent with the finding that growth rate is not constant across growth stages (Hérault *et al.*, 2011). There was no apparent fragmentation effect but growth is known to be variable and such results are common (e.g., Verdu and Traveset, 2005). Consistent with the expectation that fragmentation will reduce seed dispersal of animal-dispersed species (Cordeiro and Howe, 2001), realized dispersal was overall greater in LCBS than in SF (Table 4.3). Seed size and light requirements are often correlated, with typical pioneer species associated with small seeds (Poorter *et al.*, 2008). The results here were consistent with the prediction that reduction in dispersal is especially severe for large-seeded species (Melo *et al.*, 2010). Realized dispersal largely decreased with increasing seed size, which is ranked as: *F. tonduzii* < *L. aggregatum* < *D. brownii* < *Q. aurantiocalyx* < *T. mexicana*. Herbivory affected survivorship of SS individuals, but herbivory and biotic infection damage varied by species and year. While biotic infection may be enhanced in the environments of larger forests (Hood *et al.*, 2004), fragmentation effects on herbivory vary across studies (reviewed in Ruiz-Guerra *et al.*, 2010). Indeed, there were no consistent patterns, and differences between site groups in herbivory were only significant for two species; higher severe herbivory frequency in SF than LCBS for *D. brownii* in 2010 and *vice versa* for *Q. aurantiocalyx* in 2011. For the focal species in this study, levels of biotic damage or effects of forest fragmentation were not concordant with light requirements although a previous study showed lower herbivory in fragmented forests for shade-tolerant species (Ruiz-Guerra *et al.*, 2010). Variation in herbivory can be high even intraspecifically (Coley, 1983b) and while methods employed here might partly explain some of these patterns, it does not seem to change the main findings (Appendix B). To summarize, fragmentation effects were limited to certain metrics and stages assessed and simple comparisons based on few metrics may lead to misleading conclusions. To identify stages and processes that are affected by fragmentation and act as bottlenecks for species regeneration, multi-stage assessments on multiple metrics is essential. Additionally, the general prediction that species with low light requirements are more vulnerable to forest fragmentation did not apply at an individual-species level.

Stage-specific fragmentation effects were most evident in edge effects (Fig. 4.2). SS individuals of *T. mexicana*, which was the most shade-tolerant species, were most negatively affected by proximity to the edge (Fig. 4.2d). Yet, this pattern disappeared by the second year (Fig. 4.2e). Negative edge effects on early stages of *T. mexicana* are primarily caused by high mortality of seeds near edges (Chapter 2). Based on such results, one may expect edge effects to be most prominent for early stages, and that later stages will not be affected if not affected earlier. However, that was not the case. Such pattern was observed for *F. tonduzii* (Fig. 4.2g) and *Q. aurantiocalyx* (Fig. 4.2i), although sample size was small for *F. tonduzii*. While no edge effects were observed for SS individuals (Fig. 4.2d), number of individuals increased linearly with distance from the edge for non-SS individuals of *Q. aurantiocalyx*. Because survivorships for any of the species were not linearly related to distance from the edge (data not shown), such patterns may result from how and when measurements were taken. Note that the number of regenerating individuals and survivorship are not identical. Since most SS individuals in 2010 were 1-yr seedlings and survivorship of *Q. aurantiocalyx* was relatively high, overall patterns for

SS individuals were likely generated by germination. In contrast, patterns for non-SS individuals were generated over many years. Thus, even a weak positive relationship between survivorship and distance from the edge based on one year's data (data not shown) may lead to greater number of individuals away from the edge over time. While edge effects act on both early (Benitez-Malvido, 1998) and later growth stages (Oliveira *et al.*, 2008), whether impact of edge effects on a given species may change ontogenetically has rarely been considered (but see Kotze and Lawes, 2007). If potential ontogenetic shifts or habitat associations are not recognized, use of one stage (e.g., seed, seedling) for species restoration may not be fruitful at specific environments (e.g., open habitat) (Chapter 2).

Results from this study underline the importance of considering a wide range of individual sizes and various metrics in assessing effects of forest fragmentation on species regeneration. The general prediction that species with higher light requirements are more negatively affected by fragmentation was not valid at an individual-species level and potential ontogenetic effects of forest fragmentation were revealed. Among the five species, *T. mexicana* was most shade-tolerant and *F. tonduzii* was a pioneer species. Two major traits that define a pioneer species are that seeds only germinate in canopy gaps, and that plants cannot survive in the shade (Swaine and Whitmore, 1988; Clark and Clark, 1992). Along with some traits for *T. mexicana* that were not expected for a shade-tolerant species (e.g., defense against biotic damage; Appendix B), established seedlings of *F. tonduzii* can survive under closed forest (Chapter 6) although it has traits associated with typical pioneer species (e.g., has minute seeds that germinate and grow quickly under full sun). Thus, although light requirement defines the species functional groups in the classical sense, it was not consistent throughout the life span even for the two extreme species in this study. Different light requirements even for congeners are common (e.g., Uriarte *et al.*, 2004), which makes simple predictions on fragmentation effects even harder. In assessing effects of forest fragmentation, lack of consistent effects of fragmentation is common, even for experimentally controlled studies (Debinski and Holt, 2000). Such results may be partly explained if ontogenetic effects are considered and functional groups are redefined accordingly. Ontogenetic shifts with regard to light environment may occur in different directions in different species, with shifts that may not even be unidirectional over time (Poorter *et al.*, 2005). Since assessment of the impacts of fragmentation based on one stage may not be applicable for another stage, consideration of wide size range is critically important for fruitful conservation efforts. Additionally, the use of a single index for evaluating fragmentation effects is insufficient and the use of appropriate methods is an important consideration (Baraloto *et al.*, 2010), which is supported here from contrasting results for *T. mexicana* based on plot and transect data. In evaluating impacts of such a complex phenomenon, understanding of species, multiple metrics for evaluation and use of appropriate methods are all critical considerations.

4.6. Tables

Table 4.1
Mean tree and fruit size for the five focal species. All trees measured here were fruiting when they were measured in 2009. N is number of trees and fruits used to measure tree and fruit size, respectively. Errors represent SD.

Species	Family	Tree size				N	Fruit size			N
		Height (m)	DBH (cm)	Mean crown size (m)	Max crown size (m)		Length (cm)	Width (cm)	Fresh weight (g)	
Drypetes brownii	Putranjivaceae	24.6 ± 5.9	43.2 ± 18.7	4.1 ± 1.5	6.8	13	2.45 ± 0.61	2.26 ± 0.41	6.26 ± 3.31	107
Ficus tonduzii	Moraceae	17.5 ± 6.1	30.6 ± 15.3	4.5 ± 1.6	9.3	10	2.91 ± 0.54	3.35 ± 0.60	13.91 ± 6.97	118
Lacistema aggregatum	Lacistemaceae	9.5 ± 3.3	11.3 ± 3.4	2.6 ± 0.8	5.8	14	0.96 ± 0.19	0.65 ± 0.11	0.24 ± 0.09	994
Quararibea aurantiocalyx	Malvaceae	16.6 ± 3.7	20.2 ± 7.4	3.4 ± 1.0	5.3	17	3.05 ± 0.36	2.45 ± 0.42	8.37 ± 2.74	202
Tapirira mexicana	Anacardiaceae	26.5 ± 4.5	44.3 ± 16.8	4.9 ± 1.9	10.0	16	1.85 ± 0.20	1.22 ± 0.14	1.52 ± 0.14	390

Table 4.2
Five fragments and locations of focal trees used in this study. N indicates number of plots randomly established in each site. Errors represent SD.

Site group	Site	Primary forest (ha)	Elevation (m)	Distance from the edge (m)	N
LCBS	LCBS	209	1170 ± 58	302.8 ± 103.4	10
SF	AGZ	33	1196 ± 16	84.9 ± 30.9	4
	CED	27	1156 ± 8	132.1 ± 47.2	4
	LL	19	1152 ± 9	66.6 ± 8.3	3
	PC	4	1197 ± 33	39.4 ± 13.3	2

Table 4.3

Effects of forest fragmentation on size distributions (g_1 calculated without SS individuals), species abundance (density and commonness), survivorship, growth (relative growth and increase in leaf number), and realized dispersal between site groups. Data for g_1 and species abundance were collected in plots and all the rest were collected from transects. Asterisks indicate significant intraspecific difference between site groups. Errors represent SE.

Species	Site groups	g_1	Density per hectare		Commonness (%)		Survivorship (%)		Relative growth (%)		Increase in leaf number†	Realized dispersal (%)†
		Non-SS only	SS	Non-SS	SS	Non-SS	SS	Non-SS	SS	Non-SS		
Drypetes brownii	LCBS	1.01 ± 0.19	430 ± 204	50 ± 13	90.0	80.0	44.4 ± 3.9	78.6 ± 5.9	18.5 ± 0.8	10.3 ± 2.3	0.90 ± 0.04	16.5 ± 7.5
	SF	1.90 ± 0.26	308 ± 156	183 ± 49*	71.4	92.3	37.6 ± 12.1	84.1 ± 5.4	6.7 ± 0.5	10.3 ± 2.0	0.98 ± 0.05	18.7 ± 3.5
Ficus tonduzii	LCBS	0.35 ± 0.60	0	48 ± 15	0	60.0	60.0 ± 37.5	43.8 ± 20.6	11.4 ± 8.8	7.4 ± 4.7	-	80.0 ± 12.5
	SF	-0.93 ± 0.31	58 ± 41	85 ± 21	30.8	76.9	50.0 ± 50.0	100 ± 0	5.6 ± 5.6	2.2 ± 1.7	3.00 ± 2.12	75.0 ± 25.0
Lacistema aggregatum	LCBS	0.46 ± 0.35	1340 ± 1038	68 ± 46	20.0	40.0	54.0 ± 16.8	75.0 ± 9.1	12.0 ± 2.0	10.0 ± 9.1	0.23 ± 0.05	84.5 ± 18.7
	SF	0.09 ± 0	129 ± 100	25 ± 12	46.2	38.5	28.6 ± 7.1	88.9 ± 0	32.3 ± 1.9	4.1 ± 1.5	0.54 ± 0.06	9.3 ± 10.8
Quararibea aurantiocalyx	LCBS	1.30 ± 0.66‡	378 ± 186*	405 ± 264**	60.0	70.0*	83.5 ± 2.0	95.7 ± 1.7	8.5 ± 1.8	5.9 ± 1.0	0.90 ± 0.09	18.9 ± 16.6
	SF	-	0	4 ± 2	0	15.4	65.5 ± 9.2	97.7 ± 2.2	10.3 ± 0.7	7.3 ± 1.8	0.70 ± 0.04	2.5 ± 2.9
Tapirira mexicana	LCBS	-0.15 ± 0	65 ± 25	23 ± 25	70.0	20.0	50.4 ± 10.8	77.8 ± 8.4	50.1 ± 1.3	10.6 ± 5.0	1.01 ± 0.04	12.1 ± 14.3
	SF	2.17 ± 0.48	877 ± 456	56 ± 27	61.5	46.2	66.4 ± 17.4	89.3 ± 6.3	64.3 ± 1.6	15.9 ± 4.6	0.53 ± 0.03	9.1 ± 37.4

* $P < 0.05$

** $P < 0.005$

†Only considered SS individuals.

‡Statistical tests could not be conducted.

Table 4.4
Herbivory and biotic infection of SS individuals occurring in transects between site groups for both study years 2010 and 2011. Asterisks indicate significant intraspecific difference between site groups. Errors represent SE.

Species	Site groups	Intact (%)		Severe herbivory frequency (%)		Severe herbivory area (%)		Biotic infection (%)	
		2010	2011	2010	2011	2010	2011	2010	2011
Drypetes brownii	LCBS	53.3 ± 8.5	11.6 ± 2.6	3.8 ± 1.3	22.2 ± 4.8	1.3 ± 0.4	1.5 ± 0.6	1.1 ± 0.1	3.1 ± 1.3
	SF	39.0 ± 2.1	8.7 ± 1.5	11.0 ± 0.3*	17.6 ± 0.6	0.8 ± 0.3	1.0 ± 0.5	0.6 ± 0.7	2.1 ± 1.1
Ficus tonduzii	LCBS	60.0 ± 37.5	-	0 ± 0	-	0 ± 0‡	-	0 ± 0‡	-
	SF	0 ± 0	0 ± 0‡	25.0 ± 25.0	0 ± 0‡	0 ± 0	0 ± 0‡	0 ± 0	0 ± 0‡
Lacistema aggregatum	LCBS	31.9 ± 18.8	21.3 ± 1.9	4.4 ± 5.1	19.7 ± 10.2	1.3 ± 1.7	0.8 ± 0.4	1.8 ± 1.7	4.9 ± 2.5
	SF	35.0 ± 0.6	29.6 ± 5.9	2.2 ± 0.9	1.6 ± 2.6	0.5 ± 0.2	0.4 ± 0.2	1.5 ± 1.2	8.9 ± 3.9
Quararibea aurantiocalyx	LCBS	6.6 ± 1.4	0.6 ± 0.4	42.9 ± 8.0	69.4 ± 6.9*	0 ± 0	1.3 ± 0.5	1.4 ± 0.7	0.6 ± 0.4
	SF	15.1 ± 6.0	9.5 ± 3.4	29.0 ± 8.6	31.5 ± 8.6	2.1 ± 0.8	0.4 ± 0.2	1.5 ± 0.3	2.3 ± 0.8
Tapirira mexicana	LCBS	9.5 ± 4.4	1.1 ± 0.9	17.1 ± 8.0	60.3 ± 8.5	14.7 ± 5.7	3.6 ± 1.4	2.2 ± 1.9	8.2 ± 1.9
	SF	12.0 ± 6.3	8.1 ± 4.2	14.8 ± 4.5	19.6 ± 5.9	6.4 ± 1.9	2.6 ± 1.4	2.6 ± 1.4	3.9 ± 2.0

* $P < 0.05$
‡Statistical tests could not be conducted.

4.7. Figures

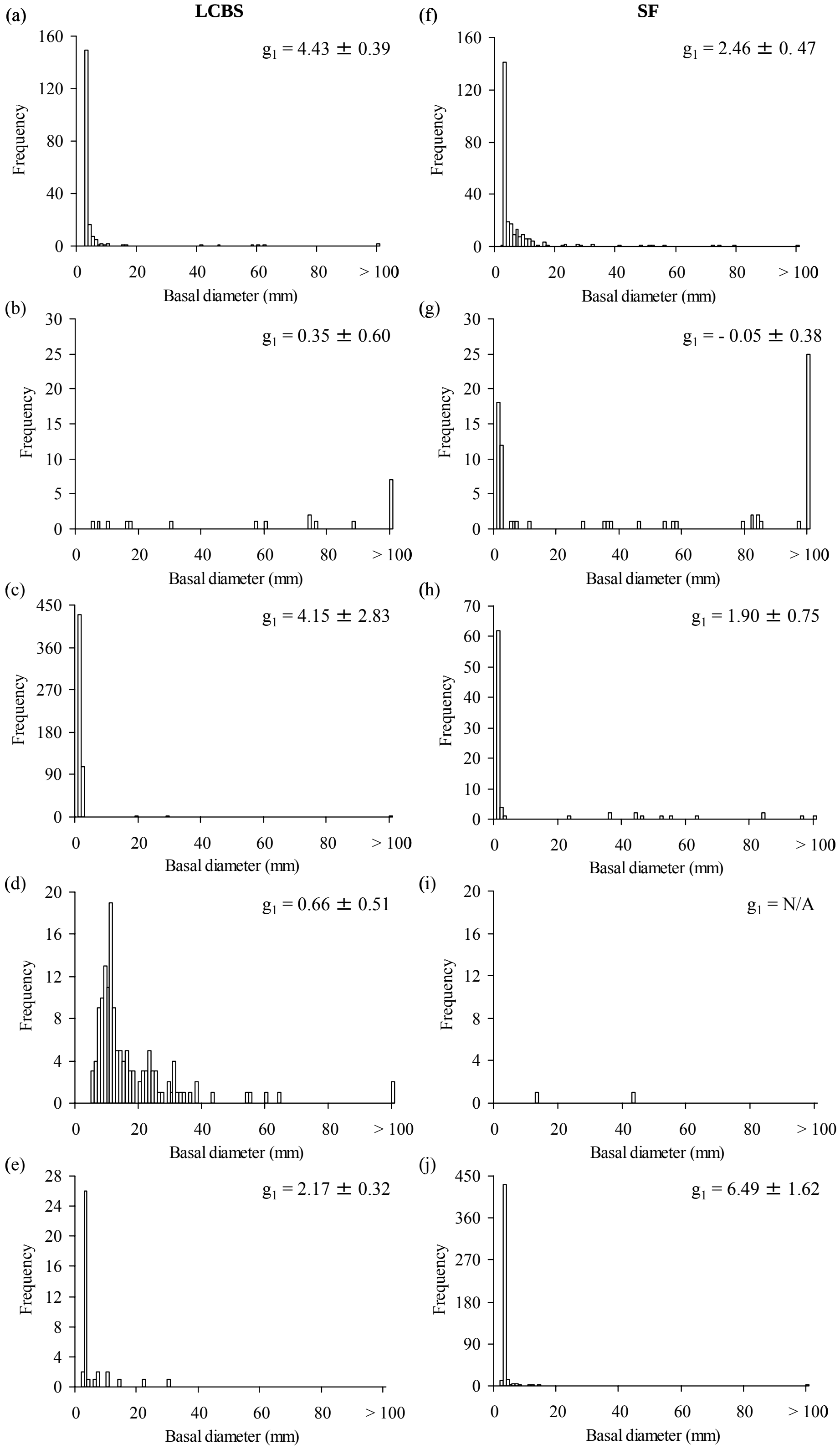

Figure 4.1

Size distribution and coefficient of skewness (g_1) of the focal species based on basal diameters of all individuals. Large g_1 value is associated with relatively higher degree of shade tolerance interspecifically, and relatively stable population persistence intraspecifically. Left column ((a) through (e)) and right column ((f) through (j)) show results for LCBS and SF, respectively. Species are sorted in the alphabetical order from the top to the bottom; (a) and (f): *D. brownii*, (b) and (g): *F. tonduzii*, (c) and (h): *L. aggregatum*, (d) and (i): *Q. aurantiocalyx*, and (e) and (j): *T. mexicana*. Since there were only two individuals, g_1 could not be calculated for (i). While actual g_1 was calculated per plot, histogram was constructed based on data from all the plots for each site group for visibility. Similarly, although individuals with basal diameter > 100 mm are pooled for visibility, actual calculation was based on raw data. See Table 4.5 for g_1 calculated only using non-SS values.

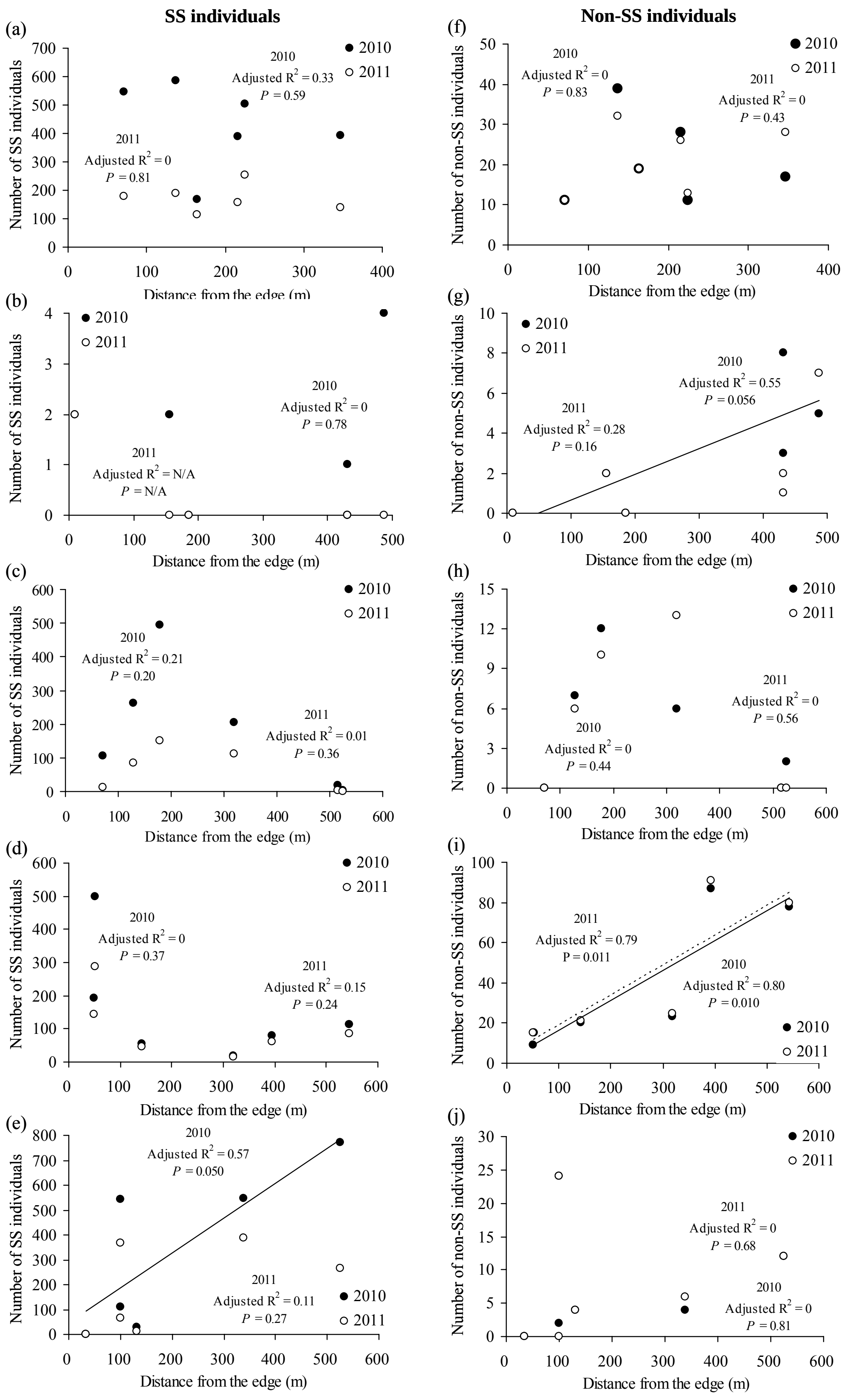
SS individuals
Non-SS individuals
(a)
2010
Adjusted $R^2 = 0.33$
$P = 0.59$
2011
Adjusted $R^2 = 0$
$P = 0.81$
● 2010
○ 2011
Number of SS individuals
Distance from the edge (m)
(b)
2010
Adjusted $R^2 = 0$
$P = 0.78$
2011
Adjusted R^2 = N/A
P = N/A
(c)
2010
Adjusted $R^2 = 0.21$
$P = 0.20$
2011
Adjusted $R^2 = 0.01$
$P = 0.36$
(d)
2010
Adjusted $R^2 = 0$
$P = 0.37$
2011
Adjusted $R^2 = 0.15$
$P = 0.24$
(e)
2010
Adjusted $R^2 = 0.57$
$P = 0.050$
2011
Adjusted $R^2 = 0.11$
$P = 0.27$
(f)
2010
Adjusted $R^2 = 0$
$P = 0.83$
2011
Adjusted $R^2 = 0$
$P = 0.43$
Number of non-SS individuals
(g)
2010
Adjusted $R^2 = 0.55$
$P = 0.056$
2011
Adjusted $R^2 = 0.28$
$P = 0.16$
(h)
2010
Adjusted $R^2 = 0$
$P = 0.44$
2011
Adjusted $R^2 = 0$
$P = 0.56$
(i)
2011
Adjusted $R^2 = 0.79$
$P = 0.011$
2010
Adjusted $R^2 = 0.80$
$P = 0.010$
(j)
2011
Adjusted $R^2 = 0$
$P = 0.68$
2010
Adjusted $R^2 = 0$
$P = 0.81$

Figure 4.2

Relationship between number of individuals regenerating around each focal tree at various distances from the edge. Left column ((a) through (e)) and right column ((f) through (j)) are SS and non-SS individuals, respectively. Species are sorted in the alphabetical order from the top to the bottom; (a) and (f): *D. brownii*, (b) and (g): *F. tonduzii*, (c) and (h): *L. aggregatum*, (d) and (i): *Q. aurantiocalyx*, and (e) and (j): *T. mexicana*. Filled and open circles are for 2010 and 2011, respectively. Actual statistical procedures were conducted on transformed data when raw data did not meet the normality assumptions. Note that there was some increase in number of individuals from 2010 to 2011 for a same tree due to shifts in size classes for some individuals. Due to low sample size statistics could not be calculated for 2011 of (b).

4.8. Appendices

Appendix A: List of known frugivores for the focal species

Drypetes brownii

Rodents (e.g., Central American agouti (*Dasyprocta punctata*), variegated squirrel (*Sciurus variegatoides*)).

Ficus tonduzii

Rodents (e.g., Central American agouti, variegated squirrel), white-faced capuchin monkeys (*Cebus capucinus*), bats (e.g., *Artibeus jamaicensis*, *Artibeus lituratus*), and birds (e.g., Chestnut-mandibled Toucan (*Ramphastos swainsonii*), crested guan (*Penelope purpurascens*)).

Lacistema aggregatum

Rodents (e.g., Central American agouti, variegated squirrel), white-faced capuchin monkeys, and birds (e.g., blue-grey Tanager (*Thraupis episcopus*), Cherrie's Tanager (*Ramphocelus costaricensis*), Silver-throated Tanager (*Tangara icterocephala*) and more species in families of Parulidae, Thraupidae, and Vireonidae).

Quararibea aurantiocalyx

Rodents (e.g., Central American agouti, variegated squirrel), white-faced capuchin monkeys, and birds (e.g., white-crowned Parrot (*Pionus senilis*)).

Tapirira mexicana

Rodents and birds (e.g., blue-crowned motmot (*Momotus momota*), crested guan (*Penelope purpurascens*), clay-colored thrush (*Turdus grayi*), fiery-billed aracari (*Pteroglossus frantzii*), and Swainson's thrush (*Catharus ustulatus*)).

All the dispersers are known to occur in all the fragments (F. Oviedo, *personal communication*) but their relative abundance is unknown. Information is based on F. Oviedo, *personal communication* and A. Sugiyama, *personal observation*.

Appendix B: Additional species information and discussion of observed patterns

Drypetes brownii

Species abundance of *D. brownii* was relatively high, and both SS and non-SS individuals were common in the field due to its high fecundity (A. Sugiyama, *personal observation*). As a result, size distribution was represented by many small individuals, which suggests that *D. brownii* is more of a shade-tolerant species. To further support such assertion, *D. brownii* is a canopy tree which reaches 30 m height at maturity (Table 4.1) and its seeds (an average of two seeds per fruit) can germinate after dormant period under both forest floor and full sun (A. Sugiyama, *unpublished data*). Thus, while typical pioneer species require intense light for their seeds to germination, the opposite does not necessarily apply for shade-tolerant species.

One should note that the smallest SS individuals of *D. brownii* recorded in 2010 were roughly a year younger than those of other species due to dormancy of seeds produced in 2009. In contrast to the rest of the species except for *F. tonduzii*, reproductive trees of *D. brownii* did not have an obvious fruiting peak (A. Sugiyama, *personal observation*). Instead, some reproductive trees fruited again the following dry season in 2010, although fruit crop was generally lower than that during the wet season of 2009. The seeds produced during the dry season germinated in about a month, apparently without dormancy (A. Sugiyama, *personal observation*). Such dormancy in tropical species is common (Garwood, 1983). Thus, the data from 2011 will be comparable with 2010 data of other species for transect surveys assuming that other environmental factors were constant. Because time since germination was much shorter for *D. brownii* than for other species, the number of SS individuals was more likely to be higher, which partly explains the high g_1 value for *D. brownii* (Table 4.3; Fig. 4.1). Similarly, such time since germination explains high proportion of intact seedlings and low severe herbivory in 2010 (Table 4.4). Considering such time lag, severe herbivory area in 2011 was very severe (Table 4.4), and was not consistent with the expectation that shade-tolerant species are more resistant against herbivores (Coley, 1983a; Coley and Barone, 1996; Ruiz-Guerra *et al.*, 2010). Although seedlings of *D. brownii* are relatively large due to its large seed size, 97.6% of the SS individuals still maintained one or two cotyledons until the second year. Such observations may suggest that seedlings of *D. brownii* are physiologically still undeveloped against biotic agents. Additionally, 0.8% of all the seedlings in 2010 were albino, all of which died by 2011. All the albino seedlings emerged from two of the focal trees in LCBS (0.3% and 3.8% of all SS individuals for each tree), which suggests that these two trees were heterogeneous for the albino gene.

Ficus tonduzii

F. tonduzii was the only species which had higher non-SS density compared to SS density and negative g_1, both of which suggest that *F. tonduzii* is a pioneer species, although some observational facts suggests that *F. tonduzii* might not be a typical pioneer species (see 4.5. Discussion in the main text) throughout its entire life (Poorter *et al.*, 2005).

F. tonduzii has small copious seeds, which are produced continuously (annual fruiting as a species but the same individual trees do not produce fruits annually). This is also a characteristic of typical pioneer species (Swaine and Whitmore, 1988; Kitajima, 1994; Hamann and Curio, 1999; da Silva and Tabarelli, 2000). It should be stressed, however, that results reported here (Table 4.3; Table 4.4) for *F. tonduzii* must be viewed with caution. Sample size for regenerating individuals were very low that it often led to extreme values compared to those of other species, which might not necessarily result from difference in life-history traits or responses to forest fragmentation.

Lacistema aggregatum

Species abundance and size distribution of *L. aggregatum* were both intermediate among the five species. Since one plot in LCBS included several reproductive trees, mean density of SS individuals in LCBS was extremely high (Table 4.3), while commonness and number of individuals in the transects around the focal trees were not particularly high (Table 4.3; Fig. 4.2). Field observations suggest that reproductive trees of *L. aggregatum* are associated with high light availability, such as along the trails (A. Sugiyama, *personal observation*), and in fact, while canopy openness for plots was not assessed, the plot that included some reproductive trees had high light availability (A. Sugiyama, *personal observation*). *L. aggregatum* is classified as a small shade-tolerant understory tree (Uriarte *et al.*, 2004) that produces small fruits (Table 4.1) whose seeds can germinate under full sun (Chapter 3). Such trends may partly explain the negative trend in relationship between number of individuals and distance from the edge (Fig. 4.2) although seed quality of *L. aggregatum* was decreased with distance from the edge (Chapter 3).

Reflecting the small seed size, seedlings of *L. aggregatum* are small, with small thin leaves. A year after germination, 36.9% of SS individuals still maintained cotyledons while it dropped to 0.6% after two years. *D. brownii* and *L. aggregatum* were the only two species that maintained cotyledons after a year after germination. While herbivory was relatively low among the five species in both years, biotic infection was relatively high instead among the five species.

Quararibea aurantiocalyx

Light requirement based on size distribution was intermediate for *Q. aurantiocalyx*. Supporting the results here, another congener *Q. asterolepis* had g_1 that was 50th percentile in 73 of the canopy trees studied by Wright *et al*. (2003), although life-history traits of species can vary greatly even within a genus as discussed in the main text. *Q. aurantiocalyx* has large fruit and seed size which can germinate under both forest floor and full sun, and has high survivorship for both SS and non-SS individuals, presumably due to its large seed size (Walters and Reich, 2000; Westoby *et al.*, 2002; Moles and Westoby, 2004a).

While individual density was relatively high in LCBS, again due to the existence of reproductive trees included in plots, both density and commonness were very rare in SF. In fact, *Q. aurantiocalyx* was the only species that had significant difference in density (both size classes) and commonness (non-SS only, due to no presence in SF). Thus, besides the fact that it is already listed on the IUCN red list, forest fragmentation effects were most severe on regeneration of *Q. aurantiocalyx* among the five species. Such negative impacts of forest fragmentation appear to result from reduced dispersal in small fragments (Table 4.3) and negative edge effects on later growth stages (Fig. 4.2i). Since *Q. aurantiocalyx* is not a tall tree without large crown (Table 4.1), most seeds will fall near the maternal tree without seed dispersers, and those seeds are often consumed by rodents (A. Sugiyama, *personal observation*). Mammals and large birds that disperse large seeds of *Q. aurantiocalyx* (Appendix A) may also be less in SF than in LCBS. Reduced dispersal will not only result in potentially higher seed/seedling mortality as expected from the Janzen-Connell hypothesis (Janzen, 1970; Connell, 1971), but also reduce chances of species range expansion and reaching safe sites (Uriarte *et al.*, 2010). Particularly in small forest fragments, severe seed limitation can have serious demographic consequences.

Tapirira mexicana

Despite the rank for light requirement based on g_1, *T. mexicana* had some traits that are not typically associated with shade-tolerant species. The most evident one was herbivory and biotic infection. While typical shade-tolerant species are more resistant against herbivores than pioneer species (Coley, 1983a; Coley and Barone, 1996; Ruiz-Guerra *et al.*, 2010), thin leaves of *T. mexicana* suffered the highest levels of herbivory and biotic infection among the five species (Table 4.4). Additionally, relative growth was overall the highest, which is more characteristic of a pioneer species (Condit *et al.*, 1996). Yet, *T. mexicana* is a canopy tree that reaches 30 m in height and over 10 m in crown size (Table 4.1), and its seeds have short longevity (Chapter 2), which is a character for typical shade-tolerant species (Swaine and Whitmore, 1988). All of these discrepancies, along with potential ontogenetic shifts for this species, suggest that typical pioneer or shade-tolerant species are presumably rather rare. Currently, *T. mexicana* commonly occurs throughout Latin America and the highest species abundance of SS individuals (Table 4.3) is supported by its high fecundity and long fruiting period. However, germination and pre-dispersal predation are negatively affected by edge effects, and future population may decline as more forests are fragmented (Chapter 2).

Potential caveats

Assessment of herbivory and biotic infection

In this study, assessments were conducted at two time points after germination in 2010 and 2011. Thus, if herbivory or biotic infection is so severe that an individual dies or the leaves are entirely lost before being initially recorded, potential herbivory or biotic infection might have been underestimated. A large insect hole might also have been a result of more than one event, which will underestimate the frequency. Herbivory on seedling stems were not recorded in this study, but it may reveal more direct impacts on seedling performance (Dalling and Hubbell, 2002). While completely intact individuals were rather rare (Table 4.4), insect galls or fungal infection was not always lethal (A. Sugiyama, *unpublished data*) but if mortality caused by disease is substantial after germination before the initial recording (Augspurger, 1983b; Coley, 1983a), the low biotic infection rates reported here might have been highly underestimated. Regardless, the methods employed here do not seem to have biased the main results based on the results for *D. brownii*, most of which germinated in 2010 when initial recording was conducted.

Assessment of realized dispersal

Since realized dispersal was assessed from the spatial distribution of the SS individuals there is one caveat as existence of a seedling is a combined effect of (primary and secondary) seed dispersal and survival/establishment. If there is a disproportionally higher survivorship for dispersed individuals, as expected by the Janzen-Connell (J-C) hypothesis (Janzen, 1970; Connell, 1971), only for certain trees, such disproportionally high survivorship may mask the actual dispersal patterns, although the comparison was between trees at different distances from forest edges, and not between the dispersal categories. Such scenario may occur when trees near forest edges, which are more likely to be in smaller fragments with potentially less dispersers, show disproportionally higher survivorship for dispersed individuals. However, one of the few studies that compared the J-C effect in sites with different disperser abundance showed the opposite pattern. Seedling survivorship was higher for seeds near the closest reproductive conspecific trees, which are less likely dispersed, in sites with less dispersers (Wright and Duber, 2001).

Tradeoff in plot size and number in studies assessing size distribution

While there was evident edge effects on SS individuals of *T. mexicana* (Fig. 4.2e), g_1 was higher in SF due to several plots in SF that included a reproductive tree. Such possibility was expected, and such an issue could be overcome by increasing the number of plots. However, the challenge is a tradeoff between plot size and number. While studies focusing on seedlings may employ numerous small plots (e.g., 20,000 1 m^2 plots; Comita *et al.*, 2010), studies assessing size distributions require larger plot size to include large individuals. Previous studies assessing size distribution has typically considered only established older individuals (e.g., dbh > 1 cm), and have been conducted in few large plots (e.g., one 50 ha plot; Condit *et al.*, 1998; Wright *et al.*, 2003), or more plots of smaller sizes for those comparing different sites (e.g., 10 subplots within 10 × 10 m plots per site depending on the individual size class; de Souza *et al.*, 2010). While increasing the sample size might have led to significant results between site groups in this study, g_1 obtained here seems to be valid despite the limitations and data from different perspectives aided in interpreting the exceptional results due to methodology mentioned above.

CHAPTER 5

ESTABLISHMENT DISTANCE SHIFTS OUTWARD FROM THE CLOSEST REPRODUCTIVE CONSPECIFIC WITH INCREASING TREE SIZE IN TROPICAL PREMONTANE WET FORESTS[4]

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5.1. Abstract

Spatial distribution of plants may change over time in a predictable manner but most studies have focused on spatial aggregation at one point in time. I empirically tested whether median distance of individuals from the closest reproductive conspecific (establishment distance) shifted outward with increasing size class for five animal-dispersed tree species in Costa Rican premontane wet forests. Establishment distance shifted outward with increasing size class for the four non-pioneer tree species, indicating eventual regeneration success for seeds dispersed away from reproductive conspecifics. Such outward shifts resulted from positive distance-dependent survivorship, supporting the Janzen-Connell hypothesis, but only for the smallest size class. However, herbivory or mean light availability did not explain such distance-dependent survivorship while heterogeneity in light environment increased with distance from reproductive trees. These results reinforce the importance of seed dispersal, and indicate that distance-dependent survivorship may play a greater role in plant spatiotemporal dynamics than previously recognized.

5.2. Introduction

Spatial distributions of sedentary plants are critical because where such organisms occur has a major influence on the environment they experience for the rest of their lives. Concurrently, their spatial distribution affects interactions among residing organisms (Stoll and Prati, 2001) and ecosystem function (Manning *et al.*, 2006). Because seed dispersal from seed sources (maternal plants) determines the initial distributions of plants, which eventually determines the distributions of seed sources and subsequent plant distributions, seed dispersal has been a major research topic in ecology (Howe and Smallwood, 1982; Hubbell, 2001; Wang and Smith, 2002).

However, seed dispersal is only the first stage of regeneration and fully understanding species regeneration requires knowing where individuals survive. Direct observation of regeneration of long-lived adults such as trees is not practical in a short-term study. Alternatively, long-term data on the temporal dynamics of plant spatial distributions are restricted to a few well studied sites. Still, life spans of trees are typically much longer than the duration of the research and information for small individuals (e.g., dbh < 1 cm) is scarce. One way to characterize how dispersal affects spatial regeneration patterns is to make the assumption that tree size is a proxy for tree age because difference in size can be roughly translated into differences in the time elapsed since a given seed dispersed and germinated. Given this assumption, the presence of individuals of a given size and location reflects their cumulative history of success in their respective local environments until the present (Picard *et al.*, 2009). Thus, differences in spatial distributions of different-sized individuals may reflect changes in the suitability of the local environment for species regeneration over time. Furthermore, comparing different-sized individuals is useful in understanding whether regeneration niches change across life stages (Kanagaraj *et al.*, 2011).

Many studies on plant spatial distributions, however, have focused on spatial aggregation (clustering, spacing) or autocorrelation, even in studies of spatial distributions of different-sized individuals (Condit *et al.*, 2000; Picard *et al.*, 2009). Independent of spatial aggregation, where a plant population occurs may shift over time in a predictable manner. A few studies have shown that the median distance of individuals from the closest reproductive conspecific (hereafter referred to as “establishment distance”) shifts outward as cohorts grow older (Augspurger, 1983a; Wyatt and Silman, 2004) or increase in their size class (Wada and Ribbens, 1997; Barot *et al.*, 1999; Jansen *et al.*, 2008). Establishment distance is the center of a spatial distribution in relation to the closest reproductive conspecific, independent of other coexisting species. The underlying cause for such outward shifts has not been fully explained (but see Barot *et al.*, 1999 for relative abundance of individuals at different distances). Figure 5.1 shows that, in theory, consistent outward shifts in establishment distance with increasing size class (substituted by time in Fig. 5.1) occur when survivorship of all sizes increases with distance from the closest reproductive conspecific, without specifying dispersal modes or species characteristics. In contrast, if germination and subsequent survivorship are equal at all distances from the closest reproductive conspecific, the population recruitment curve should closely follow the dispersal curve.

One proposed mechanism for such positive distance-dependent survivorship is the Janzen-Connell (J-C) hypothesis (Janzen, 1970; Connell, 1971). It predicts that mortality will be higher close to the reproductive conspecific where seed and seedling densities are high due to higher seed predation, herbivory, or pathogen infection. It was originally proposed to explain high species coexistence in tropical forests because this distance/density-dependent mortality does not allow conspecific offspring to occupy the space near the reproductive tree. Another non-mutually exclusive mechanism is the colonization hypothesis (Howe and Smallwood, 1982). It predicts that seeds have a higher chance of arriving in a favorable environment, both in space and time, when dispersed away from the parent. These hypotheses, especially the former, have been tested for many tropical and temperate species with mixed results. However, their tests have been predominantly on early stages (seed, seedling) and have rarely been tested across life stages (Carson *et al.*, 2008), even though underlying mechanisms may be highly stage-dependent (Blundell and Peart, 2004). Conversely, local neighborhood effects (e.g., conspecific density, relative plant size) on long-term tree survival have excluded the early stages (Hubbell *et al.*, 2001; Ahumada *et al.*, 2004), although processes that act during the early stages may be strong (Harms *et al.*, 2000).

The goal of this study was to assess spatiotemporal dynamics of regeneration near reproductive trees. While the importance of rare long-distance dispersal is recognized (Nathan, 2006), most seeds end up in the vicinity of maternal trees for many species. If few individuals near maternal trees make it through the reproductive stage, failure in seed dispersal may immediately mean failure of regeneration, although existence of pre-reproductive stages may be meaningful for its processes (e.g., as density). A discrepancy between seed shadow and seedling/sapling establishment locations has been shown (Schupp and Fuentes, 1995; Rey and Alcantara, 2000; Swamy *et al.*, 2011), but empirical evidence for the later stages is still limited. Here, I empirically studied spatiotemporal dynamics of all post-germination sizes of five animal-dispersed tree species in tropical premontane wet forests. While the closest reproductive conspecific may not be the true parent (Sezen *et al.*, 2009), the focus here was on spatiotemporal dynamics of individuals near each reproductive tree. If mechanisms underlying distance/density-dependent survivorship are those proposed by the J-C hypothesis, genetic identity should not matter (Valenta and Fedigan, 2010). I tested the following four hypotheses: (1) establishment distance will shift outward from the closest reproductive conspecific with increasing size class; (2) if (1) is true, then the outward shifts in establishment distance are caused by positive distance-dependent survivorship of all sizes; (3) herbivory and biotic infection rates of small individuals will be high near reproductive conspecifics; and (4) light availability and heterogeneity will increase with distance from the closest reproductive conspecific. I collected data over a year to test the first and second hypotheses and to test whether shifts in establishment distance are directly observable for the smallest size class during the study period.

The third and fourth hypotheses test two potential underlying mechanisms for distance-dependent survivorship. I limited the individuals to the smallest size class to test the third hypothesis because early stages are most critically affected (Coley, 1983a; Gilbert *et al.*, 1994). The fourth hypothesis provided a potential mechanism that may apply to individuals of all sizes of many species. Because the light environment is one of the most important factors that influences plant performance of all sizes (Montgomery and Chazdon, 2002), I assessed light availability and heterogeneity along the distance gradient from reproductive trees. Under its crown, light availability for individuals should be lower than for those beyond the crown. Then, due to a greater radius, area increases with distance from each reproductive tree and as expected by the colonization hypothesis, the probability of environment being more heterogeneous and encountering a light gap should increase.

5.3. Methods

Study system

I based the study at Las Cruces Biological Station (LCBS; 8° 47' N, 82° 58' W), a field station of the Organization for Tropical Studies in Coto Brus county, southern Costa Rica (Fig. 1.1). The research area is located in a highly deforested region of Costa Rica in which the forest reserve at LCBS (209 ha) is the largest remaining primary forest fragment in the immediate area. Holdridge *et al.* (1971) classified the natural vegetation of the area as tropical premontane wet forest with elevation ranges between 1,000 and 1,400 m. Temperature ranges between 14 and 29°C (mean: 20°C), and there is typically a dry season from December through March. Annual precipitation ranges between 4,000 and 5,000 mm but 2009 (El Niño year) was an unusually dry year (3,063 mm) that it affected fruit production of many tree species during the early wet season

(A. Sugiyama, *personal observation*). To facilitate location of the focal trees in such a low crop year, I used trees in smaller primary forest fragments (4-33 ha) in addition to those in the largest forest reserve at LCBS. All five forest fragments are within 8 km of each other, with similar elevations and climate, and have not had recent major disturbances.

As focal species, I selected five animal-dispersed tree species, *Drypetes brownii* (Putranjivaceae), *Ficus tonduzii* (Moraceae), *Lacistema aggregatum* (Lacistemataceae), *Quararibea aurantiocalyx* (Malvaceae), and *Tapirira mexicana* (Anacardiaceae). I selected them based on an abundance of reproductive trees and fruit production between May and August 2009. Among the five species, *F. tonduzii* is the only pioneer species. All species produce fleshy fruits dispersed by animals. The seeds do not possess dormancy under full sun, except for *D. brownii* seeds that germinate the following wet season (A. Sugiyama, *personal observation*).

Field data collection

From trees fruiting in 2009, I randomly selected a total of six focal trees per species (LCBS: n = 3, smaller fragments: n = 3). Focal trees were at least 100 m away from each other to minimize overlapping seed shadows. I established two 5 × 40 m transects in random directions radiating out from each focal tree (n = 2 transects × 6 trees × 5 species). Between the end of April and early June 2010, I recorded and tagged individuals of all sizes conspecific to the focal tree within the transects for basal diameter, dbh (when height > 3 m), and distance class (0-40 m, 1 m interval) from the focal tree (i.e., the closest reproductive conspecific). I classified each individual into one of six size classes; SS (small seedlings; < 50 cm tall), SD (seedlings; 0.5-1 m tall), SP (small saplings; 1-3 m tall), LP (large saplings; > 3 m tall, < 5 cm dbh), UN (understory; 5-10 cm dbh), and CP (canopy; > 10 cm dbh). I was able to identify the smallest individuals by conducting germination trials on seeds collected prior to this study. For the smallest size class SS, I also recorded the existence of cotyledons and the number of leaves. Because number of individuals was proportionally biased toward SS, I further classified size classes other than SS as non-SS, and, hereafter, I refer to these two categories as “size groups” (i.e., SS vs. non-SS).

To test the first hypothesis, I calculated establishment distance for each size class. Between May and early June 2011, I conducted a recensus of all the tagged individuals to measure survivorship and growth to test the second hypothesis. I calculated growth as $(d_2 - d_1)/(t_2 - t_1)$, where d_1 and d_2 are basal diameters, and t_1 and t_2 are the measurement times (days) when the recording was conducted in 2010 and 2011, respectively. Because no focal trees fruited in 2008 or 2010 (A. Sugiyama, *personal observation*), no new seeds were added from focal trees after fruiting in 2009 during the study period. This facilitated tracking the dynamics of the 2009 cohort of the smallest SS individuals, and excluded any intraspecific interactions of newly emerging seedlings. Most SS individuals in this study were from the 2009 cohort based on the number and developmental stage of leaves and the growth data. Furthermore, there were very few SS individuals in 2009 when focal trees were fruiting. Therefore, initial recording on SS individuals in 2010 largely reflected 1-yr seedling establishment except for *D. brownii*.

Invertebrate herbivory and biotic infection assessment

To test the third hypothesis, I recorded evidence for invertebrate herbivory and biotic infections (e.g., galls, fungal infection) non-destructively for all SS individuals in 2010 when they were tagged. I assessed invertebrate herbivory via both frequency (number of holes or bites) and area consumed because herbivory may affect the seedling performance via induced responses to herbivory (Kessler and Baldwin, 2002). Such responses may be cumulative with its frequency of occurrence or the total leaf area affected. In evaluating herbivory frequency, I categorized each individual into: intact (no herbivory), some frequency of herbivory (< 10 herbivory events), and severe herbivory frequency (≥ 10 events). To evaluate herbivory area, I categorized each individual into: intact (no herbivory), some herbivory area (< 50% of the area of all existing leaves consumed), and severe herbivory area (≥ 50% consumed). I only report one figure for intact individuals because seedlings classified as intact for frequency of herbivory events were also fully intact for leaf area. I also classified individuals binomially by whether or not their leaves showed evidence of biotic infection, independent of herbivory status. To infer impacts of herbivory and biotic infection on seedling survivorship, I recorded the status of leaves again in the recensus in 2011.

Light environment characterization

I tested the fourth hypothesis by assessing the relationship between leaf area index (LAI) and distance from the focal tree. LAI is a major determinant of light transmittance in forests and strongly affects regeneration processes (Kalacska *et al.*, 2005). Between the end of April and early June 2011, I took photographs under overcast conditions with a Nikon Coolpix 885 camera (Nikon Corporation, Tokyo, Japan) equipped with a leveled fisheye converter (Nikon FC-E8 0.21×) at 1m height every 5 m along the length of the two transects for each focal tree ($n = 8$ distances × 2 transects × 6 trees × 5 species). I used an automatic thresholding function on SideLook 1.1 (Nobis, 2005) for binarization (black: white) of photographs and then calculated LAI (5 rings) from the binarized photos using Gap Light Analyzer (GLA) 2.0 (Frazer *et al.*, 1999). This two-step process was necessary because GLA does not allow automatic thresholding of pixels. In addition to light availability, I also examined whether heterogeneity of light environment increased with distance from the focal tree by calculating the coefficient of variation (Désilets and Houle, 2005) of LAI.

Realized dispersal

Because I did not manipulate seed numbers in 2009, I did not experimentally study the effect of seed dispersal on seedling establishment and survival directly. To infer the role of dispersal on individual performance, I assessed seed dispersal indirectly as realized dispersal. Individuals that occurred beyond the crown periphery plus 3 m were classified as having germinated from "dispersed" seeds. Because crown shapes were irregular and the crown extent often differed by direction, I measured crown size above each transect to determine the threshold distance. Since the presence of an individual is a result of both dispersal and survival/establishment, SS individuals predominantly reflect dispersal, while non-SS individuals reflect survivorship/establishment. Thus, the difference in proportion of dispersed individuals between the two size groups reflected whether dispersed individuals were more likely to be established.

Statistical analyses

For each focal species, I calculated establishment distance across focal trees, and compared among size classes or size groups with a nonparametric Kruskal-Wallis test. To assess whether shifts in establishment distance were indeed caused by positive distance-dependent survivorship, I calculated survivorship per 5 m for each size group. I treated focal tree as an experimental unit, and pooled two transects per focal tree to represent a focal tree. Then, I conducted simple linear regressions between survivorship and distance from the focal tree.

Because survivorship may be affected by various factors, I conducted stepwise model selection (entry and stay levels: 0.05) to identify important variables in predicting survivorship. In addition to distance from the focal tree, I included the following as predictor variables in the model: density per 5 m^2 in 2010, growth, proportion of intact/severe herbivory frequency/herbivory area/biotic infection, and LAI. For model selections, I only considered individuals that occurred within 1 m of the location where I took hemispherical photographs at 5 m intervals (e.g., individuals at 4, 5, and 6 m from the focal tree were considered to experience the light environment of 5 m). To test whether there was a general pattern that light availability or heterogeneity increased with increasing distance from focal trees across species, I regressed light availability and its coefficient of variation against distance from the focal tree with all species pooled. I also compared survivorship, herbivory and biotic infection levels, and size groups between dispersed and non-dispersed individuals using generalized linear mixed models (GLIMMIX) with focal trees as random effects (binomial distribution and logit link).

For all statistical procedures, I used SAS 9.2 software (SAS Institute Inc., 2009) and for all data that did not meet the normality assumption, I log-transformed them (angular-transformed for proportion data) prior to the analyses. When there was a quasi-complete separation problem in GLIMMIX with binomial distribution, I used logistic regression instead with Firth's penalized maximum likelihood estimation (SAS Institute Inc., 2009). I report adjusted R^2 values here and errors are SE, unless otherwise indicated.

5.4. Results

Population recruitment curve

In 2010, I recorded and tagged a total of 2,712 *D. brownii*, 27 *F. tonduzii*, 1,116 *L. aggregatum*, 1,191 *Q. aurantiocaly*x, and 2,049 *T. mexicana* individuals within the 40 m transects of all focal trees of each species. In all but one pioneer species (*F. tonduzii*), shapes of population recruitment curves for all size classes combined resembled those expected from seed dispersal curves (Fig. 5.2). Such leptokurtic population recruitment curves resulted from a disproportionate contribution by the smallest size class SS. In species with population recruitment curves that were highly skewed to the right, number of individuals declined near the focal tree substantially after a year without any further seed input.

First hypothesis: establishment distances shift outward

The first hypothesis was supported for all species except F. tonduzii. Outward shifts in establishment distance with increasing size class were observed for all species with a leptokurtic population recruitment curve (Fig. 5.3). Particularly between the two smallest size classes, SS and SD, which were also the size classes with the highest number of individuals, the difference in establishment distance was significant for all those species for both years. As a result, establishment distance of non-SS was significantly greater than that of SS for all species except *F. tonduzii* (Table 5.1). Approximate age for each size class (Fig. 5.4) and extent of the shift and whether establishment distance consistently shifted outward with increasing size class varied among species. For *L. aggregatum*, establishment distance of SS individuals actually shifted outward between 2010 and 2011 (Table 5.1; $K = 361$, df = 27, $P < 0.0001$).

Second hypothesis: positive distance-dependent survivorship

Survivorship increased with distance from the closest reproductive conspecific for species that showed outward shifts in establishment distance (Fig. 5.5), consistent with theoretical expectations (Fig. 5.1). However, no distance-dependent survivorship occurred for non-SS individuals. Thus, mean survivorship of SS individuals was higher for germinants from dispersed than non-dispersed seeds for all five species (Fig. 5.6). Between the two size groups, proportion of individuals from dispersed seeds was higher for non-SS than for SS for all species except *F. tonduzii* (Table 5.2).

Results from model selections revealed that distance from the focal tree was significant in predicting survivorship of SS individuals for *D. brownii* ($F = 21.8$, $P < 0.0001$), *L. aggregatum* ($F = 8.7$, $P = 0.015$), and *T. mexicana* ($F = 10.9$, $P = 0.0026$). In contrast, density was more important in predicting survivorship of both SS and non-SS individuals for *F. tonduzii* ($F = \infty$, $P < 0.0001$) and non-SS individuals for *D. brownii* ($F = 10.4$, $P = 0.0037$) and *Q. aurantiocalyx* ($F = 8.0$, $P = 0.013$). Growth was the only significant variable in predicting survivorship of SS individuals for *Q. aurantiocalyx* ($F = 23.6$, $P = 0.0007$), and there was no significant predictor for non-SS individuals of *L. aggregatum* and *T. mexicana*.

Third hypothesis: herbivory and biotic infection levels decrease with distance

Contrary to predictions of the third hypothesis, individuals from dispersed seeds did not escape herbivory or biotic infection better than those from non-dispersed seeds (Table 5.3). One exception was the level of severe herbivory area in *T. mexicana* in 2010, for which there were significantly higher levels of herbivory in individuals from non-dispersed seeds. However, this difference disappeared by 2011. For the rest of the species and categories, levels of herbivory were higher for dispersed than non-dispersed seeds or there was no difference between the two (Table 5.3). Results from model selections revealed that, in addition to distance from focal tree, herbivory was also significant for survivorship of SS individuals in *D. brownii* (proportion of intact individuals: $F = 13.2$, $P = 0.0007$) and *L. aggregatum* (proportion of individuals with severe herbivory area: $F = 7.5$, $P = 0.021$).

Fourth hypothesis: light availability and heterogeneity increase with distance

The fourth hypothesis was partly supported. LAI increased with distance from the focal trees, in contrast to expectations (Fig. 5.7a). As shown from the results of model selection, light availability was not significant for individual survivorship for either size group. While light availability decreased with distance from the focal tree, heterogeneity in light environment increased, supporting the hypothesis (Fig. 5.7b).

5.5. Discussion

Establishment distance shifted outward from the closest reproductive conspecific and positive distance-dependent survivorship was empirically shown for most species. However, some of the underlying mechanisms were contrary to expectations. The first hypothesis was supported for all four non-pioneer species with leptokurtic population recruitment curves. Although outward shifts in establishment distance did not always occur consistently, especially for some larger size classes with few individuals (e.g., *T. mexicana*), overall, there were outward shifts. Such a shift was documented directly for SS individuals for *L. aggregatum*. Because of such outward shifts in establishment distance with increasing size class, establishment distance was consistently higher for non-SS than for SS individuals for species except *F. tonduzii* (Table

5.1). Although pre-germination stages were not considered in this study, such a discrepancy in spatial distribution including the seed stage has been documented for different cohorts (Augspurger, 1983a; Wyatt and Silman, 2004) or different-sized individuals (Barot *et al.*, 1999; Jansen *et al.*, 2008; see Dovčiak *et al.* 2001 for opposite directional shifts). While focal species were all animal-dispersed trees in this study, such spatiotemporal dynamics is not restricted to certain dispersal strategies (Swamy *et al.*, 2011) or trees (e.g., palms; Wyatt and Silman, 2004). However, the exception in this study, the only pioneer species *F. tonduzii*, suggests that such spatiotemporal dynamics may not apply to all life histories. Although seed input was not directly assessed, *F. tonduzii* had high seed input close to the focal trees as occurred for the other species (A. Sugiyama, *personal observation*). Thus, while the seed dispersal curve of *F. tonduzii* may be similar to the other focal species, their differences in the population recruitment curves may result from differences in their life histories. Recruitment of pioneer species depends on canopy gaps, and recruits do not perform well in the shade (Clark and Clark, 1992). While focal trees of *F. tonduzii* presumably recruited in canopy gaps initially, none of its focal trees were currently in gaps, which presumably led to very low numbers of conspecific individuals near reproductives of *F. tonduzii*. Additionally, pioneers suffer negative density-dependent survivorship more strongly than non-pioneers (Hubbell *et al.*, 2001; Ahumada *et al.*, 2004; Comita *et al.*, 2010). Such a mixture of species with different life histories at the community level may result in complex spatiotemporal plant dynamics. However, some fraction of species may be predictable based on individual-based models (Hubbell *et al.*, 2001; Picard *et al.*, 2009) as shown here.

The second hypothesis based on theoretical expectations was partly supported. While survivorship generally increased with distance from the closest reproductive conspecific for all species that showed outward shifts in establishment distance, supporting the J-C hypothesis, it was restricted to SS individuals (Fig. 5.5). Such size-dependent support for the J-C hypothesis is also reported in a few studies that included all life-history stages (Connell *et al.*, 1984; Silva Matos *et al.*, 1999; Gilbert *et al.*, 2001). In theory, individual survivorship of all sizes needs to increase with distance from the closest reproductive conspecific for outward shifts in establishment distance to occur (Fig. 5.1). A possible explanation of the discrepancy between observed outward shifts in establishment distance for larger size classes and positive distance-dependent survivorship restricted to SS individuals may be due to the methods used and the assignment of the closest reproductive conspecific. Survivorship was calculated based on 1-yr data between 2010 and 2011. Thus, while 1-yr survival data largely reflect population recruitment curves of young SS individuals (Fig. 5.2), that was not the case for non-SS individuals. Due to the substantial time lag between SS and non-SS individuals (Fig. 5.4), even a weak positive relationship between survivorship and distance from the focal tree based on 1-yr data may lead to outward shifts in establishment distance of non-SS individuals over the years. Alternatively, the same phenomenon will arise if the current closest reproductive conspecific for larger individuals is farther from what was the closest reproductive conspecific when it germinated because the initial closest reproductive has died. Individuals of larger size classes are older than SS individuals and the closest reproductive conspecific then is more likely to be dead. Such probability of the closest reproductive conspecific being dead is estimated to be 17-34% at Barro Colorado Island over a 30-year period (S. Hubbell, *personal communication*). Thus, individuals of larger size classes are more likely be assigned to a more distant reproductive conspecific based on spatial distribution of currently existing closest reproductive conspecific.

Invertebrate herbivory or biotic infection as an underlying mechanism for positive distance-dependent survivorship, as tested in the third hypothesis, was not supported. Herbivory was generally either higher for dispersed than non-dispersed individuals or not different between the two, which was also true for biotic infection (Table 5.3). Thus, while there was positive distance-dependent survivorship for most species, two possible underlying mechanisms of the J-C hypothesis were not supported. However, individuals were overall less likely to survive when leaf area loss to herbivory was severe (Chapter 4). Additionally, as a result of positive distance-dependent survivorship of SS individuals (Fig. 5.5), there was a general trend that survivorship was higher for dispersed individuals (Fig. 5.6). One caveat is that seedling status was not tracked until one year after germination and assessments were based on two time points. Thus, if herbivory or biotic infection was so severe that the individual died or the leaves were entirely lost before being recorded, potential herbivory or biotic infection might have been underestimated, especially if such damage occurred shortly after germination (e.g., Augspurger, 1983b). However, this did not explain the patterns at least for *D. brownii* which germinated a year later. Alternatively, analogous to predator satiation for seeds (e.g., Crawley and Long, 1995), there may also be herbivore satiation. Rates of herbivory were analyzed (Table 5.3) and absolute number of individuals affected by herbivory was actually higher near focal trees. Although the narrow host-specificity of enemies assumed by the J-C hypothesis is rather rare (Basset, 1999), if invertebrate herbivores or pathogens with limited mobility are satiated with high individual density near reproductive trees, rates may be lower or comparable between dispersed and non-dispersed individuals. Additionally, because existing individuals farther away from focal trees are more likely to be older, dispersed individuals may show higher levels of herbivory on remaining leaves, as shown from the increased level of herbivory in 2011 from 2010 (Table 5.3).

Light availability and its heterogeneity was another potential underlying mechanism explaining positive distance-dependent survivorship of all sizes. However, there was only partial support for the hypothesis, i.e., increasing heterogeneity in the light environment with distance. LAI was generally greater away from focal trees across species (Fig. 5.7a), although the predictive power was low ($R^2 = 0.047$). Although a 1% difference in light availability may affect seedling growth (Howe *et al.*, 1985), growth and survivorship were not correlated for species in this study (Chapter 4). Thus, light availability did not appear to cause the positive distance-dependent survivorship of SS individuals, similar to results reported elsewhere (Wada and Ribbens, 1997). In contrast, heterogeneity in light environment increased with distance from focal trees as hypothesized. Heterogeneity in light environment is important across species (Montgomery and Chazdon, 2002) and while an increase in heterogeneity does not necessarily guarantee higher light availability, it gives support for the colonization hypothesis. Growing evidence from other studies suggest that there are important interactions between light and other factors on individual performance. While herbivory or biotic infection levels were not correlated with light environment in this study (Sugiyama, *unpublished data*), a reduced-light environment can increase herbivory load and/or effects (DeWalt *et al.*, 2004; Norghauer *et al.*, 2008), as well as pathogen infection (Augspurger, 1983a, 1983b, 1984). Soil nutrients, which were not assessed here, is another major factor affecting plant performance of all sizes. The importance of soil nutrients, relative to light availability, is being recognized (Holste *et al.*, 2011) and their interactions may also explain the patterns observed here. Topography and habitat types (Kanagaraj *et al.*, 2011) also play important roles in structuring plant communities. Although the population recruitment curve can be mechanistically determined by seed dispersal and survivorship without specifying particular causes of mortality (Nathan and Casagrandi, 2004), interactions with surrounding individuals, such as density-dependence (e.g., Harms *et al.*, 2000) and identity of neighbor (e.g., Uriarte *et al.*, 2004), may also explain the underlying processes and unexpected patterns observed.

In this study, I assessed realized dispersal, which results from the cumulative effects of multiple factors, including seed dispersal and seedling survivorship. Individuals were more likely to achieve larger size classes when they were far from the closest reproductive conspecific, which led to outward shifts in establishment distance (Fig. 5.3). Additionally, if mature and intact seeds have a higher chance of dispersal (Wada and Ribbens, 1997), dispersed seeds may have a higher chance of establishment inherently. Because I did my analyses in relation to the distance to the closest reproductive conspecific (i.e., focal tree), estimates of dispersal were rather conservative, especially for small individuals. Although only post-germination stages were considered here, the importance of the difference between seed arrival sites and later-stage sites has been recognized (Schupp and Fuentes, 1995; Rey and Alcantara, 2000; Swamy *et al.*, 2011). Such results, along with those shown here, reinforce the importance of seed dispersal because, regardless of how fecund the parent tree is, those seeds dispersed away will more likely survive to the reproductive stage. If spatiotemporal dynamics of non-pioneer species act as shown here at the community level, current scheme of modeling or predicting spatiotemporal dynamics of plants may be improved. Distance-dependent survivorship effects on shifts in establishment distance, along with seed dispersal, may thus play greater roles in plant spatiotemporal dynamics than previously recognized.

5.6. Tables

Table 5.1
Establishment distance of SS and non-SS individuals for the focal species. First and third quartiles are given in parentheses. Different letters indicate significant difference in establishment distance between size groups for each year at $\alpha = 0.05$. For both years, establishment distance was greater for non-SS than SS except for *F. tonduzii* that did not have the leptokurtic population recruitment curve that resembled expected seed dispersal curve. For *L. aggregatum*, actual shift in establishment distance of SS individuals was observed between 2010 and 2011.

Species	Year	Establishment distance	
		SS	Non-SS
Drypetes brownii	2010	3.0^b (1.0, 6.0)	12.0^a (4.0, 25.0)
	2011	3.0^b (1.0, 6.0)	10.0^a (4.0, 25.0)
Ficus tonduzii	2010	24.0^a (8.0, 35.0)	11.5^a (5.0, 15.8)
	2011	19.5^a (11.8, 27.3)	19.5^a (8.8, 35.0)
Lacistema aggregatum	2010	2.0^b (0.0, 5.0)	9.0^a (3.5, 29.5)
	2011	3.0^b (1.0, 10.0)	9.0^a (4.0, 32.0)
Quararibea aurantiocalyx	2010	1.0^b (0.0, 3.0)	3.0^a (1.0, 8.0)
	2011	1.0^b (0.0, 3.0)	3.0^a (1.0, 8.0)
Tapirira mexicana	2010	2.0^b (1.0, 4.0)	22.5^a (7.0, 35.0)
	2011	2.0^b (1.0, 2.0)	21.5^a (9.3, 33.8)

Table 5.2
Proportion of individuals from dispersed seeds for SS and non-SS individuals. Different letters indicate significant difference between size groups in proportion of individuals from dispersed seeds for each year at α = 0.05. For all species and for both years except for *F. tonduzii* in 2010 and 2011 and *T. mexicana* in 2011, proportion of individuals from dispersed seeds was significantly higher for non-SS than SS. Errors represent SE. See main text for the classification of dispersed seeds in this study.

Species	Year	Dispersed (%)	
		SS	Non-SS
Drypetes brownii	2010	$17.6^{b} \pm 3.7$	$63.2^{a} \pm 7.8$
	2011	$22.7^{b} \pm 3.9$	$61.2^{a} \pm 9.2$
Ficus tonduzii	2010	$77.8^{a} \pm 8.3$	$77.8^{a} \pm 7.1$
	2011	$50.0^{a} \pm 0.0$	$83.3^{a} \pm 11.8$
Lacistema aggregatum	2010	$24.9^{b} \pm 14.2$	$59.3^{a} \pm 13.3$
	2011	$38.1^{b} \pm 16.2$	$62.1^{a} \pm 16.1$
Quararibea aurantiocalyx	2010	$6.2^{b} \pm 9.8$	$29.7^{a} \pm 11.9$
	2011	$6.4^{b} \pm 9.5$	$31.2^{a} \pm 12.1$
Tapirira mexicana	2010	$11.2^{b} \pm 15.0$	$75.0^{a} \pm 6.7$
	2011	$12.0^{a} \pm 13.8$	$80.4^{a} \pm 12.1$

Table 5.3

Mean percentage (± SE) of SS individuals from dispersed and non-dispersed seeds with different levels of herbivory and biotic infection for the focal species. Different letters indicate significant difference between dispersed and non-dispersed seeds for each year at $\alpha = 0.05$. N shows total number of SS individuals. See text for classification of dispersed seeds and definition of severe herbivory frequency and area in this study.

Species	Year	Intact (%)		Severe frequency (%)		Severe area (%)		Biotic infection (%)		N
		Non-dispersed	Dispersed	Non-dispersed	Dispersed	Non-dispersed	Dispersed	Non-dispersed	Dispersed	
Drypetes brownii	2010	$48.8^{a} \pm 4.8$	$33.3^{b} \pm 2.6$	$6.3^{b} \pm 1.9$	$13.2^{a} \pm 2.8$	$1.0^{a} \pm 0.2$	$1.3^{a} \pm 0.6$	$0.8^{a} \pm 0.4$	$1.3^{a} \pm 0.5$	2587
	2011	$10.7^{a} \pm 1.7$	$8.5^{a} \pm 2.0$	$19.6^{b} \pm 3.4$	$21.7^{a} \pm 3.1$	$1.3^{a} \pm 0.5$	$1.3^{a} \pm 1.4$	$2.4^{a} \pm 0.8$	$3.4^{a} \pm 1.3$	1034
Ficus tonduzii	2010	$0^{a} \pm 0$	$42.9^{a} \pm 25.0$	$0^{a} \pm 0$	$14.3^{a} \pm 12.5$	$0^{\dagger} \pm 0$	$0^{\dagger} \pm 0$	$0^{\dagger} \pm 0$	$0^{\dagger} \pm 0$	9
	2011	$0^{\dagger} \pm 0$	$0^{\dagger} \pm 0$	$0^{\dagger} \pm 0$	$0^{\dagger} \pm 0$	$0^{\dagger} \pm 0$	$0^{\dagger} \pm 0$	$0^{\dagger} \pm 0$	$0^{\dagger} \pm 0$	2
Lacistema aggregatum	2010	$34.8^{a} \pm 12.0$	$32.8^{a} \pm 10.3$	$1.8^{b} \pm 1.6$	$5.2^{a} \pm 16.0$	$0.6^{a} \pm 1.2$	$0.7^{a} \pm 0.2$	$1.5^{a} \pm 1.3$	$1.9^{a} \pm 0.5$	1089
	2011	$33.0^{a} \pm 10.0$	$25.4^{a} \pm 8.0$	$0.5^{b} \pm 0.3$	$10.9^{a} \pm 4.6$	$1.0^{a} \pm 0.3$	$2.2^{a} \pm 1.8$	$8.1^{a} \pm 3.5$	$8.0^{a} \pm 4.4$	362
Quararibea aurantiocalyx	2010	$13.6^{a} \pm 3.9$	$10.2^{a} \pm 17.1$	$31.5^{b} \pm 6.6$	$42.4^{a} \pm 12.0$	$2.2^{a} \pm 0.9$	$0^{a} \pm 0$	$1.3^{a} \pm 0.4$	$3.4^{a} \pm 1.3$	959
	2011	$7.8^{a} \pm 3.1$	$0^{a} \pm 0$	$38.9^{a} \pm 10.3$	$73.2^{a} \pm 16.9$	$0.5^{a} \pm 0.2$	$2.4^{a} \pm 1.0$	$2.5^{a} \pm 0.7$	$2.4^{a} \pm 9.1$	638
Tapirira mexicana	2010	$9.3^{a} \pm 3.3$	$12.1^{a} \pm 5.0$	$16.9^{a} \pm 6.3$	$12.9^{a} \pm 4.5$	$13.2^{b} \pm 3.7$	$5.4^{a} \pm 1.8$	$1.0^{a} \pm 0.8$	$2.7^{a} \pm 1.4$	2001
	2011	$3.5^{a} \pm 1.7$	$2.3^{a} \pm 1.3$	$47.3^{a} \pm 8.8$	$42.0^{a} \pm 10.1$	$3.7^{a} \pm 1.0$	$0.8^{a} \pm 0.4$	$6.9^{a} \pm 1.7$	$6.1^{a} \pm 2.8$	1095

[†] Statistical tests could not be performed due to no variation between dispersal categories.

5.7. Figures

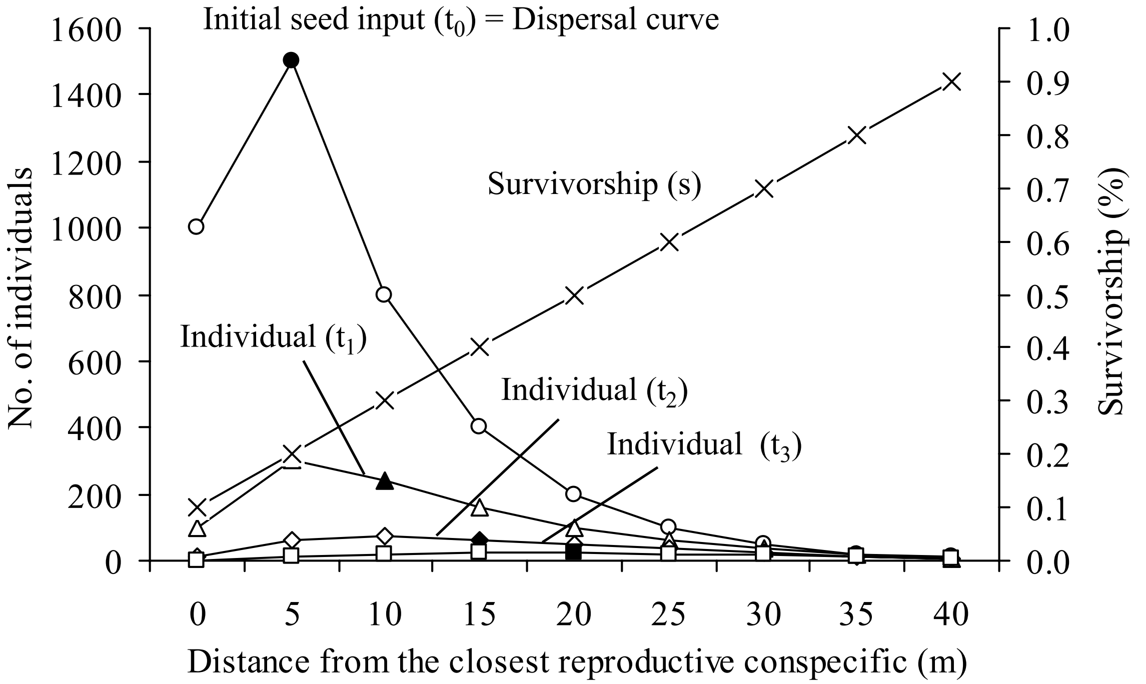

Figure 5.1
Theoretical explanation of outward shifts in establishment distance by consistent positive distance-dependent survivorship over time. Theoretical population recruitment curves for seed dispersal (dispersal curve) and individuals at different stages are shown. Here, the slope of survivorship (s) is held constant over time and increases linearly with distance for simplicity. Number of established individuals at each time point is $t_n = s \times t_{n-1}$. Filled symbols indicate establishment distance (median distance). Note that median differs from mode.

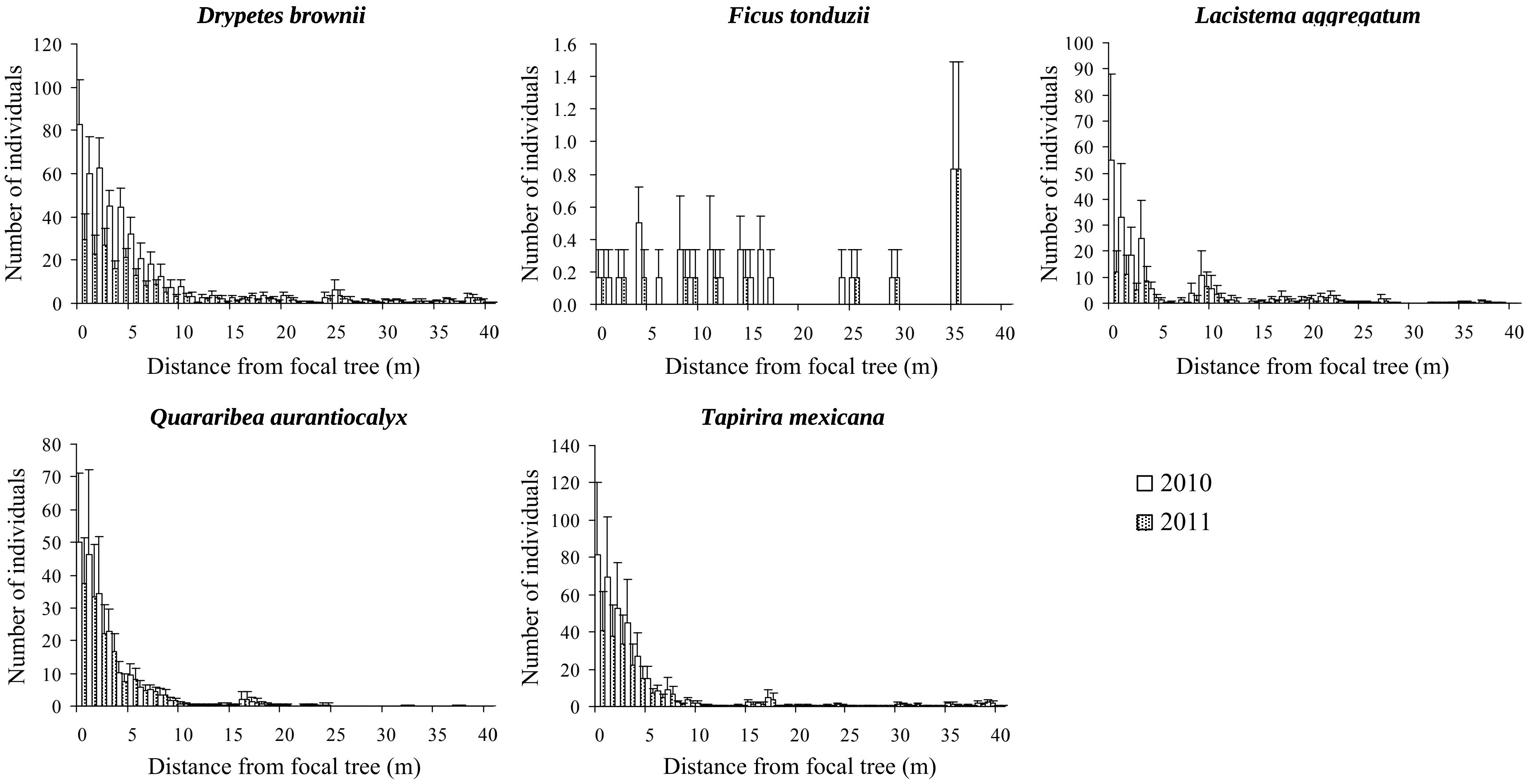

Figure 5.2
Population recruitment curves of each focal species. X-axes are distance from the focal trees, which were fruiting trees in 2009 with no apparent overlapping seed shadows. Y-axes are number of individuals of all size classes conspecific to the focal trees in transects. There was no additional seed input between years. With an exception of a pioneer species *F. tonduzii*, population recruitment curves that were highly skewed to the right as expected from seed dispersal curves. Error bars represent SE for the six focal trees for each species.

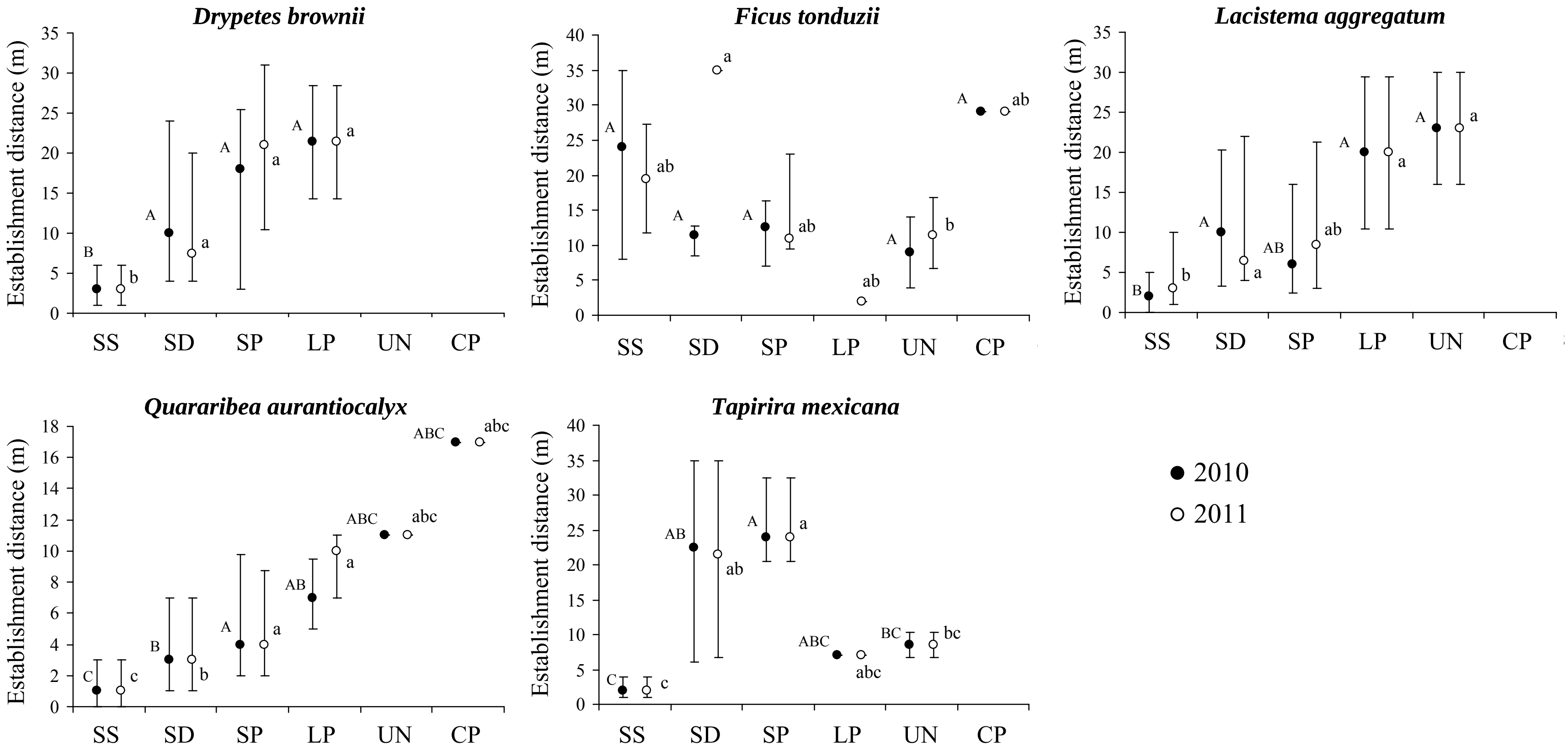

Figure 5.3
Establishment distance of each size class for the focal species. Size classes are: SS (< 50 cm tall), SD (0.5-1 m tall), SP (1-3 m tall), LP (> 3 m tall, < 5 cm dbh), UN (5-10 cm dbh), and CP (> 10 cm dbh). Different upper and lower cases indicate significant difference at α = 0.05 from Kruskal-Wallis tests for 2010 and 2011, respectively. Error bars represent first and third quartile. Note that differences between some larger and smaller size classes were not statistically significant due to small sample size for larger size classes. For some species, individuals of some larger size classes did not exist within 40 m radius of the focal trees.

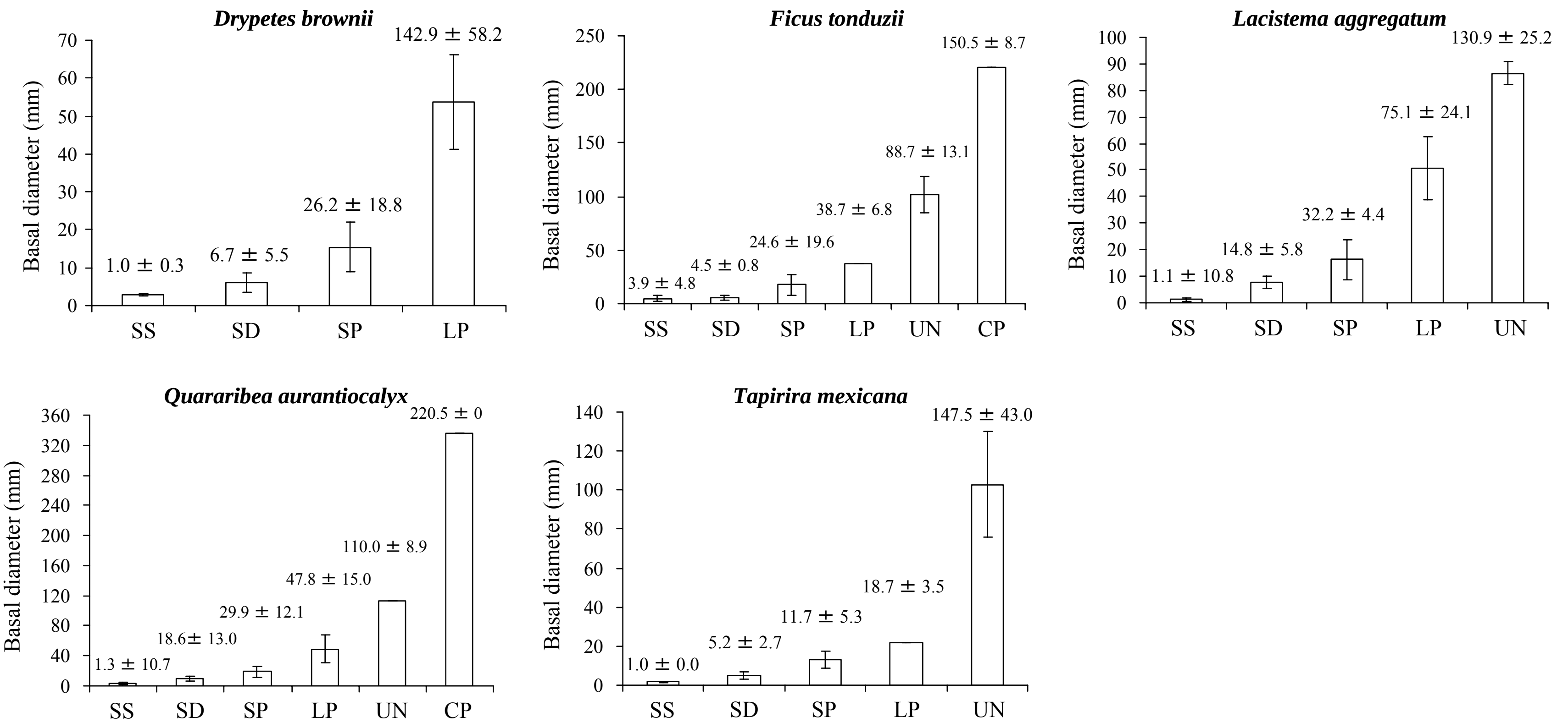

Figure 5.4
Mean basal diameter for each size class and rough mean age estimate of each size class for the focal species. Mean estimated age (year ± SD) are given above bars for each size class. Age of each size class was estimated based on mean initial basal diameter of germinants and average growth rate between the two adjacent size classes for each size class. See Materials and methods for size class designations.

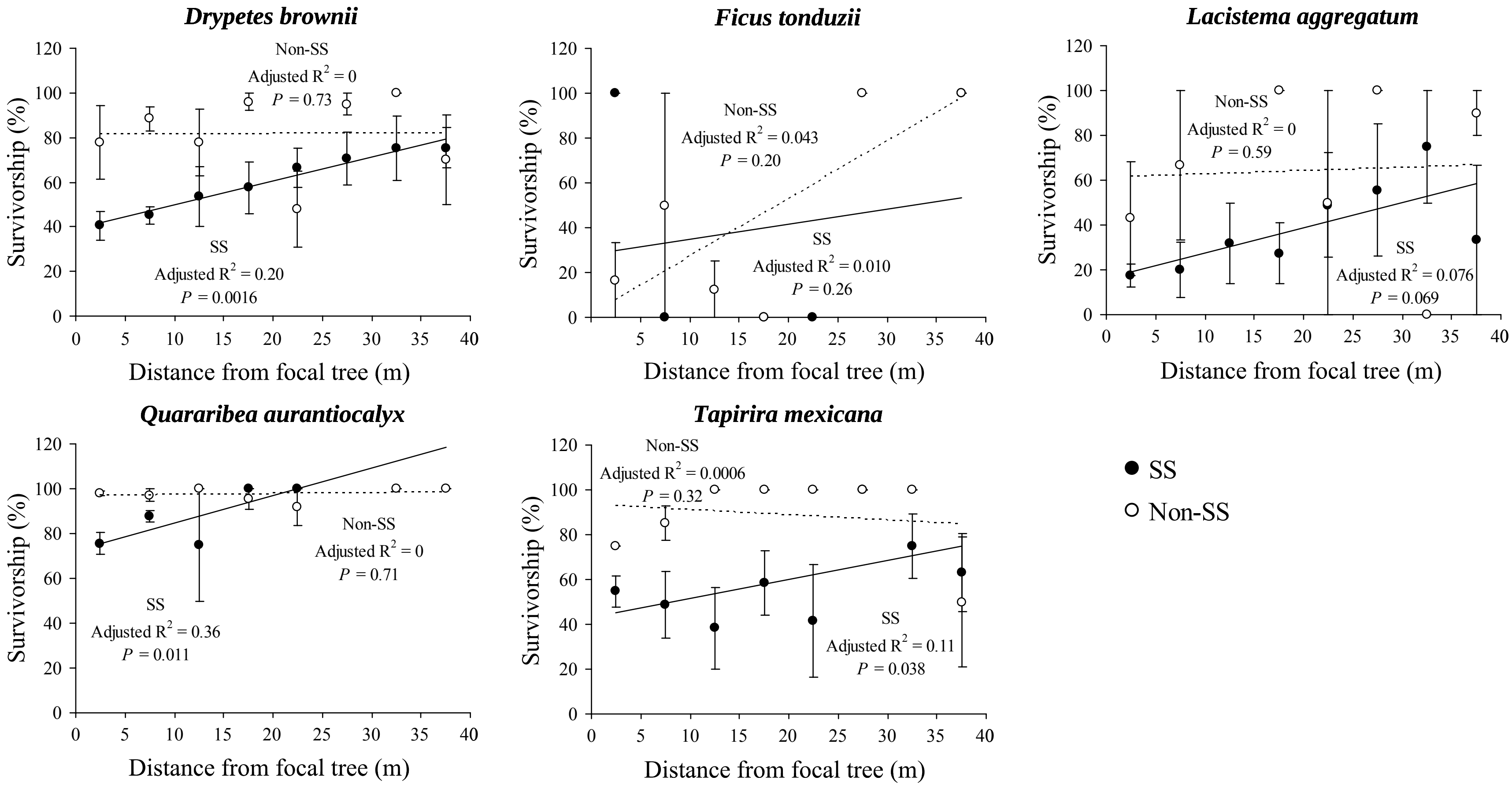

Figure 5.5
Percent survival at each distance from the focal tree for the focal species. Solid and dotted line show fitted line from linear regressions for SS and non-SS individuals, respectively. Adjusted R^2 and P-values from linear regressions are given for each size group on each panel. Error bars represent SE. Actual analyses were performed on transformed data.

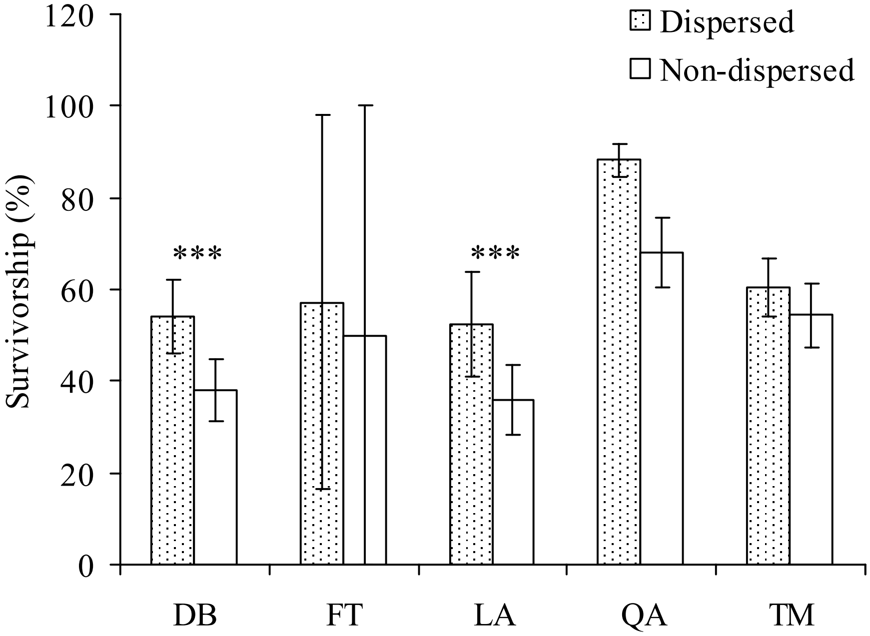

Figure 5.6
Proportion of survived SS individuals for the focal species. Abbreviations for species are: DB, *D. brownii*; FT, *F. tonduzii*; LA, *L. aggregatum*; QA, *Q. aurantiocalyx*; and TM, *T. mexicana*. Asterisks indicate significant difference in survivorship between germinants from dispersed vs. non-dispersed seeds at α = 0.0001. Error bars represent SE. See main text for classification of dispersed seeds in this study.

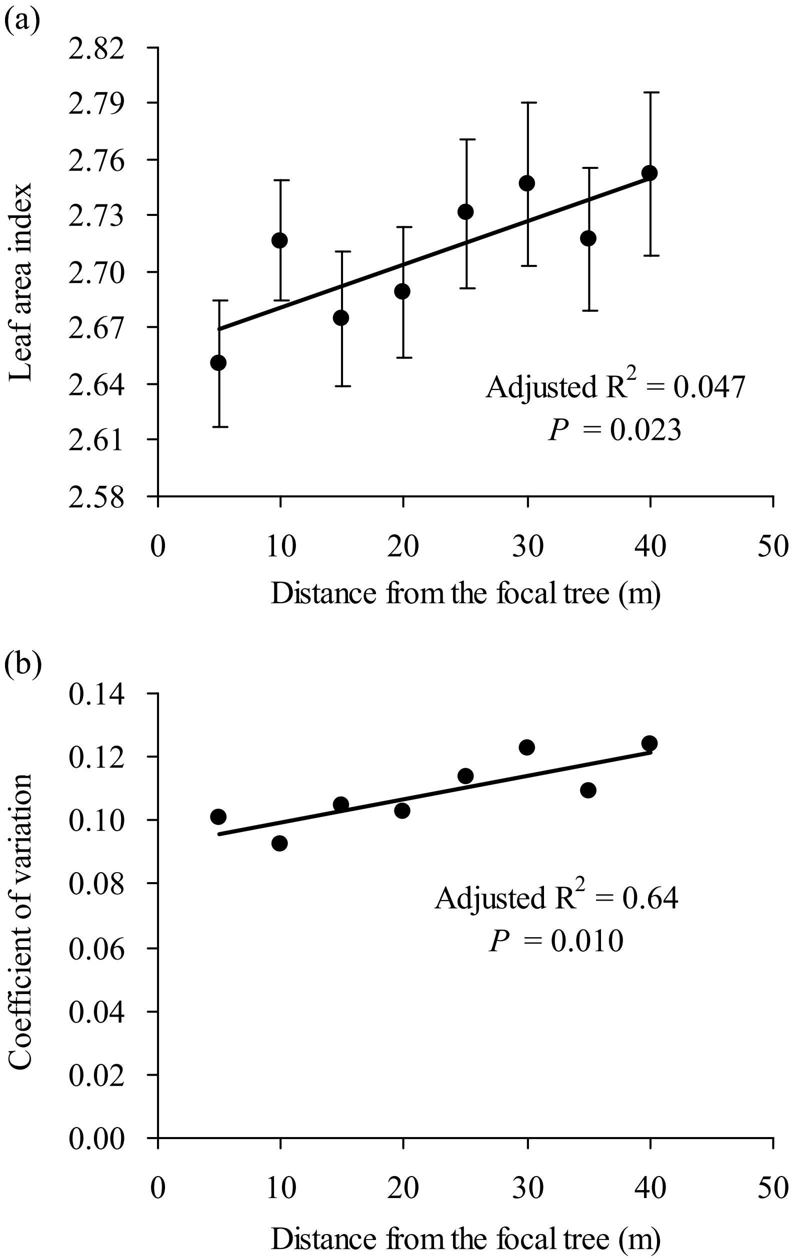

Figure 5.7
Light environment and distance from the focal tree for all the species collective. (a) Leaf area index (LAI) and (b) coefficient of variation of LAI.

CHAPTER 6

EXPERIMENTAL TEST OF THE JANZEN-CONNELL HYPOTHESIS: RELATIVE IMPORTANCE OF DISTANCE, FOREST FRAGMENTATION, MATERNAL ORIGIN, AND ENVIRONMENT[5]

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[5] Sugiyama, A. and Peterson, C. J. Submitted to *Plant Ecology*, 04/21/2012.

6.1. Abstract

Pioneer fig species play an important role in tropical forests but forest fragmentation may threaten their persistence. Species regeneration is affected by various factors such as maternal origin, environment, initial plant size, inherent growth rate, and distance from the maternal tree, as expected from the Janzen-Connell (J-C) hypothesis. However, how such factors may be altered in fragmented forests is unknown. Using an animal-dispersed, pioneer tree *Ficus tonduzii* (Moraceae), the J-C hypothesis was tested in premontane wet forest fragments in Costa Rica by conducting transplant experiments. The goals of this study were to: 1) assess forest fragmentation effects on seedling survivorship and growth, 2) test whether forest fragmentation affects the J-C effects, and 3) identify the relative importance of different factors that determine the performance of the transplants. Seedlings from different maternal origins were grown *ex situ* before being transplanted at two distance classes from maternal trees in different-sized forest fragments, to both maternal trees (home) and non-maternal reproductives (away). As a result, only environment affected both seedling survivorship and growth. Such environmental effects were likely a result of forest fragmentation. In contrast, initial plant size, inherent growth rate, home vs. away categories, and the J-C effect did not affect seedling survivorship or growth, although inherent growth rates recorded *ex situ* were also affected by maternal origin. Results here emphasize the importance of considering both environment and maternal origin at various growth stages for successful restoration practices, and suggest that the J-C effect may be less likely for pioneer species.

6.2. Introduction

Tropical forests support high species diversity and ecosystem functioning but are threatened by forest fragmentation globally (Gascon *et al.*, 2000). Forest fragmentation may cause various negative impacts on species regeneration (Laurance *et al.*, 2002) but such impacts are not equivalent across species. While shade-tolerant species decline in relative abundance in small fragments, pioneer species often increase rapidly mediated by edge effects (Laurance *et al.*, 2006b). Such rapid growth of pioneer species, as a response to forest fragmentation, are known to play important roles in conservation by mitigating genetic erosion (Mathiasen *et al.*, 2007), supporting epiphytic bryophytes (Vanderpoorten *et al.*, 2004), and supporting various ecosystem functioning, such as maintaining pollinators (Samejima *et al.*, 2004). Many rare species are pioneer species in the tropics (Welden *et al.*, 1991) and structural and compositional conditions of such early-successional forests may form unique habitats utilized by many wildlife in some ecosystems (Brooks, 2003). However, pioneer species, particularly animal-dispersed species, may be reduced in forest fragments as a result of dispersal limitation (Cordeiro *et al.*, 2009) due to local extinction of dispersers in fragmented forests (Terborgh *et al.*, 2008). Additionally, seedlings of pioneer species may actually be scarce in some disturbed habitats (Sanchez-Gallen *et al.*, 2010). Thus, the fact that a species is a pioneer does not guarantee its persistence in forest fragments, especially when it is an animal-dispersed tree species (Benitez-Malvido and Martinez-Ramos, 2003; Terborgh *et al.*, 2008).

When plant regeneration is considered in the context of forest fragmentation, various factors affect individual survivorship. Early growth stages are of special concern because they act as bottlenecks for species regeneration. One of the major consequences of forest fragmentation is edge effects (Laurance, 2000). Near forest edges, abiotic environments, such as temperature, humidity, and light intensity (Kapos, 1989) change drastically compared to those in forest interior. Increased light availability in small fragments may enhance survivorship and growth rate, particularly for pioneer species (Wright *et al.*, 2010). Forest fragmentation may also affect maternal trees. Reduced outcrossing rates can lead to reduced germinability of seeds produced in fragments or by isolated trees (Cascante *et al.*, 2002). Conversely, enhanced outcrossing (Mathiasen *et al.*, 2007) and abiotic environment in small fragments may result in higher germinability of seeds produced in small fragments (Chapter 3). Thus, both abiotic environment *per se* and environment-mediated effects on maternal trees may be important in determining progeny performance in forest fragments.

Survivorship of early life-history stages, such as seedlings, may also be affected by factors independent of forest fragmentation. Both in naturally regenerating seedlings (Gilbert *et al.*, 2001) and transplants (Benitez-Malvido *et al.*, 2005), seedling size may affect seedling performance and regeneration processes. As expected by the Janzen-Connell (J-C) hypothesis (Janzen, 1970; Connell, 1971), distance from the maternal tree or density of conspecific or related individuals can also affect individual survivorship (Liu *et al.*, 2012). The J-C hypothesis predicts that mortality will be high close to the maternal tree where seed and seedling densities are high, due to higher seed predation, herbivory, or pathogen infection. It was originally proposed to explain high species coexistence in tropical forests since this distance/density-dependent mortality precludes conspecific offspring from recruiting near the maternal tree. The J-C hypothesis has been tested in many tropical and temperate species with mixed results (Carson *et al.*, 2008). However, tests of the J-C hypothesis on pioneer tree species (only 1.2% of species reviewed in Carson *et al.*, 2008), especially experimental tests, are scarce. Furthermore, tests of the J-C hypothesis are biased towards sites in low elevations (Carson *et al.*,

2008) and it has rarely been tested in the context of forest fragmentation (but see Wright and Duber, 2001). Considering that forest fragmentation is ubiquitous (Gascon *et al.*, 2000), applying insights from a few well-studied low-elevation sites to somewhere else may be misleading since fragmentation effects may differ regionally (Bouroncle and Finegan, 2011) and different processes may act at higher elevation ranges. There is also a bias towards small fragments (< 10 ha) and more studies on medium-sized fragments are needed (Zuidema *et al.*, 1996) because some forest fragmentation impacts may not be a simple function of fragment size (Chapter 3).

To address these issues, experimental tests of the J-C hypothesis were conducted in tropical premontane wet forest fragments using an animal-dispersed, pioneer tree *Ficus tonduzii* Standley (Moraceae). Figs are known as one of the classic examples of keystone species (Lambert and Marshall, 1991) and play an important role in tropical forest ecosystem functioning and services (Harrison, 2005). Previous studies from the same study sites have revealed that the seedling density of *F. tonduzii* is very low in the forest (Chapter 4). Seedlings of *F. tonduzii* did not show the J-C pattern in an observational study (Chapter 5). However, low sample size in the natural setting precluded a rigorous test of the J-C hypothesis while such low density facilitates testing the J-C hypothesis experimentally because conspecific density will not be altered by naturally occurring individuals. While no positive distance-dependent survivorship (Chapter 5) or fragmentation effects were detected on individual establishment (Chapter 4) due to low recruitment of naturally occurring seedlings, positive edge effects were detected for germination ability of seeds collected from trees in small fragments (Chapter 3). Thus, while germination environment in small fragments may not be suitable (Bruna, 1999) for recruitment of *F. tonduzii*, seedlings originating from small fragments may have high probability of success as transplants once they are germinated *ex situ*. Three objectives of this study were to: 1) assess forest fragmentation effects on seedling survivorship and growth, 2) test whether forest fragmentation affects the J-C effects, and 3) identify the relative importance of different factors that determine the performance of the transplant. Seedlings from known maternal trees were transplanted at different distances around both maternal trees where seeds were collected (home) and non-maternal conspecific reproductive tree (away). Seeds were originally germinated and grown *ex situ* for about a year under the same environment before being transplanted. Thus, effects of maternal origin of the seedling, initial seedling size, and inherent growth rate on seedling performance could be considered together. With such background information on each seedling, the relative importance of maternal origin, forest fragmentation, the J-C effect, and environment on seedling performance was tested.

6.3. Methods

Study sites

This study was conducted in three primary forest fragments in Coto Brus county, southern Costa Rica (Fig. 6.1). The area is situated in a highly deforested landscape, such that the forest reserve at Las Cruces Biological Station (LCBS; 8° 47'N, 82° 58'W) of the Organization for Tropical Studies (OTS) is the largest remaining primary forest fragment (209 ha) in the immediate area. Overall forest cover within a 15 km radius of the station is estimated at 27% (Daily *et al.*, 2001). Evidence from aerial photographs show that LCBS forest was finally isolated as a fragment around 1978 to 1979, with partial isolation beginning in the 1960s (R. Zahawi, *personal communication*). Natural vegetation in the area is classified as tropical premontane wet forest (Holdridge *et al.*, 1971) with elevation ranging between 1,000 and 1,400 m. Temperature ranges between 14 and 29°C (mean: *ca.* 20°C) with a distinct dry season from

December through March. Annual precipitation was 4,223 mm and 5,011 mm in 2008 and 2010, respectively, while 2009 (El Niño year) was an unusually dry year with annual precipitation of 3,063 mm, which negatively affected fruit production of species at the community level during the early wet season of 2009 and 2010 (A. Sugiyama, *personal observation*). In contrast, 2011 was a La Niña year with wet dry season and dry wet season. Similar to LCBS, the smaller fragments AGZ (33 ha) and PC (4 ha) have not had recent major disturbances or newly created forest edges.

Comparisons between continuous or large forest and small fragments are common (e.g., Bruna, 1999; Cascante *et al.*, 2002). Here, site comparison was conducted between the largest fragment LCBS and the smaller fragments (SF; combined two smaller fragments AGZ and PC), due to the distinct division in fragment sizes. These categories will be referred to as "site groups" (i.e., LCBS vs. SF) hereafter. Such comparison facilitated balancing the sample size between sites in such a drought year.

Focal species

Ficus tonduzii is a long-lived pioneer tree (Chapter 4) pollinated by small fig wasps (Agaonidae). It produces fleshy fruits with many small seeds all year round but the same individual trees do not produce fruits annually. Fruits are dispersed by rodents, monkeys, bats, and birds (Chapter 4) but their relative abundances are unknown for each forest fragment (F. Oviedo, *personal communication*). While seeds quickly germinate under full sun, few recruits are observed in the forest floor even around maternal trees where the highest density is expected (Chapter 4).

Three trees that produced fruits in 2009 were randomly selected as focal trees in each site group (n = 3 trees × 2 site groups). Focal trees were at least 150 m away from each other to minimize overlapping seed shadows, and none of the focal trees produced fruits in 2008, 2010, or 2011. For each focal tree, two transects (5 × 40 m), radiating in random directions from the base, were established. Distance from the nearest forest edge, dbh, crown size above each transect, mean fruit size produced, and germination rates (when available, Chapter 3) were recorded for each focal tree.

Seedling preparation

In June 2009, germination tests were conducted in a screen house at LCBS with natural irradiation and day lengths. Fruits for germination tests were collected from five different maternal trees (LCBS: n = 2, SF: n = 3). To minimize seed-shadow overlap, focal trees of at least 500 m apart from other reproductive conspecifics were randomly chosen in the primary forest area of each fragment. Fruits were sown intact the day of collection in sterilized soil to avoid fungal infection from the soil or potential microbial effect on germination or early mortality of germinants (e.g., Bever *et al.*, 2010). Since none of the seeds collected from trees in LCBS germinated, only seedlings from non-LCBS trees were used.

At the end of the field season in August 2009, all germinated seedlings were transplanted to a larger planting space within the same screen house using the same sterilized soil. In January 2010, those seedlings were tagged and transplanted to individual plastic bags with drainage holes to allow further growth. A total of 315 seedlings from three maternal trees were obtained from germination tests. Before the transplant experiments, basal diameter and height were recorded. All the seedlings were first ordered by basal diameter and height and six largest and 21 smallest individuals were excluded from use in the experiment. To prepare equally distributed seedling sizes for each batch, seedlings were then alternatively assigned in the order of seedling size to site groups, then to distance class (see below), and then to transect. As a result, there was no bias in seedling sizes among batches (basal diameter: $F_{23,264} = 0.19$, $P = 1.00$; height: $F_{23,264} = 0.48$, $P = 0.98$).

Transplant experiment

To experimentally test the J-C hypothesis in forest fragments, seedlings grown under the same environment for about a year were transplanted to two distance classes (5 m and 20 m) from three focal trees in each site group (n = 12 individuals × 2 distance classes × 2 transects × 3 focal trees × 2 site groups) in early June 2010 (Fig. 6.2). Where these seedlings were transplanted, there were no naturally regenerating individuals, and thus, density was equal among batches. After two weeks, all the seedlings were revisited and it was confirmed that transplanting itself did not kill them. The following wet season in 2011, all the focal trees were revisited to record seedling survivorship and growth rates. Growth rate was calculated as $(g_t - g_{t-1})/(d_t - d_{t-1})$ where g_t is growth (basal diameter or height) at time t and d_t is growing days at time t when the measurement took place. The seedlings grown under full sun in the screen house were expected to reflect inherent growth rate. Since each transplant had been tagged, growth rate in the screen house and the forest could be compared directly.

Light availability assessment

Canopy openness was determined for each batch to assess whether light availability affects survivorship and growth of individuals. Hemispherical photos were taken with a leveled fisheye converter (Nikon FC-E8 0.21×) mounted on a Nikon Coolpix 885 camera (Nikon Corporation, Tokyo, Japan). Under overcast conditions, photographs were taken at 1m height above each batch (n = 2 distance classes × 2 transects × 3 trees × 2 site groups) between the end of April and early June 2011. Canopy openness was calculated using Gap Light Analyzer (GLA) 2.0 (Frazer *et al.*, 1999). Since automatic thresholding of pixels is not possible on GLA, binarization of photographs was conducted using automatic thresholding function on SideLook 1.1 (Nobis, 2005) prior to image analyses on GLA.

Statistical analyses

To test whether survivorship differs among maternal trees, distance from the tree, and site group to which they were transplanted (all fixed effects), a generalized linear mixed model (binomial distribution and logit link) was used, where focal trees were nested within site group as a random effect. Similarly, difference in growth rates (basal diameter and height), both in the screen house (inherent growth rate) and in the forest, were assessed with Gaussian distribution and identity link. For differences among fixed effects, the Tukey-Kramer adjustment was conducted for multiple comparisons. To identify the factors that affect survival and growth rate of transplants, stepwise model selections were conducted (entry and stay levels: 0.05). Variables that reflect the maternal origin, forest fragmentation effect, environment, the J-C effect, and seedling size were included in the models (Fig. 6.2). Since basal diameter and height of the seedlings showed similar performance, only seedling basal diameter was considered as predictor variable for model selections for growth rate and initial size. To assess the effect of maternal origin for survivorship and growth, maximum crown size (maternal identity), fragment size (site identity), mean germination rate of seeds, and inherent basal diameter growth rate in the screen house were included in the models as predictor variables. Distance from the edge for focal tree to which seedlings were transplanted was included for assessing effects of forest fragmentation. Environmental effects considered were crown size of the focal tree and canopy openness. Distance from the tree to which seedlings were transplanted was included for considering the J-C effect, and initial seedling basal diameter was also included as a predictor. Finally, for two maternal trees which had sufficient seedlings (FT2 and FT4), whether being transplanted to its maternal tree (e.g., FT2 seedlings to FT2 tree) or to another focal tree (i.e., home vs. away) affects survivorship was tested by G-tests. All analyses were performed on SAS 9.2 software (SAS Institute Inc., 2009). When necessary, data were log- or angular-transformed prior to analyses and batch was treated as experimental unit.

6.4. Results

Survivorship of transplanted seedlings

In predicting transplant survivorship, maternal origin, fragmentation effect, environmental effects, initial plant size, and the J-C effect were considered in model selections. However, only one of the environmental effects, namely, the focal tree where 1-yr seedlings were transplanted was significant ($F_{1,59} = 11.1$, $P = 0.0015$) in predicting seedling survivorship. When survivorship of transplants was compared among trees where they were transplanted, their survivorship was significantly lower when transplanted to FT4 than those transplanted elsewhere, independent of distance class (Fig. 6.3; $F_{5,282} = 5.7$, $P < 0.0001$). Thus, survivorship of transplants did not differ by maternal origin, fragmentation effects (distance from the forest edge and site group transplanted to) or the J-C effects (distance from the maternal tree). Similarly, because seedlings suffered significantly low survivorship only when they were from FT4 and also transplanted to FT4 ($F_{1,286} = 12.9$, $P = 0.0004$), seedling survivorship was not associated with general home or away categories ($G = 0.91$, df = 1, $P = 0.34$).

Growth rate of seedlings in the forest and screen house

Based on model selection, the tree to which 1-yr seedlings were transplanted ($F_{1,54} = 8.3$, $P = 0.0057$) was the only variable left as significant in predicting basal diameter growth rates of the transplant, which was lowest when transplanted to FT4 (Fig. 6.4; $F_{5,222} = 4.9$, $P = 0.0003$). In contrast, no variables were significant in predicting growth of seedling height. Thus, as for survivorship of transplants, growth rates of transplants did not differ by maternal origin (Table 6.1), fragmentation effects or the J-C effects.

In contrast to the results from seedling growth rates in the field, inherent growth rates recorded in the screen house were also affected by maternal origin for both basal diameter and height (Table 6.1). Seedlings originating from FT4 had lower inherent growth rates than from other maternal trees (basal diameter: $F_{2,285} = 12.2$, $P < 0.0001$; height: $F_{2,285} = 4.8$, $P = 0.0094$). Thus, seedlings transplanted to FT4 suffered not only lower survivorship but also lower growth rates, and even had lower inherent growth.

6.5. Discussion

Basic understanding of how the J-C effect may be altered as a result of forest fragmentation, and identifying factors that are critical for survivorship and growth of seedlings in rapidly proliferating forest fragments are important for successful conservation efforts. This study assessed the relative importance of maternal origin, forest fragmentation, environment, and the J-C effects by experimentally transplanting seedlings of known origin grown under the same environment for about a year. Two major findings from this study are: 1) environment was the only important variable for seedling survivorship and seedling growth, and 2) there was no evidence for the J-C effect.

Among different variables included in model selections, the only significant variable was identity of the tree to which seedlings were transplanted (environment). Seedlings transplanted to FT4 had significantly lower survivorship (Fig. 6.3), independent of maternal origin, the distance from the focal tree (the J-C effect), initial seedling size, or inherent growth rate. Similarly, growth rates of seedlings transplanted to FT4 were significantly lower than those transplanted elsewhere (Fig. 6.4). Furthermore, inherent growth rates in the screen house for seedlings from FT4 were significantly lower than those from different maternal origin, although growth rates of transplants in the forest originating from FT4 were overall comparable to those from different maternal origins (Table 6.1). Thus, while neighbors of different genetic makeup can affect individual performance (Augspurger and Kitajima, 1992 and references therein) and beneficial mycorrhizae associated with specific tree or site may not exist away from its maternal origin (Allen *et al.*, 2005), survivorship was, in general, not affected by home vs. away categories in this study. The lack of home-advantage in this study may be a result of seedlings being germinated and grown *ex situ* under the same environment before being transplanted. In fact, while growth rates of seedlings from FT4 were inherently lower, they did not have such disadvantage in growth if transplanted to a non-home tree.

Negative maternal and environmental effects detected for FT4 may result from FT4 being the only tree from the smallest forest fragment (PC). However, edge effects were not specifically detected based on the results from model selection, presumably due to non-linear survivorship of seedlings transplanted to focal trees at different distances from edges. Two transects for FT4 were very close to the forest edge (< 50 m). Edge effects often act up to 150 m from the edge (Laurance, 2000) and even for established seedlings of a pioneer species, those transplanted around FT4 might have experienced high mortality due to drought (Pearson *et al.*, 2003). Yet, since FT4 was the only reproductive *F. tonduzii* individual within PC, tree and site or fragment size could not be separated. This issue highlights the difficulty of finding sufficient sample size in small fragments (Zuidema *et al.*, 1996) or having multiple forest fragments of similar sizes at a landscape scale without altering the traits associated with fragments, such as elevation and species composition.

While environment, potentially a forest fragmentation effect (identity of tree to which seedlings were transplanted), was important for seedling survivorship, light availability was not significant in predicting survivorship or growth of the transplants. For a pioneer tree, light difference among batches (6.7-17.4%) might have been too small to detect any effect since none of the locations where seedlings were transplanted were in light gaps. Similarly, initial size did not affect survivorship or growth of the transplants. While one might expect large individuals have better chance of survival (Gilbert *et al.*, 2001), it can be species-dependent (Benitez-Malvido *et al.*, 2005).

There was no support for the J-C hypothesis for either survival (Fig. 6.3) or growth (Fig. 6.4), although such results are fairly common (Carson *et al.*, 2008). Thus, forest fragmentation did not influence the J-C effects in this study. Such a result is consistent with a previous observational study on naturally occurring individuals of *F. tonduzii*, which was due to overall very low seedling density in the forest (Chapter 5) since seeds of many pioneer species stay dormant in the soil as a seed bank until canopy gaps are created (Swaine and Whitmore, 1988). If density is more important than distance, density of seedlings transplanted at two distance classes from trees might have been too low in this study to elicit response from enemies. The J-C effects may also be restricted to small size classes. Since 1-yr seedlings used as transplants were well-developed after growing in a full sun environment, they might have been too large to be influenced by processes that are responsible for the J-C effects. Such use of well-established seedlings resulted in overall high survivorship (84.7 ± 13.8% SD; Fig. 6.3) for transplanted 1-yr seedlings in the forest (Chapter 4). Mortality of transplants was largely due to physical damage such as falling branches (A. Sugiyama, *personal observation*) while major causes of mortality for naturally occurring seedlings, presumably less developed, are known to be herbivory, drought, and fungal attack (Moles and Westoby, 2004b). Stage-specific support for the J-C hypothesis is also reported elsewhere (Connell *et al.*, 1984; Silva Matos *et al.*, 1999; Gilbert *et al.*, 2001), which is not restricted to pioneer species. All four non-pioneer species that showed positive distance-dependent survivorship in these study sites (i.e., LCBS, AGZ, PC) were also restricted to individuals in the smallest size class but were all less developed than the 1-yr seedlings used here (Chapter 5).

Based on the results here, both biological and conservational implications arise. First, while there was no direct evidence for the J-C hypothesis even for experimentally transplanted seedlings, the results here are still concordant with its implications on high species diversity in tropical forests. This is because whatever the processes, seedlings of *F. tonduzii* occurred in very low density a year after fruiting (Chapter 4) although seed input was high close to the maternal trees as for many other species (A. Sugiyama, *personal observation*). Thus, while there was no positive distance-dependent or negative density-dependent survivorship, offspring of *F. tonduzii* did not occupy the space around the maternal tree after seed dispersal. Since tests of the J-C hypothesis specifically using pioneer species are still scarce (Carson *et al.*, 2008), more studies are needed to assess whether species with such regeneration patterns or life histories generally show no support for the J-C hypothesis. Still, creation of light gaps plays an important role in determining seedling recruitment patterns, which may involve mechanisms proposed by the J-C hypothesis (Augspurger, 1983a). Additionally, results here reinforce the importance of seed dispersal, not necessarily for the reasons postulated by the J-C hypothesis. While density-dependent mortality or intraspecific competition (Moles and Westoby, 2004b) may be rare for *F. tonduzii* with such low density, germination of pioneer species depend on light gaps (Swaine and Whitmore, 1988). Thus, dispersal away from the maternal tree can increase the chance to land in an (eventually) suitable environment as proposed by the colonization hypothesis (Howe and Smallwood, 1982).

In conservation, results here revealed potentially complex forest fragmentation effects on both maternal trees and the environment. Survivorship and growth of transplants were primarily affected by environmental factors, which were likely a result of forest fragmentation. Earlier in regeneration, inherent growth rate was affected by maternal origin. Thus, while carryover of maternal effect on seedling performance was observed (Baskin and Baskin, 1973), environmental factors were relatively more important for later stages or in the forest for *F. tonduzii*. Although post-germination stages were all negatively affected potentially by forest fragmentation, positive fragmentation-mediated maternal effects were detected for *ex situ* germination of *F. tonduzii*, where germination was consistently greater for seeds collected from SF than LCBS (Chapter 3). Thus, as a whole, better *ex situ* germination did not guarantee successful *in situ* seedling survivorship in small fragments. Forest fragmentation diminishes environmental quality for seed germination (Bruna, 1999) and where they are transplanted was an important consideration, even using well-developed seedlings. Alternatively, while seeds collected from LCBS did not germinate (Chapter 3), LCBS could serve as suitable environment for survival of transplants (Fig. 6.3). Therefore, both maternal origin and environmental effects on different stages are important considerations. For restoration, environment, such as vegetation cover and type, and strategies to enhance recovery have been an active topic (Holl *et al.*, 2000) but stage-specific maternal effects have been given less attention outside purely biological studies. While *F. tonduzii* is a pioneer tree, which is expected to survive poorly under the forest canopy (Swaine and Whitmore, 1988), well-developed 1-yr seedlings overall maintained relatively high survivorship. Thus, for *F. tonduzii*, a combination of seed collection from small fragments (Chapter 3), *ex situ* germination, and transplanting in suitable environments seem promising for restoration. The next step forward is to identify the factors determining suitable environments in the forest and applying insights obtained here to sites outside the forest where actual restoration practices likely take place for *F. tonduzii* and species with similar life history traits.

6.6. Tables

Table 6.1
Mean size and growth rates of seedlings used for transplant experiments shown by different maternal origin (site). Seedling sizes and growth rates are shown for both screen house (inherent) and forest conditions (field). Inherent growth rates is growth since germination in the screen house recorded in 2010, and growth rates in the field is based on the measurements in the forest in 2011. Thus, size for inherent and field conditions show initial and final seedling size, respectively. Different superscript letters show difference among different maternal trees for each condition and column at α = 0.05. Note that significance shown by some letters did not match the order of seedling size or growth rates due to small sample size for FT9. There was no difference in final seedling size and field growth rate by maternal origin. Errors are SD and N indicates sample size.

Site	Maternal origin	Condition	Basal diameter		Height		N
			Size (mm)	Growth (mm/day)	Size (cm)	Growth (cm/day)	
AGZ	FT2	Inherent	$4.6^{a} \pm 1.1$	$0.014^{a} \pm 0.003$	$15.2^{a} \pm 5.2$	$0.048^{a} \pm 0.015$	125
		Field	$5.2^{a} \pm 1.2$	$0.0017^{a} \pm 0.0021$	$21.1^{a} \pm 7.3$	$0.015^{a} \pm 0.014$	113
PC	FT4	Inherent	$3.9^{b} \pm 1.1$	$0.012^{b} \pm 0.003$	$13.6^{b} \pm 4.5$	$0.043^{b} \pm 0.014$	150
		Field	$4.8^{a} \pm 1.2$	$0.0022^{a} \pm 0.0021$	$19.6^{a} \pm 6.3$	$0.014^{a} \pm 0.011$	120
CED	FT9	Inherent	$4.6^{a} \pm 0.8$	$0.015^{a} \pm 0.014$	$12.7^{ab} \pm 2.1$	$0.041^{ab} \pm 0.007$	13
		Field	$4.9^{a} \pm 1.1$	$0.0018^{a} \pm 0.0014$	$18.3^{a} \pm 5.2$	$0.014^{a} \pm 0.012$	11

6.7. Figures

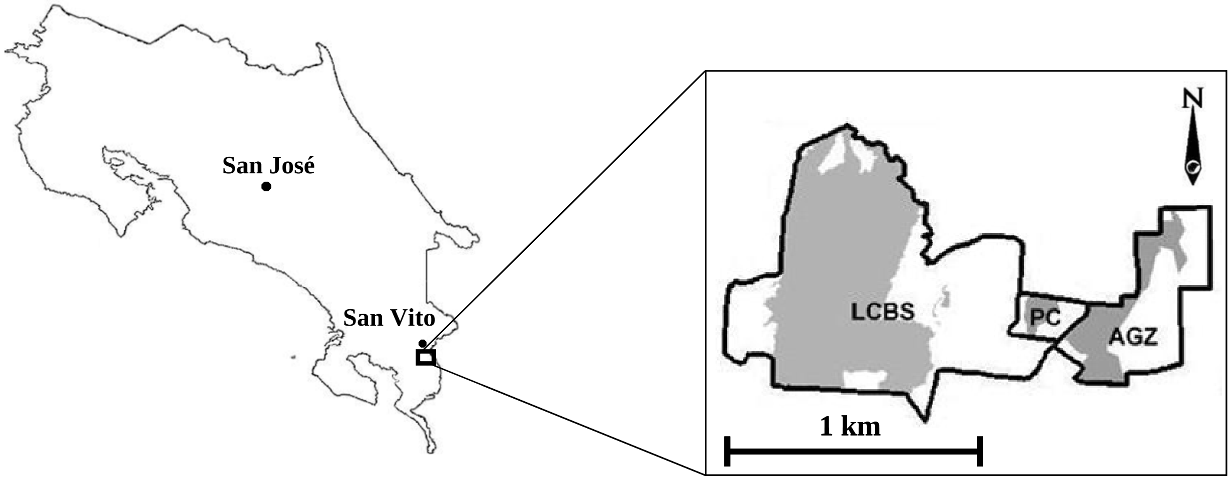

Figure 6.1
Map of the study region and configuration of three forest fragments. The closest town to Las Cruces Biological Station (LCBS) is San Vito. Configuration of primary forest covers where the focal trees were located are shown in gray coloring within each site. Primary forests of the three forest fragments, LCBS, PC, and AGZ are distinctly isolated by roads, pastures, and garden areas.

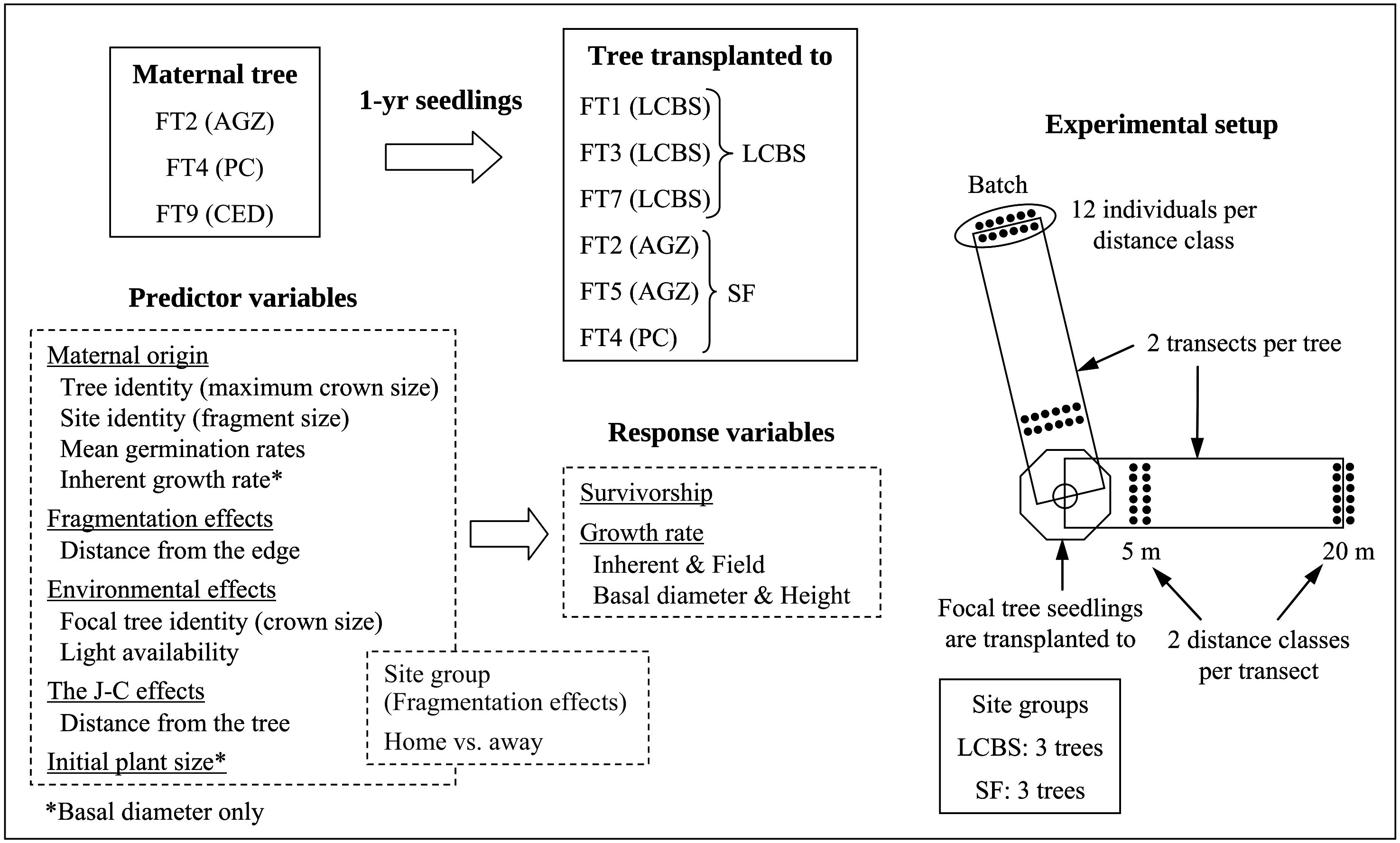

Figure 6.2
Conceptual and experimental setup of this study. The left side shows trees used as maternal trees and to which seedlings were transplanted, and predictor and response variables. The right side shows a schematic example of the experimental setup for two batches in a given tree to which seedlings were transplanted.

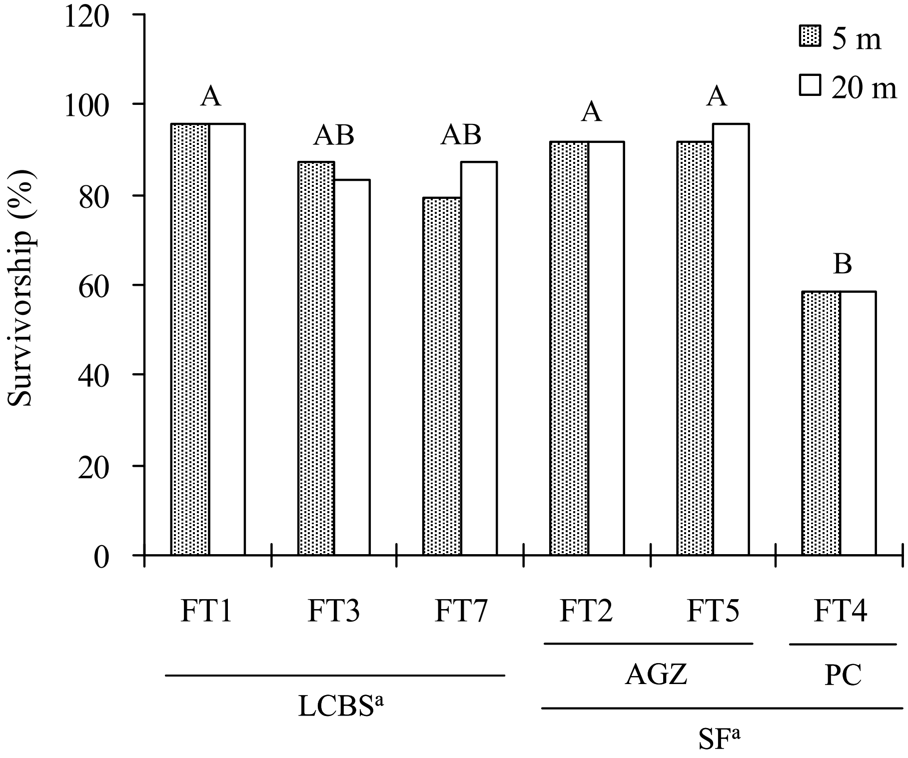

Figure 6.3
Survivorship of the transplants among different focal trees to which they were transplanted, distance from the tree, and site groups. Filled and open bars show 5 m and 20 m, respectively. Different letters above both bars show difference in survivorship among trees to which seedlings were transplanted at $\alpha = 0.01$ (adjusted *P*). Horizontal bars below focal trees show trees from each site and site group. There was no significant difference in transplant survivorship between site groups to which they were transplanted at $\alpha = 0.05$.

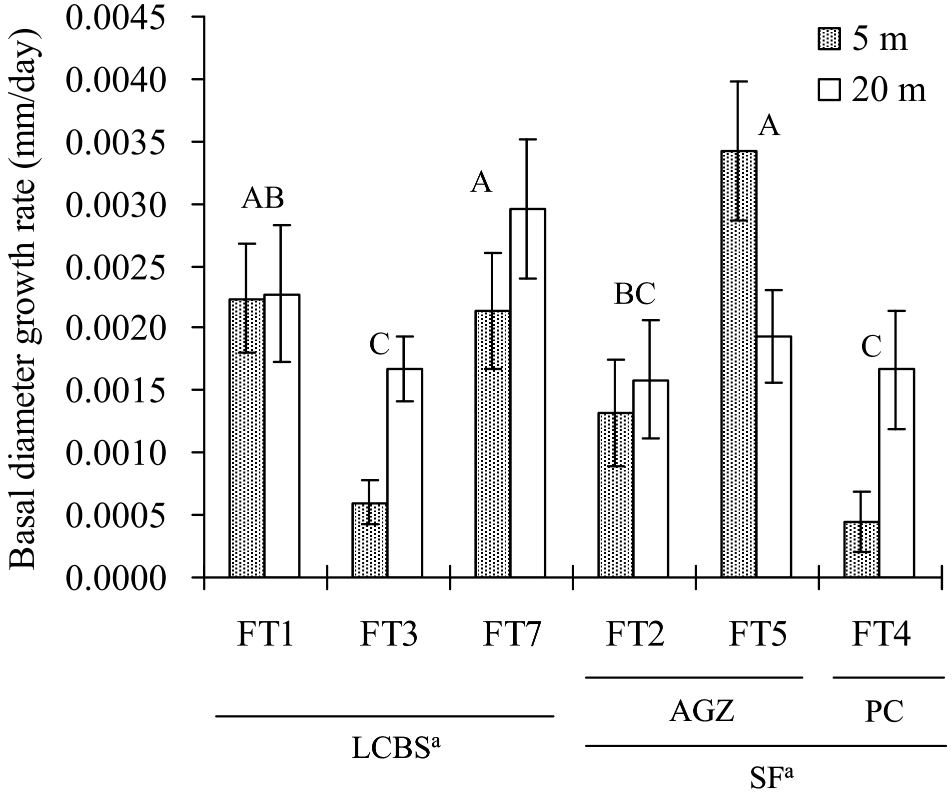

Figure 6.4
Basal diameter growth rate of transplants in the forest among different focal trees to which they were transplanted, distance from the tree, and site groups. Filled and open bars show 5 m and 20 m, respectively. Different letters above both bars show difference in survivorship among trees to which seedlings were transplanted at α = 0.05 (adjusted *P*). There was no significant difference in transplant growth rate between site groups to which they were transplanted for both basal diameter and height at α = 0.05.

CHAPTER 7

CONCLUSION

Ecosystem functioning and immense numbers of species are supported by rapidly disappearing tropical forests around the world. Since forest fragmentation is a fairly recent but drastic phenomenon compared to the long time scales of species regeneration of many long-lived tropical trees, typically spanning over several to many decades, there is an urgent need for assessing various aspects of the consequences of forest fragmentation. Yet, there is a major bias in existing studies towards few well-studied sites (Laurance *et al.*, 2002; Laurance *et al.*, 2011) and lowland forests (Turner and Corlett, 1996b; Carson *et al.*, 2008) while forest fragmentation is a ubiquitous phenomenon (Gascon *et al.*, 2000; Riitters *et al.*, 2000). My dissertation work was conducted in underrepresented tropical premontane wet forests and included a size class of fragments that are generally underrepresented, i.e., medium-sized forest fragment (Turner and Corlett, 1996b; Zuidema *et al.*, 1996; Debinski and Holt, 2000). Among different functional groups, animal-dispersed trees constitute major part of tropical forests (Howe and Smallwood, 1982) yet are one of the groups most threatened by forest fragmentation (Tabarelli *et al.*, 1999; Metzger, 2000; Benitez-Malvido and Martinez-Ramos, 2003; Terborgh *et al.*, 2008). Thus, I assessed consequences of forest fragmentation and species regeneration using five animal-dispersed tree species as focal species, with particular focus on considering various life- and growth-stages. Additionally, throughout my dissertation, bridging insights from my dissertation between both basic and applied science was my underlying goal. In chapters where the study was more applied, broader insights for basic importance were sought, and *vice versa* for studies with more emphasis on the theoretical side of ecological understanding.

One of the key findings from my dissertation research is the importance of considering various stages of regeneration. Since early stages can act as bottlenecks for species regeneration, they are certainly important but forest fragmentation impacts may be very process- or stage-specific as demonstrated in Chapter 2 and 4. In Chapter 2, I assessed impacts of edge effects on various early stages of regeneration of *T. mexicana*. Although *T. mexicana* is a currently common species, it is animal-dispersed and shade-tolerant canopy tree, that are traits expected to be negatively affected by forest fragmentation (Benitez-Malvido, 1998; Benitez-Malvido and Martinez-Ramos, 2003; Laurance *et al.*, 2006a; Terborgh *et al.*, 2008). However, negative impacts of forest fragmentation on its regeneration occurred at very specific times and on specific processes, i.e., between pre-dispersal stage and a year after germination. Later stages evaluated in Chapter 4 also confirmed those results. Thus, if only later stages are considered, negative consequences of edge effects would be entirely missed and might mistakenly lead to a conclusion that there are no negative consequences. This is especially true for large adults because they might have existed before forest fragmentation occurred and may not reflect current conservation status or expected future trends. Such stage-specificity was also evident in forest fragmentation-mediated maternal effects as demonstrated in Chapter 3 and 6. Impacts of forest fragmentation, both positive and negative, on maternal effects assessed *ex situ* were different for germination and subsequent seedling growth. Thus, forest fragmentation effects were more complex than what could be observed in the field. Results from Chapter 3 and 6 also underlined the importance of considering potentially substantial inter-individual variation in maternal effects. All of these results have important implications on conservation or restoration practices because which stage to employ for such practices is an important consideration for fruitful outcomes. For example, use of seeds for restoration of degraded pasture will not be successful for *T. mexicana* while larger seedlings may be, as demonstrated in Chapter 2 and 4. Therefore, consideration of a broad array of different life-stages and processes is indeed important for a comprehensive understanding of complex consequences of forest fragmentation.

Similarly, my results highlighted the importance of considering a wide spectrum of individual sizes for both biological and conservational understanding. Ontogenetic shifts (Clark and Clark, 1992; Dalling *et al.*, 2001; Wright *et al.*, 2003; Niinemets, 2006), which have been recognized for tropical trees, may be more common than had been previously thought. Assessments of forest fragmentation impacts at the level of functional groups typically employ classification based on one growth stage or based on another species within a genus (Primack and Lee, 1991; Davies *et al.*, 1998; Slik, 2005; Chazdon *et al.*, 2010; Kirika *et al.*, 2010) but that may not always be correct. Thus, either long-term studies or consideration of a wide spectrum of individual sizes is essential for correctly evaluating the impacts of forest fragmentation although the vast majority of studies considering ontogenetic shifts are limited to purely biological studies. As demonstrated in Chapter 4, effects of forest fragmentation may not be apparent in very early growth stages and may be apparent in later stages or *vice versa*, potentially as a result of ontogenetic shifts. Such change in response to light or temperature may occur at very early stages, as demonstrated in Chapter 2, which are typically not considered in well-studied sites where most long-term data come from. Such well-studied sites are still restricted in regions, elevations, and forest types (Carson *et al.*, 2008). Considering a wide spectrum of individual sizes has also revealed different regeneration niches across life stages (Comita *et al.*, 2007; Kanagaraj *et al.*, 2011), which may lead to the stage- or size-dependence for some of the underling mechanisms that are responsible for species regeneration or dynamics of communities in the forest. In Chapter 5, my results suggested that Janzen-Connell effects (Janzen, 1970; Connell, 1971) may be restricted to early growth stages, such as seeds and seedlings, as tested in most studies (Clark and Clark, 1984; Carson *et al.*, 2008), and also to non-pioneer species. However, tests of the Janzen-Connell hypothesis considering all sizes are very limited (but see Connell *et al.*, 1984; Silva Matos *et al.*, 1999) and using pioneer species are still rare (Carson *et al.*, 2008). Effects of forest fragmentation have been assessed separately for both early (Benitez-Malvido, 1998; Herrera and Garcia, 2010; Uriarte *et al.*, 2010) and later growth stages (Laurance *et al.*, 2000; Nascimento and Laurance, 2004; Oliveira *et al.*, 2008). However, more studies on a broad array of individual sizes are indeed needed for considering both potential effects of forest fragmentation and biological processes which may be stage- or size-dependent.

Another implication from my dissertation work is the importance of considering underrepresented systems. Some of the really important and novel findings from my dissertation might be attributable to the fact that studies have not been conducted in a similar setting. For example, in Chapter 3, I demonstrated a potential case where effects of forest fragmentation may be positive and may not be a simple function of fragment size. Based on the results from previous studies, forest fragmentation effects on germination ability were consistently negative, whether due to maternal effects *ex situ* (Nason and Hamrick, 1997; Rocha and Aguilar, 2001; Cascante *et al.*, 2002; Henriquez, 2004; Valdivia and Simonetti, 2007) or environment where seeds germinate *in situ* (Bruna, 1999, 2002). However, my results were consistently opposite for all the assessed species, and inter-annually. When such results were compared to the few studies considering medium-sized fragments (Mathiasen *et al.*, 2007; Gonzalez-Varo *et al.*, 2010), a possibility that such results may result from comparison between small and medium-sized fragments arose. Whether such results are general trends observed between many small and medium-sized fragments is yet to be tested but such results highlight the importance of studies at a wide range of systems. While contributions from long-term data at well-studied sites are invaluable, more studies on currently underrepresented study systems may reveal underlining causes for apparently contradictory or inconsistent results among studies.

Finally, bridging biological understanding and conservation applications is one of the major themes in studies assessing forest fragmentation and species regeneration in the tropics. A solid scientific foundation is critical for policy makers and conservation practitioners. Findings from Chapters 3 and 6 have direct implications for conservation and restoration based on biological findings for the species and the system. Results from Chapter 2 demonstrated that current population abundance may not guarantee future species persistence although currently common species are typically not of conservation interest. In contrast, general predictions of forest fragmentation effects based on species life histories at one stage may not be valid as suggested from results in Chapter 4. Ontogenetic effects of forest fragmentation may be much more common than had been previously thought and findings from Chapter 4 stress the importance of biological understanding of species for conservational applications. Additionally, the general perception that small forests are of low quality when compared to large continuous forests often led to the conclusion that small forest fragments are not worth conserving (Ghazoul, 1996; Turner and Corlett, 1996a, 1996b). Yet, results from Chapter 3 revealed previously unrecognized potential of small fragments as seed sources for some species, further reinforcing the assertion that small forest fragments should be conserved (Turner and Corlett, 1996b; Tscharntke *et al.*, 2002; Arroyo-Rodriguez *et al.*, 2009). Likewise, small fragments serve as important stepping stones to maintain genetic connectivity across landscapes (Nason and

Hamrick, 1997; Cascante *et al.*, 2002; Gonzalez-Varo *et al.*, 2010; Fuchs and Hamrick, 2011). A number of studies have also shown that small fragments actually maintain considerable species diversity (Obbens *et al.*, 2001; Yates *et al.*, 2007; Higuchi *et al.*, 2008; Gibson *et al.*, 2011). As more forests exist predominantly as small fragments globally (Gascon *et al.*, 2000; Riitters *et al.*, 2000), small fragments are not simply tenuous leftovers detached from large expanses of forest but an important part of our ecosystems in a rapidly changing world (Wright, 2005). In conserving such forests, good understanding of the species residing in those forests and underlining mechanisms for their species regeneration processes are essential, even in highly diverse tropical forests.

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