EFFECTS OF HABITAT FRAGMENTATION ON THE REPRODUCTIVE ECOLOGY AND CONSERVATION GENETICS OF THE ALMENDRO (*Dipteryx panamensis*), A KEYSTONE RAINFOREST TREE

A Dissertation

Presented in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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Graduate School

Centro Agronómico Tropical de Investigación y Enseñanza

by

Thor Hanson

December, 2006

Major Professor (UI): Jo Ellen Force, Ph.D.
Major Professor (CATIE): Bryan Finegan, Ph.D.

AUTHORIZATION TO SUBMIT DISSERTATION

This dissertation of Thor Hanson submitted for the degree of Doctor of Philosophy with a major in Natural Resources and titled “Effects of Habitat Fragmentation on the Reproductive Ecology and Conservation Genetics of the Almendro (*Dipteryx panamensis*), a Keystone Rainforest Tree,” has been reviewed in final form. Permission, as indicated by the signatures and dates given below, is now granted to submit final copies to the College of Graduate Studies at the University of Idaho (UI), and to the Postgraduate School at Centro Agronómico Tropical de Investigación y Enseñanza (CATIE) for approval.

Major Professor ________________________________ Date ________________
Jo Ellen Force (UI)

Co-Advisor ________________________________ Date ________________
Bryan Finegan (CATIE)

Committee Members ________________________________ Date ________________
Nilsa A. Bosque-Pérez (UI)

________________________________ Date ________________
Olle Pellmyr (UI)

________________________________ Date ________________
Lisette Waits (UI)

Department
Administrator (UI) ________________________________ Date ________________
Jo Ellen Force

Discipline’s College
Dean (UI) ________________________________ Date ________________
Steven Daley Laursen

Final Approval and Acceptance by the College of Graduate Studies (UI)

________________________________ Date ________________
Margrit von Braun

And by the Postgraduate School (CATIE)

________________________________ Date ________________
Glenn Galloway

ABSTRACT

Deforestation in the tropics creates a mosaic of forest patches embedded in a human-dominated matrix. Maintaining biodiversity in these fragmented landscapes relies on their ability to sustain ecological processes. This study examined the effects of habitat fragmentation on the reproductive ecology and genetic diversity of *Dipteryx panamensis*, a canopy emergent tree considered a keystone species in the Atlantic lowland forests of Central America. Populations of *D. panamensis* were mapped, sampled and genotyped for nine microsatellite loci in a protected forest, two forest patches and adjacent pastures, and two isolated pasture sites in northern Costa Rica. Additionally, seed dispersal, seed predation and seedling density were assessed along transects placed systematically at the protected forest and fragment sites. Through paternity analysis of progeny arrays we found the shortest mean pollen dispersal distances among protected forest trees, moderate distances for fragment and adjacent pasture populations, and distances of up to 2.3 km in isolated pastures. Pollen moved between pasture and adjacent fragment trees, as well as between fragments across intervening pasture. Seeds also moved from pasture trees into fragments at distances of up to 853.0 m, as determined by genetic analysis of maternal endocarp tissue. The rate of seed dispersal did not vary between protected forest and fragments and there was strong evidence of bat-mediated dispersal at all sites. We found no difference in genetic diversity between adults and progeny in any population, but outcrossing rates decreased for pasture and isolated pasture trees and there were trends towards increased genetic structure and spatial autocorrelation among their progeny. Our results suggest that high rates of gene flow via pollen and seeds may help ameliorate the negative effects of fragmentation for *D.*

panamensis populations, but that pasture and isolated pasture populations are still at risk of long-term genetic erosion. Also, we noted lower seed predation and higher seedling densities in fragments than in protected forest, an altered recruitment pattern with implications for the spacing of future *D. panamensis* generations, as well as the species diversity of forest fragment tree communities.

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This work is dedicated to the memory of Steven J. Brunsfeld, a dedicated scientist, a great mentor, and a true friend.

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DISSERTATION INTRODUCTION

The most pressing questions in the natural sciences occur at the interface between human-dominated and natural systems, where social and political context is as important as any ecological process. It is a landscape that is both literally and figuratively fragmented: patches of native habitat set in a human-dominated matrix and influenced by a disparate patchwork of forces, from ecological to economic. Biodiversity conservation in this context requires input and cooperation from a wide range of scientists, professionals and citizens. In the face of this need for integrated thinking, however, most graduate programs remain highly compartmentalized. Recognizing this, faculty at the University of Idaho and CATIE (Centro Agronómico Tropical de Investigación y Enseñanza) established the Doctoral Program in Biodiversity Conservation and Sustainable Production in Tropical and Temperate Fragmented Landscapes. With funding from the National Science Foundation's IGERT (Integrative Graduate Education and Research Traineeship) Program, the UI/CATIE project created an international community of scientists and students from multiple disciplines, focused on collaboration and integrated research. The work described herein was conducted in this interdisciplinary setting and benefited from continuous interaction with colleagues of differing expertise.

This dissertation is presented in three chapters formatted for journal submission, followed by a collective list of references. The project introduction and conclusions offer an overview of the research context and its implications, as well as directions for future efforts.

An additional journal-formatted paper is included as an appendix. Though different in research focus from the rest of the document, it is united by the theme of habitat fragmentation and represents an important goal of the UI/CATIE program: integrated team research and co-authorship with other doctoral fellows. It should be read as a stand-alone product, however, and is not referenced elsewhere in the dissertation.

CONTEXT

Tropical countries lose an estimated 12 million hectares of forest cover every year (F.A.O. 2000), leaving behind a mosaic of pastures, small farms and forest patches (Meyers 1984). Maintaining biodiversity within these fragmented landscapes presents a major challenge for policy makers, natural resource managers and landowners. Studies of species-level fragmentation effects have often focused on changes in the local abundance of mammal, bird and plant taxa (see Laurance & Bierregaard 1997). Recent innovations in molecular genetic techniques allow new insights in the study of pollen and seed dispersal (reviewed in Wang & Smith 2002). These techniques can be applied to help define patterns of gene flow for plant populations in forested and fragmented habitats (e.g. Aldrich & Hamrick 1998; Aspit *et al.* 2001; Dick *et al.* 2003).

Keystone plant resources are defined as those species exerting a strong influence on other groups of organisms at various trophic levels, particularly through copious fruit production (Nason *et al.* 1998). Their extirpation causes changes in habitat structure and community composition, with the potential for a cascade of local extinctions (Nason et al 1998; Lennartson 2002). Keystone plants in tropical forests are often large, fruiting trees vulnerable to increased windthrow and other effects associated with habitat fragmentation (Nason *et al.* 1998; Peres 2001). Many also have commercial or cultural value, exposing them to logging pressure and other human disturbances. Preserving viable populations of keystone plants is a fundamental aspect of biodiversity conservation in fragmented landscapes.

D. panamensis is a large canopy emergent tree occurring in lowland wet tropical forests of the Caribbean slope, from Colombia to Nicaragua (Flores 1992). Once a characteristic and widespread species at low elevations, *D. panamensis* has suffered from habitat loss through conversion to commercial banana and pineapple production, selective logging, and clearing for pasture and small-scale farming. It is currently listed as an Appendix III species by CITES (UNEP-WCMC 2006). In Costa Rica, remaining populations are concentrated in the northeastern corner of the country from the Río San Juan south to Braulio Carrillo National Park. Though little forest remains undisturbed in this region, many *D. panamensis* individuals were left standing in fragments or pastures due to the extremely dense nature of its wood. Improved saws and milling technology were introduced in the 1980s and remnant trees began rapidly disappearing from the landscape. Recognizing the biological importance of the species, the Costa Rican government placed a temporary moratorium on harvest in 2003, pending an environmental review.

A member of the pea family (Fabaceae; Papilionoideae), *D. panamensis* bears compact, single-seeded drupes during the dry season when relatively few other trees are in fruit. Sixteen species of mammals (Bonaccorso *et al.* 1980) and approximately 100 species of birds (Flores 1992) have been observed visiting the trees and feeding on its fruits and seeds. Suspected pollinators include at least nineteen different bees (Perry & Starrett 1980; Thiele 2002), while the seeds are dispersed by large bats, primates and the scatterhoarding habits of several small mammals (Flores 1992; Forget 1993). Among the associated fauna, the endangered great green macaw (*Ara ambiguus*) has been the subject of considerable conservation attention (Arias & Chassot 2003).

Once ranging from Ecuador to Honduras, the great green macaw has suffered from widespread habitat destruction and capture for the pet trade, and its conservation status was recently changed from globally vulnerable to endangered (IUCN 2006). In Costa Rica, fewer than thirty breeding pairs remain (GGMRCP 2003) and the species is recognized as critically endangered (Ramirez & Benavides 1998). The macaw relies on *D. panamensis* fruits for up to 80% of its diet during the dry season and nests almost exclusively in cavities within mature *D. panamensis* (GGMRCP 2003). Conservation efforts in Costa Rica have focused on an area in the northeast known as the San Juan-La Selva Biological Corridor. The proposed Maquenque National Park would protect core macaw habitat along the border with Nicaragua, while environmental service payments, reforestation and other conservation activities would help maintain habitat connectivity south to the La Selva Biological Reserve and Braulio Carrillo National Park. Maintaining viable populations of *D. panamensis* is recognized as a critical aspect of this effort, and the relationship between the tree and the macaw features heavily in local conservation education efforts.

As a prominent member of the Mesoamerican flora, *D. panamensis* has been a focal species for studies ranging from seedling survival (De Steven & Putz 1984; Clark & Clark 1987; De Steven 1988) to long-term demographic trends (e.g. Clark & Clark 1992, 2001). Significant questions remain, however, about pollen dispersal, seed dispersal, genetic diversity, seed predation and seedling recruitment, and the potential effects of habitat fragmentation on these processes.

OBJECTIVES

This project combines molecular genetics and field observations to describe the reproductive ecology of *D. panamensis* in a fragmented landscape in Canton Sarapiqui, Costa Rica. Patterns of pollen and seed dispersal are documented in protected forest, forest fragments and pastures to reveal the impacts of habitat fragmentation on the mechanisms of gene flow. Analysis of genetic diversity among populations and between parent and offspring generations offers insight into the long-term consequences of altering gene flow processes. Additionally, seed predation and seedling density are examined to better understand the recruitment environment for *D. panamensis* in forest fragments. The project seeks to further our understanding of fragmentation effects on the reproduction and population genetics of rainforest trees, while providing specific information on a species considered crucial to conservation efforts in the region.

CHAPTER ONE: Effects of Forest Fragmentation on Pollen Dispersal and Genetic Diversity of *Dipteryx panamensis,* a Keystone Rainforest Tree

Thor Hanson
Department of Forest Resources
University of Idaho
P.O. Box 441133
Moscow, Idaho 83844-1133
thor@rockisland.com

Steven Brunsfeld
Department of Forest Resources
University of Idaho
P.O. Box 441133
Moscow, Idaho 83844-1133

Bryan Finegan
Departamento de Recursos Naturales y Ambiente
Centro Agronómico Tropical de Investigación y Enseñanza (CATIE)
7170 Turrialba
Costa Rica

Lisette Waits
Department of Fish and Wildlife Resources
P. O. Box 441136
University of Idaho
Moscow, Idaho 83844-1136

ABSTRACT

In the face of widespread tropical deforestation, the conservation of rainforest trees relies increasingly on their ability to maintain reproductive processes in fragmented landscapes. Here we used molecular genetic paternity analysis to determine pollen dispersal distances for the canopy emergent tree *Dipteryx panamensis* in protected forest, forest fragments, pastures adjacent to fragments and isolated pastures. Due to the polyploid nature of the tree, microsatellite data were transformed for analysis using dominant methods, and were also analyzed using the software TETRASAT, a codominant tool recently developed for tetraploids. We found the shortest pollen dispersal distances among protected forest trees, moderate distances for fragment and adjacent pasture populations, and distances of up to 2.3 km in isolated pastures. Outcrossing rates were lower in pasture and isolated pasture populations than in fragments and protected forest, and spatial autocorrelation showed increased scale and magnitude of the correlation coefficient (r_c) for pasture and isolated pasture progeny. Neither dominant or codominant analyses demonstrated a significant difference in genetic diversity between adult and progeny populations, but codominant results indicated a trend towards lower heterozygosity (H_e), and Shannon-Weiner gene diversity (H') in the progeny generations of pasture and isolated pasture trees. Similarly, codominant results suggest increased genetic structure (G_{st}) among progeny populations from pasture and isolated pasture, while no consistent pattern appeared in the equivalent dominant metric (Φ_{pt}). Reproductively dominant individuals were present in all but the isolated pasture populations, and we found high rates of pollen flow from pastures into adjacent fragments. While pollen-mediated gene flow appears to bolster the genetic diversity of fragmented populations, results from pasture and isolated pasture trees suggest a risk of long-term genetic erosion. In addressing the challenges of polyploid analysis, the dominant approach with microsatellites provided enough resolution for paternity assignment and spatial autocorrelation, but TETRASAT appeared to detect more subtle trends in genetic structure and diversity.

KEYWORDS

Costa Rica, *Dipteryx panamensis*, genetic diversity, microsatellites, paternity analysis, polyploidy, pollen dispersal, spatial autocorrelation, TETRASAT

INTRODUCTION

Habitat loss is widely recognized as the greatest threat to global biodiversity (Myers *et al.* 2000; Novacek & Cleland 2001). In tropical forests, home to more than two-thirds of the world's species (Raven 1988), deforestation rates are estimated at 12 million hectares per year (F. A. O. 2000). While large-scale agriculture and development projects can lead to complete habitat conversion, forest clearing often occurs more sporadically, leaving behind a mosaic of pastures, small farms and forest patches (Myers 1984). Maintaining biodiversity within these fragmented landscapes presents a major challenge for policy makers, natural resource managers and landowners.

Keystone plants in tropical forests are often large, fruiting trees vulnerable to increased windthrow, disrupted pollen and see dispersal, and other effects associated with habitat fragmentation (Nason *et al.* 1998; Laurance *et al.* 2000). Many also have commercial value, exposing them to logging pressure and other human disturbances. Keystone trees exhibit a strong influence on other groups of organisms at various trophic levels, particularly through copious fruit production (Nason *et al.* 1998). Their extirpation causes changes in habitat structure and community composition, with the potential for a cascade of local extinctions (Nason *et al.* 1998; Lennartsson 2002). Preserving viable populations of keystone trees is a fundamental aspect of biodiversity conservation in fragmented landscapes, and requires detailed knowledge of their responses to habitat fragmentation.

Innovations in molecular genetics have led to new insights in the study of pollen dispersal and the reproductive ecology of plant populations (reviewed in Nason & Hamrick 1997; Ward *et al.* 2005). By applying these techniques to tropical trees, we can track specific patterns of pollen-mediated gene flow in fragmented landscapes (e.g. Aldrich & Hamrick 1998; White *et al.* 2002) and better understand issues of connectivity, population viability and the long-term genetic consequences of habitat fragmentation (reviewed in Lowe *et al.* 2005). Emerging trends highlight signs of inbreeding (Aldrich & Hamrick 1998; Cascante *et al.* 2002), reduced reproductive output (Hall *et al.* 1996) and decreased progeny vigor (Nason & Hamrick 1997; Cascante *et al.* 2002) in fragmented populations. On the other hand, studies have also shown increased pollen dispersal distances (White *et al.* 2002; Dick *et al.* 2003) and higher seed production (Dick 2001) in fragmented landscapes. Two studies have noted reproductive dominance by a few fecund individuals (Aldrich & Hamrick 1998; Sezen *et al.* 2005), which could lead to genetic bottlenecks in fragmented or regenerating habitats. Also, regeneration in forest fragments has in some cases been dominated by outside sources, either trees in adjacent pasture (Aldrich & Hamrick 1998) or trees in distant fragments and continuous forest (Nason & Hamrick 1997).

In a recent review, Lowe *et al.* (2005) note that few studies of tropical trees have found signs of decreased genetic diversity in fragmented populations, but that such changes could take generations to appear. Questions remain about how widespread the observed trends are in other species and systems, and about the impact of altered pollen flow on the genetic diversity and structure of progeny populations. Also, research to date has been limited to diploid species due to the challenges of analyzing polyploid genetic data. While the ploidy level of most tropical trees remains unknown, polyploids account for more than half of all plant species (Raven *et al.* 1992) and there is need to include them in this growing field of inquiry.

In this study we examined pollen dispersal distances for the canopy emergent tree *Dipteryx panamensis* (Fabaceae), a putative tetraploid and a keystone species in the Atlantic lowland forests of Central America. Though reduced by harvest and forest clearing over much of its range, *D. panamensis* persists in fragments and pastures and remains a conservation priority. It is listed as a CITES Appendix III species (UNEP-WCMC 2006) and was recently designated as vulnerable to extinction in an evaluation of Costa Rican plant species using IUCN Red List criteria (Estrada-Chavarria *et al.* 2005). Additionally, the endangered great green macaw (*Ara ambiguus*) depends on *D. panamensis* as nesting habitat and a critical food source (Juniper & Parr 1998), a relationship that figures prominently in environmental education and conservation efforts throughout the region. Understanding the effects of forest fragmentation on the reproduction and genetic diversity of this species has important management and conservation implications.

D. panamensis adults and progeny were studied in four habitat types: protected forest, forest fragments, pastures adjacent to fragments and pastures isolated from forest by > 1.5 km. The reproductive adults at all sites dated from the continuous, pre-fragmentation forest, while the progeny reflected current, post-fragmentation reproductive conditions. Fragmentation impacts on pollinator communities and the spacing of adult trees were expected to influence pollen dispersal distances and the genetic diversity of progeny. We predicted that the mean pollen dispersal distance would vary among habitat types, increasing in fragmented areas. We expected outcrossing rates to decline in fragmented habitats, particularly for trees in pasture. Additionally, we predicted a decline in genetic diversity and increased genetic structure and spatial autocorrelation for the progeny of highly fragmented populations. We tested for signs of reproductive dominance at the individual and site level, as well as between trees in forest fragments and adjacent pastures. Finally, we explored the utility of both codominant and dominant methods for analyzing microsatellite markers in a polyploid species.

METHODS AND MATERIALS

Study Area

Study sites for this project lie in Cantón Sarapiqui, Heredia Province, Costa Rica, at elevations of 30 – 70 m. The natural vegetation community consists of lowland wet tropical forest with a mean annual rainfall of ~4000 mm (measured at La Selva Biological Station) (McDade & Hartshorn 1994). Since roads first penetrated the area in the early 1950s, rapid settlement has replaced much of the original forest cover with a network of fragments and wooded riparian strips, set in an agriculturally-dominated matrix of pastures, banana, pineapple and palm plantations, small towns and family farms (Butterfield 1994).

This study was carried out in a private forest reserve, two forest fragments exposed to hunting and selective logging, two actively-grazed pastures adjacent to forest fragments, and two actively-grazed pasture areas isolated from forests by > 1.5 km (Fig. 1). Study sites were separated by a mean distance of 18.4 km and were grouped for analysis into four habitat types: protected forest, forest fragments, pastures and isolated pastures.

La Selva Biological Station is a 2500 ha private reserve that includes 700 ha of lowland rainforest protected from logging and hunting for at least the last 20-30 years. It adjoins Braulio Carrillo National Park, forming the largest tract of intact habitat in the region (described in detail in McDade *et al.* 1994). We established two 65 ha focal study areas in the unlogged interior portion of this forest, separated from one another by 500 m and containing populations of mature *D. panamensis* at 0.97/ha and 0.68/ha.

Pineda (40 ha) and Ladrillera 3 (52 ha) are privately held remnants surrounded by pastures and managed under sustainable forestry principles in partnership with the Fundación para el Desarrollo y Conservación de la Cordillera Volcánica Central (FUNDECOR), a local non-governmental organization. Ladrillera 3 consists of primary forest that was selectively logged in 2002 at an intensity of 1.5 trees/ha for a total volume of 351.2 m^3 (FUNDECOR, unpubl. data). In the Pineda fragment, 15.5 ha were selectively logged in 2001 at an intensity of 4.32 trees/ha for a total volume of 291.93 m^3 (FUNDECOR, unpubl. data). Additional acreage in Pineda was selectively logged during the 1990s, but no specific harvest data are available. Both fragment sites maintain a diverse forest canopy with populations of adult *D. panamensis* at 0.58/ha in Pineda and 0.21/ha in Ladrillera 3. Pastures surrounding both fragments are actively grazed and contain remnant *D. panamensis* as shade trees at densities of 0.19/ha for Pineda and 0.25/ha for Ladrillera 3.

The Rio Frio sites (east and west) include small populations of isolated remnant trees in pastures located > 1.5 km from protected forest or forest fragments, as determined by extensive ground surveys, interviews with landowners, and examination of 2001 Landsat satellite imagery (1000 m^2/pixel). Density of adult *D. panamensis* in the Rio Frio landscape is < 0.04/ha.

Study Species

Dipteryx panamensis (Pittier) Record & Mell (Fabaceae; Papilionoideae) is a large canopy emergent tree occurring in lowland wet tropical forests of the Caribbean slope, from Colombia to Nicaragua (Flores 1992). Considered a keystone species for its copious fruit production, *D. panamensis* bears single-seeded drupes for an extended period during the low-rainfall season, outside the peak fruiting period for this forest type (Frankie *et al.* 1974). Sixteen species of mammals (Bonaccorso *et al.* 1980) and approximately 100 species of birds (Flores 1992) have been observed in and around fruiting trees. Suspected pollinators include nineteen different medium to large native bees (Thiele 2002), while seed dispersal is carried out by primates and frugivorous bats, and through the scatterhoarding habits of several small mammals (Bonaccorso *et al.* 1980). The tree is monoecious and has been suggested as an obligate outcrosser (Perry & Starrett 1980), flowering asynchronously for six-week periods from May through August (Frankie *et al.* 1974; Perry & Starrett 1980). Individuals mature and begin bearing fruit at approximately 30 cm dbh (T. Hanson, pers. obs.).

No previous genetic work has been published on *D. panamensis* and its chromosome number remains unknown. Over the course of this study, however, eight of nine microsatellites behaved like tetraploid loci, while one showed additional signs of gene doubling. The Amazonian congener *D. odorata* also exhibits signs of tetraploidy with multisomic inheritance (C. Vinson, pers. comm.).

Sampling

Adult trees (dbh > 30cm) at Pineda, Ladrillera 3 and Rio Frio were located and GPS-mapped through exhaustive ground surveys and interviews with landowners during site visits in August 2004 and January – April, 2005. Trees at La Selva had been previously mapped and were sampled during the 2005 monitoring visits for an ongoing demographic study (*e.g.* (Clark & Clark 1987, 1999). Samples of leaf or cambium tissue were collected from each individual and stored immediately in silica gel for later extraction and analysis. Progeny arrays were gathered concurrently and during additional visits in 2006 and consisted of 5 seeds or leaves of seedlings gathered from directly beneath mature trees.

Genetic Markers

Nine microsatellite primer pairs were designed from loci developed for the Amazonian species *Dipteryx odorata* (Vinson 2004). Sequences and PCR characteristics are summarized in Table 1.1. DNA from plant tissues was extracted using Qiagen DNEasy Plant Mini kits, standardized to a concentration of 25 ng/µl, and stored in Qiagen AE buffer at -20 degrees Celsius. Primers were triplexed in 25 µl PCR reactions containing 10 pmols of each primer, 25 ng DNA template, 10 µmols of each DNTP, 1 µg BSA, 2.5 µl 10x Promega PCR buffer and 0.15 µl Promega Taq polymerase (5 units/µl), using the $MgCl_2$ concentrations and thermocycler profiles detailed in Table 1.1. Each forward primer was labeled with a 5' fluorescent dye and fragments were sized on an Applied Biosystems 3130xl capillary sequencer using Applied Biosystems' GeneScan 500 Liz size standard. Fragments were scored using GeneMapper v. 3.7 software (© 1999-2004 Applied Biosystems). Genotyping error rates were determined by re-extracting and re-running a subset of 90 samples (16% of all samples) for all nine loci.

The application of microsatellite markers in polyploid species is limited by the challenge of identifying true genotypes for partial heterozygotes. In a tetraploid, for example, the genotype ABBC produces the microsatellite result ABC, and cannot be reliably differentiated from AABC or ABCC. The analysis software TETRASAT computes all possible allele combinations for partial heterozygotes and reports mean values for expected Hardy-Weinberg heterozygosity (H_e), the Shannon-Weiner diversity index (H'), and Nei's measure of population differentiation (G_{st}) (Markwith *et al.* 2006). Similar techniques do not exist, however, for paternity analysis, spatial autocorrelation, or the calculation of additional diversity statistics. For these analyses we transformed our codominant microsatellite data into a dominant dataset following the methods of Rodzen *et al.* (2004). This technique treats microsatellite alleles as loci, creating binary genotypes that can be used with a variety of programs designed for dominant markers. This approach for polyploid data has been used successfully for parentage analysis (e.g. Rodzen *et al.* 2004) as well as studies of genetic diversity and structure (Mengoni *et al.* 2000).

Paternity Analysis

Maximum likelihood paternity analysis was performed using the dominant algorithms and default settings of the software FAMOZ (Gerber *et al.* 2003). For each population, we used the Delta criterion (Marshall *et al.* 1998) from 10,000 simulations to assign paternity among multiple likely fathers with strict 95% confidence. Where one father was identified, we determined an exact pollen dispersal distance between the maternal and paternal trees using Euclidian distance matrices calculated from their GPS locations. When no likely father was found, we measured a minimum pollen dispersal distance to the nearest edge of the sampled area. For progeny with two likely fathers, both minimum and maximum pollen dispersal distances were calculated. Progeny with ambiguous paternity (*i.e.* multiple fathers with low delta scores) were left unassigned. Pollen dispersal distances from one-father, two-father or no-father assignments were compared among habitat types in analyses of covariance (ANCOVA), using the Tukey-Kramer procedure (Tukey 1953; Kramer 1956) for multiple comparisons and the least square means method (Ott & Longnecker 2001) to compare individual treatments. To account for the spatial clustering of potential fathers around maternal trees, we included the median distance to the five nearest adults as a covariate for each pollen dispersal assignment.

Outcrossing Rate

The multilocus outcrossing rate (t_m) was measured as the proportion of progeny in each array that contained non-maternal alleles. We also measured the assignment outcrossing rate (t_a) as the proportion of paternity assignments attributed to non-maternal trees. Assignments outside the population (no likely father present) were considered outcrossing events for the determination of t_a. Calculations for both t_m and t_a accounted for genotyping error rates and were compared between habitat types using the Kruskal – Wallis test (Kruskall & Wallis 1952) for t_m and a chi-square test for t_a. Due to the low incidence of self-pollination events in protected forest and forest fragments, habitat types were combined for a comparison of all forest and fragment trees vs. trees in pastures and isolated pastures.

Reproductive Dominance

We examined reproductive dominance at the scale of individual trees, as well as among progeny arrays and habitat types. Dominant individuals were identified by calculating a reproductive index (R) for each adult tree, based on the number of outcrossing events assigned to them during paternity analysis (R = single-father assignments + ½ two-father assignments). Individuals whose reproductive index was higher than 95% of their peers (> 2 SD above mean) were considered dominant. For each progeny array, the ratio of observed fathers to expected fathers under a random, equal-contribution mating model was calculated and compared among habitat types using a Kruskal-Wallis test (Kruskall & Wallis 1952).

Finally, we used chi-square tests to compare the proportions of pollination events originating inside and outside of each habitat at the Ladrillera 3 and Pineda sites, testing for reproductive dominance between trees in adjacent habitats.

Genetic Diversity and Structure

Codominant Analysis: We used TETRASAT to calculate Hardy-Weinberg heterozygosity (H_e) and the Shannon-Weiner diversity index (H') for all populations, as well as pairwise Nei's population differentiation index (G_{st}) among populations (Markwith *et al.* 2006). Intense computational demands limit the number of partial heterozygotes TETRASAT can process at one time (Markwith *et al.* 2006). We therefore performed 10 iterations of each analysis on random subsamples (10 individuals) from each population, using the program's default subsampling routines and settings. Mean G_{st} values for adults and progeny generations in each habitat were compared using paired t-tests, blocked by locus. H_e and H' values were also blocked by locus and compared between adult and progeny generations using paired t-tests for normal data and Wilcoxon signed rank sum tests if the data failed a Shapiro-Wilk test for normality (Shapiro & Wilk 1965). The t-tests were two-tailed for protected forest populations, but one-tailed for other habitat types where we expected lower diversity and higher structure for the progeny generation.

Dominant Analysis: For the dominant dataset, POPGENE v. 3.2 (Yeh & Boyle 1997) provided estimates of Nei's (Nei 1973) gene diversity (*h*), the Shannon information index (I) (Lewontin 1972) and the percent polymorphic loci (PPL). Additionally, we calculated among-population phi-statistics (Φ_{pt}), an F_{st} equivalent (Excoffier *et al.* 1992), from an analysis of molecular variance (AMOVA) in the program GENALEX v. 6 (Peakall &

Smouse 2006). Significance levels for the AMOVA tests were determined from 9999 permutations.

For comparisons between adult and progeny generations, populations were grouped by habitat and 50 iterations of the analysis were performed on equal random subsamples from each population, controlling for sample size and the relatedness of progeny. Mean h and I values were then compared between generations using paired t-tests, blocked by locus. As for the codominant analysis, t-tests were two tailed in protected forest and one-tailed in fragmented habitat types.

Spatial Autocorrelation

Using the dominant dataset in the program GENALEX, we performed spatial autocorrelation analysis on the parental and progeny generations in each habitat type. GENALEX follows the multivariate approach described by Smouse & Peakall (1999), and tests for significance by establishing upper and lower 95% confidence boundaries for the autocorrelation coefficient (r) based on 999 random simulations of the null (no autocorrelation) model. Similar confidence intervals are established around the estimate of r using 999 bootstraps, and structure is inferred for non-overlapping positive ranges. We chose the multiple distance class option in GENALEX to output graphs of the combined autocorrleation coefficient (r_c) for increasing distance size classes (100 – 2500 m). The spatial limit of genetic structure is indicated by the size class at which r_c is no longer significant. Rather than a traditional correlogram, which can be strongly affected by the interplay between true genetic structure, the chosen distance classes, and unequal samples within those classes, the cumulative model is less prone to parameter errors and may provide a more reliable estimate of the spatial extent of genetic structure (Double *et al.* 2005).

Statistical Analyses

We used SAS version 9.1 (©2002-2003, SAS Institute Inc.) for all standard statistical tests (*e.g.* ANCOVA, t-tests). Paternity analysis, genetic diversity and spatial autocorrelation analyses were carried out using the specialized programs detailed above. Non-parametric tests (e.g. Kruskal-Wallis, Wilcoxon) were used only when data or log-transformed data failed tests of normality.

RESULTS

Sampling and Genetic Markers

A total of 218 adults were located, mapped and sampled from the four habitat types, as well as 325 progeny representing 65 maternal trees. All tissue samples were analyzed at nine microsatellite loci. The number of alleles per locus ranged from 3 to 13 with a mean of 7.11 (Table 1.1). Genotyping error rates from 90 duplicate samples (16.6% of total) ranged from 0.0% to 2.78% per locus (Table 1.1).

Paternity Analysis

Maximum likelihood paternity assignment found a single likely father or two likely fathers for more than half of all progeny tested (Table 1.2). An additional 38 progeny had no likely fathers within the population, leaving less than 30% of the progeny without pollen dispersal estimates. We found the shortest pollen dispersal distances in protected forest, moderate distances in forest fragments and adjacent pastures, and distances of up to 2.3 km among isolated pasture trees. This pattern was consistent for exact distances determined from single likely fathers as well as the minimum (nearest tree) and maximum (farthest tree) distances from assignments to two likely fathers (Figure 1.2). Distances from no father/outside population assignments were less clear due to a lack of data from protected forest (Figure 1.2). We documented pollen moving among trees within habitats and also found pollen moving from forest fragments to pastures, from pastures to fragments and between fragments across an intervening pasture.

The difference in dispersal distances among habitats was significant in all ANCOVA tests except for the single likely father assignments, where only a slight trend was observed (Table 1.3). Where tests were significant, least square means comparisons found that pollen traveled shorter distances in protected forests than in all other habitats, and that there was no difference between dispersal distance in forest fragments and adjacent pastures (Table 1.4). Pollen generally traveled farther in isolated pastures, but this trend was not significant in all comparisons (Table 1.4). The covariate accounting for the clustering of potential fathers was significant only for distances from fathers outside the population (Table 1.3), which may reflect the large contribution of widely-spaced (< 0.04 adults/ha) isolated pasture trees to this model (25 of 38 assignments). Otherwise, pollen dispersal distances appeared largely unaffected by the clustering of adults. Interaction effects between the covariate and habitat were not significant in any test ($p > 0.46$ for all models), upholding the ANCOVA assumption of equality of slopes for treatment and covariate effects.

Outcrossing Rate

Outcrossing rates were lower for pasture and isolated pasture trees than for trees located in protected forest and forest fragments (Fig 1.3). The differences were marginally significant ($p < 0.10$) for both the multilocus outcrossing rate (t_m) ($X^2 = 2.7418$, d.f. = 1, $p = 0.0978$), and the assignment outcrossing rate (t_a) ($X^2 = 3.4583$, d.f. = 1, $p = 0.0629$).

Reproductive Dominance

Reproductively dominant individuals were present in similar numbers in protected forest (n = 4), forest fragments (n = 2) and pastures (n = 3), but none were detected in isolated pastures. At the Ladrillera 3 forest fragment, one tree contributed pollen to 23.1% of all assigned outcrossing events (n = 26). Collectively, these nine dominant individuals accounted for 18.0% of all outcrossing events recorded in the study (n = 164). For progeny arrays, we found no difference among habitats in the ratios of observed fathers to fathers expected under random equal mating ($X^2 = 0.9954$, d.f. = 3, $p = 0.8024$), suggesting similar rates of reproductive dominance across sites. Where pastures and forest fragments were adjacent, however, there was inequality in pollen flow between habitats ($X^2 = 8.6722$, d.f. = 1, $p = 0.0032$). In fragments, 41.9% of outcrossed progeny (n = 43) were pollinated by trees in neighboring pastures, while in pastures only 13.6% of outcrossed progeny (n = 44) received pollen from fragment trees.

Genetic Diversity

Codominant Analysis: We found moderately high levels of expected heterozygosity (H_e) and slight variation among populations, ranging from 0.604 in the adults of the Rio Frio isolated pasture to 0.520 in the progeny of Rio Frio East (Table 1.5). Similarly, the Shannon-Weiner diversity index (H') varied moderately, from 1.543 in the La Selva West adults to 1.293 in the pasture population at Pineda (Table 1.5). No significant differences were found in H_e or H' between parental and progeny generations in any habitat (Table 1.6), though both measures trended slightly lower for progeny in pastures and isolated pastures.

Dominant Analysis: Variation among populations and habitats in the percentage of polymorphic loci (PPL), Nei's gene diversity (*h*) and the Shannon information index (I) were closely linked to population size (Table 1.5). After adjustments to control for sample size and relatedness of progeny, the means from fifty iterations of the analysis found no difference between adult and progeny generations in any habitat (Table 1.6).

Genetic Structure

Codominant Analysis: Mean pairwise Nei's differentiation index (G_{st}) indicated moderate structure among all populations ($G_{st} = 0.0540$) (Table 1.7). Comparisons between adult and progeny populations did not vary in protected forest ($t = -0.4548$, $p = 0.6630$), forest fragments ($t = 0.5609$, $p = 0.5923$) or pastures ($t = -0.6507$, $p = 0.5360$), but there was a marginally significant (one-tailed) increase in differentiation among progeny than adults in isolated pastures ($t = -2.5495$, $p = 0.0381$).

Dominant Analysis: AMOVA showed significant structure (Φ_{pt}) among adult populations and among progeny populations in all habitats, with the exception of progeny in protected forest ($p = 0.2230$) (Table 1.7). There were no clear patterns, however, in comparisons between adult and progeny generations, with similar Φ_{pt} values for adults and progeny in forest fragments (0.069 vs 0.074) and isolated pastures (0.103 vs. 0.117), but less differentiation between the progeny (0.037) than the adults (0.063) in pastures (Table 1.7).

Spatial Autocorrelation

Significant spatial autocorrelation existed in adult populations in protected forest, forest fragments and adjacent pastures, but no pattern was detected in isolated pastures (Figure 1.4). In protected forest, the combined autocorrelation coefficient (r_c) measured 0.034 in the first 100 m and remained significant to 300 m. In fragments, r_c reached 0.077 at 100 m and maintained significance to 400 m. Pasture trees showed no autocorrelation at 100 m, perhaps due to small sample size in that distance class. Their r_c values resembled forest fragment trees at 400 m (0.031), but remained significant at very low magnitude (< 0.01) up to 1000 m. The lack of signal in isolated pastures probably stemmed from small sample size and spatial distributions beyond the scale of the measurable genetic structure.

Progeny populations in all habitats displayed significant spatial autocorrelation (Figure 1.5). The peak magnitude and scale of the autocorrelation rose in increasingly fragmented habitats, from a peak r_c value of 0.184 (100 m) and significance up to 500 m in protected forest to magnitude 0.274 at 100 m and significance to 2000 m in isolated pastures.

DISCUSSION

Pollen Dispersal and Outcrossing Rates

Patterns of pollen movement in *D. panamensis* matched our predictions, with increased dispersal distances in fragments and pastures compared to protected forest, and the longest distances in isolated pastures. These results resemble findings from other fragmented populations of bee-pollinated tropical trees (Dick 2001; White *et al.* 2002; Dick *et al.* 2003). While lower adult densities in disturbed habitats must inherently lead to longer pollination distances if outcrossing is to take place, our ANCOVA results show that for *D. panamensis*, pollen dispersal distance increases with fragmentation in spite of the proximity of potential fathers. A major shift in pollinator behavior or the community of pollinators in fragmented areas would explain this trend. In Brazil, Dick (2001) observed non-native Africanized honeybees (*Apis mellifera*) dominating the pollinator community of *Dinizia excelsa* in fragmented landscapes. He and his colleagues (2003) also documented longer pollen dispersal distances among isolated trees than in continuous forest where honeybees were largely absent. Similar processes may be at work in our study area, where *A. mellifera* is widespread (Rincon *et al.* 2000), but has not been observed visiting *D. panamensis* flowers in our protected forest site (Thiele 2002).

Long-distance pollen flow may increase the genetic neighborhood of trees in fragmented areas and counteract some negative effects of habitat degradation (Lowe *et al.* 2005). In *D. panamensis*, however, we also observed lower outcrossing rates for pasture trees (0.806 - 0.865) than forest trees (0.896 - 0.929). So while pollen is moving farther, it appears to be moving less often. This could reflect lower incidence of pollination in general, or different pollinator behavior (e.g. spending complete foraging bouts in one tree). Our results contradict earlier suggestions of obligate outcrossing in *D. panamensis* (Perry & Starrett 1980), but reflect similar changes in outcrossing rates observed in other pasture trees (Cascante *et al.* 2002; Dick *et al.* 2003). Increases in pollen-mediated gene flow for fragmented *D. panamensis* populations must therefore be interpreted in the context of increased inbreeding, which can have an immediate impact on heterozygosity and fitness (Nason & Hamrick 1997; Lowe *et al.* 2005).

Genetic Diversity

From our codominant analysis, the moderately high levels of H_e and H' in adult populations indicate high rates of historic (pre-fragmentation) gene flow throughout the study area. There was no difference between adult and progeny populations in either protected forest or forest fragments, but both H_e and H' trended lower for pasture and isolated pasture progeny. Though neither change was significant, the trends follow from our observed drop in outcrossing rates and hint at the long-term genetic consequences for these populations. Because the adults in our study date from the pre-fragmentation period, their progeny represent the first generation to reflect post-fragmentation reproductive conditions. Only a severe bottleneck would be expected to produce large declines in diversity in one-generation (Frankham *et al.* 2002), but allelic diversity measures from microsatellites can often detect even moderate bottleneck events (Spencer *et al.* 2000). Simulations indicate that a breeding population of 20 trees with an outcrossing rate of 0.80, similar to our isolated pasture sites, would produce declines of < 10% in allelic richness and expected heterozygosity in one generation, but would lead to more than 50% loss of alleles and a 25% drop in heterozygosity after 10 generations (Lowe *et al.* 2005). As a tetraploid, *D. panamensis* should fare better in preserving allelic diversity by carrying more copies per individual, but these populations still face long-term risks of genetic erosion and the corresponding decline in fitness. While individual adults maintain diversity levels of a pre-fragmentation forest, their effective breeding population may be reduced in pasture settings in spite of long pollen dispersal distances. Other studies have documented the loss of rare alleles in fragmented populations (White *et al.* 1999), but most analyses of diversity have been inconclusive (Lowe *et al.* 2005). Research spanning multiple generations, a challenge for long-lived tree species, may be necessary before the true genetic impacts of fragmentation can be known.

Our dominant analysis also showed moderate to high levels of diversity in adult populations, but detected no trends between adult and progeny generations in any habitat. We suspect this discrepancy resulted from differences in the sensitivity of the analysis methods, as discussed below.

Genetic Structure and Spatial Autocorrelation

Codominant analysis found increased genetic structure among progeny populations in isolated pastures, an expected result and logical extension of our outcrossing and genetic diversity analyses. Spatial autocorrelation analysis produced a similar but more revealing trend, as the magnitude of the correlation coefficient for progeny increased steadily with increasing fragmentation, from protected forest to fragments, pastures and isolated pastures. The scale of the correlation increased as well, a reflection of the longer pollen dispersal distances in fragmented habitats. These patterns follow theoretical predictions for the genetic effects of fragmentation, an increase in differentiation among populations over successive generations (Nason & Hamrick 1997). We are unaware of other studies examining spatial autocorrelation for tropical trees in forests and pastures, but Pither *et al.* (2003) did document higher differentiation among naturally-fragmented *Terminalia amazonia* populations in Belize, while Cascante *et al.* (2002) found higher levels of relatedness within seed arrays of isolated *Samanea saman* in Costa Rica.

Our dominant analysis failed to produce coherent results for estimates of genetic structure. Again, we feel this inconsistency is a reflection of analysis resolution with 60 dominant loci. Hollingsworth and Ennos (2004), for example, estimated that 50 dominant loci were sufficient to accurately detect high levels of population differentiation ($F_{st} > 0.15$), but that up to 250 loci were necessary to identify low levels of structure ($F_{st} < 0.10$), such as those in our study.

Reproductive Dominance

Reproductive dominance has been noted in the disproportionate parental contribution of a few pasture trees to the seedling cohorts of adjacent fragments (Aldrich & Hamrick 1998) and by several forest palms in the colonization of regenerating secondary habitat (Sezen *et al.* 2005). Just as demographics and mating behaviors have major impacts on effective population size for vertebrates (Storz *et al.* 2002), dominance of a few fecund individuals can strongly influence the genetic variability of plant populations. We noted reproductively dominant individuals in similar numbers in continuous forest, forest fragments and pastures adjacent to fragments. Collectively, these seven individuals accounted for 18% of the outcrossing events in the study. In any habitat, this trend reduces the effective population size for *D. panamensis*, but it may be particularly important in pastures, where lower outcrossing rates may already be affecting genetic diversity. In combination, dominance and increased self-pollination could lead to faster loss of variability than either process alone.

The high rates of pollen flow we observed from pastures to adjacent fragments echo the findings of Aldrich and Hamrick (1998), where pasture trees were responsible for the majority of regeneration in fragments. High fecundity in pasture trees may be a response to reduced competition and high light conditions, allowing a greater allocation of resources to flowering and fruit production. Such dominance can effectively cause a bottleneck, sharply reducing the effective number of breeding adults in the population (Aldrich & Hamrick 1998). For *D. panamensis*, additional studies will be needed to determine whether seeds from pasture trees are dominating regeneration in fragments, but high rates of germination have been observed directly beneath adult fragment trees, suggesting otherwise (Hanson *et al.* 2006). Additionally, we observed pollen arriving not from a dominant few but from many pasture individuals (15 at Pineda and 10 at Ladrillera 3) which may in fact enhance the genetic diversity of fragment populations and underscores the importance of pasture trees to landscape-level gene flow.

Codominant and Dominant Methods of Microsatellite Analysis for Polyploids

Our results demonstrate the utility of dominant and codominant approaches for analyzing microsattelite data in polyploid species, but also highlight limitations and differences in resolution between the methods. For paternity assignment, we achieved good results using dominant data with a total of 60 loci (microsatellite alleles). Rodzen *et al.* (2004) used the same methods and a total of 40 loci for parentage assignment in a captive population of nine octoploid sturgeon (*Acipenser transmontanus*). For single-parent assignments they used 82 loci and achieved 96% accuracy. In spite of much larger parental populations (7 – 63 individuals), we still made exact (37.2%), two likely father (21.8%), or no father (11.7%) paternity assignments for more than 70% of the progeny in our study. The accuracy of dominant markers for paternity analysis reflects the number of potential fathers, the number of loci used and their frequency within the population (Lewis & Snow 1992; Gerber *et al.* 2000). As a general rule, 50 polymorphic dominant loci have been recommended for paternity analysis (Lewis & Snow 1992), though successful studies have ranged from 32 (Fowler *et al.* 1998) to 125 (He *et al.* 2004) polymorphic loci.

For assessing genetic diversity, dominant markers (AFLPs, RAPDs) can be sensitive to sample size (Gaudeul *et al.* 2004) and often produce lower estimates of within-population diversity than codominant microsatellites (reviewed in Nybom 2004). Several comparison studies have also found that microsatellites have better resolution than AFLPs for detecting fine-scale genetic structure (Ross *et al.* 1999; Lougheed *et al.* 2000), though other among-population comparisons have been congruent (Nybom 2004). Such marker-related discrepancies are attributed to different mutation rates and to the relative number of markers used (Nybom 2004; Gaudeul *et al.* 2004). Since our datasets derive from the same microsatellite genotypes they should reflect the same mutation rate. We therefore attribute inconsistencies between our dominant and codominant results to the relative number of markers: for our data, eight codominant microsatellites had greater resolution to detect changes in diversity and structure than 60 dominant loci.

Interestingly, spatial autocorrelation analysis with dominant data produced patterns in line with our expectations and complementary to the G_{st} estimates from our codominant (TETRASAT) analysis (*i.e.* greater structure among pasture and isolated pasture progeny). It may be that the inherently multivariate autocorrelation approach (Smouse & Peakall 1999) is better able to ascertain subtle patterns than the calculations for Φ_{pt}, or that the true patterns in structure simply become more identifiable in a spatial context.

Codominant analysis using TETRASAT produced results consistent with our expectations for all diversity and structure analyses, and appeared better able to detect subtle patterns in the data. Markwith *et al.* (2006) found close concordance between TETRASAT and allozyme results, but we are unaware of any other study comparing TETRASAT results to those from a dominant analysis of the same data. The software faces computational limits, but we found that these can be overcome for large datasets by using multiple runs with random subsamples.

Limitations

Though eight loci for in this study behaved in a tetraploid fashion, the ploidy history and inheritance patterns for *D. panamensis* remain unconfirmed. In reality, most polyploid species fall somewhere on a continuum between true allopolyploids and autopolyploids, since hybridization events are typically between closely-related species with similar genomes. We therefore present these results with the caution that in addition to assumptions of Hardy-Weinberg equilibrium, we have assumed random chromosomal segregation at all loci.

CONCLUSIONS

Low population densities and animal-mediated pollen and seed dispersal make many tropical trees vulnerable to habitat disturbance and fragmentation (Nason *et al.* 1997). On the other hand, their natural ability to maintain gene flow over wide areas may help them persist in fragmented settings (Sork & Smouse 2006; Hamrick 2004), provided that systems for pollen and seed dispersal can adapt to the new conditions. Pollen dispersal distances for *D. panamensis* appear to increase dramatically in fragmented areas and measures of genetic diversity in progeny remain generally high. Long-distance pollination among isolated pasture trees and between pastures and forest fragments emphasize the importance of remnant trees in maintaining landscape-level gene flow, but this trend will be ephemeral unless pasture populations are replanted or allowed to regenerate. In spite of heightened pollen flow, however, we observed lower outcrossing rates for pasture trees as well as slightly lower diversity and increased genetic structure among their progeny, suggesting that fragmented populations are still at risk form long-term genetic erosion.

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Table 1.1 Sequences and characteristics of nine *Dipteryx panamensis* primers designed from microsatellite regions developed for *D. odorata* (Vinson 2004), and utilized on 549 individuals.

Locus	GenBank Accession no.[*]	Primer sequences (5' – 3')	Repeat motif	Size range (bp)	T_a	$MgCl_2$	Multi-plex[†]	E_s	N_a	N_i
Do3*	AY535410	F: GGGCTTGTAGCTATTGAGTG R: GTTT AAAAGGGGATTAAGACCTTG[§]	$(CT)_{13}$	223-241	52	1.7mM	A	2.12%	10	4
Do5	AY535411	F: GGCCAAGTAAGCTAGAA R: TTTGAAGTTGAAGCTTGGAT	$(GA)_{18}$	188-214	52	3.4mM	B	0.63%	13	7
Do8*	AY535412	F: TCTGTAGCTCTCTGCCTCTC R: GTT TCGAATGAGTAATGTTGTGC[§]	$(CT)_{14}$	152-170	54	3.4mM	C	2.50%	8	4
Do17	AY535413	F: CTGTCGGTTCTCCATATATTTT R: ATTTAAACCTCTGCGTTGAA	$(GA)_{13}$	141-149	52	3.4mM	B	0%	5	4
Do18*	AY535414	F: TCTCTCCCCCTTTGTCTC R: GTTT AGGTTGGCAGTGAAGGTG[§]	$(CT)_{12}$	99-103	52	3.4mM	B	1.25%	3	3
Do20*	AY535416	F: GTCTTCGCCAACAGTAACTC R: GTT TATAGTGGAAGGGTGGATTG[§]	$(TC)_{17}$	155-175	54	3.4mM	C	0%	6	4
Do24*	AY535417	F: CAGGATCTAGCCAAAAGAAA R: GTTT CAAGCTAACTGGTCTCCCTA[§]	$(GA)_{14}$	132-152	52	1.7mM	A	2.78%	8	4
Do25	AY535418	F: AAAACGGAAGAGGAAGATTT R: GAGACTTCGATGTCAGGGTA	$(GA)_9$	173-195	54	3.4mM	C	1.41%	7	4
Do39	AY535421	F: TAATGGATGCCTGAATGAAT R: GCTTAAAAGCGAGTTGTCAT	$(CT)_{11}$	179-207	52	1.7mM	A	2.56%	4	4

T_a – annealing temperature (Celsius), E_s – genotyping error rate, N_a – total alleles, N_i – maximum observed alleles/individual

[*] Original *D. odorata* microsatellite region Accession numbers (Vinson 2004); original locus names also retained.

[§] Tail added to reverse primer to increase adenylation for consistent scoring (Brownstein *et al.* 1995).

[†] Thermocycler Profiles for multiplexes: A & B - (45 cycles: 94º 30sec, 52º 30sec 72º 35sec), C - (45 cycles: 94º 30sec, 54º 30sec 72º 30sec).

Table 1.2 Maximum likelihood paternity analysis for 325 *D. panamensis* progeny from protected forest, forest fragments, pastures adjacent to fragments and pastures isolated from forest by > 1.5 km in Cantón Sarapiqui, Costa Rica.

Site	Habitat*	Adults N (# potential pollen donors)	Progeny N (# arrays)	Loci	Missing data	Exclusion probabilities (paternity/ parentage)	Delta criterion (95% confidence threshold from 10,000 simulations)	Paternity assignments			
								1 likely father	2 likely fathers	No father – outside population	Ambiguous/ unassigned
La Selva West	PF	63 (63)	21 (5)	51	1.89%	(84.5%/96.3%)	2.08	6	8	0	7
La Selva East	PF	41 (41)	29 (6)	47	1.16%	(85.1%/96.8%)	2.29	11	5	0	13
Pineda Forest	FF	26 (52)	74 (15)	49	0.34%	(86.7%/97.4%)	2.02	17	25	1	31
Ladrillera 3 Forest	FF	14 (50)	49 (10)	47	1.37%	(86.1%/97.1%)	2.34	26	7	0	16
Pineda Pasture	P	26 (52)	50 (10)	49	0.56%	(86.3%/97.4%)	2.26	23	11	1	15
Ladrillera 3 Pasture	P	36 (50)	58 (12)	47	1.47%	(86.1%/97.1%)	1.92	21	13	11	13
Rio Frio West	I	5 (5)	15 (3)	38	1.75%	(83.5%/96.2%)	1.52	4	0	11	0
Rio Frio East	I	7 (7)	29 (6)	40	1.42%	(74.7%/90.1%)	1.21	13	2	14	0
							totals:	121 (37.2%)	71 (21.8%)	38 (11.7%)	95 (29.2%)

*PF (protected forest), FF (forest fragment), P (pasture), I (isolated pasture)

Table 1.3 Results of ANCOVA comparing pollen dispersal distances assigned by paternity analysis of 325 *D. panamensis* progeny in four habitat types: protected forest, forest fragments, pastures adjacent to fragments and isolated pastures. The covariate is a distance measure accounting for the spatial clustering of potential fathers.

Paternity Assignment Source of Variation	Dispersal Distance	N d.f. err. d.f. eff.	F	*P*
1 Likely Father (95% confidence)	exact	121		
Habitat		3	1.86	0.1403
Covariate		1	1.81	0.1808
2 Likely Fathers (95% confidence)	minimum	71		
Habitat	(near father)	3	4.64	0.0053
Covariate		1	0.42	0.5189
2 Likely Fathers (95% confidence)	maximum	71		
Habitat	(far father)	3	11.08	<0.001
Covariate		1	0.02	0.8879
No Fathers/Outside Population	minimum	38		
Habitat		2	14.86	<0.001
Covariate		1	5.92	0.0052

Table 1.4 Least square means comparisons of pollen dispersal distances for *D. panamensis* in protected forest, forest fragments, pastures adjacent to fragments, and isolated pastures. Data are from significant ANCOVA models with Tukey-Kramer adjustment for multiple comparisons.

	P- values for tests of unequal LS means		
Comparison	Two Likely Fathers – minimum distance	Two Likely Fathers – maximum distance	No Likely Fathers – minimum distance
Protected forest vs. forest fragments	0.0080	<0.0001	-
Protected forest vs. pastures	0.0098	0.0002	-
Protected forest vs. isolated pasture	0.0046	0.0003	-
Forest fragments vs. pasture	0.9989	0.9996	0.7369
Forest fragments vs. isolated pasture	0.1500	0.0037	0.0222
Pasture vs. isolated pasture	0.1345	0.0030	<0.0001

Table 1.5 Genetic diversity from codominant and dominant analyses of microsatellite data for adult and progeny populations of *D. panamensis* in protected forest, forest fragments, pastures and isolated pastures.

			POPGENE (dominant analysis)			TETRASAT (codominant tetraploid analysis)	
Population*	Site	N	PPL	h ± SD	I ± SD	H_e ± SD	H' ± SD
PFA1	La Selva west	63	78.33%	0.2414 ± 0.2102	0.3593 ± 0.2882	0.5984 ± 0.0080	1.5428 ± 0.0330
PFA2	La Selva east	41	75.00%	0.2212 ± 0.1970	0.3357 ± 0.2745	0.5802 ± 0.0157	1.4348 ± 0.0452
PFP1	La Selva west	21	63.33%	0.2288 ± 0.2102	0.3379 ± 0.2963	0.5754 ± 0.0150	1.4782 ± 0.0423
PFP2	La Selva east	29	65.00%	0.2154 ± 0.2005	0.3237 ± 0.2833	0.5797 ± 0.0189	1.4609 ± 0.0630
FFA1	Pineda	26	73.33%	0.2249 ± 0.1994	0.3394 ± 0.2783	0.5916 ± 0.0281	1.4819 ± 0.0610
FFA2	Ladrillera3	14	61.67%	0.2158 ± 0.2101	0.3199 ± 0.2957	0.5426 ± 0.0153	1.3826 ± 0.0515
FFP1	Pineda	75	73.33%	0.2227 ± 0.2018	0.3354 ± 0.2812	0.5707 ± 0.0281	1.4017 ± 0.0830
FFP2	Ladrillera3	54	70.00%	0.2376 ± 0.2045	0.3542 ± 0.2855	0.5648 ± 0.0116	1.4549 ± 0.0508
PA1	Pineda	26	71.67%	0.2203 ± 0.1972	0.3335 ± 0.2765	0.5252 ± 0.0310	1.3477 ± 0.1169
PA2	Ladrillera3	36	68.33%	0.2385 ± 0.2037	0.3549 ± 0.2863	0.5490 ± 0.0574	1.4311 ± 0.1202
PP1	Pineda	50	68.33%	0.2093 ± 0.1995	0.3165 ± 0.2804	0.5226 ± 0.0478	1.2933 ± 0.1198
PP2	Ladrillera3	58	76.67%	0.2434 ± 0.1965	0.3658 ± 0.2742	0.5916 ± 0.0281	1.4819 ± 0.0610
IA1	Rio Frio west	5	46.67%	0.1638 ± 0.1983	0.2518 ± 0.2860	0.6040 ± 0.0029	1.5302 ± 0.0167
IA2	Rio Frio east	7	51.67%	0.1994 ± 0.2185	0.2909 ± 0.3081	0.5657 ± 0.0045	1.4256 ± 0.0048
IP1	Rio Frio west	15	58.33%	0.2217 ± 0.2110	0.3264 ± 0.2992	0.5791 ± 0.0173	1.4212 ± 0.0352
IP2	Rio Frio east	29	60.00%	0.2104 ± 0.2082	0.3123 ± 0.2948	0.5200 ± 0.0171	1.3092 ± 0.0407
Population* (combined)		N	PPL	h ± SD	I ± SD	H_e ± SD	H' ± SD
PFA	-	104	83.33%	0.2396 ± 0.2066	0.3584 ± 0.2835	0.5893 ± 0.0152	1.4888 ± 0.0679
PFP	-	50	71.67%	0.2281 ± 0.2003	0.3432 ± 0.2799	0.5775 ± 0.0164	1.4696 ± 0.0519
FFA	-	40	80.00%	0.2333 ± 0.1989	0.3528 ± 0.2742	0.5671 ± 0.0335	1.4323 ± 0.0748
FFP	-	129	76.67%	0.2379 ± 0.2042	0.3561 ± 0.2822	0.5678 ± 0.0193	1.4283 ± 0.0713
PA	-	62	75.00%	0.2386 ± 0.2011	0.3577 ± 0.2796	0.5608 ± 0.0442	1.4244 ± 0.1177
PP	-	108	80.00%	0.2365 ± 0.1950	0.3578 ± 0.2708	0.5358 ± 0.0522	1.3622 ± 0.1352
IA	-	12	66.67%	0.2177 ± 0.1921	0.3301 ± 0.2738	0.5848 ± 0.0205	1.4779 ± 0.0563
IP	-	44	63.33%	0.2224 ± 0.2051	0.3316 ± 0.2889	0.5496 ± 0.0351	1.3652 ± 0.0691

*Population codes: PFA (protected forest adults), PFP (protected forest progeny), FFA (forest fragment adults), FFP (forest fragment progeny), PA (pasture adults), PP (pasture progeny), IA (isolated pasture adults), IP (isolated pasture progeny)

PPL - % polymorphic loci, h – Nei's (1973) gene diversity, I – Shannon information index (Lewontin 1972), H_e – Hardy-Weinberg expected heterozygosity, H' – Shannon-Weiner diversity index (Markwith *et al.* 2006).

Table 1.6 Comparisons of genetic diversity between adult and progeny generations of *Dipteryx panamensis* in protected forest, forest fragments, pastures and isolated pastures.

Analysis 1: Adult vs. progeny in protected forest – Paired t-test (*h*, I), Wilcoxon signed rank sum test (H_e, H').

Diversity Estimator	d.f.	t	S	*P*
h^*	59	1.1586	-	0.2513
I^*	59	1.0962	-	0.2774
$H_e^\dagger$	7	-	-3.0	0.7422
$H'^\dagger$	7	-	-1.0	0.9453

Analysis 2: Adult vs. progeny in forest fragments – Paired t-test (*h*, I, H'), Wilcoxon signed rank sum test (H_e).

Diversity Estimator	d.f.	t	S	*P*
h^*	59	-0.7101	-	0.4778
I^*	59	-0.4023	-	0.6922
$H_e^\dagger$	7	-	2.0	0.8438
$H'^\dagger$	7	0.25	-	0.8069

Analysis 3: Adult vs. progeny in pastures adjacent to forest – Paired t-test (*h*, I, H_e, H').

Diversity Estimator	d.f.	t	S	*P*
h^*	59	0.3454	-	0.7380
I^*	59	-0.0229	-	0.9880
$H_e^\dagger$	7	1.5587	-	0.1630
$H'^\dagger$	7	1.2333	-	0.2573

Analysis 4: Adult vs. progeny in isolated pastures – Paired t-test (*h*, I, H'), Wilcoxon signed rank sum test (H_e).

Diversity Estimator	d.f.	t	S	*P*
h^*	59	-0.3740	-	0.7097
I^*	59	-0.0850	-	0.9325
$H_e^\dagger$	7	-	8.0	0.3125
$H'^\dagger$	7	1.7814	-	0.1180

h – Nei's (1973) gene diversity index
I – Shannon information index
H_e – Hardy-Weinberg expected heterozygosity
H' – Shannon-Weiner diversity index
[*] From 50 iterations of dominant analysis, controlling for unequal sample size and relatedness of progeny.
[†] From 10 iterations of codominant tetraploid analysis, controlling for unequal sample size and relatedness of progeny.

Table 1.7 Analysis of molecular variance (AMOVA) and G_{st} estimators of genetic structure for adult and progeny populations of *Dipteryx panamensis* in protected forest, forest fragments, pastures and isolated pastures.

	AMOVA (dominant analysis)				TETRASAT (codominant tetraploid analysis)
Populations	d.f.	Φ_{pt}[*]	% total	*P* value	G_{st}[§] ± SD
All samples					
Among populations	15	0.080	8.0%	0.01	0.0558 ± 0.0023
Within populations	542		92.0%		
PFA (protected forest adults)					
Among populations	1	0.062	6.2%	<0.001	0.0499 ± 0.0139
Within populations	100		93.8%		
PFP (protected forest progeny)					
Among populations	1	0.026	4.6%	0.233	0.0495 ± 0.0084
Within populations	12		95.4%		
FFA (forest fragment adults)					
Among populations	1	0.069	6.9%	<0.001	0.0657 ± 0.0090
Within populations	39		93.1%		
FFP (forest fragment progeny)					
Among populations	1	0.074	7.4%	0.003	0.0700 ± 0.0221
Within populations	24		92.6%		
PA (pasture adults)					
Among populations	1	0.063	6.3%	<0.001	0.0676 ± 0.0165
Within populations	61		93.7%		
PP (pasture progeny)					
Among populations	1	0.037	3.7%	0.044	0.0842 ± 0.0180
Within populations	21		95.3%		
IA (isolated pasture adults)					
Among populations	1	0.103	10.3%	0.013	0.0460[**] ± 0.0035
Within populations	11		89.7%		
IP (isolated pasture progeny)					
Among populations	1	0.117	11.7%	0.027	0.0649[**] ± 0.0069
Within populations	6		88.3%		

[*] Analogous to F_{st} at the molecular level (Excoffier *et al.* 1992).

[§] Mean of pairwise values from 10 iterations of TETRASAT analysis with random subsamples.

[**] Significant difference ($p = 0.038$)

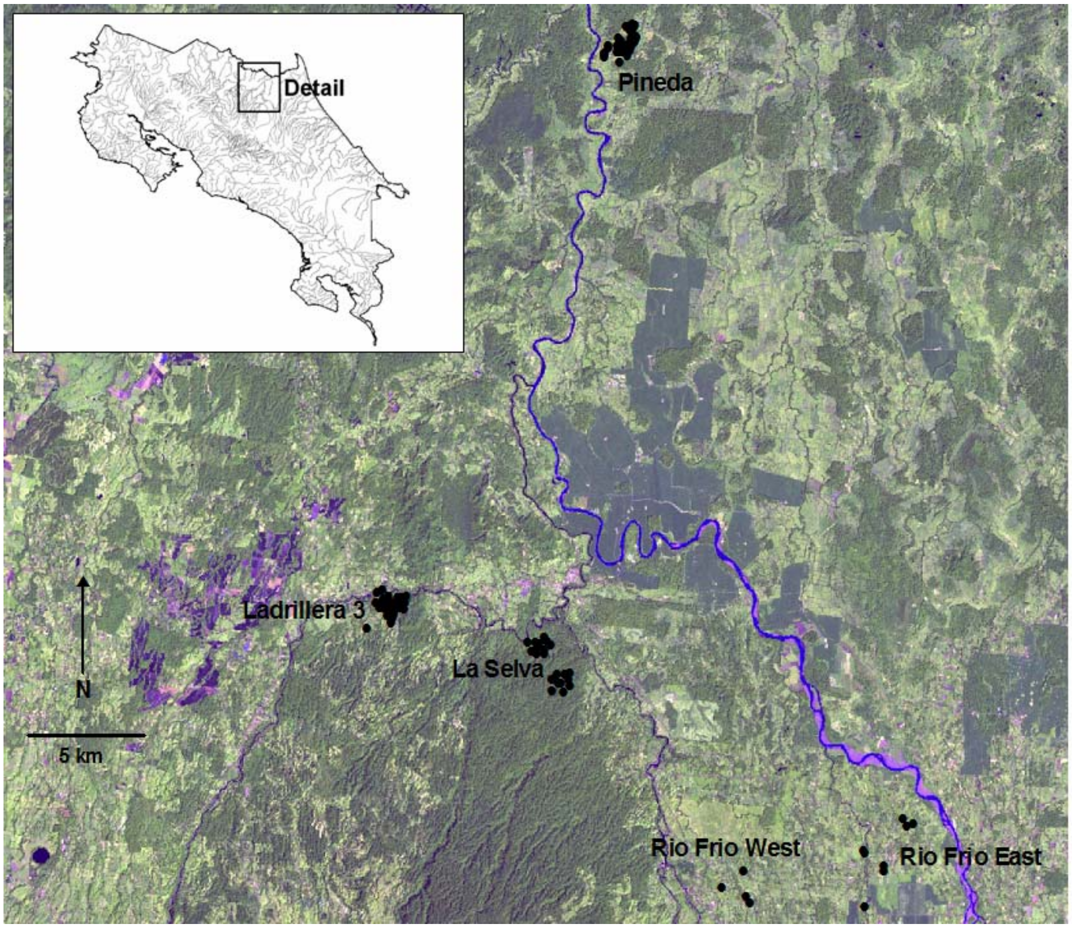

Figure 1.1 Landsat (2001) image showing study sites, adult *D. panamensis* (•) and major features in the fragmented landscape of Cantón Sarapíqui, Heredia Province, Costa Rica.

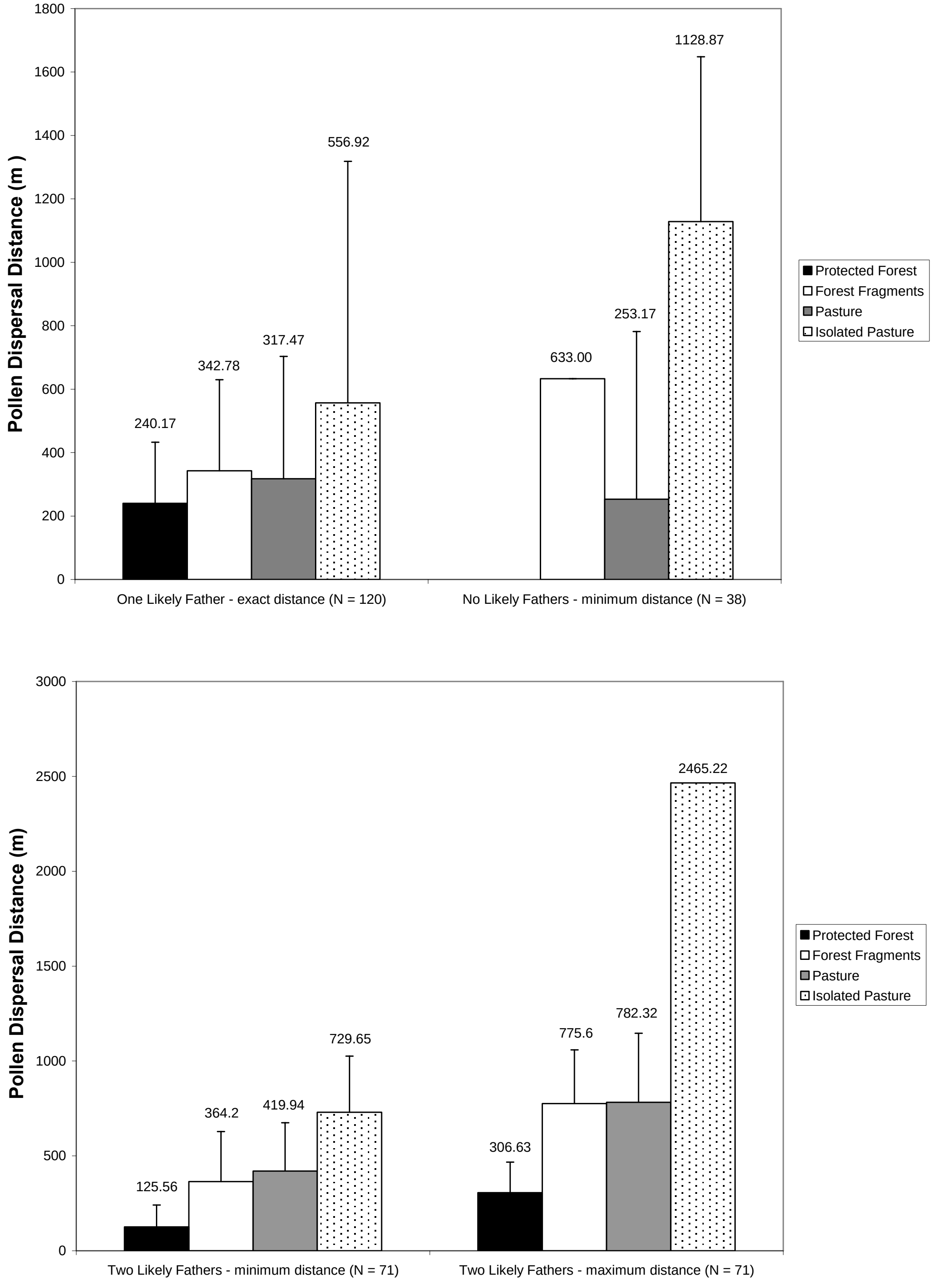

Figure 1.2 Mean pollen dispersal distances (+ SD) for *D. panamensis* progeny assigned by paternity analysis to one likely father (exact distance) or two likely fathers (minimum and maximum distances), or with no likely fathers in the population (minimum distance to nearest edge of site).

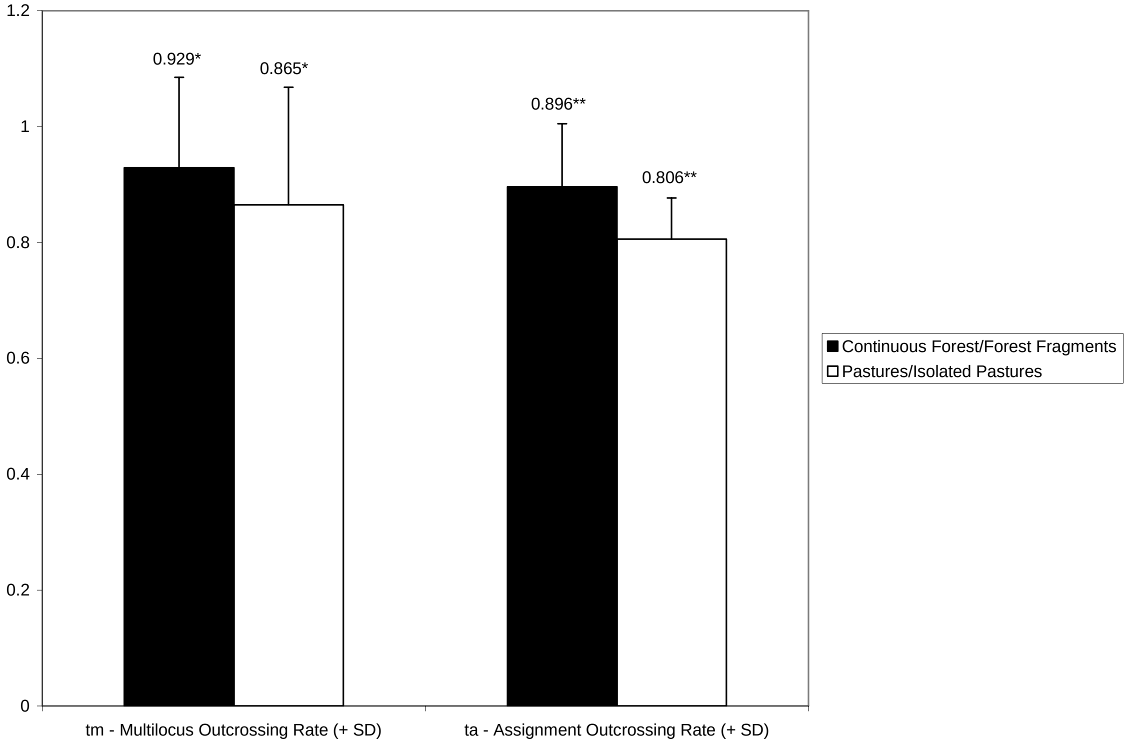

Figure 1.3 Two measures of outcrossing rate for *D. panamensis* in protected forest and forest fragments vs. pastures and isolated pastures. The multilocus outcrossing rate (t_m) is derived from the presence of non-maternal alleles in progeny arrays. The assignment outcrossing rate (t_a) is the proportion of paternity assignments ascribed to non-maternal trees. ($^{*}p = 0.0978$; $^{**}\ p = 0.0629$).

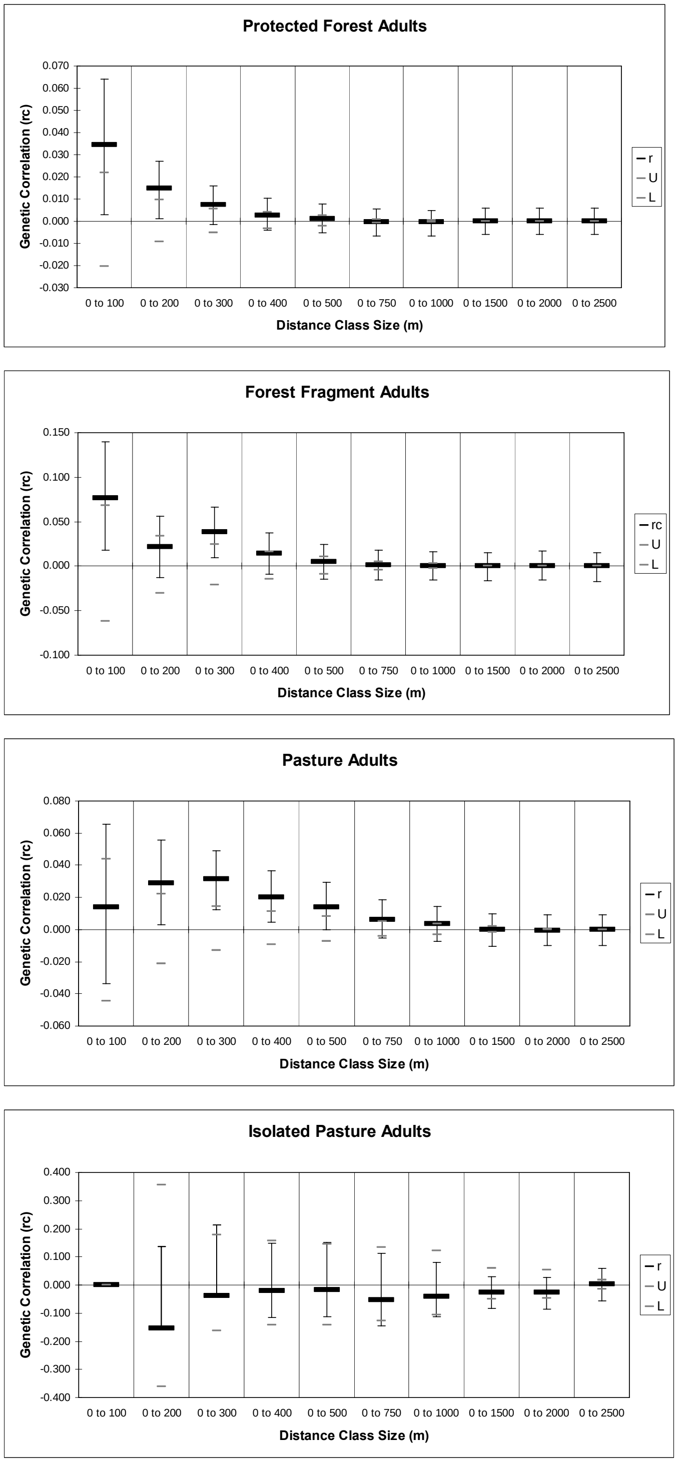

Figure 1.4 Combined spatial autocorrelation coefficient values (r_c) for adult populations of *D. panamensis* across increasing size classes in protected forest, forest fragments, pastures and isolated pastures. Upper and lower 95% confidence boundaries from 999 simulations; 95% confidence bars around r_c from 999 bootstraps.

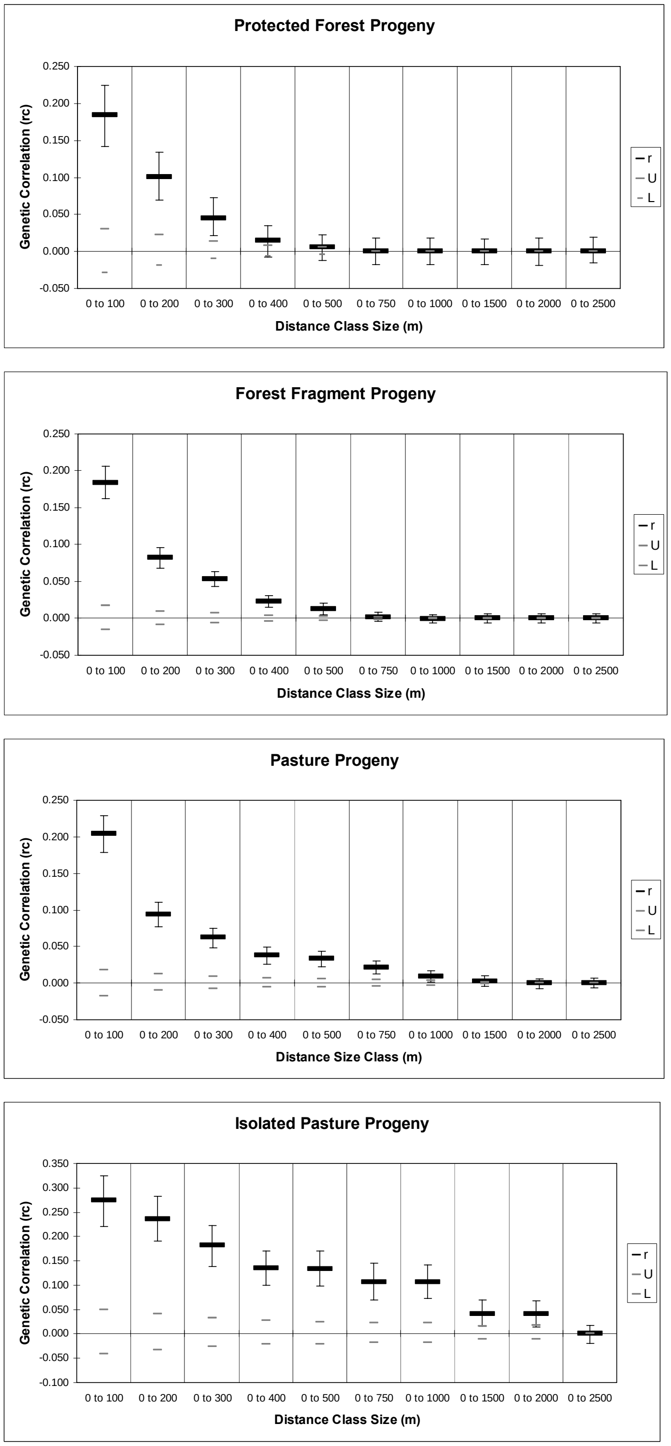

Figure 1.5 Combined spatial autocorrelation (r_c) for progeny populations of *D. panamensis* across increasing distance class sizes in protected forest, forest fragments, pastures and isolated pastures. Upper and lower 95% confidence boundaries from 999 simulations; 95% confidence bars around r_c from 999 bootstraps.

CHAPTER TWO: Conventional and Genetic Measures of Seed Dispersal for *Dipteryx panamensis* (Fabaceae) in Protected and Fragmented Rainforest

Thor Hanson
Department of Forest Resources
University of Idaho
P.O. Box 441133
Moscow, Idaho 83844-1133
thor@rockisland.com

Steven Brunsfeld
Department of Forest Resources
University of Idaho
P.O. Box 441133
Moscow, Idaho 83844-1133

Bryan Finegan
Departamento de Recursos Naturales y Ambiente
Centro Agronómico Tropical de Investigación y Enseñanza (CATIE)
7170 Turrialba
Costa Rica

Lisette Waits
Department of Fish and Wildlife Resources
University of Idaho
P.O. Box 441136
Moscow, ID 83844-1136

ABSTRACT

The effects of habitat fragmentation on seed dispersal mechanisms can strongly influence the diversity and evolutionary potential of tropical forest plant communities. Here we document seed dispersal distances for the emergent tree *Dipteryx panamensis* in protected forest and two forest fragments in Cantón Sarapiqui, Costa Rica. *D. panamensis* occurs in the Atlantic lowlands of Mesoamerica from Colombia to Nicaragua, and is considered a keystone species for its copious fruit production. Dispersed seeds were located, mapped and gathered from 100 m x 4 m transects at La Selva Biological Reserve (n = 42) and two forest fragments (n = 35) surrounded by active pastures. Site-specific distance matrices were calculated between the locations of seeds (n = 3016) and adult *D. panamensis* (n = 283). The number of seeds dispersed > 25 m from adult trees did not vary between the two fragments ($t = 0.589$; $p = 0.588$), or between fragments and protected forest ($t = -0.389$; $p = 0.717$). The distribution of seeds in distance classes away from adult trees varied among sites ($X^2 = 23.25$, $p = 0.007$), showing a more erratic dispersal pattern at fragment sites. The maximum distance a seed was recovered away from an adult tree (the minimum dispersal distance) was 243.4 m in protected forest and 251.5 m in fragments. We also determined exact dispersal distances for a subset of seeds (n = 14) from which viable maternal DNA could be extracted from the endocarp. Genotypes for up to nine microsatellite markers were generated and matched with the genotypes of potential maternal trees, documenting dispersal distances of up to 852.96 m. Dispersal was noted between pasture trees and adjacent forest fragments, and we found a single tree dominating the seed cohort at one site. There was a strong correlation between the location of dispersed seeds and the location of overstory palms favored as bat feeding roosts in protected forest ($X^2 = 39.1201$, $p < 0.0001$) and fragments (X^2

$= 10.4121$, $p = 0.0013$; $X^2 = 8.9745$, $p = 0.0027$), and no difference in the frequency of clustered seeds among sites ($X^2 = 0.3441$, $p = 0.8419$). Our results support the theory that large fruit bats (*Artibeus* spp.) are important medium and long-distance seed dispersers for *D. panamensis*, and suggest that these bats remain active dispersal agents in fragmented areas. Combined with earlier work on pollen dispersal, we confirm that pasture trees expand the genetic neighborhood for fragmented *D. panamensis* populations, and suggest that mechanisms for maintaining gene flow remain intact in a fragmented landscape.

KEY WORDS

Costa Rica, *Dipteryx panamensis*, forest fragmentation, gene flow, microsatellites, reproductive dominance, seed dispersal

INTRODUCTION

Animal-mediated seed dispersal exerts a strong influence on the distribution and dynamics of tropical forest tree communities (reviewed in Nathan & Muller-Landau 2000; Wright 2002). Dispersal limitation can promote species diversity by reducing competition and competitive advantage at recruitment sites (Hubbell *et al.* 1999), while high mortality near parent trees encourages long-distance dispersal and the wide spacing of con-specifics (Janzen 1970; Connell 1971). At the species level, dispersed seeds carry both maternal and paternal genetic information, giving seed dispersal twice the evolutionary weight as pollination in measures of gene flow (Hamilton 1999).

Disruption of seed dispersal mutualisms is predicted to alter recruitment patterns and reduce plant diversity in fragmented tropical forests (Cordeiro & Howe 2003). For individual species, evolutionary potential may erode as the loss of gene flow partitions genetic variation among increasingly isolated, inbred and vulnerable populations (Young *et al.* 1996). Low population density and high dependence on animal-vectored dispersal makes many tropical trees particularly vulnerable to these effects (Nason *et al.* 1997). With deforestation rates reaching 12 million ha annually in the tropics (F. A. O. 2000), the degree to which forest remnants maintain seed dispersal and other ecological processes has far-reaching implications for biodiversity conservation (Laurance & Bierregaard 1997).

Although widely recognized as critical, documenting seed dispersal distances in the field has proven challenging, particularly for long-distance dispersal events (Cain *et al.* 2000; Sork & Smouse 2006). The generally-predicted dispersal pattern is leptokurtic (Howe & Smallwood 1982), with the frequency of seeds dropping steeply with distance from the maternal plant to a long, narrow tail. Studies of tropical trees have modeled dispersal curves based on seed rain data (e.g. Harms *et al.* 2000), the composition of progeny cohorts (Webb & Peart 2001), and estimates of frugivore movements and gut-passage time for seeds (Westcott & Graham 2000). Idiosyncratic disperser behaviors, however, and a lack of species-specific data have hampered accurate modeling of the narrow, long-distance tail of the dispersal curve (Cain *et al.* 2000). Innovations in molecular genetics offer new tools for measuring exact dispersal distances (reviewed in Cain *et al.* 2000; Wang & Smith 2002; Sork & Smouse 2006) and estimating seed pool contributions (Grivet *et al.* 2005). Initial research has shown extensive movement of seeds from pasture trees into adjacent fragments (Aldrich & Hamrick 1998) and from continuous forest into regenerating pasture (Sezen *et al.* 2005). There is also evidence that long-distance dispersal events may be more frequent than previously expected (Hardesty *et al.* 2006).

Here we examine the effects of habitat fragmentation on seed dispersal of *Dipteryx panamensis* (Fabaceae), a canopy emergent tree considered a keystone species in the Caribbean lowland forests of Mesoamerica. Though reduced by harvest and forest clearing over much of its range, *D. panamensis* persists in fragments and pastures and remains a conservation priority. It is listed as a CITES Appendix III species (UNEP-WCMC 2006) and was recently designated as vulnerable to extinction in an evaluation of Costa Rican plant species using IUCN Red List criteria (Estrada-Chavarria *et al.* 2005). Additionally, the endangered great green macaw (*Ara ambiguus*) depends on *D. panamensis* as nesting habitat and a critical food source (Juniper & Parr 1998), a relationship that figures prominently in environmental education and conservation efforts throughout the region. Understanding the effects of forest fragmentation on the reproductive ecology of *D. panamensis* has important management and conservation implications.

This study documents seed dispersal distances in protected forest and two forest fragments in Cantón Sarapiqui, Costa Rica. We expected altered dispersal processes in fragments to be manifest in different quantities and spatial patterns of dispersed seeds. Transect-based dispersal data were augmented with genetic analysis to compare minimum and exact dispersal distances, and to look for the movement of seeds between pasture trees and forest fragments. Finally, we documented the relationship of dispersed seed locations to the distribution of large, pinnate palms favored as feeding roost sites by fruit bats of the genus *Artibeus*, the putative primary medium to long-distance dispersal agents for *D. panamensis* (Bonaccorso *et al.* 1980).

MATERIALS AND METHODS

Study Area

One protected forest and two forest fragments in Cantón Sarapiqui, Costa Rica, served as study sites for this project. La Selva Biological Station is a 2500 ha private reserve that includes 700 ha of lowland rainforest protected from logging and hunting for at least the last 20-30 years. It adjoins Braulio Carrillo National Park, forming the largest tract of intact habitat in the region (described in detail in McDade *et al.*1994). Pineda (40 ha) and Ladrillera 3 (52 ha) are privately held remnants surrounded by pastures and managed under sustainable forestry principles in partnership with the Fundación para el Desarrollo y Conservación de la Cordillera Volcánica Central (FUNDECOR), a local non-governmental organization. The fragment sites and the focal area at La Selva are separated by a mean distance of 17.9 km and lie in primary wet tropical forest at elevations of 40 -75 m, with annual rainfall of ~4000 mm (McDade & Hartshorn 1994). Ladrillera 3 and Pineda have been selectively logged, but maintain diverse canopies with populations of adult *D. panamensis* at 0.21/ha and 0.58/ha respectively. The core La Selva study area (~85 ha) is in unlogged, interior forest with adult *D. panamensis* at 0.45/ha.

Study Species

Dipteryx panamensis (Pittier) Record & Mell (Fabaceae; Papilionoideae) is a large canopy emergent tree occurring in lowland wet tropical forests of the Caribbean slope, from Colombia to Nicaragua (Flores 1992). Initial genetic analysis suggests a polyploid origin for the species, with eight of nine microsatellite markers behaving in a tetraploid fashion (Hanson *et al.* in prep.). Considered a keystone species for its copious fruit production, *D. panamensis* bears single-seeded drupes for an extended time during the low-rainfall season, outside the peak fruiting period for this forest type (Frankie *et al.* 1974). Sixteen species of mammals (Bonaccorso *et al.* 1980) and approximately 100 species of birds (Flores 1992) have been observed in and around fruiting trees. The fruit consists of a large seed encased in a thick stony endocarp and covered by a thin, fleshy mesocarp. The fruit bat *Artibeus lituratus* is thought to be the major long-distance disperser, with occasional dispersal by primates (Bonaccorso *et al.* 1980). Agoutis (*Dasyprocta punctata*), squirrels (*Sciurus* spp.), and other small mammals are primarily seed predators, but also carry out important secondary and short-distance dispersal, particularly through scatterhoarding (Bonaccorso *et al.* 1980; Forget 1993). Recruitment patterns appear to follow the inverse relationship between progeny mortality and dispersal distance predicted by Janzen (Janzen 1970) and Connell (1971) (Clark & Clark 1984), although there is evidence this relationship may be disrupted in forest fragments (Hanson *et al.* 2006). Individual trees mature and begin bearing fruit at approximately 30 cm dbh (T. Hanson, pers. obs.).

Sampling

We searched for dispersed seeds along 100 x 4 m transects (dispersal transects) with pre-selected start points and directions inside the La Selva Grid System (described in Clark 1998) (n = 42) and along GPS-referenced transects in the fragments (Ladrillera 3, n = 19; Pineda, n = 16). Transects were spatially arranged for a systematic sample of each site and their numbers reflect the relative size and shape of sites. Within each transect we located, mapped and gathered all fresh *D. panamensis* fruits, as well as their distinctive woody endocarps, which can persist on the forest floor through at least two fruiting seasons (T. Hanson, pers. obs.). Endocarps and fruits were dried immediately and stored in silica gel for later DNA extraction. Seedlings in the transects were also noted and included in dispersal estimates. Recovered endocarps had often been split, either through natural drying and germination or through foraging by seed predators. To prevent an over-estimation of dispersal rates, individual half-endocarps received only half credit in all counts of dispersal events. For the purpose of estimating seed clustering, fruits or endocarps found with 100 cm of one another were considered grouped. All dispersal transects were completed between January and April, 2005. (Note: in our descriptions below we use the term 'seeds' in reference to both dispersed endocarps and fruits).

To determine the minimum dispersal distance, *i.e.* the distance to the nearest adult tree, we calculated distance matrices between all dispersed seeds and the adult *D. panamensis* at each site. All adult trees (> 30 cm dbh) in the forest and adjacent pastures at Pineda and Ladrillera 3 were located and mapped as part of a parallel study of pollen dispersal (described in Hanson *et al.* in prep.). Trees at La Selva had been mapped as part of an ongoing, long-term demographic study (e.g. Clark & Clark 1999; 2001).

To evaluate the role of fruit bats in seed dispersal, we studied the spatial relationship between dispersed seeds and the canopy or sub-canopy palms often used as roosting sites by the genus *Artibeus* (Morrison 1978; Evelyn & Stiles 2003). At the location of each dispersed seed or group of seeds, we assessed the overstory directly (90º) above with a clinometer, noting the presence or absence of large pinnate palms, including the genera *Welfia, Iriartea, Socratea, Bactris* and *Euterpe*. This protocol was also repeated from survey points (n = 1421) located at five-meter intervals along transects to generate a systematic estimate of palm distribution for each site.

Data analysis

For this study we defined seed dispersal as the transport of diaspores away from the adult plant (Begon *et al.* 2005) and therefore restricted our analyses to seeds > 25 m from the nearest mature tree. Crowns of *D. panamensis* can be extremely large, and the area < 25 m from the base of the tree is usually carpeted with fruits and seeds that have fallen directly from the crown. Though they may have been dropped or locally re-arranged by animals, they were not considered dispersed away from the adult for the sake of this study.

Dispersed seeds were grouped into five distance classes to capture the expected leptokurtic distribution: 25-50 m, 50-75 m, 75-100 m, 100-150 m and > 150 m. We compared the number of dispersed seeds/m^2 between fragment sites and between fragments and La Selva using t-tests (two-tailed). We also compared the distribution of seeds in distance classes among sites using chi-square tests for independence, with the longest two distance classes collapsed to account for small sample sizes. To test the relationship between seed location and overstory palms at each site, we compared the frequency of palms above seed locations to the frequency of palms in the canopy using 2 x 2 contingency tables and a chi-square test. Finally, we also used contingency tables and chi-square tests to examine the prevalence of grouped vs. single seeds among sites and examined their distribution among distance classes. All statistical analyses were performed using SAS version 9.1 (©2002-2003, SAS Institute Inc.).

Genetic Markers

For genotyping adult trees and endocarps, we used nine microsatellites designed from markers for the Amazonian congener *Dipteryx odorata* (Vinson 2004). The primers, PCR characteristics and results from adult genotyping are described in detail elsewhere (Hanson *et* *al.*, in prep.). In this study we used the same extraction procedures and PCR protocols to genotype endocarps and endocarp material from whole fruits. Due to the low yields and poor quality of DNA from endocarp tissue, amplification success was extremely low (< 10%). The addition of a preamplification step designed to increase template quality (Piggott *et al.* 2004) had no effect, nor did additional sample purification through alcohol precipitation. To verify the accuracy of our limited results, we repeated the extraction process and up to four duplicate PCR runs for each successful sample. Because *D. panamensis* is a polyploid, partial heterozygotes cannot be accurately discerned from microsatellite data (Markwith *et al.* 2006). In a tetraploid, for example, the genotype AAAB produces the microsatellite result AB and cannot be differentiated from AABB or ABBB. We therefore treated our microsatellite alleles as dominant loci, creating binary genotypes following the methods of Rodzen *et al.* (2004). An overall error rate was calculated from the duplicate runs (23 samples) and only clearly reproducible genotypes were used for further analysis.

Maternity Analysis

Since endocarp material is maternally-derived, its DNA can be used to match dispersed seeds directly to their tree of origin (Godoy & Jordano 2001). We conducted a maternity analysis on the small number (n = 19) of successful endocarp amplifications by visual inspection of the genotypes and simple exclusion, comparing them to the genotypes of all adults at each site (La Selva, n = 181; Pineda, n = 52; Ladrillera 3, n = 50). To determine the veracity of each assignment, we calculated the probability of identity ($P_{(ID)}$) for each match, based on the loci used and their frequency in the adult population (Waits *et al.* 2001). $P_{(ID)}$ is the probability that a given genotype will match one drawn at random from the population, and is calculated for each dominant locus with the equation $P_{(ID)} = p^2 + (2pq)^2 + (q^2)^2$, multiplied across all loci (Waits *et al.* 2001). This theoretical $P_{(ID)}$ often underestimates observed $P_{(ID)}$ in natural populations that deviate from random mating, so we also calculated a stricter measure based on the $P_{(ID)}$ equation for full siblings ($P_{(ID)sib} = 1 - \{(3/2p)(q^2)\}$) (Waits *et al.* 2001). Maternity assignments with no genotypic mismatches and $P_{(ID)}$ scores of < 0.001 were assigned with confidence. This value (1/1000) is conservative as it is considerably lower than the number of adult plants in each study area. $P_{(ID)sib}$ scores are also reported as a guideline in the unlikely event that adult populations were made up of closely-related individuals (*i.e.* siblings). We used distance matrices to determine the exact dispersal distance for each maternity assignment, and calculated the proportion of assignments made to the closest adult tree.

RESULTS

Dispersal Transects

A total of 3016 seeds were recovered from our dispersal transects, of which 253 (8.4%) were located > 25 m from the nearest adult *D. panamensis* (La Selva n = 143; Ladrillera 3 n = 68; Pineda n = 42). We found no difference in the number of dispersed seeds/m^2 between Ladrillera 3 and Pineda ($t = 0.589$; $p = 0.588$), or between La Selva and the fragments ($t = -0.389$; $p = 0.717$). The distribution of seeds among distance classes varied significantly among all sites ($X^2 = 23.2481$; $p = 0.007$). La Selva displayed the predicted leptokurtic distribution, with the number of dispersed seeds declining rapidly as the distance from adult trees increased (Figure 2.1). The pattern was similar but more erratic in the fragments. The number of seeds peaked in the second distance class (50-75 m) at Ladrillera 3, while no seeds at all were found beyond 100 m in Pineda (Figure 2.1). The longest minimum dispersal distances from our transect data were 243.4 m at La Selva, 251.5 m at Ladrillera 3, and 87.6 m at Pineda.

We completed 1421 systematic point surveys for pinnate palm coverage in the overstory and compared the results to palm occurrence above 165 seed dispersal events. Dispersed seeds occurred disproportionately more often under palms at La Selva ($X^2 = 39.1201, p < 0.0001$), Ladrillera 3 ($X^2 = 10.4121, p = 0.0013$), and Pineda ($X^2 = 8.9745, p = 0.0027$) (Figure 2.2), suggesting an association with bat dispersal at all three sites. Similarly, the occurrence of grouped seeds common at bat feeding roosts did not vary among sites (La Selva - 29.2%, Ladrillera 3 – 26.5%, Pineda – 24.3%) ($X^2 = 0.3441, p = 0.8419$). We found grouped seeds in similar proportions in the shorter distance classes among sites, but did not observe any among seeds > 150 m from adult trees.

Maternity Analysis

Of the nine microsatellite markers used in genotyping, two failed to amplify for endocarp DNA and were discarded from the analysis (Do18, Do39). The seven remaining markers (Do3, Do5, Do8, Do17, Do20, Do 24, Do25) produced dominant genotypes of up to 57 loci (microsatellite alleles) with an overall error rate of 6.1%. Endocarps that produced repeatable genotypes for at least four markers (≥ 25 loci) were included in maternity assignment tests (n = 19). Not surprisingly, endocarps from fresh fruit provided the most reliable DNA, constituting 89.5% of successful, reproducible amplifications. DNA from older endocarps performed inconsistently or not all. Maternity was unambiguously assigned to 14 endocarps (Table 2.1). The majority of successful assignments were to one tree within the Ladrillera 3 forest fragment, although we also documented three cases of seed dispersal from adjacent pastures into fragments (Table 2.1, Figure 2.3). No dispersal events were assigned at La Selva, probably as a result the lack of fresh fruit recovered at that site (< 1.0% of dispersed seeds). Maximum dispersal distances from the maternity analysis were 355.92 m at Pineda and 852.96 m at Ladrillera 3, and the closest adult tree was the true maternal tree in only six (42.9%) of the assignments. In one instance where seeds were grouped, at least two individuals contributed to the seed pool.

Among the unassigned endocarps, four individuals lacked enough genotypic resolution to assign maternity while one from the Pineda site contained combinations of alleles not found in any potential source tree, suggesting dispersal from outside the known adult population (minimum distance = *ca*. 550 m).

DISCUSSION

Dispersal Patterns

The majority of seeds recovered in this study (91.6%) had not been dispersed > 25 m from the maternal tree, fitting the general prediction that most diaspores fall close to the source (Howe & Smallwood 1982). This concentration sets the stage for the high rates of density-dependent mortality near parent trees predicted by Janzen (1970) and Connell (1971), and observed in an earlier study of *D. panamensis* (Clark & Clark 1984). The number of seeds that were dispersed > 25 m from adult trees did not vary among sites, suggesting similar dispersal rates in fragments and protected forest. The longest minimum dispersal distances were also similar, exceeding 240 m for both forest types. These results belied our predictions, but complement two earlier studies of dispersal in fragmented landscapes. Aldrich and Hamrick (1998) found high rates of bat-mediated dispersal for the tree *Symphonia globulifera* between pastures and fragments in southeastern Costa Rica, while Chapman *et al.* (2003) noted evidence of long-distance, inter-fragment dispersal for several tree species dispersed by hornbills (*Ceratogymna subcylindricus*) and chimpanzees (*Pan troglodytes*) in Uganda. On the other hand, Cordeiro and Howe (2003) found lower dispersal and recruitment for the tree *Leptonychia usambarensis* in fragments with low abundance of its avian frugivore dispersers. Clearly, fragmentation effects on seed movement are determined by impacts on the vectors, and the dispersal agents of *D. panamensis* appear to remain active in fragmented landscapes.

Though the number of dispersed seeds did not vary, we did find differences in the distribution of seeds among distance classes. The pattern of dispersed seeds at La Selva followed the classic leptokurtic dispersal curve, with the number of seeds dropping rapidly to a ‘long tail’ in distance classes far from parent trees (Howe & Smallwood 1982; Cain *et al.* 2000). This pattern was similar but more erratic in the fragments, with higher dispersal at medium distances in Ladrillera 3 and no long distance (> 100 m) events whatsoever in Pineda. These patterns may simply reflect inherent variety among sites, but may also point to subtle changes in dispersal activity in fragments. The fauna of Neotropical forests are often highly impacted by fragmentation and hunting (Wright 2003). While we suspect that the main bat dispersers of *D. panamensis* persist in fragmented landscapes (see below), the community of terrestrial dispersers is almost certainly depauperate. Working in nearby forest fragments, Guariguata *et al.* (2002) noted few or no signs of squirrels or agoutis, both important scatterhoarders and dispersers of *D. panamensis* (Bonaccorso *et al.* 1980; De Steven & Putz 1984; Forget 1993). Changes in squirrel and agouti populations, or in the abundance of occasional dispersers including primates and peccaries (*Pecari tajacu*), may account for the less predictable distribution of seeds in fragments.

Maternity Analysis

The limited amount of viable DNA recovered from endocarps severely restricted this analysis, but we did gain useful insights from the small number of successful assignments. In more than half of these dispersal events, the true source tree was not the nearest tree. Hardesty *et al.* (2006) found a similar but more pronounced pattern for *Simarouba amara* at Barro Colorado Island, Panama, where only 8.2% of seedlings assigned with the highest confidence came from the nearest tree. These results imply that minimum dispersal distances from our transect data (up to 251.5 m) regularly underestimated the actual movement of seeds. The maximum exact dispersal distance from our genetic data, for instance, was 852.96 m, over three times as high as the upper estimate from transect data.

Maternity analysis also documented three dispersal events from pasture trees into fragments, in one case crossing an intervening river. Additionally, we documented one seed at Pineda from an unknown pasture or fragment tree outside the study area (min. distance 550 m). From such a limited sample, these results strongly suggest that long-distance seed dispersal is relatively common for *D. panamensis* in fragmented landscapes. Aldrich and Hamrick (1998) reported frequent long-distance movement of seeds from pastures into fragments, where the seedling cohort was so dominated by a few fecund pasture trees that it represented a potential genetic bottleneck. We too found evidence of reproductive dominance, but in a forest tree at the Ladrillera 3 fragment (84.6% of all assignments for that site). It's possible that this individual simply had the freshest fruits at the time of our sampling, allowing a greater number of successful DNA extractions. The same tree, however, exhibited dominance in pollen contributions (23.1% of all outcrossing events) in a parallel study of pollen dispersal (Hanson *et al.*, in prep.). Reproductive dominance has also been documented in the colonization of abandoned pasture by the canopy palm *Iriartea deltoidea* (Sezen *et al.* 2005), but was not noted in two studies conducted in primary forest (Hardesty *et al.* 2006 and reference therein). Our data are too limited to draw conclusions, but excessive reproductive dominance has the potential to counter the positive genetic effects of long-distance seed and pollen dispersal.

Bat-mediated Dispersal

Artibeus lituratus has been mist-netted while carrying *D. panamensis* fruit and is assumed to be a major medium to long-distance seed disperser (Bonaccorso *et al.* 1980; Flores 1992). Though smaller, *A. jamaicensis* also visits *D. panamensis* and may be capable of occasionally carrying its fruit (Bonaccorso *et al.* 1980). When foraging, these bats generally remove fruit to feeding roosts where they are less vulnerable to owls, bat hawks and other predators (Morrison 1978). There they consume the fleshy mesocarp and drop the unopened endocarps below. Two studies have noted that *Artibeus* bats often choose large pinnate palms as feeding roosts, perching under the midrib of a leaf where they are out of sight from avian predators, and where any terrestrial predator would inadvertently shake the frond in warning as it climbed (Morrison 1978; Evelyn & Stiles 2003). Although many bat species are adversely affected by habitat fragmentation (Cossons *et al.* 1999), both *A. lituratus* and *A. jamaicensis* have been captured repeatedly in highly-fragmented landscapes within 25 km of our study sites (Esquetini 2006) and are known from other agriculturally dominated landscapes in the region (Medina *et al.* in press). Our data show a clear correlation between the location of dispersed seeds and the location of large, pinnate palms in the overstory at all sites, strongly suggesting bat-mediated dispersal. The frequency of dispersed seeds in distance classes away from adult trees also complements known *Artibeus* behaviors. Morrison (1980) found that foraging *A. lituratus* make multiple trips to feeding roosts located < 100 m from fruiting trees, and periodically move to roosts farther away (150 - 350 m) to rest. *A. jamaicensis* are known to transport fruit up to 750 m to day roost areas (Morrison & Morrison 1981), and *A. lituratus* are capable of even longer dispersals (D. Morrison, pers. comm.).

Artibeus bats often use a feeding roost repeatedly, dropping one or more seeds together before moving on (Morrison 1978). Clustered seeds accounted for roughly a quarter of all dispersed seeds at our sites, another likely indication of bat-mediated dispersal. Agoutis scatterhoard seeds singly and bury them (De Steven & Putz 1984; Forget 1993), while squirrel and rodent-cached seeds are also typically out of sight (Forget 1993), not lying plainly in the open as ours were encountered. Also, while agoutis and squirrels have been sighted carrying seeds up to 100 m from adult trees (Bonaccorso *et al.* 1980) and peccaries will occasionally transport undamaged endocarps (T. Hanson, pers. obs.), these animals are primarily short-distance (< 25 m) dispersers. Their scarcity in disturbed habitat makes an even stronger case for bats as the main dispersal agents at our fragment sites.

Gene flow

Previous work at these same sites indicated longer pollen dispersal distances in pastures and fragments than at La Selva, and pointed to high levels of pollen exchange between pasture and fragment trees (Hanson *et al.*, in prep.). Here we note seed movement from pasture individuals into fragments, further evidence that pasture trees expand the genetic neighborhood of trees fragment populations. Maximum pollen dispersal distances observed at these sites (1093 m) (Hanson *et al.*, in prep.) are similar to the maximum seed dispersal distance we documented from maternity analysis (853 m). Given that seeds carry twice the genetic information as haplotypic pollen (Hamilton 1999), this suggests a potentially stronger role for seed dispersal in overall patterns of gene flow, a trend recently reported for another Neotropical tree, *Simarouba amara* (Hardesty *et al.* 2006).

The capacity for *D. panamensis* to maintain gene flow in fragmented landscapes appears high, but it should be noted that the benefits of pasture trees will be ephemeral in the absence of replanting or otherwise maintaining pasture populations. The role of reproductive dominance in disturbed landscapes (e.g. Aldrich & Hamrick 1998, Sezen *et al.* 2005) merits further investigation, however, since the bottleneck effect of a few highly-fecund individuals may counteract any positive impact of high pollen and seed dispersal.

Recruitment Environment

While our results suggest robust seed movement for *D. panamensis* in protected forest and fragments, dispersal alone does not determine the recruitment and spatial distribution of future generations. In a parallel study, we noted lower indications of seed predation and higher seedling densities in these same fragments than at La Selva, a likely result of changes in the mammalian seed predator community (Hanson *et al.* 2006). Earlier work at La Selva found that *D. panamensis* conformed to the Janzen-Connell dispersal model (Janzen 1970, Connell 1971), showing clear signs of density-dependant mortality near adults and a reliance on seed dispersal for recruitment (Clark & Clark 1984). This system may be eroding in the fragments, however, where dense carpets of seedlings often occur directly beneath adult crowns (Hanson *et al.* 2006). De Steven and Putz (1984) also noted carpets of regeneration under *D. panamensis* in a forest exposed to hunting in Panama, while other studies point to widespread changes in plant community structure in fragmented and faunally depauperate landscapes (e.g. Dirzo & Miranda 1991; Chapman & Chapman 1995). In the context of such an altered recruitment environment, the role of seed dispersal in the spatial distribution of future generations remains unclear. Studies are needed that follow *D. panamensis* seed fate through the entire cycle, from dispersal through germination and establishment.

CONCLUSIONS

Our results point to similar rates of seed dispersal for *D. panamensis* in protected forest and forest fragments and strongly suggest that fruit bats are the primary medium to long-distance dispersal agents at all sites. Though limited in scope, the exact dispersal events from maternity analysis greatly enhanced the data, indicating seed flow from pastures to fragments and underscoring the disparity between minimum estimates from transects and true dispersal distances. There is some indication of reproductive dominance and erratic dispersal activity in fragments, but gene flow via seeds for *D. panamensis* appears relatively high in the fragmented landscape. These results suggest that some tree species can maintain connectivity and evolutionary potential in fragmented landscapes, provided their dispersers can adapt to the altered landscape. Future seed dispersal efforts should expand the use of maternity analysis to other tropical trees, examine trends in reproductive dominance, and be extended across longer time frames to better elucidate the full dispersal cycle, from deposition through recruitment.

ACKNOWLEDGEMENTS

This work was supported by NSF-IGERT Grant No. 0114304, with additional assistance from the DeVlieg Foundation and the Organization for Tropical Studies. The authors thank Jo Ellen Force, Nilsa A. Bosque-Pérez and Olle Pellmyr for their input, and gratefully acknowledge the help of the many field assistants, laboratory colleagues and landowners who made the project possible.

Table 2.1 Seed dispersal distances for *D. panamensis* from exact genotype matches between maternal endocarp tissue and adult source trees in two forest fragments, Canton Sarapiqui, Costa Rica.

Seed ID	Site (habitat)	Maternal Tree (habitat)	Dispersal Distance (m)	# Loci*	# Genotype Mismatches	$P_{(ID)}$§	$P_{(ID)sib}$¥
PSD15	Pineda (fragment)	P38 (pasture)	355.92	25	0	0.0003	0.080
L3SD3	Ladrillera 3 (fragment)	L31 (fragment)	176.06	46	0	<0.0001	0.004
L3SD4	Ladrilela 3 (fragment)	L31 (fragment)	178.14	46	0	<0.0001	0.004
L3SD6B	Ladrillera 3 (fragment)	L31 (fragment)	70.60	46	0	<0.0001	0.004
L3SD6C	Ladrillera 3 (fragment)	L31 (fragment)	70.60	38	0	<0.0001	0.007
L3SD6E	Ladrillera 3 (fragment)	L31 (fragment)	70.60	38	0	<0.0001	0.007
L3SD6H	Ladrillera 3 (fragment)	L31 (fragment)	70.60	46	0	<0.0001	0.004
L3SD10	Ladrillera 3 (fragment)	L31 (fragment)	38.28	46	0	<0.0001	0.004
L3SD15A	Ladrillera 3 (fragment)	L31 (fragment)	38.74	34	0	<0.0001	0.007
L3SD15B	Ladrillera 3 (fragment)	L341 (pasture)	852.96	29	0	0.00019	0.044
L3SD18C	Ladrillera 3 (fragment)	L31 (fragment)	69.05	38	0	<0.0001	0.007
L3SD19C	Ladrillera 3 (fragment)	L31 (fragment)	74.14	46	0	<0.0001	0.004
L3SD20	Ladrillera 3 (fragment)	L31 (fragment)	142.24	46	0	<0.0001	0.004
L3SD21	Ladrillera 3 (fragment)	L349 (pasture)	567.82	26	0	0.00014	0.041

*Loci are microsatellite alleles used in dominant fashion for polyploid analysis (Rodzen *et al.* 2004)

§$P_{(ID)}$ – probability of identity (Waits *et al.* 2001)

¥$P_{(ID)sib}$ – probability of identity in the extreme case of full siblings (Waits *et al.* 2001)

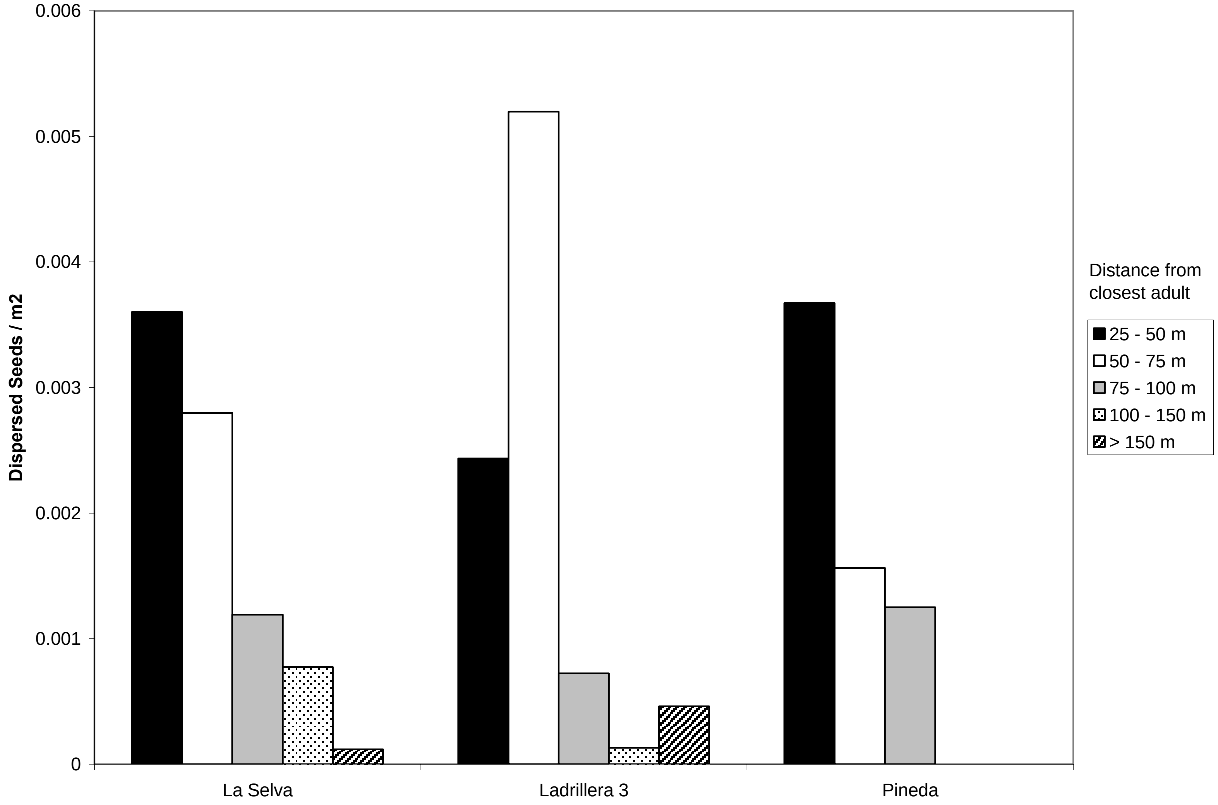

Figure 2.1 Dispersed seeds of *D. panamensis* in distance classes from the closest adult trees. Data are from surveys of protected forest and two forest fragments in Cantón Sarapiqui, Costa Rica. Proportions of seeds in distance classes vary significantly among sites ($X^2 = 23.25$, $p = 0.007$).

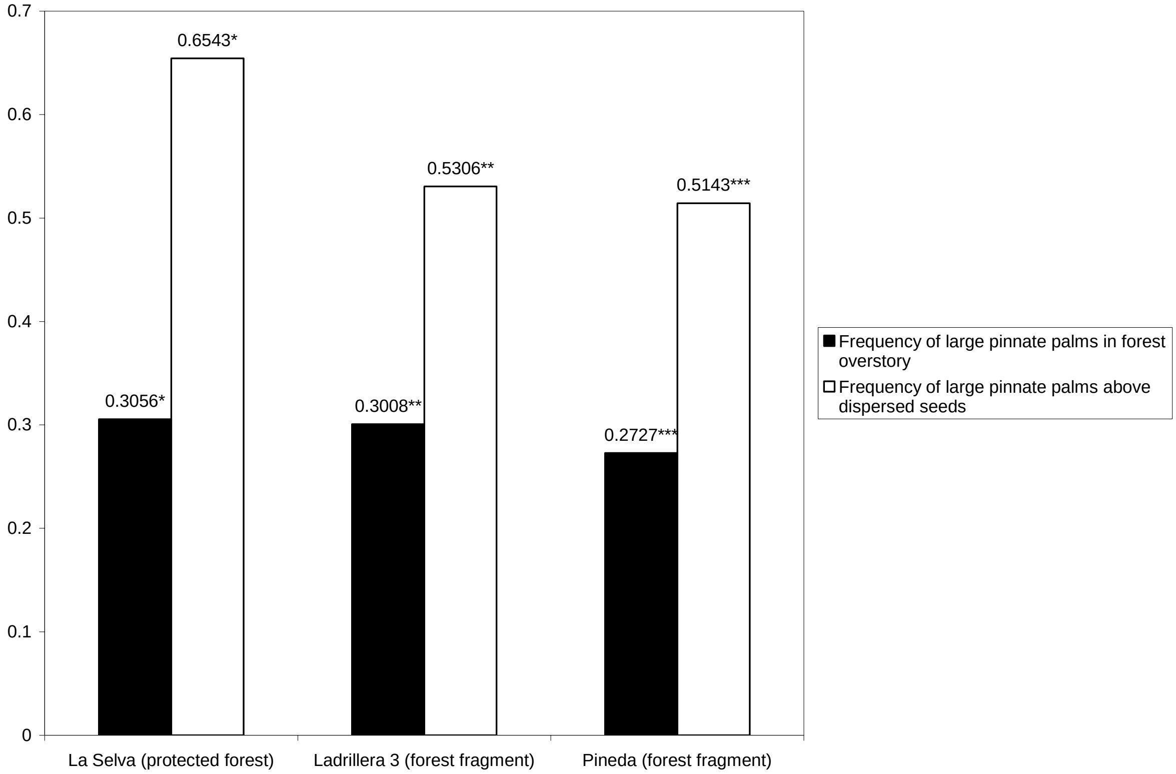

Figure 2.2 Frequency of large pinnate palms (e.g. *Welfia, Iriartea, Socratea, Euterpe*) above systematically distributed points in the forest overstory (n = 1427) and in the overstory directly above dispersed *D. panamensis* seeds (n = 165) at three sites in Cantón Sarapiqui, Costa Rica. ($^{*}X^2 = 39.1201$, $p < 0.0001$; $^{**}X^2 = 10.4121$, $p = 0.0013$; $^{***}X^2 = 8.9745$, $p = 0.0027$).

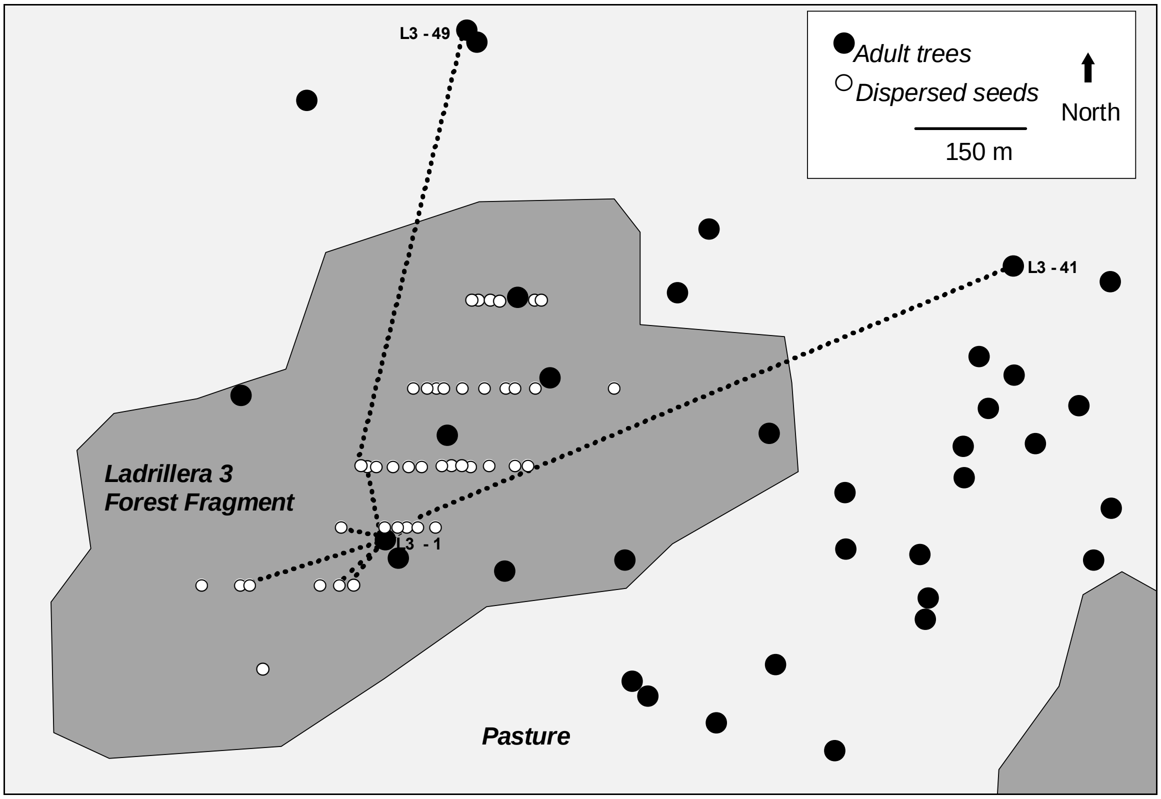

Figure 2.3 Location of adults and dispersed seeds of *D. panamensis* in the Ladrillera 3 forest fragment and adjacent pasture. Dispersal events confirmed by maternity analysis are indicated by dashed lines.

CHAPTER THREE: Variation in Seedling Density and Seed Predation Indicators for the Emergent Tree *Dipteryx panamensis* in Continuous and Fragmented Rainforest

Thor Hanson
Department of Forest Resources
University of Idaho
P.O. Box 441133
Moscow, Idaho 83844-1133
thor@rockisland.com

Steven Brunsfeld
Department of Forest Resources
University of Idaho
P.O. Box 441133
Moscow, Idaho 83844-1133

Bryan Finegan
Departamento de Recursos Naturales y Ambiente
Centro Agronómico Tropical de Investigación y Enseñanza (CATIE)
7170 Turrialba
Costa Rica

ABSTRACT

Seedling density and the condition of stony endocarps of the tree *Dipteryx panamensis* were assessed in protected continuous forest and two forest fragments exposed to hunting and selective logging. Seedling density was higher in forest fragments than in continuous forest, while more whole endocarps and fewer chewed and half endocarps were found in fragments, indicating lower seed predation at fragment sites. These findings appear to contradict two earlier *D. panamensis* studies and we discuss methodological differences that could account for our disparate results. Hunting and fragmentation effects on mammal populations are suggested as a cause for the altered recruitment pattern in fragments.

KEYWORDS

Costa Rica; *Dipteryx panamensis*; forest fragmentation; hunting; seedling density; seed predation; wet tropical forest.

INTRODUCTION

Forest loss in the tropics exceeds 15 million hectares annually (Whitmore 1997), leaving behind a mosaic of forest patches in a human-dominated matrix (Dale & Pearson 1997; Laurance & Bierregaard 1997). Many remnants exist on private land and face continued pressure from hunting and resource extraction. It can be challenging to differentiate between strict fragmentation effects and the influence of ongoing human activities (Terborgh 1992), but forest fragments typically experience significant changes in their faunal communities (Peres 2001). Hunters often target known seed predators and seedling browsers, including forest ungulates and large-bodied rodents (Peres 2001). Hunting pressure has been implicated in altered rates of seed predation for tropical trees, either through direct loss of important frugivores and granivores (De Steven & Putz 1984; Wright & Duber 2001; Wyatt & Silman 2004), or through the ecological release of non-target species (Guariguata *et al.* 2002). Spatial patterns of seed and seedling mortality are thought to influence the high diversity and spatial heterogeneity of tropical forest tree communities (Janzen 1970; Connell 1971). Changing these patterns and processes will probably change forest community characteristics and may exacerbate the long-term loss of species diversity in fragmented and disturbed tropical forest (Putz *et al.* 1990; Terborgh 1992; Wright & Duber 2001).

Dipteryx panamensis (Pittier) Record & Mell (Fabaceae; Papilionoideae) is a large canopy emergent tree occurring in lowland wet tropical forests of the Caribbean slope, from Colombia to Nicaragua (Flores 1992). Considered a keystone species for the timing and quantity of its fruit production, *D. panamensis* bears single-seeded drupes during the low-rainfall season when few other trees are in fruit (Frankie *et al.* 1974). The seeds are encased in a thick woody endocarp that can persist on the forest floor for two years or more (pers. obs.). Primary seed dispersal is by frugivorous bats and occasionally primates or other mammals (Bonaccorso *et al.* 1980). Known seed predators include agoutis (*Dasyprocta punctata*), squirrels (*Sciurus* spp.), peccaries (*Tayassu* spp.) and the great green macaw (*Ara ambigua*) (Bonaccorso *et al.* 1980; Flores 1992). Scatterhoarding by agoutis probably also contributes to seed dispersal (Bonaccorso *et al.* 1980, Forget 1993). The very high seed and seedling mortality near parent trees (De Steven & Putz 1984; Clark & Clark 1984) may promote wide spacing in natural populations (Bonaccorso *et al.* 1980; Clark & Clark 1984). Though reduced by harvest and forest clearing over much of its range, *D. panamensis* persists in fragments and pastures and remains a conservation priority. It is listed as a CITES

Appendix III species (UNEP-WCMC 2006) and was recently designated as vulnerable to extinction in an evaluation of Costa Rican plant species using IUCN Red List criteria (Estrada Chavarría *et al.* 2005). Understanding the effects of forest fragmentation on seed predation and seedling recruitment has important management and conservation implications.

This study addresses two questions: 1.) Does the density of seedlings differ between continuous forest and medium-sized forest fragments? 2.) Do the proportions of whole, half and chewed endocarps vary between continuous forest and medium-sized fragments? Seeds and seedlings can experience different predation pressures when dispersed away from adult trees than when dispersed close to or beneath adults (Janzen 1970; Connell 1971). To assess both situations, our research questions are addressed at a distance > 20 m from all potential parents, as well as directly below adult crowns. With the expectation that fragmented forests contain reduced populations of known *D. panamensis* seed predators, we predict higher seedling density and lower seed predation at forest fragment sites.

METHODS AND MATERIALS

Study Area

One continuous forest and two medium-sized fragments in northeastern Costa Rica served as the study sites for this project. La Selva Biological Station (1600 ha) adjoins Braulio Carrillo National Park, forming the largest intact forest in the region (described in detail in McDade *et al.* 1994). Pineda (40 ha) and Ladrillera 3 (52 ha) are privately held remnants surrounded by pastures and managed under sustainable forestry principles in partnership with the Fundación para el Desarrollo y Conservación de la Cordillera Volcánica Central (FUNDECOR), a local non-governmental organization. The fragment sites and the focal area at La Selva are separated by a mean distance of 17.9 km, lying in primary wet tropical forest at elevations of 40 -75 m, with annual rainfall of ~4000 mm (measured at La Selva) (McDade & Hartshorn 1994). Ladrillera 3 and Pineda maintain diverse canopies with populations of adult (>30 cm dbh) *D. panamensis* at 0.21/ha and 0.58/ha respectively, determined by extensive ground surveys and GPS-mapping of all adult individuals at each site. Personal observations of hunting activity and conversations with landowners and local residents indicate that both fragments experience continuous hunting pressure. The focal study area at La Selva (~85 ha) is in unlogged continuous old-growth forest protected from hunting for at least the last 20-30 years, with adult *D. panamensis* populations at 0.45/ha. Adult density and location data at La Selva are from an ongoing demographic study by Clark and Clark (1984; 1985; 1987; 2001).

Sampling

To assess predation pressures away from adult trees, endocarps and seedlings were surveyed within 100 x 4 m transects placed at pre-selected start points and directions within the La Selva Grid System (described in Clark 1998) (n = 33) and along GPS-referenced transects in the fragments (Ladrillera 3, n = 16; Pineda, n = 12). These transects were spatially arranged for a systematic sample of each site and their numbers reflect the relative size and shape of sites. Transects passing within 20 m of the bole of an adult *D. panamensis* were discarded. All *D. panamensis* seedlings in the transects were measured at the base of the stem and classified as < 5 mm or 5 – 10 mm in diameter, following size classes established by Clark and Clark (1987). All *D. panamensis* endocarps were individually inspected and classified as either whole, half, or chewed. Whole endocarps included those with the dorsal and ventral sutures intact and no markings breaching the inner seed cavity. They were considered non-predated. Chewed endocarps were empty and bore the piercing tooth marks of agoutis, squirrels or other animals. They were considered predated. Half endocarps included those split along the sutures, which could occur through germination or natural drying, or through predation by peccaries or great green macaws. Their predation status is discussed below. Transects away from adult trees were carried out from January – March, 2005 at La Selva, and in the fragments from February – April, 2005.

To assess predation pressures near adult trees, *D. panamensis* seedling density and endocarp condition were similarly surveyed in a second set of transects located directly under the crowns of adult *D. panamensis* (La Selva, n = 10; Ladrillera 3, n = 4; Pineda, n = 3). These transects measured 20 x 4 m and were sited to radiate in randomly-selected directions from the trunks of focal trees. Focal trees were chosen to reflect similar site conditions: closed-canopy interior forest on alluvial soils with moderate slope (< 10%). All near-adult transects were completed in July, 2005.

Data Analysis

Limited sample size prevented separate analysis of seedling size classes, so count data for both classes were combined and compared between La Selva and the two fragment sites using Wilcoxon signed-rank tests. Count data for endocarp condition at La Selva and the fragments were compared using Chi-Square Tests for Independence. Data from near-adult and away-from-adult sampling were analyzed separately. All analyses were performed with SAS software, version 9.1 (©2002-2003, SAS Institute Inc.).

RESULTS

Density of seedlings was significantly higher in the fragments than at La Selva near adult trees ($Z = 3.6456$, $P = 0.003$), and trended higher away from adults ($Z = 1.8148$, $P = 0.0696$) (Figure 3.1.) The number of half, whole and chewed endocarps differed between La

Selva and the fragments near adults ($X^2 = 630.829$, $P < .0001$) and away from adults ($X^2 = 56.491$, P <.0001) (Figure 3.2). In both cases chewed endocarps and half endocarps were more common at La Selva, while more whole endocarps were found at fragment sites. Though not analyzed separately, large seedlings (5 – 10 mm diam) were present in fragments both near adults (n = 21) and away from adults (n = 7), while none were encountered anywhere at La Selva. All trends between La Selva and the fragments were similar near and away from adult trees, suggesting causal factors that affect both settings.

DISCUSSION

Results of this study support the premise that forest fragmentation and human disturbance can strongly influence seed predation and seedling recruitment. Hunting pressure is particularly high in this landscape (pers. obs.), and Guariguata *et al.* (2000; 2002) found a depauperate mammal fauna in other fragments nearby. They noted few or no signs of squirrels, agoutis, or peccaries, three of the most important *D. panamensis* seed consumers. The lower proportion of chewed endocarps and the increase in whole endocarps in the fragments met our expectations and suggest less seed predation in Pineda and Ladrillera 3 than at La Selva (Figure 3.2). Half-endocarps can result from seed predation, but may also be the product of natural splitting during germination. Guariguata *et al.* (2002), however, found no difference between germination rates for surface-sown or buried *D. panamensis* seeds in nearby fragments and at La Selva, while De Steven and Putz (1984) found similar germination rates among surface sown laboratory treatments and surface-sown and buried field treatments. Assuming similar germination patterns among our sites, the reduced number of half-endocarps in the fragment site may again indicate less seed predation, particularly by peccaries. Data from our seed predation indicators represent at least two fruiting periods, due to the long persistence of *D. panamensis* endocarps on the forest floor.

Altered mammal populations also offer an explanation for the higher densities of *D. panamensis* seedlings found at our fragment sties (Figure 3.1). In a review of hunting effects on vertebrate and plant populations, Wright (2003) supported this view, noting that more seeds may survive to germination in defaunated sites, and that post-germination survival may benefit from reduced vertebrate herbivory on seedlings. Alternatively, among-site differences in seedling density could simply be a reflection of site-specific adult density. But in our study we found higher seedling densities in the fragments, where the adult density was similar to or lower than at La Selva. The presence of larger (5-10 mm diam) seedlings in the fragments suggests that this increased density may be more than an ephemeral trend. These larger, more robust individuals may represent fruiting events several years in the past, as Clark and Clark (1987) found that a five-year-old cohort of *D. panamensis* seedling averaged only 5 mm in diameter.

Our results agree with several recent studies that have documented decreased seed predation in heavily hunted and fragmented forests (Wright & Duber 2001; Wyatt & Silman 2004). Dirzo and Miranda (Dirzo & Miranda 1991) noted greatly reduced herbivory in a hunted Mexican rainforest, accompanied by high seedling density and low understory diversity. De Steven and Putz (1984) observed lower seed predation and increased seedling recruitment at sites in Panama exposed to hunting. Wright *et al.* (Wright *et al.* 2000) found altered seed predation patterns and increased seedling density for two palm species in forests with increased levels of hunting. Other research, however, has shown an increase in seed predation in fragments (Guariguata *et al.* 2002) and increased seed predation and seedling predation on small islands (Asquith *et al.* 1997; Guariguata *et al.* 2002).

Working in the same landscape as the present study, Guariguata *et al.* (2002) reached nearly opposite conclusions for *D. panamensis*, noting higher seed predation rates in fragments than at La Selva. They suggested that hunting pressure on larger mammals may have allowed an ecological release of smaller (< 1 kg) seed consumers, which follows the Asquith *et al.* (1997) observation of high seed predation rates for *D. panamensis* on islands in Panama where spiny rats (*Proechimys semispinosus*) were the only resident fruit and seed consumers. The disparity between these results and the present study may arise in part from differences in methodology. Both Guariguata *et al.* and Asquith *et al.* defined seed predation as the removal of whole endocarps that were experimentally placed on the forest floor, which may overestimate the contribution of small mammals that eat the fleshy mesocarp but do not breach the endocarp to reach the seed, or that scatterhoard endocarps intact, some of which may later be forgotten. Conversely, our technique of assessing endocarp damage may underestimate predation by mammals that bury or cache endocarps out of sight.

Additional findings from these studies, however, also point to high predation pressure in fragments and on islands and cannot be explained by methodological differences. Asquith *et al.* (1997) followed the fate of *D. panamensis* seedlings at their small island sites and found extremely high rates of mortality from herbivory, presumably by spiny rats. Guariguata *et al.* (2002) measured seed dispersal and individual seed survival, noting higher seed dispersal and survival rates at La Selva than in fragments. Many authors have noted the complexity of interactions between defaunation and plant populations, suggesting that the impacts may be specific to the sites and biota involved (Asquith *et al.* 1997; Guariguata *et al.*

2002; Wright 2003; Wyatt & Silman 2004). Site and biota variation may be a factor here, as the fragments used by Guariguata *et al.* were considerably larger (> 110 ha) than those used in our study (< 55 ha), while the islands studied by Asquith *et al.* have experienced major ecological shifts since their isolation from continuous forest (Putz *et al.* 1990), and may not be comparable with mainland fragments.

We suggest that hunting and fragmentation effects on mammalian seed predator populations offer a credible explanation for our results, but other factors may be involved. Natural fluctuations in seed predator abundance can also impact predation rates and seedling recruitment (Asquith *et al.* 1997; DeMattia *et al.* 2004). While our data reflect at least two fruiting periods, they may still represent a temporary disparity between predation activity in La Selva and the fragments. The timing of our sampling effort may also be relevant. At Barro Colorado Island in Panama, fruits mature from mid-December through early March (Bonnacorso *et al.* 1980) and seedling germination peaks several weeks after heavy rain events in late March or April (De Steven & Putz 1984). Our transects away from adults were conducted from mid-January through early April, so it is possible that early sampling did not reflect a full season's fruit crop and that fruits had not long been exposed to predators, while later sampling may have captured more fruit-of-the year seedlings. Endocarps from previous years would not be affected by this variation, nor would the near-adult transects, which were all carried out in July. Reduced predation activity may account for the higher seedling density we observed in fragments, but *D. panamensis* seedling survival is also influenced by light conditions (De Steven & Putz 1984; De Steven 1988), invertebrate herbivory and litterfall (Clark & Clark 1985) and fungal pathogens (De Steven & Putz 1984). Further research is needed on the influence of these factors in fragments, as well as on site-specific predator abundance and compensatory predation rates that could clarify our results in light of previous work.

CONCLUSIONS

Seed and post-germination predation by mammals has been called the most significant barrier to seedling recruitment for *D. panamensis* (De Steven & Putz 1984). The higher density of seedlings in fragments suggests that this barrier may be breaking down, both near and away from adult trees, while the presence of larger individuals suggests it is more than an ephemeral trend. If so, it has implications for the spacing and genetic diversity of *D. panamensis* populations and may negatively impact the diversity of forest fragment tree communities.

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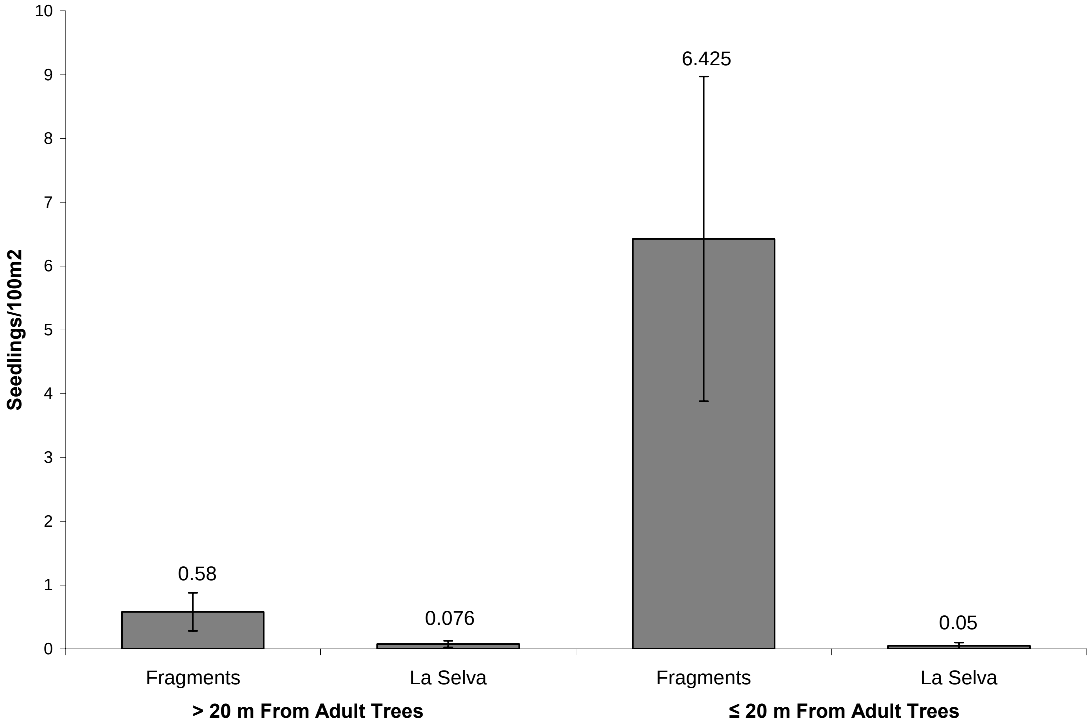

Figure 3.1 Mean number of Dipteryx panamensis seedlings per 100 m2 sampled away from adult trees (> 20 m) and near adult trees (≤ 20 m) in forest fragments and at La Selva Biological Station, Costa Rica. Error bars indicate one SE; numbers indicate mean seedlings/100 m2.

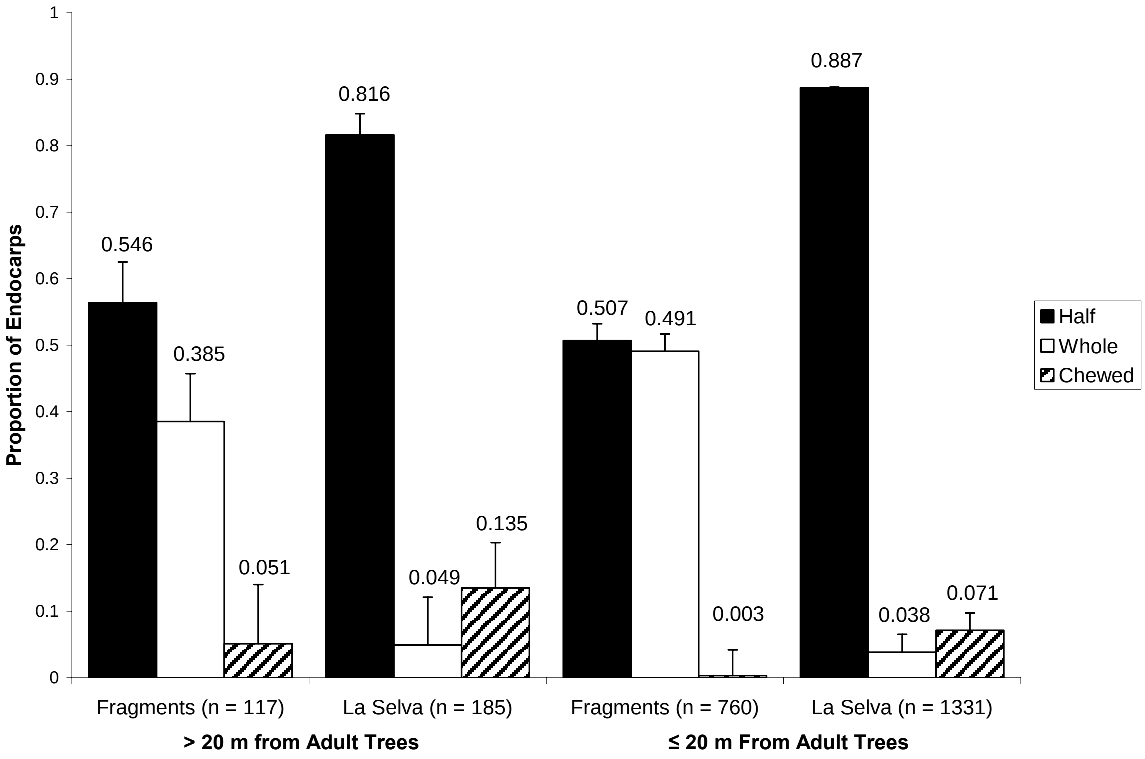

Figure 3.2 The proportions of whole, half and chewed endocarps of *Dipteryx panamensis* encountered away from adult trees (> 20 m) and near adult trees (≤ 20 m) in two forest fragments and at La Selva Biological Station, Costa Rica. Error bars indicate one SE; numbers indicate exact proportions; n = number of endocarps.

DISSERTATION CONCLUSIONS

SUMMARY

The studies in this dissertation consistently point to high rates of both pollen and seed-mediated gene flow for *D. panamensis* in fragmented landscapes. Mean pollen dispersal distances increased in fragments, pastures and isolated pastures, while seed dispersal appeared largely the same in fragments and protected forest. Pollen dispersal results suggest a change in the behavior or community of pollinators in fragmented areas, while rates of bat-mediated seed dispersal were similar in all sites. Pollen and seeds move extensively between pastures and adjacent forest fragments, enlarging the genetic neighborhood for isolated fragment populations and increasing landscape-level connectivity. These benefits of pasture trees will be ephemeral, however, without active replanting or otherwise ensuring the regeneration of pasture populations.

Though genetic diversity of adult and progeny generations was similar in all sites, self-pollination increased for pasture and isolated pasture trees and there were signs of increased genetic structure among their progeny. This trend suggests a risk of long-term genetic erosion in pasture populations, which could also impact adjacent forest through subsequent dispersal of seeds and pollen. We found reproductively dominant individuals in protected forest, fragment and pasture populations, though there was no clear trend towards the overwhelming dominance seen in other studies (Aldrich and Hamrick 1998; Sezen *et al.* 2005).

High rates of gene flow appear to bolster *D. panamensis* against the negative genetic consequences of fragmentation, a pattern observed for several other tropical trees (reviewed in Sork & Smouse 2006). Changes in seed predation and seedling density, however, point to an altered recruitment environment in fragments with negative implications for species diversity. Low predation indicators and increased seedling density in fragments suggest that density-dependent barriers to recruitment may be breaking down. This pattern could lead to mono-specific seedling cohorts near adults and an overall decline in species diversity for fragmented tree communities.

FUTURE DIRECTIONS

In pursuing our original project objectives, we opened doors to a range of additional research questions. All aspects of this study would benefit from a broader temporal scale to extend our observations over multiple flowering and fruiting seasons. Seed dispersal in particular merits a longer-term effort, following seed cohorts through the entire cycle from dispersal through germination and recruitment. It should be noted that our work dealt primarily with potential gene flow. We documented seed and pollen dispersal in various landscapes, but, as our seed predation results indicate, other factors may determine which progeny are actually recruited into the next generation. Specific impacts of fragmentation on recruitment and the relationship between potential and actual gene flow should be further explored.

The presence of reproductively dominant individuals has now been noted in several studies and should be investigated in other species and in other populations, both fragmented and undisturbed. If common, such “alpha trees” have important implications for the effective population size, genetic diversity and evolutionary potential of tropical tree populations.

Methodologically, the application of maternity analysis to endocarp material was problematic for *D. panamensis*, but merits further effort in tropical systems. Concentrating on fresh tissue and looking for other species with likely seed characteristics offers hope of expanding the use of this promising technique.

Collaborative opportunities are being pursued with several other ongoing studies. A doctoral candidate at Duke University has developed multispectral images of *D. panamensis* crowns throughout the San Juan-La Selva Biological corridor (S. Chun, pers. comm.). Combining these images with pollen and seed dispersal data offers the chance to predict gaps in gene flow and set conservation and management priorities for *D. panamensis* populations in the corridor. Christina Vinson, who developed the microsatellites used in this project, is now at Oxford continuing her work on *D. odorata*, and we have discussed collaborating on ploidy history and segregation patterns within the genus. Finally, our study area at La Selva overlaps with the TREES project founded by David and Deborah Clark. Combining genetic data with over twenty years of demographic data for *D. panamensis* presents a rare chance to explore the connections between relatedness, recruitment, growth rates and other factors.

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APPENDIX A: Soil and Site Characteristics Influence the Diversity and Composition of Palouse Prairie Plant Communities

Thor Hanson
Department of Forest Resources
P.O. Box 441133
University of Idaho
Moscow, ID 83844-1133

Yaniria Sanchez-de Leon
Division of Soil and Land Resources
Department of Plant, Soil and Entomological Sciences
P.O. Box 442339
University of Idaho
Moscow, ID 83844-2339

Jodi Johnson-Maynard
Division of Soil and Land Resources
Department of Plant, Soil and Entomological Sciences
P.O. Box 442339
University of Idaho
Moscow, ID 83844-2339

Steve Brunsfeld
Department of Forest Resources
P.O. Box 441133
University of Idaho
Moscow, ID 83844-1133

ABSTRACT

The structure of grassland communities can vary widely in response to heterogeneous habitat variables. In this study we document the plant communities, soil types and site characteristics of twelve Palouse prairie remnants in southeastern Washington and northern Idaho. General linear models were used to test the predictive value of six biophysical variables (slope, aspect, distance from edge, edge type, vegetation structure and soil type) on three plant community metrics: species richness, Simpson's diversity index and the dominance of exotic species. All models included a class variable accounting for inherent site-level variability and we used Akaike's Information Criterion (AIC) to select the best model for each metric. Site, soil type and aspect had significant effects in all models, while vegetation structure was also a significant predictor of species richness and diversity. Distance from the edge had predictive value for species diversity, while distance from edge and the type of adjacent matrix habitat influenced the dominance of exotic species. Additional soil sampling failed to clarify the relative importance of soil characteristics on species richness and diversity, but the depth of the A-horizon and depth to restrictive layer had significant impacts on the dominance of exotic species. This study confirms the importance of biophysical characteristics in shaping local plant community structure and offers tools to help set research and conservation priorities for isolated and un-inventoried Palouse prairie remnants.

KEYWORDS

Palouse prairie, grasslands, plant communities, soil type, mixed-effects linear models, plant-soil interactions, exotic species

INTRODUCTION

Habitat heterogeneity exerts a strong influence on the structure of biotic communities, affecting species composition and diversity at multiple scales (Greig-Smith 1979; Steiner & Kohler 2003; Bennett *et al.* 2006). Individual species respond to a range of biophysical variables, often forming distinct assemblages where their habitat suitability overlaps (Fischer *et al.* 2004). Though patterns vary by taxa (Atauri & de Lucio 2001), physically diverse landscapes typically support a wider range of communities, increasing levels of regional diversity (e.g. Pino *et al.* 2000; Wilsey *et al.* 2005). Even subtle changes in environmental variables can shift local species composition (e.g. Huenneke *et al.* 1990; Steinauer & Collins 1995; Bowles *et al.* 2005) and lead to higher site-level diversity. Finding links between biophysical characteristics and species composition is a fundamental aspect of understanding and managing biodiversity in complex landscapes.

Worldwide declines in native grasslands have led to increased research and conservation attention (Samson & Knopf 1996; White *et al.* 2000). Recent studies of North American prairies have focused on relict populations (Wilsey *et al.* 2005), invasive species (e.g. Sperber *et al.* 2003; Vinton & Georgen 2006) and restoration efforts (e.g. Polley *et al.* 2005; McLachlan & Knispel 2005). Links between biophysical variables and species assemblages have been shown across climatic clines (Brye *et al.* 2004) and among sites with different topography and geomorphology (Wilsey *et al.* 2005). Interactions between plants and soil characteristics have received considerable attention (reviewed in Burke *et al.* 1998), with trends showing that water availability, nutrient levels and soil depth can be significant predictors of plant community composition (Bliss & Cox 1964; Wilson *et al.* 1996; Baer *et al.* 2005) and the prevalence of invasive species (e.g. Vinton & Georgen 2006). In a study of

Iowa's tallgrass prairie, Wilsey *et al.* (2005) found that differences in topography and soil types contributed to high differentiation among patches, and that even very small remnants (< 1.5 ha) could harbor meaningful levels of regional native plant diversity. Patterns of plant diversity can be very site-specific (Greig-Smith 1979), however, and the influence of environmental variables merits further research in communities of high conservation value.

The Palouse prairie of southeastern Washington and northern Idaho has been called one of the most endangered ecosystems in North America (Noss *et al.* 1997). Defined by its rolling hills, moderate climate and a vegetation community of bunchgrasses, forbs and shrubs (Daubenmire 1970), the region suffered a rapid and almost complete conversion to commercial agriculture in the late 19th and early 20th centuries. As much as 99.9% of the original prairie was lost with remnants persisting only on steep, untilled slopes, rocky ridge-tops and field margins, where they still face threats from development, off-road vehicle use and invasive species (Lichthardt & Moseley 1997). In a re-analysis of 35 – 50 year old permanent transects, Klepeis (2001) noted significant declines in native species cover and frequency within several prairie remnants, particularly for the native graminoids. Nascent conservation efforts include restoration and public outreach (Weddell & Lichthardt 1998; Weddell 2001), but the remaining habitat is largely undescribed, scattered in small patches across privately-held farmland. Understanding the influence of biophysical characteristics on plant diversity offers a means to predict habitat quality and set research and conservation priorities for these important but un-catalogued prairie patches.

Here we examine the influence of soil and site characteristics on plant diversity and the dominance of native species in twelve Palouse prairie remnants. We test the hypothesis that biophysical metrics (e.g. soil type, slope, aspect, vegetation structure) predict plant community characteristics (species richness, Simpson's diversity index and dominance of exotic species), and use model testing to identify the factors associated with high native plant diversity. The relationship between soil type and plant communities is explored in further detail, testing the predictive value of several readily-measured field characteristics (e.g. depth of the A-horizon, depth to restrictive layer) on the same diversity and dominance response variables. The potential application of these results to Palouse prairie conservation is also discussed.

MATERIALS AND METHODS

Study Area

Ecoregional descriptions of the Palouse often include nearby river canyons and shrub steppe habitat stretching far into eastern Washington (Noss *et al.* 1997). Here we focus on the biophysical heartland of the Palouse prairie, an area of rolling hills confined to southeastern Washington and the adjacent Idaho panhandle, where wind-blown loess accumulated in thick, hilly deposits throughout the inter-glacial periods of the Pleistocene (McDonald & Busacca 1988). Derived from the sediments of post-glacial lakes and flooding, this loess gave rise to deep, productive topsoil and a native plant community of bunchgrass prairies interspersed with wetlands and patches of forest. The prairie community has been described as a *Symphorocarpus alba*/*Festuca idahoensis* association (Daubenmire 1970), with the shrub *S. alba* as the dominant woody species and *F. idahoensis* the dominant bunchgrass. The soil and climatic conditions so favorable for native grasses, however, were also ideal for the production of grains. Conversion to agriculture was swift and nearly complete following European settlement and today the Palouse is a working landscape dominated by large, commercial farms. Extant prairie is limited to small isolated patches on steep, untilled slopes, rocky ridge-tops and field margins, as well as pioneer cemeteries and other historical sites (Lichthardt & Moseley 1997; Weddell & Lichthardt 1998). Little was known about the original plant and animal communities and the distribution and condition of the remaining patches are poorly understood.

Study Sites

Twelve prairie remnants in Whitman County, WA and Latah County, ID were sampled in this study. Since most prairie patches lie on private land, site selection was determined in part by permission for access. While not a random sample, the sites represent a range of sizes (1.2 – 165.4 ha) and settings (e.g. ridge-tops, field slopes) including four matrix types and 15 different soil map units (Table A.1). Plant data were collected during the peak flowering period (April – June) in 2004 and 2005. Soil data were collected in September and October, 2006.

Plant Communities and Biophysical Characteristics

Within each prairie remnant, twenty-five 50 x 20 cm plots were randomly located and mapped using a Garmin GPS unit. Percent cover was recorded for every species present in each plot frame, following the methods standardized by Daubenmire (1959). Specimens were identified in the field, or collected and pressed for comparison with herbarium specimens. From the species occurrences and percent cover estimates we calculated three plant community variables for each of the 300 total plots: species richness, Simpson's diversity index (Simpson 1949), and the dominance of exotic species (proportion of total cover).

Biophysical characteristics recorded for each plot included slope, aspect, distance from edge, edge type (defined as the closest adjacent matrix habitat: Conservation Reserve

Program field, active agricultural field, forest, exurban home site), and a vegetation structure variable based on the dominant vegetation type: grass/forb, low-shrub (woody species < 0.5 m, e.g. *Symphorocarpus alba*), or high-shrub (woody species > 0.5 m, e.g. *Rosa nutkana, Cratageous douglasii*). Soil map units for each plot were determined from the Natural Resource Conservation Service Soil Survey Geographic Databases for Latah, ID and Whitman, WA Counties (NRCS 2005a, 2005b). Map units describe soils based on their surface texture, topographic position and other general features and may include different taxonomic soil classes if they are closely juxtaposed in the landscape (Buol *et al.* 2003). For simplicity, however, we refer to map units as “soil types” for the remainder of this document.

Additional soil data were collected at six sites, chosen to include multiple examples of the most common soil map units (NRCS 2005a, 2005b). Ten plant community sample plots were relocated at each site with a Garmin GPS unit. From each plot, we recorded presence of rocks in the surface and percent cover in a 50 cm x 50 cm area. We used a 7 cm diameter soil auger to a depth of 50 cm to record: depth of A-horizon, depth to change in color, depth to restrictive layer and type of restrictive layer (rock or bedrock contact, dense clay layer, or unknown).

Data Analysis

General linear models (GLMs) were used to estimate the effects of biophysical characteristics on plot-level species richness, Simpson’s diversity index, and the dominance of exotic species. Since our dependent variables were measured at the plot scale (n = 300), we included site of origin as a categorical variable in all models to account for inherent site-level variability (e.g. site size, disturbance history). For each of the three response variables, the full model included site, soil type, edge type and vegetation structure as categorical variables. Aspect was included as an ordinal variable in increments from true north (0° – 45°; 46° – 90°; 91° – 135°; 136° – 180°), reflecting the plot's annual exposure to direct sunlight. Slope and distance from edge were included as continuous variables and we included an interaction term for distance from edge and edge type. From the full model we used Akaike's Information Criterion (AIC) (Akaike 1974) to test reduced models and find the most efficient model. AIC evaluates each model using the equation AIC = n {ln(SSE/n)} + 2p, where SSE is the model's error sum of squares (goodness-of-fit) and p is the number of parameters in the model. It gauges each model's goodness-of-fit in relation to its number of parameters, allowing one to remove superfluous terms from the full model until the most efficient and applicable combination is identified.

For the sixty plots with additional soil data, we fitted new linear models to test the predictive value of soil characteristics alone. These models included site and soil type as above, with ordinal variables for depth of A-horizon, depth to the first color change and depth to restrictive layer (0 – 10 cm, 11 – 20 cm, 21 – 30 cm, 31 – 40 cm, 41 – 50 cm, >50 cm). The percentage of visible rock fragments in the surface layer was included as a continuous variable and we added a categorical variable for the type of restrictive layer (rock, clay, unknown). AIC was again used to select the most efficient model.

All statistics were performed with the software SAS version 9.1 (©2002-2003, SAS Institute Inc.)

RESULTS

We identified a total of 114 native species and 47 exotics in the prairie remnants. Mean richness, diversity and dominance of exotic species values for categorical biophysical variables are reported in Table A.2. Inherent site-level variability was significant in nearly all general linear models, as noted below, but a range of plot-level biophysical characteristics also had predictive value for species richness and diversity and the dominance of exotic species.

Species Richness

The full GLM for species richness was highly significant (d.f. = 33, $F = 4.45$, $P < 0.0001$), but five factors comprised the most predictive and efficient model (d.f. = 28, $F = 5.15$, $P < 0.0001$) (Table A.3). Site, soil type, aspect and vegetation structure were all significant parameters for predicting species richness in the best model (Table A.3). Sample plots on northern-facing slopes had the highest mean richness values, as did those in grass/forb dominated communities (Table A.2). The most species-rich soil types included Schumacher Silt Loam, Palouse Silt Loam and Schumacher Variant Loam. Slope, distance from edge, edge type and the interaction of edge type and distance were not significant predictors of species richness.

Simpson's Diversity Index

The full GLM for Simpson's diversity was also highly significant (d.f. = 33, $F = 5.06$, $P < 0.0001$), and a reduced, five-parameter model again proved the most efficient (d.f. = 27, $F = 6.12$, $P < .0001$) (Table A.3). Site, soil type, aspect, vegetation structure and distance from edge all had significant effects on species diversity in the best model. The highest mean diversity values were again found in northern-facing, grass-forb communities, while soil types with high mean diversity included Schumacher Silt Loam and Palouse Silt Loam (Table A.2). While distance from edge was significant in the context of the model, a post-hoc regression analysis could not establish a stand-alone linear relationship between edge distance and diversity (d.f. = 299, $F = 0.49$, $P = 0.4832$). Slope, edge type and the interaction between edge type and distance from edge had no impact on diversity.

Dominance of Exotic Species

The full GLM for dominance of exotic species was highly significant (d.f. = 33, $F = 6.34$, $P < 0.0001$), and a six-factor reduced model had the best fit to the data (d.f. = 29, $F = 7.07$, $P < 0.0001$) (Table A.3). Site, soil type and aspect were significant in the best model, as were the distance from edge, edge type and their interaction. The lowest mean dominance of exotic species occurred on northern facing slopes, while soil types with low dominance of exotics included Naff Silt Loam, Palouse Silt Loam and Thatuna Silt Loam (Table A.2). Plots with agricultural fields as the nearest matrix habitat had the lowest mean dominance of exotics (Table A.2), but the significant interaction term implies this effect is intertwined with distance from edge. Slope and vegetation structure had no impact on the dominance of exotics.

Soil Characteristics

Soil characteristics alone had did not have predictive value in GLMs for either species richness (d.f. = 25, $F = 1.00$, $P = 0.4890$) or Simpson's diversity (d.f. = 25, $F = 1.75$, $P = 0.0643$) (Table A.4). For the dominance of exotic species, however, both the full model (d.f. = 25, $F = 4.95$, $\mathrm{P} < 0.0001$) and a reduced, four-parameter model (d.f. = 21, $F = 6.03$, $P < 0.0001$) were highly significant. The best model included site, soil type, depth to A-horizon and depth to restrictive layer as significant parameters (Table A.4). Low mean dominance of exotics occurred in several soil types characterized by deep surface layers (NRCS 2005a; 2005b), including Palouse Silt Loam and Thatuna Silt Loam.

DISCUSSION

Our results met the prediction that biophysical characteristics influence the structure of plant communities in Palouse prairie. We found several biophysical variables that had significant power to predict plot-scale species richness, Simpson's diversity and the dominance of exotic species. Additional soil data failed to clarify the role of specific soil features in our species richness and diversity results, but confirmed that two soil-depth characteristics influenced the dominance of exotic species. These findings may be useful in predicting the habitat quality of the scattered, isolated, and largely un-documented remnants of endangered Palouse prairie.

Biophysical Characteristics

After controlling for inherent site-level variability, our models found that soil type, aspect and vegetation structure were the most important predictors of plant community richness and diversity, and that soil type (and associated depth characteristics), aspect and edge type had strong impacts on the dominance of exotic species. Plots on northern-facing slopes with several different silt-loam soil types appeared to have the most diverse and species rich plant communities, with the lowest incidence of exotic species. Grass/forb-dominated plots were more diverse than those dominated by shrubs, and we also found a lower proportion of exotic species on plots where agricultural fields were the nearest adjacent habitat.

Numerous studies have documented associations between aspect, soil type and prairie plant communities (e.g. Albertson 1937; Hanson & Whitman 1938). In recent work, the influence of these variables on grassland diversity is often closely related to the incidence of invasive species, but the trends are sometimes contradictory. Harrison (1999) documented higher native plant diversity and lower incidence of exotics on north-facing plots in California grasslands, but Hutchings (1983) found no difference in diversity associated with aspect in English chalk grasslands, the same habitat where Bennie *et al.* (2006) proposed that south-facing slopes are more resistant to invasives. It is likely that patterns are landscape-specific and influenced by local factors, including disturbance history (Collins *et al.* 2002; Maestre 2004). In Palouse prairie, many of the south-facing sites are on larger, ridge-top remnants with a history of grazing, while small north-facing patches can be embedded in agricultural fields with a much different disturbance regime. Incidence of *Bromus tectorum*, one of the most common exotic species in this study, has also been strongly associated with south-facing slopes (Rickard 1975). Soil type was also a strong predictor in all of our models and specific soil characters are often linked to plant diversity and the prevalence of exotic species, as discussed in detail below. Slope, an important parameter for some plant communities (e.g. Bennie *et al.* 2006), appears relatively unimportant to the community structure in Palouse prairies.

Vegetation structure is often linked to the diversity of plant species (e.g. Baer *et al.* 2005; Lett & Knapp 2005), and our finding that grass/forb communities are more diverse than shrub-dominated sites matches earlier descriptions of Palouse prairie (Daubenmire 1970; Klepeis 2001). Our results also suggest that grass/forb, high-shrub and low-shrub communities are equally susceptible to invasion by non-natives, however. The type of adjacent matrix habitat also impacted exotic species dominance, with lower prevalence of natives in remnants adjacent to active agricultural fields. This contradicts an earlier study of hedgerows and woodlot margins (Boutin & Jobin 1998), where intensive farming practices were associated with increases in weedy exotics in neighboring habitats. At our sites, however, it appears possible that the control of weeds in agricultural fields may be helping to prevent their establishment in adjacent prairie, a possibility worthy of further study.

Additional Soil Characteristics

Soil type was a consistently significant predictor of variation in our models, influencing species richness, diversity, and the dominance of exotic species. The addition of data on several depth and restrictive layer characteristics, however, failed to clarify what specific soil qualities were affecting the species richness and diversity of the plant community. We did determine that the depth of the A-horizon and the depth to a restrictive layer had strong implications for the dominance of exotic species, with deeper soils supporting communities less dominated by exotics. A-horizon depth has also been linked with plant community structure in tallgrass prairie (Bliss & Cox 1964), but Baer *et al.* (2005) found no direct link between soil depth and plant diversity in experimental prairie treatments, and MacDougall *et al.* (2006) found no relationship between soil depth and the prevalence of invasive species in California savanna grasslands.

It is likely that other soil properties are involved in the predictive power of soil type in our analyses. Numerous studies have demonstrated the importance of water and nutrient availability to plant community structure (see Greig-Smith 1979; Burke *et al.* 1998) and the prevalence of invasive species (e.g. Vinton & Georgen 2006). Soil characteristics that can affect water content and nutrient availability include clay percent, textural class, mineralogy, organic matter content, nutrient status, drainage and soil depth. Soil biological factors can also influence plant diversity (Bever *et al.* 1997), and the presence of cryptogamic crusts has been shown to influence nitrogen fixation, soil hydrological processes and the establishment of exotic species (Hilty *et al.* 2004). Our soil measurements did not include any of these parameters, but descriptions in the NRCS Soil Survey Data base (NRCS 2005a, 2005b,) suggest that they can vary widely among Palouse prairie soil types. The north-facing silt loam soils we associated with high plant diversity, for example, are very likely to provide greater available water than coarser soils facing south. These variables clearly deserve further consideration in future studies.

Limitations

Results of this study should be viewed as preliminary, since twelve sites, 300 vegetation plots and 60 soil samples cannot fully represent the variation among Palouse prairie remnants. The emerging trends merit further effort, however, and we believe the standard sampling and statistical methods used herein can be easily replicated in additional sites.

Another note of caution is the lack of a metric for site disturbance history in our models. Palouse prairie remnants have been impacted by a variety of human disturbances (e.g. grazing, herbicide drift) at varying intensities (Weddell & Lichthardt 1998). Disturbance has repeatedly been shown to impact plant community structure in grasslands (e.g. Coffin & Lauenroth 1988; Maestre 2004), and in some cases may overwhelm the effects of biophysical variables (Naeem *et al.* 2000). The impacts of disturbance history on Palouse prairie remnants remain unknown and should be included in any future efforts.

CONCLUSIONS

Northern-facing plots in grass/forb communities on silt loam soils had the highest species richness and diversity and the lowest dominance of exotic species in this study. Dominance of exotics was also lower for plots near active agricultural fields and on soils with deep surface layers. Though preliminary, these results may be helpful in identifying conservation priorities for the endangered and little-studied Palouse prairie ecosystem.

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Table A.1 Locations and site characteristics of twelve Palouse prairie remnants in southeastern Washington and northern Idaho used for plant and soil sampling from April 2004 through October, 2006.

Site	**Size (ha)**	**Location (Lat/long)**	**Ownership**	**Matrix[a] (edge type)**	**Soil map units[b] present**	**Soil sample plots**	**Plant sample plots**
Black Butte	100.3	Whitman Co., WA (N46˚37.9.1′ W117˚05.7′)	Private	CRP	Tekoa stony silt loam 25/40	-	25
Campus Prairie	2.7	Whitman Co., WA (N46˚44.1′ W117˚08.4′)	Washington State Univ.	CRP	Palouse silt loam 7/25 Palouse silt loam 3/7	10	25
Dumrose Prairie	2.0	Latah Co., ID (N46˚39.7′ W116˚59.6′)	Private	CRP HOME	Southwick silt loam 25/35 Naff-Palouse silt loams 7/25	-	25
Ernie's Prairie	1.4	Whitman Co., WA (N46˚35.5′ W117˚12.4′)	Private	AG	Palouse silt loam 7/25	-	25
Kamiak Butte	165.4	Whitman Co., WA (N46˚51.6′ W117˚10.1′)	Whitman County Parks/Private	CRP FOR	Tekoa gravelly silt loam 25/55 Tekoa stony silt loam 25/40 Schumacher silt loam 15/25	10	25
Kramer Prairie	11.8	Whitman Co., WA (N46˚34.9′ W117˚12.8′)	Washington State Univ.	AG CRP	Palouse-Thatuna silt loams 7/25 Palouse silt loam 7/25 Calouse silt loam 25/40	10	25
McCrosky Ridge	18.5	Latah Co., ID (N47˚05.2′ W116˚59.0′)	Idaho State Parks	CRP FOR	Schumacher variant loam 15/55 Schumacher silt loam 10/35	-	25
Paradise Ridge	85.1	Latah Co., ID (N46˚40.3′ W116˚58.3′)	Private	CRP FOR	Schumacher variant loam 15/55	-	25
Schulteiss Prairie	5.2	Whitman Co., WA (N46˚34.2′ W117˚110.7′)	Private	AG	Thatuna silt loam 40/55 Naff silt loam 25/40	10	25
Smoot Hill	15.4	Whitman Co., WA (N46˚49.2′ W117˚14.4′)	Washington State Univ.	CRP	Tekoa stony silt loam 25/40 Palouse silt loam 40/55	10	25
Tomer Butte	11.9	Latah Co., ID (N46˚42.5′ W116˚55.9′)	Private	CRP FOR	Schumacher variant loam 15/55 Larkin silt loam 12/35	10	25
Wheelan Cemetery	1.2	Whitman Co., WA (N46˚46.7′ W117˚07.0′)	Private	AG	Palouse silt loam 7/25	-	25

[a] CRP – Conservation Reserve Program fields; AG – active agricultural fields; FOR – forest; HOME – exurban home site

[b] Soil map units from Natural Resource Conservation Service Soil Survey Geographic Database (NRCS 2005 a,b).

Table A.2 Species richness, Simpson's diversity index and the dominance of exotic species from 300 vegetation plots at twelve Palouse prairie remnants in southeastern Washington and Northern Idaho, sampled in April – June, 2004 and 2005. Means are calculated for four biophysical variables: soil type, vegetation structure, aspect and edge type (nearest adjacent habitat).

Biophysical variable	n (# plots)	Species Richness ± SD	Simpson's diversity[a] ± SD	Dominance of Exotic Species[b] ± SD
Soil Type				
Calouse silt loam 25/40	2	6.000 ± 1.41	0.287 ± 0.05	0.978 ± 0.03
Larkin silt loam 12/35	3	9.667 ± 3.79	0.758 ± 0.08	0.339 ± 0.31
Naff silt loam 25/40	1	9.000 ± 0.00	0.642 ± 0.00	0.014 ± 0.00
Naff-Palouse silt loam 7/25	9	8.333 ± 1.94	0.658 ± 0.08	0.256 ± 0.22
Palouse silt loam 3/7	11	9.727 ± 5.12	0.753 ± 0.09	0.305 ± 0.20
Palouse silt loam 40/55	12	11.417 ± 3.12	0.703 ± 0.11	0.088 ± 0.12
Palouse silt loam 7/35	65	10.708 ± 3.62	0.720 ± 0.16	0.207 ± 0.24
Palouse-thatuna silt loams 7/25	22	10.182 ± 3.69	0.709 ± 0.16	0.141 ± 0.21
Schumacher silt loam 10/35	16	10.438 ± 1.36	0.824 ± 0.07	0.514 ± 0.20
Schumacher silt loam 15/25	4	17.500 ± 4.78	0.788 ± 0.14	0.288 ± 0.28
Schumacher variant loam 15/55	56	10.607 ± 2.51	0.726 ± 0.14	0.386 ± 0.30
Southwick silt loam 25/35	16	9.938 ± 3.19	0.727 ± 0.16	0.155 ± 0.20
Tekoa gravelly silt loam 25/55	20	10.050 ± 3.07	0.746 ± 0.08	0.284 ± 0.21
Tekoa stony silt loam 25/40	39	10.324 ± 3.31	0.698 ± 0.17	0.184 ± 0.19
Thatuna silt loam 40/55	24	9.708 ± 2.85	0.739 ± 0.11	0.059 ± 0.07
Aspect				
North	90	10.744 ± 3.38	0.742 ± 0.13	0.122 ± 0.15
Northwest/northeast	52	10.462 ± 3.85	0.707 ± 0.18	0.177 ± 0.20
Southwest/southeast	52	10.192 ± 3.41	0.715 ± 0.16	0.326 ± 0.26
South	106	9.943 ± 3.16	0.706 ± 0.16	0.344 ± 0.29
Vegetation structure				
Grass/forb dominated	221	10.851 ± 3.19	0.742 ± 0.14	0.284 ± 0.27
Low-shrub dominated	60	9.350 ± 2.90	0.683 ± 0.13	0.142 ± 0.13
High-shrub dominated	17	8.000 ± 4.23	0.618 ± 0.21	0.141 ± 0.26
Edge Type (nearest adjacent habitat)				
Conservation Reserve Program field	133	10.481 ± 3.40	0.721 ± 0.13	0.27 ± 0.23
Agricultural field (active)	100	10.480 ± 3.55	0.719 ± 0.16	0.178 ± 0.25
Forest	61	10.000 ± 2.61	0.734 ± 0.13	0.306 ± 0.30
Exurban home site	4	10.750 ± 3.86	0.732 ± 0.15	0.329 ± 0.33

[a] Simpson's (1949) diversity index.

[b] Proportion of total plant cover attributed to non-native species.

Table A.3 Full and best general linear models for the influence of biophysical variables on plot-level (n = 300) plant species richness, Simpson's diversity and the dominance of exotic species in twelve Palouse prairie remnants. Values of $P < 0.05$ are highlighted in bold.

	Full Model				Best Model			
	AIC[d]	df	*F*	*P*	AIC[d]	df	*F*	*P*
Species Richness								
model	662.91	33	4.45	**<0.0001**	656.89	28	5.15	**<0.0001**
site	-	8	6.43	**<0.0001**	-	8	6.53	**<0.0001**
soil type	-	9	2.21	**0.0221**	-	9	2.25	**0.0194**
aspect	-	3	10.24	**<0.0001**	-	3	10.61	**<0.0001**
vegetation structure	-	3	149.87	**<0.0001**	-	3	18.63	**<0.0001**
edge type	-	2	3.31	**0.0382**	-	2	2.69	0.0698
distance from edge	-	1	0.06	0.8106	-	-	-	-
edge*distance[a]	-	3	1.06	0.3645	-	-	-	-
slope	-	1	0.3	0.5828	-	-	-	-
Simpson's Diversity[b]								
model	-1200.99	33	5.06	**<0.0001**	-1207.18	27	6.12	**<0.0001**
site	-	8	2.26	**0.0239**	-	8	2.22	**0.0209**
soil type	-	9	2.78	**0.0040**	-	9	3.65	**0.0003**
aspect	-	3	7.54	**<0.0001**	-	3	7.01	**0.0001**
vegetation structure	-	3	27.86	**<0.0001**	-	3	28.92	**<0.0001**
edge type	-	2	0.66	0.5202	-	-	-	-
distance from edge	-	1	0.02	0.8912	-	1	4.29	**0.0393**
edge*distance[a]	-	3	0.87	0.4568	-	-	-	-
slope	-	1	0.80	0.3709	-	-	-	-
Dominance of Exotic Species[c]								
model	-931.83	33	6.34	**<0.0001**	-935.10	29	7.07	**<0.0001**
site	-	8	6.33	**<0.0001**	-	8	6.34	**<0.0001**
soil type	-	9	3.63	**0.0003**	-	9	3.70	**0.0002**
aspect	-	3	3.02	**0.0304**	-	3	3.56	**0.0147**
vegetation structure	-	3	1.34	0.2628	-	-	-	-
edge type	-	2	6.09	**0.0026**	-	2	6.34	**0.0020**
distance from edge	-	1	5.43	**0.0205**	-	1	5.79	**0.0168**
edge*distance[a]	-	3	3.13	**0.0263**	-	3	3.31	**0.0207**
slope	-	1	0.26	0.6125	-	-	-	-

[a] term for the interaction of edge type and distance from edge.

[b] Simpson's (1949) diversity index.

[c] Proportion of total plant cover attributed to non-native species.

[d] Akaike's (1974) Information Criterion.

Table A.4 Full and best general linear models for the influence of soil characteristics on plot-level (n = 60) plant species richness, Simpson's diversity and the dominance of exotic species in six Palouse prairie remnants. Values of $P < 0.05$ are highlighted in bold.

	Full Model				Best Model			
	AIC[c]	df	*F*	*P*	AIC[c]	df	*F*	*P*
Species Richness								
model	108.74	25	1.00	0.4890	-	-	-	-
Simpson's Diversity[a]								
model	-267.79	25	1.75	0.0643	-	-	-	-
Dominance of Exotic Species[b]								
model	-225.86	25	4.95	**<0.0001**	-228.40	21	6.03	**<0.0001**
site	-	3	2.07	0.1223	-	3	3.04	**0.0404**
soil type	-	4	15.40	**<0.0001**	-	4	17.80	**<0.0001**
depth of A-horizon	-	2	1.93	0.1603	-	5	3.41	**0.0119**
depth to color change	-	3	1.07	0.3754	-	-	-	-
depth to restrictive layer	-	1	0.62	0.4370	-	4	8.12	**<0.0001**
type of restrictive layer	-	1	0.09	0.763	-	-	-	-
rock fragments	-	1	1.31	0.2607	-	-	-	-

[a] Simpson's (1949) diversity index.

[b] Proportion of total plant cover attributed to non-native species.

[c] Akaike's (1974) Information Criterion.