GENETIC DIVERSITY AND REALIZED DISPERSAL IN THE DIOECIOUS NEOTROPICAL TREE, SIMAROUBA AMARA

by

BRITTA DENISE HARDESTY

(Under the Direction of Stephen P. Hubbell)

ABSTRACT

Patterns of genetic diversity at local and large scales can provide insight into current and historical processes of gene flow, whether by pollen or seed. I used molecular markers to measure local patterns of genetic relatedness (i.e. fine scale genetic structure) in three size classes at the scale of 50 ha for Simarouba amara, in a large natural tree population on Barro Colorado Island, Panama. Simarouba amara is a widespread, dioecious, vertebrate-dispersed tropical tree species. Using multilocus genotype matching and parent-pair analyses with five microsatellite loci, I assessed the frequency of successful long-distance seedling establishment and evaluated the relative contribution of gene movement via pollen versus seed. Finally, I sampled from 14 populations of S. amara across Panama, Ecuador and French Guiana to assess levels of genetic diversity within and among a geographic expanse spanning > 3000 km. This portion of the study provides insights to the historical and evolutionary forces that may have structured current S. amara populations.

My research revealed that S. amara exhibits little fine scale genetic structure at the scale of 50 ha. Only seedlings demonstrate significant spatial autocorrelation exceeding 20 m. For juveniles and adults, individuals located close to one another are not significantly related. On Barro Colorado Island, long distance seed dispersal is commonplace, as indicated by a high frequency of seedling establishment greater than 200 m from both maternal and paternal parents. Significant directional movement was evident in pollen though not in seedling establishment and gene movement was comparable via both pollen and seed. Finally, the 14 populations of S. amara sampled across Central and South America, populations differed significantly from one another using F and R-statistics, and follow and isolation by distance model. Notably, coastal populations in Panama and Ecuador differed significantly from all other populations suggesting different evolutionary histories influenced by geographic boundaries in addition to distance.

INDEX WORDS: Amplified Fragment Length Polymorphism (AFLP), Barro Colorado Island, Ecuador, gene flow, genetic diversity, genetic structure, microsatellite markers, nuclear DNA, Panama, pollen dispersal, seed dispersal, seedling recruitment, tropical forest

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DEDICATION

For my nephews, Kai and Ken;

Live long, live well, and explore the world in its infinite possibilities

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

Introduction and Literature Review

This dissertation is a genetic study of dispersal in relation to the population genetic structure of the vertebrate-dispersed, Neotropical tree, Simarouba amara (Simaroubaceae). Seed dispersal plays a key role in determining patterns of tree diversity and distribution; yet quantifying the effect of dispersal has proven a challenge to ecologists. Dispersal is a central process driving the many hypotheses formulated to explain the maintenance and diversity of tropical forest communities (Janzen 1970, Connell 1971, Tilman 1994, Hurtt and Pacala 1995, Hubbell et al. 1999, Hubbell 2001). Dispersal underlies the spatial genetic structure within and among populations, and it reflects the complicated patterns and processes of seed arrival, germination, establishment, survival and growth in contemporary and historical time.

Seed dispersal is a fundamentally important life history stage for plants, yet we know relatively little about seed dispersal in tropical trees. In part this is because dispersal has been studied in relatively few of the many tropical tree species and their associated dispersers and in part because the patterns that have been observed have generally evaded easy generalization. Dispersal fundamentally influences plant population persistence, range expansion, and evolutionary processes. The signature of dispersal is also visible both in historic and current patterns of genetic diversity. For tropical tree species, 60-90% of woody tree species are estimated to be adapted for dispersal by vertebrates (Willson 1989). The interaction between vertebrate dispersers and the plants they disperse is diverse: animals may consume fruit; drop,

spit or defecate the seeds, carry seeds in their coats, or scatter-hoard seeds for later consumption. For vertebrate-dispersed tree species, we can presume active dispersal has taken place for seeds which arrive and survive some distance away from underneath the crown of the parent tree.

With molecular techniques we can gain insights to a wide arrange of questions that have long eluded ecologists using traditional methods. Molecular methods enable us to not only determine overall relatedness of individuals within a population, but also advance our ability to measure seed dispersal and subsequent germination and recruitment by positively identifying parents for any given seed or seedling. Dispersal and establishment of new genes into a population is critical for the maintenance and spatial distribution of genetic variation. With an increase in gene flow, we may observe a genetic homogenization across a landscape. Gene flow may also introduce novel alleles into a population and counteract the effects of drift. Insight to the level and pattern of gene flow is fundamental to our understanding of plant population dynamics, and we can use genetic techniques to further understand the complicated relationships between plants, pollinators, and dispersers both in a contemporary and historical context.

Using molecular methods, one can quantify local dispersal by assigning offspring to their maternal and paternal parents. This approach enables us to measure the precise distances of seed arrival and seedling recruitment. We can also measure the relative contribution to gene flow made by pollen and seed using genetic techniques. We can test for directional seedling establishment and pollen flow as well as determine the relative contribution of individual reproductive adults to offspring in a particular cohort or in the 'future generation' of a population.

The maintenance of genetic diversity becomes increasingly important as forest populations are fragmented. In the future, ecologists will have less and less opportunity to study

species solely in pristine, undisturbed habitats. As the landscape is increasingly fragmented and dispersers are extirpated, one can predict an increased partitioning of genetic diversity. This may ultimately lead to a loss of genetic heterogeneity, genetic drift, and isolation, particularly as distances between patch sizes increase and vertebrate dispersers are lost from the system. We can study current patterns of genetic variation within and among populations to gain insights not only about past patterns of gene movement but also about the future impact of habitat loss.

This thesis is a genetic marker-assisted study of dispersal, recruitment, and the population genetic structure of Simarouba amara Aubl. (Simaroubaceae), a dioecious, vertebrate dispersed, Neotropical tree species. The studies of dispersal and recruitment were conducted on the S. amara population in and around the 50 ha Forest Dynamics Project (FDP) plot on Barro Colorado Island (BCI), Panama ($9^0$10'N, $79^0$51'W) (Hubbell and Foster 1983). All ca. 240,000 non-liana woody plants $\geq$ 1 cm diameter at breast height (dbh) in the plot are tagged, mapped and identified to species. The plot has been censused at approximately 5 year intervals since 1980. Although individual turnover is quite high, the total number of S. amara stems remains remarkably consistent between census periods. Simarouba amara grows to approximately 35 m in height with a maximum reported dbh of 70 cm on BCI (Croat 1978). This moderate-to-high light requiring tree typically flowers from February-April. It produces large-seeded fleshy fruits in 3-5 drupes, up to 17 mm long (Croat 1978). The purplish black ripe fruits attract numerous dispersers including chachalacas, flycatchers, motmots, and thrushes (Croat 1978), tamarinds (pers. obs.), howler and spider monkeys (Hladik and Hladik 1969).

Where fine-scale genetic structure is observed within populations, this structure is the result of non-random distribution of genetically more similar (more related) individuals relative to a random sample of the population as a whole. Within a population, we expect genetic

structure where seed dispersal and subsequent establishment is limited, because dispersal limitation will result in the clumping or aggregation of related nearby individuals. In this thesis, I am particularly interested in quantifying the amount of long-distance dispersal and recruitment. In general, longer distance dispersal not only enables the offspring of a single parent to colonize a larger area, but also enables more offspring to escape from host-specific pathogens and competition with related individuals. Long-distance dispersal also allows the offspring of individual parents to sample a greater number and variety of microsite regeneration sites, in effect, a bet-hedging strategy.

The organization of this thesis is as follows. Chapter two of the dissertation describes the spatial genetic structure of three size classes of S. amara; seedlings (<1 cm dbh), saplings or juveniles (1-10 cm dbh), and adults (>10 cm dbh). Within the 50 ha FDP, we used microsatellite markers and Amplified Fragment Length Polymorphisms (AFLPs) to describe the genetic structure of 100 randomly selected individuals in each class. We did not find consistent results from using AFLP and microsatellite markers, although the direction of the results was consistent. AFLP markers did not demonstrate significant spatial autocorrelation. However, we found that S. amara presents relatively weak genetic structure using spatial autocorrelation analyses with microsatellites. Significant fine scale genetic structure exceeded 20 m only in seedlings. The lack of relatedness among nearby large individuals suggests that there is differential thinning among related individuals that were established at a result of short-term dispersal events. It also indicates that there is extensive gene movement via pollen and seed in S. amara.

It is generally presumed that tropical trees exhibit dispersal limitation (Dalling et al. 2002), with gene movement via pollen responsible for much of the gene flow, since pollen has typically been shown to move much further than seed (e.g. Nason et al. 1998). However, if all

else is equal, since pollen is haploid and seed is diploid, seed dispersal accounts for two-thirds of the movement of genetic diversity (Hamilton 1999), and maternal gene flow is completely dependent upon seed movement. In Chapter three we examine the relative contribution of gene movement via pollen and seed movement. We also make progress toward quantifying the effects of dispersal on population genetic structure. We tested the hypothesis that gene movement is more extensive for pollen than for seed, as has been found with many Neotropical tree species (e. g. Nason et al. 1998, Dick 2001). We measured seedling establishment distances and found that long distances between offspring and maternal trees are common (up to 1 km). This indicates that S. amara is not as dispersal limited as might have been expected from seed rain data. These large seedling recruitment distances also indicate that vertebrate dispersers are playing an important role for successful seed dispersal and subsequent seedling establishment in this species. Individual reproductive success was variable, and a significant compass directional bias was evident for pollen movement, though not for seedling establishment. In this chapter I also discuss our finding of comparable gene movement via pollen and seed.

We can infer past patterns of gene movement by describing current patterns of genetic variation within and among populations. The fourth chapter of my dissertation tests the hypothesis that genetic distance is dependent upon geographic distance. In this chapter, we ask whether genetic diversity is maintained predominantly within or among 14 S. amara populations sampled across Panama, Ecuador, and French Guiana. We tested whether genetic diversity is evenly distributed across a 3200 kilometer range and whether geographic boundaries play a discernible role in genetic differentiation among populations. We found that genetic diversity within populations is high and that all but one of the population pairs demonstrated significant genetic differentiation. We also found that the hypothesis of isolation by distance hypothesis is

supported, although geographic boundaries also play a notable role in genetic relatedness among populations, regardless of geographic distance.

The research in my dissertation addressed three main objectives. First, at the smallest scale, we determined the fine-scale relatedness among individuals of different age (size) classes of S. amara within a single 50 ha study area on Barro Colorado Island, Panama. Second, we quantified the frequency of long distance seedling recruitment and the measured the relative contribution of pollen and seed to long distance gene movement. Finally, we step outward in scale, both spatially and temporally, to assess the distribution of genetic diversity within and among fourteen natural populations of S. amara across a 3200 km range. From this study we can infer the historical role of geographic distance and boundaries in current genetic diversity within and among tropical tree populations.

References

Connell, JH 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rainforest trees. in PJ Den Boer and G Gradwell, eds. Dynamics of populations. PUDOC, Wageningen, the Netherlands. pp 298-312.

Croat, T (1978). Flora of Barro Colorado Island. Stanford University, California.

Dalling, JW, HC Muller-Landau, SJ Wright, and SP Hubbell 2002. Role of dispersal in the recruitment limitation of Neotropical pioneer species. Journal of Ecology 90: 714-727.

Dick, CW (2001). Genetic Rescue of Remnant Tropical Trees by an Alien Pollinator. Proceedings of the Royal Society of London Series B-Biological Sciences 268:2391-2396.

Hamilton, M. B. (1999). Tropical tree gene flow and dispersal. Nature 401:129.

Hladik, A and CM Hladik (1969). Rapports trophiques entre végétation et primates dans le foret de Barro Colorado (Panama). La Terre et la Vie. 1:25-117.

Hubbell, SP. 2001. The unified neutral theory of biodiversity and biogeography. Princeton University Press, Princeton, NJ.

Hubbell, SP, and RB Foster (1983). Diversity of canopy trees in a Neotropical forest and implications for conservation. In Sutten SL, TC Whitmore and A C Chadwick, eds. Tropical Rain Forest: Ecology and Management. Blackwell Scientific Publications, Oxford.

Hubbell, SP, RB Foster, ST O'Brien, KE Harms, R Condit B Wechsler, SJ Wright and SL de Lao.1999. Light-gap disturbances, recruitment limitation, and tree diversity in a Neotropical wet forest. Science. 283: 554-557.

Hurtt, GC and SW Pacala. 1995. The consequences of recruitment limitation: reconciling chance, history, and competitive differences in plants. Journal of Theoretical Biology 176: 1-12.

Janzen, DH. 1970. Herbivores and the number of tree species in tropical forests. American Naturalist 104: 501-528.

Nason, J.D., E.A. Herre, and J.L. Hamrick. (1998). The breeding structure of a tropical keystone plant resource. Nature 391: 695-687.

Tilman, D. 1994. Competition and biodiversity in spatially structured habitats. Ecology 75: 2-16.

Willson, M.F., Irvine, A.K., & Walsh, N.G. (1989) Vertebrate Dispersal Syndromes in Some Australian and New-Zealand Plant-Communities, with Geographic Comparisons. Biotropica, 21, 133-147.

CHAPTER 2

Spatial genetic structure of Simarouba amara Aubl. (Simaroubaceae), a dioecious, animal-dispersed Neotropical tree, on Barro Colorado Island, Panama[1]

[1] Britta Denise Hardesty, Christopher W. Dick, Antoine Kremer, Stephen Hubbell, and Eldredge Bermingham; Submitted to Heredity

Abstract

Simarouba amara (Simaroubaceae) is a vertebrate-dispersed, insect pollinated Neotropical tree found in lowland moist forest from upper Mesoamerica to the Amazon basin. Using five microsatellite loci and 155 amplified fragment length polymorphisms (AFLP), we assess the spatial genetic structure for S. amara within the 50-hectare Forest Dynamics Plot on Barro Colorado Island in the Republic of Panama. Three hundred individuals were genotyped using microsatellite markers, representing 100 individuals with a dbh ≥10 cm dbh, 100 individuals with a dbh of 1- 10 cm dbh, and 100 individuals with a dbh of <1 cm. The 200 individuals in the two larger size classes were also genotyped using AFLP loci. Spatial autocorrelation analysis using Moran's Index detected significant genotypic association at the smallest distance classes for 1-10 cm dbh (0-20 m) and >10 cm dbh (0-40 m) individuals. Significant spatial autocorrelations were detected over larger scales (0-140 m) in <1 cm dbh individuals. The relatively weak genetic structure of S. amara, in contrast to other recent studies, may be explained by pollen and seed dispersal over the 50 hectare plot, overlapping seed shadows, and post-recruitment mortality.

Introduction

Tropical forest tree species tend to exhibit spatially clumped distributions (Hubbell 1979, Hubbell and Foster 1983, Condit et al. 2000), while having low numbers of conspecific individuals per unit area. Spatially limited seed dispersal contributes to the patterns of clumping. However, in comparisons between tropical and temperate zone trees, it is unclear whether tropical trees experience greater dispersal limitation due to their preponderant reliance on animals for both pollination and seed dispersal (Bawa 1990). Moreover, it is difficult to predict patterns of gene flow in tropical trees because of the unpredictable effect of animal behavior on gene movement (Nason and Hamrick 1998; Hamrick and Loveless 1986). Models of gene flow for tropical forest trees require either 1) direct observation of animal behavior and their effectiveness as pollinators or seed dispersers (sensu Howe 1986, Schupp 1993), 2) indirect correlations based on comparative population genetic analysis of plant species with different pollination and seed dispersal syndromes (Loveless and Hamrick 1984; Hamrick et al. 1993, Degen et al. 2001a), or 3) use of genetic markers to reconstruct seed and pollen movement (Aldrich and Hamrick 1998, Chase et al. 1996, Dick 2001, Jordano and Godoy 2002, Ouborg et al. 1999) using parentage analysis. Here we contribute to an emerging body of data on spatial genetic structure in tropical trees through an analysis of spatial genetic structure of the tropical tree Simarouba amara within the 50-hectare Forest Dynamics Plot (FDP) on Barro Colorado Island (BCI), Panama, where we have two decades of demographic data based on periodic censuses of all S. amara ≥1 cm diameter at breast height (dbh).

The specific objectives of our study were: 1) to evaluate and describe the genetic diversity of a mapped population of S. amara within a lowland Neotropical forest using microsatellite and AFLP data; 2) to assess the utility of microsatellite and AFLP markers for

evaluating spatial genetic structure; 3) to describe the genetic structure of three size classes of individuals (>10 cm dbh, 1-10 cm dbh, and <1 cm dbh) at the 50 ha scale; and 4) to assess the level of heterozygosity across those three size classes. If dispersal and pollen movement is limited, as often assumed among plant populations with low densities of reproductive adults, our prediction is increased genetic relatedness among individuals in close proximity. However, if effective seed dispersal is widespread, the result should be a lack of spatial genetic structure or less than random genetic relatedness at further distances, if family structure exists at larger scales.

Materials and Methods

Study species

Simarouba amara Aubl. (Simaroubaceae) is a widespread Neotropical tree species found in lowland moist forests from upper Mesoamerica (Honduras, Nicaragua) to the lower rim of the Amazon basin (Bolivia and Peru). The wood of Simarouba amara is used for paper, furniture building, interior construction, plywood, and matches (Rodriguez 2000). Simarouba amara is dioecious, insect pollinated, and dispersed primarily by vertebrates (birds and large mammals). It grows to 35 m in height with a maximum reported diameter at breast height (dbh) on BCI of 70 cm (Croat 1978). Simarouba amara requires intermediate to high light environments, and on BCI typically flowers from the end of January through April. The unisexual flowers of S. amara occur in terminal panicles comprised of numerous, small (<1 cm long), pale yellowish flowers. Data from 15 years reporting all flowering and fruiting parts arriving in seed traps suggest that there is annual synchrony in the phenology of S. amara adults (SJ Wright, unpublished data).

Flowering persists for approximately 11-15 weeks annually within the 50 ha plot. The floral syndrome of S. amara is typical of pollination by generalist small insects such as small bees and moths (Bawa 1990), which have been observed visiting the flowers of this species (BDH pers. obs.).

Ripe fruits are produced within 1-3 months after pollination. The fruits are large-seeded and fleshy, approximately 17 mm in length, with a seed of 10-14 mm, and are presented in clusters of 3-5 drupes (Croat 1978). Seeds of S. amara do not experience dormancy. Primates and birds are the primary dispersers of S. amara. The brightly colored green to purplish black fruits attract birds such as chacalacas, flycatchers, motmots, and thrushes (Duke as cited in Croat 1978), as well as howler and spider monkeys (Hladik and Hladik 1969) and tamarins (BDH pers. obs). Leaf-cutter ants have been observed to carry the seeds, and dense seedling carpets of S. amara have been noted only at the two leaf-cutter ant dump sites in the 50 ha FDP (BDH pers. obs).

The year 2000 BCI plot census contained 1230 S. amara individuals ≥ 1 cm. The FDP contains 126 individuals ≥ 20 cm dbh, which represents the minimum reproductive size, though most individuals do not reproduce below 30 cm dbh (N = 72 individuals ≥ 30 cm dbh). Forty-eight female trees have been reproductive during the past three years according to our observations. Trees flower and fruit annually, though not every reproductive female produces fruit each year (Hardesty, unpubl. data). The mean dispersal distance for S. amara seeds is 39 m based on maximum likelihood best- fit estimates ("inverse modeling") of arrival in seed traps using known distances from reproductive-sized adults within the FDP (Muller-Landau 2001). Current inverse models do not take tree gender into consideration, however, and likely underestimate seed dispersal distances.

Study site

This research was carried out in the 50-hectare permanent forest dynamics plot (FDP) within the Barro Colorado National Monument (9º 10' N, 79º 51' W), Republic of Panama. The FDP is located on a plateau near the center of Barro Colorado Island (BCI), an island formed by the flooding of Lake Gatun following the construction of the Panama Canal in the early 1900's. The lowland moist tropical forest on BCI receives approximately 2600 mm of rainfall annually, and the island has a pronounced dry season between December and April (Leigh 1982). Every five years beginning in 1982, all woody plants within the FDP with ≥ 1 cm diameter breast height (dbh) are identified, mapped, and measured to assess growth, mortality, and recruitment of new individuals.

Sampling strategy

Using the FDP inventory data, we randomly selected 200 S. amara individuals from two size classes: (1) 1-10 cm dbh N=100), and (2) >10 cm dbh (N=100) (Figure 1). In order to assess fine level spatial structure below the 1 cm dbh class, we performed an additional sample of 100 'seedlings' that were randomly selected from approximately 500 individuals with <1 cm dbh that had been collected in a survey across nearly 40 ha of the FDP. Tissue was collected from only from healthy plants in which there was sufficient leaf material such that removal of tissue would be unlikely to cause mortality. In patches of high density (>1 seedling/m^2), tissue from every third plant was collected, whereas in lower density areas, tissue was collected from each seedling encountered.

Leaf collection and DNA extraction

Leaves were collected directly from branches or by using a sling-shot to drop leaves from the canopy of tall trees. The fresh tissue was placed in plastic bags in a cooler with ice while in the field. In the laboratory, samples were either refrigerated and the DNA extracted within two days, or they were put into labeled vials and flash-frozen in liquid nitrogen. DNA extractions using 40-50 mg of the fresh tissue were performed using DNEasy™ kits (Qiagen Corporation).

Microsatellite and AFLP genotyping

Microsatellite protocol: We tested 11 of the 18 published microsatellite primers for S. amara (Rodriguez et al. 2000), but poor or unreliable PCR amplification promoted use of the following five dye-labeled primer pairs: SA02 (TET), SA05 (FAM), SA06 (FAM), SA27 (HEX), and SA29 (FAM) (Rodriguez et al. 2000). The PCR (10.0 µl total) contained 250 µm of dNTPs, 25µm $MgCl_2$, 0.0625 µl of Taq polymerase (Qiagen Corporation), and 0.5µl of the forward and reverse primer (10µM stock). All PCRs were performed on an MJ Research PTC-200 thermal cycler, using the following protocol: 5 min at 94˚C; 25 cycles of 45 s at 94˚C, 1 min at 53˚C, and 30s at 72˚C; ending with 15 min at 72˚C. Amplification products were electrophoresed using Rox 400 size standard (Applied Biosystems Incorporated, ABI), on an MJ Research Base Station Automated DNA Analyzer. Forward primers were labeled with the same fluorescent dyes (HEX, FAM, or TET) on loci with non-overlapping size ranges to permit loading of all five loci in a single lane.

AFLP protocol: For Amplified Fragment Length Polymorphisms, we used a protocol modified from Gadeul and colleagues (2000): Digestion and ligation (11.0 µl total volume): 1.1 µl of T4 Buffer 10X; 1.1 µl of 0.5 M NaCl; 0.5 µl BSA; 1 mg/ml MseI (1 U) 0.1 µl; EcoRI (5 U)

0.25 µl; T4-Ligase (1.2 U) 0.2 µl; MseI Adaptors 1.0 µl; EcoRI Adaptors 1.0 µl; dd H_2O 0.2 µl. 5.5 µl of ≥10 mg/µl DNA was added and the reaction was incubated for 2 hrs at 37 ˚C.

Pre-selective PCR (total volume 25 µl): We used 14.9 µl ddH2O, 2.5 µl 10X Buffer; 1.5 µ$MgCl_2$ l; 2.0 µl of 25 µM dNTPs; 0.5 µl of 10 µM EcoRI (E/A); 0.5 µl of 10 µM MseI (M/C); 0.1 µl Qiagen Taq; and 3.0 µl of digested and diluted (1:10) DNA. The PCR was performed for 2 min at 72˚C; 25 cycles of 30 s at 94˚C, 1 min at 56˚C, and 30s at 72˚C; ending with 10 min at 72˚C.

Selective PCR (12.5 µl reaction volume) required: 1.25 µl of 15 mM MgCl210x Buffer; 0.5 µl 25 mM $MgCl_2$, 1.0 µl of 25 mM dNTPs; 10µM 0.05 µl EcoRI (E-AC or -ATG); 0.25 µl MseI (M-CTG or -CTT); 0.1 µl Hotstart Taq; 0.1 µl of 1 mg/ml BSA; 6.75 µl ddH2O; 2.5 µl pre-selective (20X dilution) DNA. The PCR was performed for 15 min at 95˚C; 13 cycles of 30 s at 94˚C, 1 min at 65-55.9˚C, 1 min at 72^0C (ΔT= -0.7 ˚C/cycle); 23 cycles of 30 s at 94˚C, 30 sec at 56˚C, 1 min at 72˚C; ending with 15 min at 72˚C. Two primer pairs were used for AFLP analyses; Mse1+AC/EcoR1+/CTG and Mse1+ATG/EcoR1+CTT. Amplification products were electrophoresed and analyzed using the same equipment and software described for microsatellites.

Data analyses

Control individuals were run on different gels to insure consistency between gel runs and to evaluate possible base pair shifts that might result in allele assignment errors for both microsatellite and AFLP data. For the microsatellite and AFLP loci, we had < 1% and 3% error rates respectively, in allele assignment for the 200 individuals genotyped using both techniques, comparing individuals run on multiple gels at all loci. Each of the five microsatellite loci was

scored using the program Cartographer (MJ Research) utilizing locus binning rules to avoid scoring stutter bands or erroneous alleles. Cervus assignment software (2.0, Marshall et al.1998) was used to calculate the number of effective alleles, observed and expected heterozygosity, frequency of null alleles, and exclusion probabilities at each locus with knowledge of one parent. GENEPOP (vers 3.3 Raymond and Rousset 1995) was used to calculate F_{is} according to Weir and Cockerham (1984), and departure from Hardy-Weinberg equilibrium was tested using Haldane's exact test (1954), with exact P-values estimated using the Markov chain method. Alleles were permuted among individuals within cohorts. We used 1000 random permutation, and jackknifing was performed over loci to estimate standard errors. We used one-way ANOVA and Student's t-test to compare allelic richness among the size classes, and z-test to compare H_o and H_e among cohorts.

AFLP bands were scored using Cartographer**.** After scoring, data were transformed to a 1-0 presence/absence matrix using the program Cotrix (Degen 1999). We also hand-scored all bands. To ensure consistency, bands with less than 10% of the average peak height were not included in analyses. In Cotrix, the fragment data are first sorted by length. The user then determines bounds for each fragment length category using a measure of 'coarseness', whereby fragments differ in length by the number of base pairs selected by the programmer. If coarseness is set too low, the possibility of assigning different lengths to identical fragments increases due to variation in electrophoretic mobility across gels, whereas setting coarseness too high may result in the assignment of fragments of different length to the same band class (Degen 1999). We set the band coarseness at 1.4 base pair differences possibly erring on the conservative side of false locus assignment, and we excluded individuals with fewer than 30 bands, under the assumption that these samples had amplified poorly.

After generating a presence-absence matrix for AFLP bands or tabulating microsatellite alleles, data were analyzed for the existence of spatial autocorrelation using SGS (Degen et al. 2001b). In SGS, the significance values are verified using permutation tests (n=1000) based on Monte-Carlo simulation to evaluate deviation from a spatially random distribution of each calculated measure (Manly 1997), against the null hypothesis of no spatial structure. Simulations compared observed values with the distribution obtained after permutations for each distance class using an individual's AFLP or microsatellite profile. SGS uses the Euclidean distance between pairs of data points. Distograms presenting the Tanimoto distance for AFLP's (Gregorius 1978), and Correlegrams using Moran's Index of spatial autocorrelation for microsatellite data (Sokal and Wartenberg1983) between all pairs of individuals within an assigned spatial distance class, are plotted against the distance class. At least 30 pairs of individuals must be present at each distance class to ensure statistical robustness (Legendre and Fortin 1989, Wartenberg cited in Doligez and Joly, 1997). At small distance intervals (e.g. <20 m), fewer than 30 pairs of individuals occur. Thus, our study focuses on 20 m distance classes. Due to insufficient number of <1 cm dbh pairs of individuals at some of the further distance classes, we restricted the seedling analyses to distances ≤640 m. We used the program SGS (Degen 2001b) to calculate an aggregation index based on Clark and Evans (sensu Ripley 1981) to compare spatial locations of individuals of each size class to determine whether or not S. amara individuals in this populations were generally spatially aggregated or occurred at random. Values <1 indicate a clumped and aggregated distribution and random distribution is associated with values of 1 and above.

Results

All five microsatellite loci were polymorphic with ranges of observed heterozygosity between 0.12 and 0.75 (H_e range = 0.33-0.72) (Table 1). Of the 50 alleles scored, eight (16.7%) were rare – occurring in less than 1% of the individuals genotyped. Twelve alleles were 'private alleles, occurring in only one cohort, with 5, 4, and 3 alleles occurring solely at the seedling, 1-10 cm dbh, and >10 cm dbh size classes respectively. The presence of null alleles at locus SA06 is likely based on estimates using Cervus (Table 1). The mean numbers of alleles per cohort ranged from 7.6-8.2 and were not significantly different (ANOVA, $p > 0.05$). Mean observed and expected heterozygosities (H_o and H_e) ranged from 0.502-0.524 (H_o) and 0.556-0.564 (H_e) (Table 1) and did not differ among cohorts (z test, $p > 0.05$)

Significant deviation from Hardy-Weinberg equilibrium was detected at multiple loci in each of the three cohorts, though it was most frequent at the smallest size class, occurring at four of the five loci (Table 1). The fixation index (F_{is}) was most pronounced at SA06 in all cohorts, perhaps because of putative null alleles at that locus. While there was a significant heterozygote deficit for the smallest sized individuals ($F_{is} = 0.164$), 1-10 cm dbh plants ($F_{is} = 0.118$) and >10 cm individuals ($F_{is} = 0.121$), there were no significant F_{is} difference among the three size classes.

The mean number of AFLP loci per primer per individual was 43.9 (range = 26 – 71 loci) excluding individuals that failed to amplify. In total we analyzed 155 AFLP loci for 190 individuals**.** No genetic structure was detected at short distances for the pooled population of S. amara 1-10 cm dbh and >10 cm dbh genotyped using AFLP's (not shown), though using microsatellites, structure was evident to 20 m not shown.

When analyzed separately, spatial autocorrelation was significant for the >10 cm and 1-10 cm dbh size classes from 0-40 m and 0-20 m , respectively, using Moran's Index (Figures 2a

and b). AFLP analysis for the same classes failed to detect significant spatial autocorrelation at the same distances (Figures 3a and b). The 100 seedlings <1 cm dbh exhibited significant genetic structure at distance classes of 0-40 m, 80-100, and 120-140 m using microsatellites. We observed a negative autocorrelation at distances between 480 and 540 m (Figure 2c).

An aggregation index value of 0.988, 0.982, and 0.635 for the >10 cm dbh, 1-10 cm dbh, and <1 cm dbh cohorts respectively indicate that only at the seedling cohort are individuals more aggregated than would be expected at random. The mean distance to the nearest individual for the >10 cm dbh, 1-10 cm dbh, and the <1 cm dbh cohorts is 34.1 m, 34.1 m, and 21.3 m, respectively. Combining the largest two size classes reduces the mean distance between nearest individuals to 23.5 m, though spatial aggregation remains non-significant.

Discussion

Theoretical models predict the existence of local spatial genetic structure under locally restricted gene flow (Wright 1943, 1978), and an absence of spatial structure when gene flow via seeds is extensive. Where seed dispersal is limited, we expect to observe increased relatedness at local spatial scales, particularly among plant populations with low densities of reproductive adults (Loiselle et al. 1995, Peakall and Beattie 1995, Schnabel et al. 1998), as is generally found in tropical rain forest trees (Fedorov 1966, Hubbell and Foster 1983). Even with random mating, patches of highly related individuals (half siblings) will occur. If seed dispersal is widespread and neighborhood size is large, there is typically an absence of spatial genetic structure even if pollen flow is restricted (Hamrick and Loveless 1986, Kalisz 2001).

In comparing AFLP and microsatellite utility, the two marker systems failed to yield identical results at each distance category. In fact, the AFLP analyses failed to find significant

spatial autocorrelation, though it was detected using microsatellites in all three cohorts. There has been some difference of opinion regarding the relative usefulness of AFLP's and microsatellites (Campbell et al. 2003). We found that microsatellite markers demonstrated significant results in instances where AFLP markers showed a trend in the same direction, but where results were not significant, in spite of using a sizeable number of AFLP bands for analysis. This difference may be due to the fact that microsatellite markers are codominant and thus are more informative in this study than were the dominant AFLP markers. In further discussion we refer specifically to microsatellite findings.

Simarouba amara presents a contrast in patterns, with positive spatial genetic autocorrelation at the shortest distances, and some negative spatial autocorrelation at distances several hundred meters away for each of the three size classes. The presence of significant spatial autocorrelation at short distances among size and census cohorts for S. amara suggests that some seed dispersal is quite local. The distance over which we detect significant spatial autocorrelation is similar to that found in other studies of tropical trees (Legendre and Fortin 1989, Degen et al. 2001a), and may correspond to a spatial scale of conspecific tree clusters.

We suggest that high seed fall near parent plants coupled with possible short-distance secondary seed dispersal near maternal trees accounts for the spatial genetic structure detected at the shortest distance categories for S. amara. The genetic structure pattern we observed at longer distance classes (between 480 and 600 m) for seedlings results from nearby individuals being more closely related than the random population. At further distances, other 'families' of trees are encountered, resulting in less structure than would be predicted, as fewer alleles are shared than expected by chance. Perhaps the lack of relatedness we detected at longer distance classes

reflects long-distance dispersal events which have resulted in recruitment and persistence of individuals unrelated to their neighbors.

The spatial genetic structure observed in the smallest size class is reduced in the 1-10 cm dbh and >10 cm dbh cohorts, suggesting that demographic thinning may be occurring between seed deposition, seedling establishment, and recruitment to later life history stages. Although heterozygosity deficit (F_{is}) was significant at all three cohorts, the decrease in F_{is} from the <1 cm dbh individuals to the larger size classes may further support this idea. Overall levels of heterozygosity at all five loci were remarkably consistent among the three cohorts.

The closer proximity of the <1 cm dbh plants sampled and the significant spatial aggregation apparent at this size class compared to the larger size classes may also have contributed to the presence of spatial genetic structure observed at short distances. Also, since our relatedness values for the larger two size classes does not exceed 1.2 and for <1 cm dbh individuals relatedness is even less, it seems likely that overlapping of seed shadows are present, even at the short distances. It is also possible that local seed shadows result in genetic structuring, but this decreases at the older life history stages, or it persists only at short distances, i.e. 0-40 m. The mean minimum distance between seedlings is less than that of older individuals – 21.3 m (range = 0.9-1003.5 m), compared to 34.1 m (range = 3.3-1006.1 m) and 34.1 (range = 1.3-1012.8 m) 1-10 cm dbh and for >10 cm dbh trees, respectively.

Data from the FDP censuses indicate that turnover in S. amara is rapid. Only 438 of the 1239 stems from the 1982 census were alive in the 2000 census, with the highest mortality occurring at the smallest (1-2 cm dbh) size classes. For example, between the 1995-2000 censuses, 137 1-2 cm dbh individuals (37%) died, whereas 62 (16%) of the 371 trees that died

were ≥10 cm dbh. Such thinning likely contributes to the decay of spatial genetic structure through the culling of half-sibs in aggregations, or selection against inbred individuals.

Muller-Landau (2001) estimated a mean dispersal distance of 39 m for S. amara from seed trap data, only slightly less than the mean minimum distance, 43.6 m, between any two of the 48 female trees that have been reproductive during the last three years. Nonetheless, the generally low density of >20 cm dbh S. amara on the FDP (2.4 trees per ha^{-1}) suggest that overlapping seed shadows are unlikely to be sufficiently pronounced to result in a mixture of different single tree progenies that might obscure weak spatial structure; our results of spatial genetic structure at short distances in all age classes support this. With an estimated mean dispersal distance of nearly 40 m, one would expect to observe at least some weak spatial genetic structure in S. amara at distances less than 100 m if the seed dispersal models utilized by Mueller-Landau (2001) are accurate and transferable from the seed-seedling phase of plant development. We do observe structure at smaller distance classes for all three cohorts, supporting the idea that these models may be transferable to other life history stages, at least at shorter distances. Current modeling techniques, however, likely underestimate seed dispersal distances because they assume seeds in traps arrive from the nearest adult tree. Models also generally ignore long-distance seed input that may play a substantial role in spatial genetic processes (Oddou-Muratorio et al. 2001), as well as individual variability in fecundity and survivorship (Godoy and Jordano 2001). Also, most models fail to incorporate dioecy, and thus underestimate seed dispersal because any reproductive size adult is considered a potential seed donor.

Our results contribute to a growing database suggesting that vertebrate-dispersed tropical tree species often demonstrate mixed patterns of spatial genetic structure over small spatial

scales (~1 ha. to several kilometers). Our study is comparable to that of Degen et al. (2001a), who analyzed spatial genetic structure using RAPD markers for eight canopy tree species in French Guiana using 150 m distance categories. They used the same randomized sampling strategy, but only considered trees > 10 cm dbh. Four of the animal dispersed species in their study showed no evidence of spatial genetic structure, although the other four species did exhibit spatial genetic structure. Weak spatial genetic structure for both adults and seedlings was also observed within a large population of the vertebrate-dispersed tree, Carapa procera, in French Guiana (Doligez and Joly 1997), though data were analyzed at the scale of hundreds of meter distance classes. Fine scale genetic structure, even at the shortest (≤13 m) distance class, was described as "weak at most" by Chung et al. (2000) in all five age classes of a bird-dispersed dioecious tree in southern Korea. Their focal area, however, was restricted to a very small (60x100 m) plot.

Hamrick and colleagues (1993) found that two wind-dispersed species exhibited spatial genetic structure to 30 m (Alseis blackiana) and 100 m (Platypodium elegans) on BCI, though only in the <1.0 m tall and 1-10 cm 'sapling' size classes; structure was not evident among the >10 cm dbh cohort. Similarly, in a recent study by Latouche and colleagues (2003), significant positive spatial correlation was detected for the wind-dispersed tree Dicorynia guianensis for saplings and adults from 50 - ca. 125 m and a negative association was observed for adults from 50-150 -300 m, analyzed at 50 m distance intervals.

The variety of seed and pollen dispersal syndromes among tropical trees and the effectiveness of different dispersal vectors make broad scale generalizations difficult, particularly in highly diverse tropical ecosystems. However, molecular approaches provide a useful tool for assessing both seed arrival and effective dispersal in ways that traditional

ecological studies cannot. Additional studies at multiple spatial and temporal scales will help improve our knowledge of the mechanisms and impacts of dispersal at both local and large scales.

Analyses of local and macro-geographic genetic structure in plant populations may provide insights to the relative importance of gene flow, habitat selection, and genetic drift, which in turn allow us to understand the ecological and genetic basis of microgeographic differentiation in tropical rainforest trees – a process that figures prominently in debates about the origin of the high species richness of tropical rainforest trees (Ashton 1969). Studies of mapped populations in undisturbed forests also provide baseline information about genetic processes that become increasingly important as the demographic structure and animal dispersers become disrupted by habitat fragmentation and global change. This information is also relevant in the context of tropical forest management practices, particularly for vertebrate dispersed tree species such as S. amara.

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References

Aldrich, PR and JL Hamrick. (1998). Reproductive dominance of pasture trees in a fragmented tropical forest mosaic. Science, 281:103-105.

Ashton, PS (1969). Speciation among tropical forest trees: some deductions in the light of recent evidence. Biol. J. Linn. Soc. 1:155-196.

Bawa, KS (1990). Plant-pollinator interactions in tropical rain forests. Ann. Rev. Ecol. Syst. 21:399-422.

Campbell, D, P Duchesne, and L Bernatchez (2003). AFLP utility for population assignment studies: analytical and empirical comparison with microsatellites. Molecular Ecology 12: 1979-1991.

Chase, MR, C Moller, R Kesseli, and K S Bawa (1996). Distant gene flow in tropical trees. Nature 383:398-399.

Chung, MG, MY Chung, GS Oh, and BK Epperson (2000). Spatial genetic structure in a Neolitsea sericea population (Lauraceae). Heredity 85: 490-497.

Condit, R, PS Ashton, P Baker, SS Bunyavejchewin, S. Gunatilleke, N Gunatilleke, and SP Hubbell (2000). Spatial patterns in the distribution of tropical tree species. Science 288: 1414-1418.

Croat, T (1978). Flora of Barro Colorado Island. Stanford University, California.

Degen, B (1999). CoTrix-software for inheritance analysis. In: Which marker for which purpose? Hrsg. E. M. Gillet, Hamburg: Max Wiedebusch Kommiss.-Verl., 2000, 155-156. Hamburg, Nr. 198.User's Manual. http://kourou.cirad.fr/genetique/software.html#CoTrix

Degen, B, H Caron, E Bandou, L Maggia, MH Chevallier, A Leveau, and A Kremer (2001a). Fine-scale spatial genetic structure of eight tropical tree species as analysed by RAPDs. Heredity 87:497-507.

Degen B, R Petit, and A Kremer (2001b). SGS – Spatial Genetic Software: A computer program for analysis of spatial genetic and phenotypic structures of individuals and populations. Journal of Heredity 92: 447-448.

Dick, CW (2001). Genetic Rescue of Remnant Tropical Trees by an Alien Pollinator. Proceedings of the Royal Society of London Series B-Biological Sciences 268:2391-2396.

Doligez, A, and HI Joly (1997). Genetic diversity and spatial structure within a natural stand of a Neotropical forest tree species, Carapa procera (Meliaceae), in a French Guiana. Heredity 79: 72-82.

Fedorov, AA (1966). The structure of the tropical rain forest and speciation in the humid tropics. Journal of Ecology 54:1-11.

Gadeul, M, P Taberlet, and I Till-Bottraud 2000. Genetic diversity in an endangered alpine plant, Eryngium alpinum L. (Apiaceae), inferred from amplified fragment length polymorphism markers. Molecular Ecology 9:1625-1637.

Godoy, JA, and P Jordano. (2001). Seed dispersal by animals: exact identification of source trees with endocarp DNA microsatellites. Molecular Ecology 10: 2275-2283.

Gregorius, HR (1978). The concept of genetic diversity and its formal relationship to heterozygosity and genetic distance. Mathematical Bioscience 41:253-271.

Haldane, JBS (1954). An exact test for randomness of mating. J. of Genetics 52: 631-635.

Hamrick, JL and MD Loveless (1986). The influence of seed dispersal mechanisms on the genetic structure of plant populations. In Frugivores and seed dispersal, A. Estrada and T. Fleming, Eds. 211-223. Dordrecht, The Netherlands.

Hamrick, JL, DA Murawski, and JD Nason (1993). The influence of seed dispersal mechanisms on the genetic structure of tropical tree populations. Vegetatio 107/108:281-297.

Hladik, A and CM Hladik (1969). Rapports trophiques entre végétation et primates dans le foret de Barro Colorado (Panama). La Terre et la Vie. 1:25-117.

Howe, HF (1986). Seed dispersal by fruit-eating birds and mammals. Pp 123-189 in D. R. Murray, editor. Seed dispersal. Academic Press, Sydney, Australia.

Hubbell, SP (1979). Tree dispersion, abundance, and diversity in a dry tropical forest. Science 203: 1299-1309.

Hubbell, SP, and RB Foster (1983). Diversity of canopy trees in a Neotropical forest and implications for conservation. in Sutten SL, TC Whitmore and A C Chadwick, eds. Tropical Rain Forest: Ecology and Management. Blackwell Scientific Publications, Oxford.

Jordano, P, and JA Godoy. (2002). Frugivore-generated seed shadows: a landscape view of demographic and genetic effects. Pages 305-321 in D. J. Levey, W. Silva, and M. Galetti, editors. Frugivores and seed dispersal: ecological, evolutionary, and conservation issues. CAB International, Wallingford, UK.

Kalisz S, JD Nason, FM Hanzawa, and SJ Tonsor. (2001). Spatial population genetic structure in Trillium grandiflorum: the rolres of dispersal, mating history, and selection. Evolution 55: 1560-1568.

Latouche-Hallé C, A Ramboer, E Bandou, H Caron, and A Kremer. (2003). Nuclear and chloroplast genetic structure indicate fine scale spatial dynamics in a Neotropical tree population. Heredity 91:181-190

Legendre, P. & Fortin, M.-J. (1989) Spatial pattern and ecological analysis. Vegetatio 83: 107-138.

Leigh, E (1982). The Ecology of a Tropical Forest: Seasonal Rhythms and Long-term Changes. Eds. A. Stanley Rand, Donald M. Windsor and Egbert G. Leigh Jr. Smithsonian Institution Press, Washington DC.

Loiselle, BA, VL Sork, J Nason, and C Graham (1995). Spatial genetic structure of a tropical understory shrub, Psychotria officinalis (Rubiaceae). American Journal of Botany 82(11):1420-1425.

Loveless, MD and JL Hamrick (1984). Ecological determinants of genetic structure of plant populations. Ann. Rev. Ecol. Syst. 15:65-95.

Manly, BFJ (1997). Randomization, bootstrap and Monte Carlo methods in Biology. Chapman and Hall, London.

Marshall, TC, J. Slate, LEB Kruuk, and ZJM Pemberton (1998). Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology 7:639-655.

Muller-Landau, HC (2001). Seed dispersal in a tropical forest: Empirical patterns, their origins, and their consequences for community dynamics. PhD Diss. Princeton University. Princeton, New Jersey.

Oddou-Muratorio, S, RJ Petit, B Leguerroue, D Guesnet, and B Demesure. (2001). Pollen-versus seed-mediated gene flow in a scattered forest tree species. Evolution 55: 1123-1135.

Ouborg, N J, Y. Piquot, and JM van Groenendael. (1999). Population genetics, molecular markers and the study of dispersal in plants. Journal of Ecology 87: 551-568

Peakall, R and AJ Beattie (1995). Does ant dispersal of seeds in Sclerolaena diacantha (Chenopodiaceae) generate local spatial genetic structure? Heredity 75: 351-361.

Raymond M and F Rousset. (1995) Genepop (version 1.2): population genetics software for exact tests and ecumenicism. J. Heredity 86: 248-249.

Ripley, BD. (1981). Spatial statistics. Wiley series in probability and mathematical statistics, New York .

Rodriguez von Platen H (2000a). Genetic diversity of the tropical tree Simarouba amara in fragmented forests in Costa Rica as measured by DNA microsatellites. PhD dissertation, Yale University.

Rodriguez von Platen, H, J Geistlinger, G Berlyn, G Kahl, and K Weising (2000b). Characterization of novel microsatellite loci isolated from the tropical dioecious tree Simarouba amara. Molecular Ecology Primer Notes 9(4): 498-500.

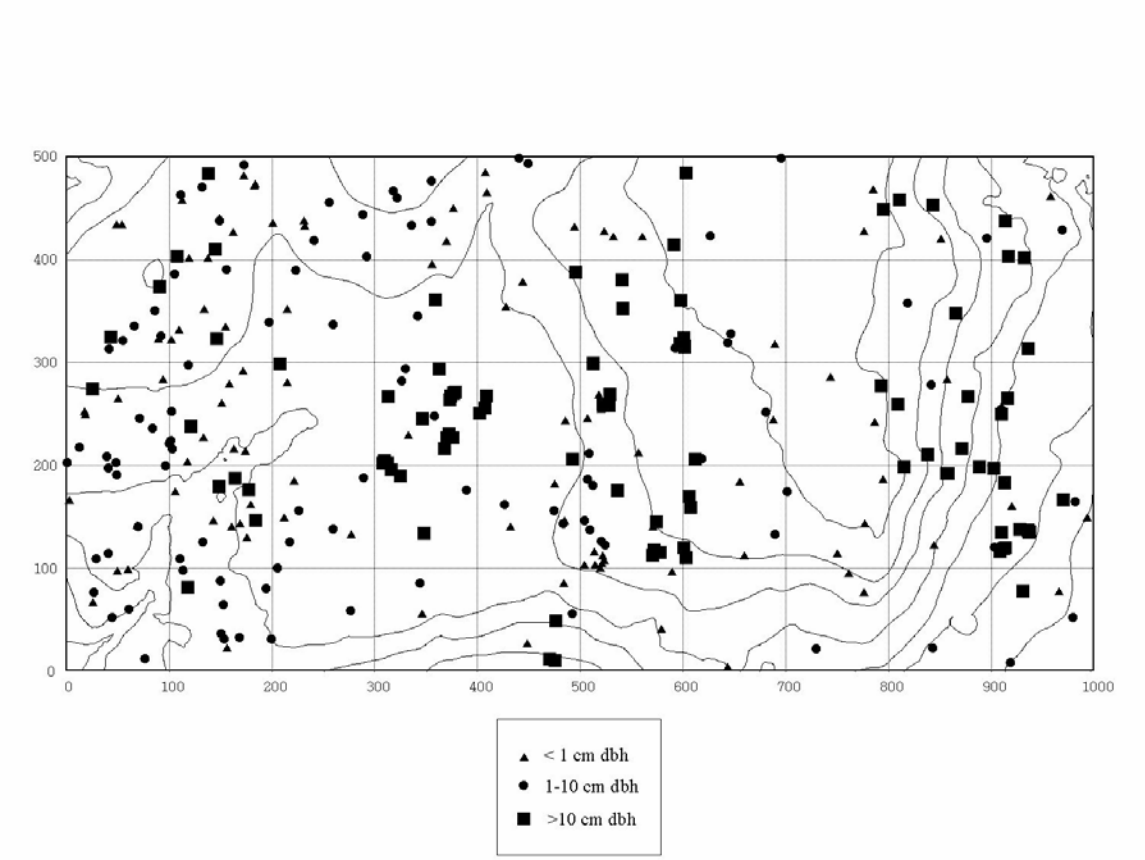
Schupp, EW (1993). Quantity, quality, and the effectiveness of seed dispersal by animals. Pages 15-29 in T. H. Fleming and A. Estrada, editors. Frugivory and seed dispersal: ecological and evolutionary aspects. Kluwer Academic Publishers, Dordrecht, Netherlands.

Schnabel, A, JD Nason, and JL Hamrick (1998). Understanding the population genetic structure of Gleditsia triacanthos L.: seed dispersal and variation in female reproductive success. Molecular Ecology 7:819-832.

Sokal RR and DE Wartenberg (1983). A test of spatial autocorrelation analysis using an isolation-by distance model. Genetics 105:219-237.

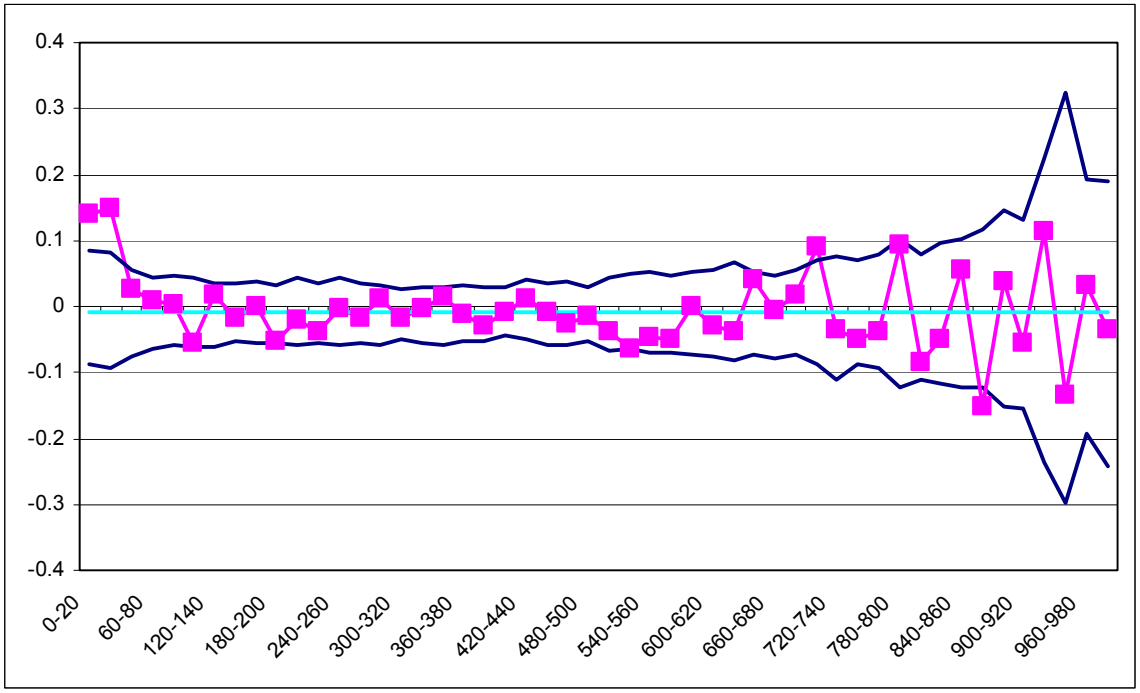
Wright, S (1943). Isolation by distance. Genetics 28: 114-138.

Wright, S (1978). Evolution and the genetics of populations, Vol. 4. Variability within and among natural populations. University of Chicago press, Chicago, Il.



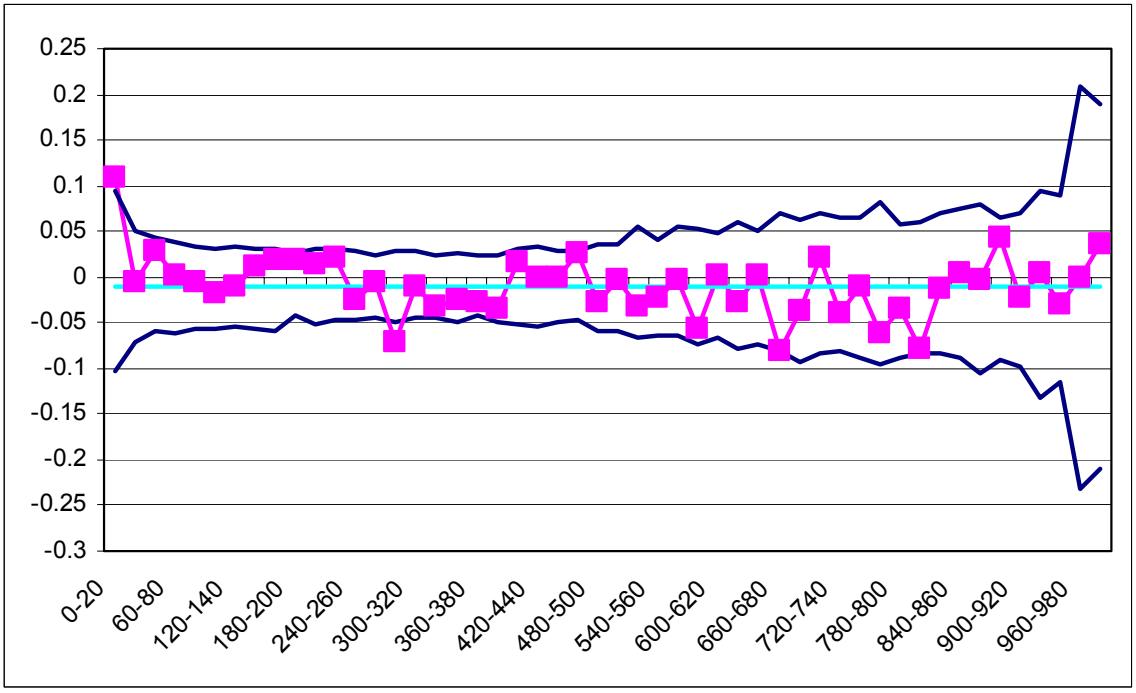
The 50 ha permanent Forest Dynamics Plot on Barro Colorado Island, with 300 S. amara randomly chosen for this study. Locations of 100 individuals of <1 cm dbh are represented with triangles, 100 individuals with a dbh of 1-10 cm are represented with circles, and 100 individuals with a dbh >10 cm are depicted with squares.

a)

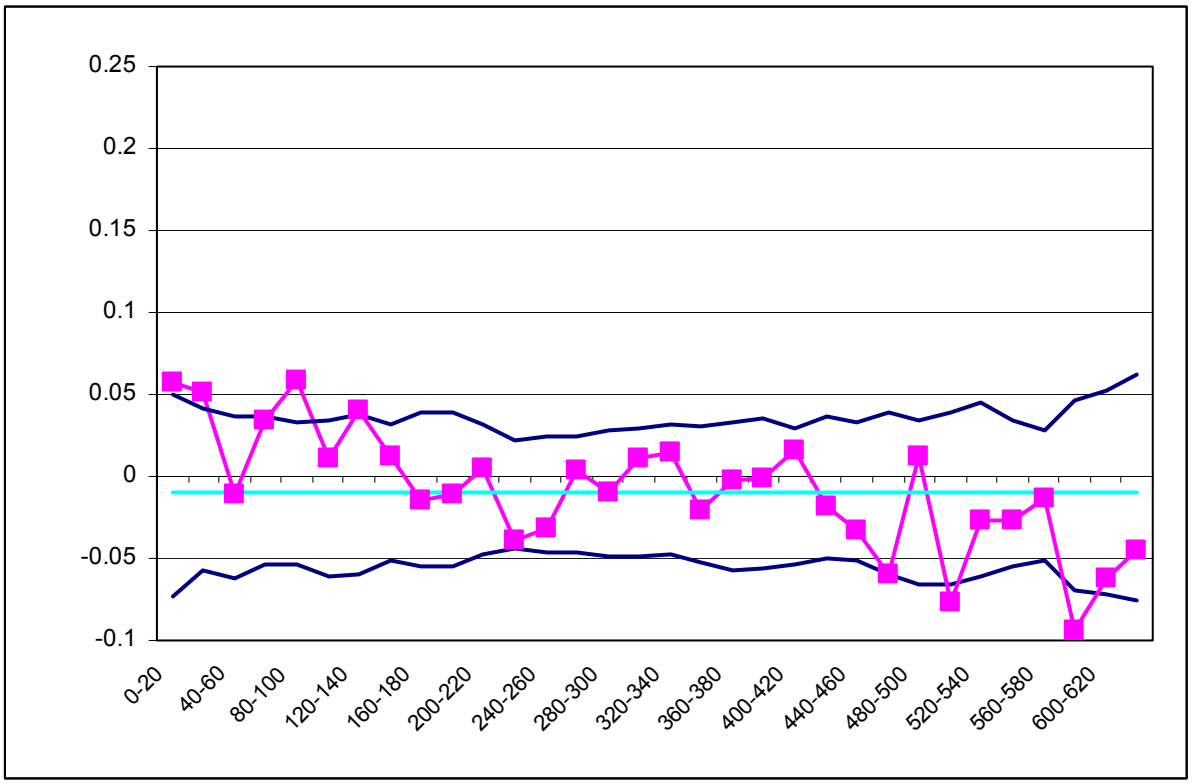


Correlogram of microsatellite data using Moran's Index to depict genetic spatial autocorrelation for 100 randomly selected S. amara individuals in the 50ha FDP representing three size categories: >10 cm dbh (a), 1-10 cm dbh (b), and < 1 cm dbh (c). The reference lines around the point estimates represent 95% confidence intervals around no relationship.

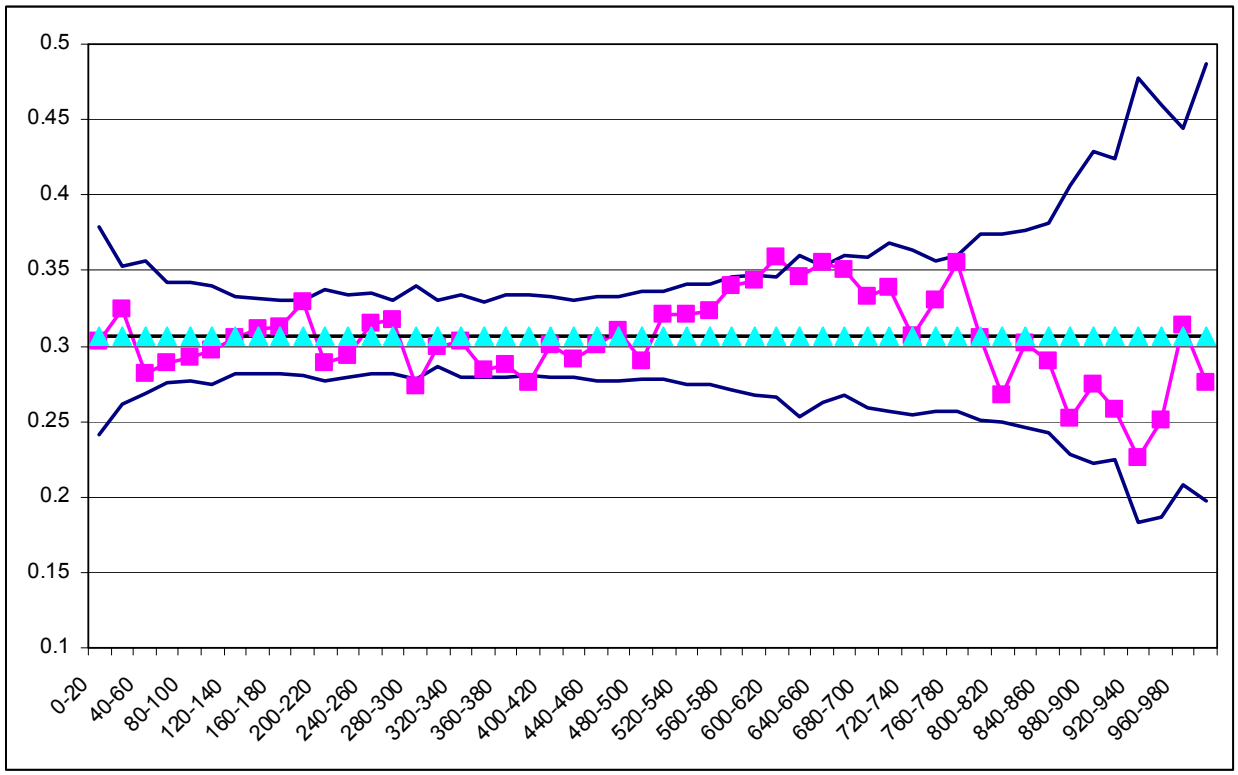
b)


0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.25
-0.3
0-20
60-80
120-140
180-200
240-260
300-320
360-380
420-440
480-500
540-560
600-620
660-680
720-740
780-800
840-860
900-920
960-980

c)


0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
0-20
40-60
80-100
120-140
160-180
200-220
240-260
280-300
320-340
360-380
400-420
440-460
480-500
520-540
560-580
600-620

a)



Distogram of AFLP data using Tanimoto Distance to depict genetic structure for S. amara individuals in two size categories: >10 cm dbh (a) and 1-10 cm dbh (b) at 20 m distance classes within the 50 ha FDP. Observed values (squares) above the reference line indicate positive spatial autocorrelation, and those below the line indicate negative spatial autocorrelation, within upper and lower 95% confidence intervals around no relationship.

b)

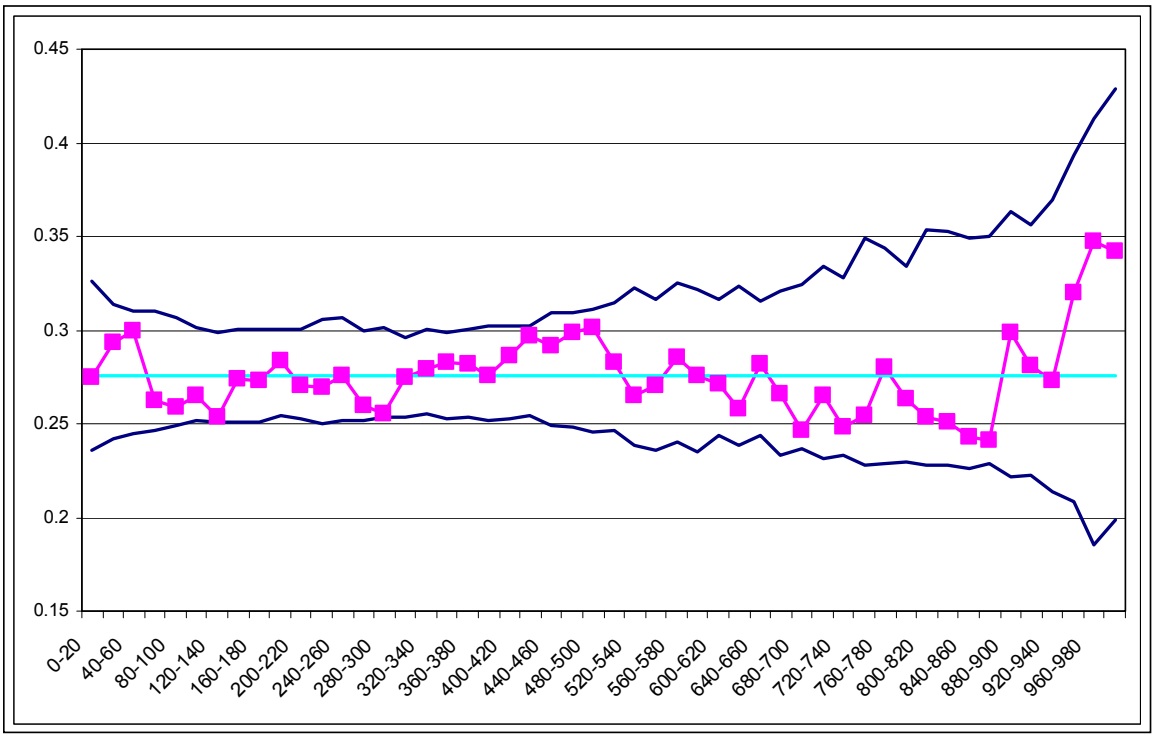

0.45
0.4
0.35
0.3
0.25
0.2
0.15
0-20
40-60
80-100
120-140
160-180
200-220
240-260
280-300
320-340
360-380
400-420
440-460
480-500
520-540
560-580
600-620
640-660
680-700
720-740
760-780
800-820
840-860
880-900
920-940
960-980

Table 2.1 Summary table for profiles of microsatellite loci of S. amara on BCI, with number of alleles (k), number of effective alleles (A_e), observed heterozygosity (H_o), expected heterozygosity (H_e), and null allele frequency (Null) calculated by the program Cervus (Marshall et al. 1998). The number of private alleles (PA) at each locus is also given. F_{is} (Standard Error) and departure from Hardy-Weinberg equilibrium was tested using Haldane's (1954) exact test and P-values were estimated (NS: P >0.05). Mean number of alleles per locus = 10.0 across all three cohorts. Each cohort estimate is based on a sample of 100 individuals.

Locus	**k**	**A_e**	**H_o**	**H_e**	**Null**	**PA**	**F_{is}**	**P-value**
<1 cm dbh								
Sa02	7	2.0	0.53	0.52	-0.04	1	-0.029 (0.0042)	0.027
Sa05	8	3.9	0.72	0.73	-0.01	1	0.029 (0.0175)	NS
Sa06	5	1.4	0.20	0.32	0.31	1	0.252 (0.0029)	0.014
Sa27	11	6.6	0.72	0.84	0.04	1	0.153 (0.0006)	0.000
Sa29	7	1.9	0.28	0.41	0.13	1	0.416 (0.0000)	0.000
All loci	7.6	3.16	0.502	0.564	0.086	5	0.164	<0.000
1-10 cm dbh								
Sa02	4	2.0	0.53	0.51	-0.02	0	-0.022 (-0.0600)	NS
Sa05	8	3.9	0.74	0.74	0.01	1	0.005 (0.0172)	NS
Sa06	8	1.5	0.15	0.27	0.13	0	0.527 (0.0000)	0.000
Sa27	13	6.2	0.83	0.85	0.08	2	0.011 (0.0279)	NS
Sa29	8	1.7	0.37	0.48	0.26	1	0.107 (0.0075)	0.020
All loci	8.2	3.06	0.524	0.556	0.092	4	0.118	<0.000
>10 cm dbh								
Sa02	6	2.3	0.60	0.56	-0.05	0	-0.076 (0.0130)	NS
Sa05	7	3.5	0.78	0.71	-0.05	0	-0.097 (0.0061)	0.038
Sa06	10	1.6	0.15	0.36	0.43	2	0.588 (0.0000)	0.000
Sa27	11	6.3	0.75	0.84	0.05	1	0.118 (0.0031)	0.003
Sa29	7	1.5	0.32	0.34	0.04	0	0.072 (0.0177)	NS
All loci	8.2	3.04	0.520	0.562	0.084	3	0.121	<0.000

CHAPTER 3

Successful long distance seedling recruitment of a vertebrate-dispersed Neotropical tree [1]

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[1] Britta Denise Hardesty, Stephen Hubbell, and Eldredge Bermingham; to be submitted to *Ecology Letters*

Abstract

We used five microsatellite markers to assign seedlings to their maternal and paternal parents in a natural population of the Neotropical tree, *Simarouba amara* (Simaroubaceae) in a forest in Panama. Our objectives were to measure dispersal by calculating distances between maternal parents and their successful seedling recruits, and to compare gene movement via seed and pollen for this vertebrate-dispersed tree. In our case, recruitment includes seed dispersal, germination and subsequent seeding establishment. We were particularly interested in documenting the frequency of long-distance recruitment events, defined operationally as recruitment occurring > 100 m from the maternal parent. In our study, fewer than 10% of seedlings were produced by the nearest reproductive female. Long distance dispersal was frequent: 74% of assigned seedlings were dispersed > 100 m. The mean dispersal distance for established seedlings was 348 m (range 9.3-1000.5 m, 214.5 m SD) from the maternal parent. Gene movement via pollen was comparable to that of seed, with a mean pollen dispersal distance (paternal to maternal parent) of 345.0 m (range 57.6-739.7 m, 157.7 m SD). Significant directional bias was detected in pollen movement, though not in seedling recruitment. These findings demonstrate the high frequency of successful long distance seed dispersal of a vertebrate dispersed tropical tree.

Introduction

The frequency of long-distance dispersal events is an important unsolved problem in dispersal biology and in biogeography (Ouborg 1999). Theoretical models show that even quite modest rates of long-distance dispersal can have large effects on rates of range expansion (Lewis 1997, Clark et al. 1999), and there are good phylogeographic data in several European tree species supporting the importance of long-distance dispersal events during postglacial recolonization (e.g., Petit et al. 1997). Long-distance dispersal can also have large effects on community composition (Hubbell 2001, Borda de Agua et al., in press) and on β diversity (Condit et al 2000).

Despite its fundamental role in forest community structure and dynamics (Clark et al. 1999, Hamilton 1999, Harms et al. 2000), seed dispersal is perhaps the least understood plant life history stage (Anderson 1991, Eriksson and Jacobsson 1999). Historically, evaluating the importance of dispersal has been hampered by the difficulty of measuring dispersal directly. Most attempts to measure seed dispersal have used indirect methods such as fruit removal rates by frugivore dispersers coupled with seed passage times (Holbrook and Smith 2002, Westcott and Graham 2000) or data on seed rain collected using traps (Muller-Landau 2001). These data have been fit with so-called inverse modeling methods to obtain estimates of the dispersal kernel (Ribbens et al. 1994, Clark et al. 1999, Nathan and Muller-Landau 2000). Although model-fit methods account for overlapping seed shadows of clumped adults, it is difficult to independently check the validity of their assumptions. Indirect methods have been applied with the greatest success to species whose seeds are dispersed by abiotic processes such as wind (Nathan and Muller-Landau 2000). However, using indirect methods to study animal-dispersed tree species is especially fraught with difficulties, because the patterns of seed dispersal are often highly patchy

and depend on the idiosyncratic, site-specific movement patterns of particular dispersal agents. Moreover, indirect methods tend to underestimate the frequency of long-distance dispersal events for all dispersal syndromes (Turchin 1998).

Terborgh (1990) has estimated that 80% or more of all tropical tree species are animal-dispersed, mostly by vertebrates; with abiotic factors such as wind, water, and ballistic dispersion comprising the remaining dispersal strategies. Therefore, in order to understand the general significance of dispersal in structuring tropical forests, it is imperative that dispersal studies be conducted on animal-dispersed species. Different animal dispersal agents may have different effects on the demography and population genetic structure of a given tree species. For example, frugivores that disperse seeds of fleshy fruited species may produce highly non-random, aggregated patterns of seed deposition due to their behavior (Howe 1989, Clark et al. 1999, Harms et al. 2000). Dispersers may differentially disperse seeds to particular sites, spatially aggregate seeds, drop seeds differentially underneath the canopy of the maternal trees, and have either a positive or negative effect on seed germination.

Where seeds are deposited can also be important to subsequent seed and seedling survival. Dispersal away from adult conspecifics and other conspecific seedlings can increase post-dispersal survival (Harms et al 2000). Thus, if frugivores transport seeds to sites away from conspecifics, this will tend to improve seed survival. However, in *Prunus mahaleb*, a Mediterranean tree, genetic markers revealed that many seeds are deposited beneath the canopy of conspecific adults other than the maternal parent as dispersers move from tree to tree (Jordano and Godoy 2002). Such dispersal may fail to improve seed survival, however, because seeds are not transported away from conspecifics.

Studying seed dispersal is also important to understanding the genetic structure of plant populations and the maintenance of genetic diversity in them. Animal generated seed shadows can affect local population genetic structure (Hamrick et al. 1993), which will subsequently influence gene movement and recruitment patterns (Jordano and Godoy 2002). Since pollen is haploid and seeds are diploid, if all else is equal, seeds contribute to two-thirds of the genetic neighborhood size, and variance in seed movement contributes twice as much as the variance in pollen movement (Hamilton 1999). Molecular techniques provide an opportunity to measure the elusive 'tail' of the dispersal curve (Clark et al. 1999, Ouborg 1999) and to improve our estimates of variance around the mean as a function of dispersal distance.

For this study, we used microsatellite genetic markers to study seedling recruitment distances (.i.e. effective dispersal) in a naturally occurring population of an animal-dispersed tropical tree, *Simarouba amara* (Simaroubaceae). Genotyping seedlings and matching them to their maternal source provides a means to resolve long-standing questions in dispersal ecology about the spatial relationships between parent trees and their established offspring. It should be noted that genotyping seedlings and assigning them to their maternal parent characterizes the recruitment kernel rather than the dispersal kernel. It combines the net effects of dispersal, post-dispersal survival and germination of the seeds, with establishment of the seedling. We were particularly interested in the role of frugivores in *S. amara* recruitment at relatively large distances from the maternal parent. For purposes of this study, we defined "long distance" to be > 100 m from the maternal parent. We can be certain that seeds were actively transported for established seedlings reaching this distance. Also, this is a long distance in relation to the distances of measured density dependent effects on seedling and sapling growth and survival, which are generally detectable only within a distance of 20 m or less (Hubbell et al. 1990,

Hubbell et al. 2001, Ahumada et al 2004, Uriarte et al. 2004). We address the following questions about the recruitment kernel in *Simarouba amara*: (1) to what distances do seeds disperse and successfully recruit as seedlings? (2) What is the frequency of long distance (>100 m) recruitment, which necessarily involves the active agency of vertebrate dispersers? (3) What is the relative contribution to gene movement made by seeds and pollen? (4) Is pollen movement and seedling establishment random with respect to compass direction? Finally, (5) how well is the recruitment kernel determined by genetic markers fit by dispersal kernels obtained from inverse modeling methods?

Study Site and Focal Species

The study was conducted in the 50 ha Forest Dynamics Project (FDP) plot on Barro Colorado Island (BCI), Panama ($9^0$10'N, $79^0$51'W) (Hubbell and Foster 1983). Within the FDP plot, all ca. 240,000 non-liana woody plants $\geq$ 1 cm diameter at breast height (dbh) have been tagged, mapped and identified to species, including our focal species, *Simarouba amara*. The plot has been censused five times at five year intervals. *Simarouba amara* Aubl. (Simaroubaceae) is a dioecious, vertebrate dispersed tree species that grows to 35 m with a maximum reported dbh of 70 cm on BCI (Croat 1978). This moderate to high light requiring, out-crossing tree typically flowers from February-April, and produces large-seeded fleshy fruits in clusters of 3-5 drupes, up to 17 mm long (Croat 1978). Observed dispersers include chachalacas, flycatchers, motmots, and thrushes (Duke as cited in Croat 1978), as well as tamarinds (pers. obs.); and howler and spider monkeys (Hladik and Hladik 1969). The 2000 BCI plot census contained 1230 individuals $\geq$ 1 cm dbh, 50 of which are reproductive females. The

minimum reproductive size of *Simarouba* is considered to be 30 cm dbh. To be conservative, we considered any individual with ≥20 cm dbh to be potentially reproductive in this study.

Sampling

In 2001 and 2002 we collected leaf tissue from the canopy layer of all reproductive individuals and from any individual ≥20 cm diameter at breast height (dbh) in the BCI 50-ha FDP (Total N = 134, comprising 50 females, 38 males, and 46 non-reproductive or unknown reproductive status adults). In addition, we mapped and collected tissue from all >20 cm dbh S. amara within a 100 m 'buffer zone' around the FDP. The buffer zone added 34 ha to the total area sampled for adults and an additional 24 reproductive females, 23 males, and 37 non-reproductive or unknown gender trees. We added the buffer zone in an attempt reduce the proportion of seedlings without assigned parentage because some seedlings may have come from parents located outside the FDP. We sexed trees based upon floral morphology and presence of fruit. If trees did not produce fruit in the course of our study, they were not considered for maternal assignment to seedlings. Trees identified as male were used for testing paternity, and individuals with >20 cm dbh whose reproductive status was 'unknown' but which could have been reproductive were also considered as potential parents.

Over two years, approximately 40 hectares were systematically searched for *S. amara* seedlings. We sampled tissue from established seedlings ≤ 50 cm tall at various distances from possible parents across the FDP. In 5 x 5 m quadrants where more than five *S. amara* seedlings were encountered, we sampled tissue from every fourth seedling in that quadrant. Otherwise, we collected leaf tissue from each seedling. We could determine that the *Simarouba* seedlings were produced in the current year from the fact that seedlings still had their cotyledons (for at most 6

months post germination) and they have distinctive stem coloration (BDH pers. obs). Because we were especially interested in the role of dispersers in successful recruitment events at long distances from the parents, we systematically searched areas ≥ 100 m from any potential parent tree for seedlings. Though we were initially concerned this could skew our results, we found only 44 seedlings with a greater than 100 m minimum distance to the nearest possible mother for which we ascertained maternal matches; this accounts for only 14% of the total seedlings sampled and 1 ha of sample area within the 50 ha FDP.

In the field we kept leaf tissue in a cooler, and upon return to the laboratory, the tissue was either flash frozen in liquid nitrogen and then stored in a -20^{o} C freezer prior to DNA extraction, or stored in a refrigerator for no more than two days prior to DNA extraction. We extracted DNA using the Qiagen DNEasy Plant kit and PCR protocols followed those described in Hardesty et al. (in press). We genotyped samples on an MJ Basestation automated DNA analyzer. By multiply loading differentially labeled primers, we were able to genotype large numbers of individuals at multiple loci efficiently and cost-effectively. To ensure reliability in scoring gels and avoid genotyping errors (see review in Bonin et al. 2004), we extracted DNA, performed PCR, and genotyped approximately 20% of all potential parents and 10% of seedlings at least twice. A single observer performed all extractions, PCRs, genotyping, and scoring of gels.

We used the Ripley's K function minus πr^2 to test for spatial aggregation (Ripley 1981). Because πr^2 is the ideal value of Ripley's function for a Poisson process (complete spatial randomness), $K - \pi r^2 > 0$ indicates aggregation (more points than expected compared to complete spatial randomness), and $K - \pi r^2 < 0$ indicates a regular distribution (fewer points than expected from a Poisson process). Because we have a finite number of points (the number of

individuals we are testing), even for a poison process (complete spatial randomness), values may not be equal to 0. Hence, we used 500 random simulations to test if the distribution we observe falls within or outside of confidence envelopes drawn from the random simulations.

Genetic diversity analyses

We used expected heterozygosity, (H_e) and the mean number of alleles per locus (A), to assess genetic diversity. Expected heterozygosity is calculated as the proportion of individuals that are heterozygous under the assumption of Hardy-Weinburg equilibrium. The mean number of alleles per locus at all five scored loci provides another measure of genetic diversity among adults and offspring.

Parentage Analyses

Analyses were restricted to those seedlings that we were able to identify unambiguously as seedlings that had germinated in the current year. Since *S. amara* has recalcitrant seeds with no dormancy or a soil seed bank (Camargo et al. 2002), seedlings that germinated in the current year must also have dispersed in the same year from the maternal tree. We used analyses of parentage to determine the seedlings' origin. Through genealogical reconstruction and exclusion of parentage, we were able to determine when seed immigration from outside the extended FDP (84ha.) occurred if a maternal parent for a seedling could not be identified, and we inferred gene flow via pollen from outside the FDP in instances where a male parent could not be discerned.

One advantage of working with a dioecious species such as *S. amara* is that the number of potential parents is essentially halved compared with a hermaphroditic species. Maternity assignment was conducted by simple exclusion based on multilocus genotypes of all female

reproductive trees. All reproductive female trees had unique genotypes, with the exception of three trees that shared an exact genotype within the population. These trees were separated by more than 100 m. For potential paternal parents, a single pair of multilocus genotype matches occurred. Again, they were separated by > 100 m. In the instances in which exclusion could eliminate all but 2-3 potential mothers, we assigned the nearest female to be the mother, thereby making the most conservative dispersal distance estimate. Since the female parent was not definite for seedlings with 2-3 mothers, for these seedlings we could not attempt parent-pair matches, although we were able to test separately for paternity. We calculated the distance from the seedling to the nearest edge of the expanded plot for all seedlings that did not have a maternal match within the study site. This calculation yields a minimum dispersal distance for seedlings whose seeds were dispersed into the 50ha FDP.

There are advantages and disadvantages to various parentage assignment techniques, a discussion of which is beyond the scope of this paper. Because we were more interested in estimating recruitment distances than relative reproductive success of male and female parents, we used exact multilocus matching coupled with likelihood estimates of parentage assignment rather than fractional assignment. We used CERVUS (Marshall et al. 1998) to perform paternity analyses so we could compare gene movement via both pollen and seed. The program uses maximum likelihood to assign a most likely parent based upon Log of the odds (LOD), and follows Meagher (1986). The program uses a 3-step method for assigning parentage. First, allele frequency data is summarized for all parents and progeny. Next, the user runs a simulation for parentage which uses the previously generated allele frequency data to define confidence limits in parentage assignment. At this step, exclusion probabilities for potential parents are calculated. These are based upon the allele frequency distribution present in the population.

Exclusion probabilities differ depending on whether the analysis seeks to identify one parent when neither parent is known or whether the user has a previously assigned maternal genotype and paternal exclusion is the goal. Finally, LOD scores are estimated for the set of parents and offspring selected. Parentage assignment techniques have been criticized because of the bias of increased likelihood of assignment for homozygous individuals (Devlin and Ellstrand 1990, Jones and Arden 2003). However, the high level of heterozygosity at four of the five loci we used (Table 1) reduces this problem. Although our exclusion probabilities for single parent (77%) and second parent (94%) were lower than we would have liked (Table 1), we were able to distinguish among candidate parents or could rule out all possible candidate parents with high confidence for several hundred seedlings, and our findings are based on seedlings with high-confidence parentage assignments. Determining both male and female parents for seedlings enabled us to separate gene movement from pollen versus seed so that we could determine the relative contribution of each to the population of seedling recruits within the BCI 50 ha FDP.

To test whether pollen flow and seed movement was random with respect to direction, we calculated direction from paternal to maternal trees (pollen flow) and from maternal trees to seedlings (seed movement). Using four quadrants of 90 degrees each (from zero to 360), we determined the number of events that fell into each quadrant. We then performed a chi-square test to determine whether the number of seed or pollen movement events that fell in each quadrant demonstrated significant directional bias.

Results

We detected a total of 65 alleles among the five microsatellite across all individuals sampled (range = 10-16 alleles per locus, Table 1). From our estimates of genetic diversity (A,

and H_e) we found that although SA06 has a large number of alleles, it is relatively uninformative, exhibits low heterozygosity, and contributes little to our probability of excluding potential parents (Table 1). This is likely due to the presence of null alleles at this locus, because SA06 exhibits heavily skewed allele frequencies. In contrast, the four other loci exhibit high levels of heterozygosity, and therefore were more useful for parentage analyses.

In total, 306 seedlings were analyzed for which we could either discern no potential mother or which we were able to match to one, two or three potential mothers (Figure 2). Forty mothers out of seventy-four reproductive females produced the 195 seedlings for which we were able to make maternal assignments. For 111 of the seedlings (36%), there was no potential maternal match within the 84 ha area sampled. The mean minimum dispersal distance (i.e. distance to extended plot edge) for these unmatched seedlings was 217.1 m ± 65.2 m SD (range 93.1-343.5 m).

The spatial distribution pattern of *S. amara* on the FDP (N=1230) differs significantly from complete spatial randomness (Figure 1a). Reproductive females also demonstrated a spatial distribution different from random (Figure 1b) and the mean distance between nearest females was 36.5 m (Figure 2). The spatial aggregation observed in seedlings was similar to that of all *S. amara* in the FDP (Figure 1c), and the mean distance was 11.3 m to the nearest seedling. Removing the 41 seedlings which were detected by searching >100 m distance from the nearest female adult does not alter these findings. With these 41 seedlings excluded, the mean distance between nearest seedling pairs was 11.1 m and the seedlings remain aggregated (Figure 1c). Male trees on the FDP were also spatially aggregated (Figure 1d), in a similar manner to that observed for female adults, and the mean distance between nearest male adult trees was 77.2 m.

For seedlings whose genotypes matched with one or more mothers, 97 (31.7%), 51 (16.7%) and 47 (15.4%) matched with one, two or three mothers, respectively. Only 17 (8.8%) of these 195 seedlings were produced by the nearest reproductive female. For the remaining 91.2% of seedlings, the nearest reproductive female was not among the potential mothers of the seedling. The mean distance from seedling to nearest reproductive female tree was 50.5 m ± 35.5 SD (range 0.76-153.1 m). The mean distance to the genetic mother was 278.6 m ± 214.5 SD (range 9.2-1000.5 m) for all seedlings, including those assigned to the nearest of 2-3 potential maternal parents. When seedlings were unambiguously matched to a unique mother (N=97), the mean dispersal distance was 348.0 m ± 238.6 SD (range 9.3-1000.5 m). See Figure 3a for the frequency distribution of distances between seedlings and maternal parents.

The 97 seedlings that matched to a unique mother were the collective progeny of 30 female trees whose numbers of genotyped offspring ranged from 1-10 seedlings. Interestingly, we found that pairwise seedling distances for half and full sibs generally exceeded 100 m (median = 187.4 m, mean = 215.8 m± 137.0 SD, range = 0.81-801.6 m) and sib distances were significantly different ($P<0.0001$, T-test) from nearest seedling distances for all 306 seedlings (median nearest seedling distance = 6.2 m, mean = 11.3 m, $\pm$15.8 SD, range = 0.34-132.8 m). Using a chi-square test, no directional bias for mother to offspring was evident ($x^2=7.701$, DF=3, $P>0.05$).

We were also able to unambiguously assign a unique father for 100 seedlings. Twenty-five (of 75 potential) fathers sired between 1- 18 seedlings each. The mean net pollen movement (i.e. the distance between the pollen parent and the seedling) for these 100 seedlings that were assigned to a single father was 373.2 m $\pm$ 243.1 m SD (range 1.4-1005.8 m) (Figure 3b). The

variance in the number of offspring produced by females was 8.0, while the variance for male reproductive success was nearly four-fold, 23.9.

We assigned the pollen parent unambiguously for one third (N=33) of the seedlings with a single mother. For these full parentage seedlings, the mean distance between parent pairs, and thus, the mean distance for pollen movement, was 334.4 m ± 231.0 m SD (range 8.0-1063.2 m, Figure 4a). In contrast, the mean distance between nearest male-female pairs was 54.1 m ± 35.6 SD (range 2.3-145.6 m). Thus, seedlings were seldom the result of pollination between neighboring reproductive trees of the opposite sex (Figure 3b). The average distance between fathers and seedlings for the parent-pairs was 326.3 m, which was slightly less than the 349.7 m distance between mothers and seedlings for the two-parent seedlings. Direction for pollen movement from paternal to maternal parent was significantly northeasterly (x^2=10.03, DF=3, P=0.018) (Figure 4b). For the remaining seedlings (N=67) for which we were unable to assign a single, unique father, pollen movement was attributed to fathers outside the 84 ha sample area.

Discussion

We infer that an overwhelming proportion of established seedlings in our population were the result of active long-distance seed dispersal. Seedling establishment was frequent near non-related adult trees, rather than adjacent to maternal parents, and gene movement via seed and pollen was of a similar magnitude. Our estimates of seed and pollen movement are conservative because they fail to account for dispersal events resulting from cryptic gene flow (Devlin and Ellstrand 1990), the false assignment of a parent to an offspring with the true parent outside of the 84 ha study area.

Because *S. amara* is insect pollinated and vertebrate dispersed, we were interested in testing whether there was a directional bias in pollen movement and successful seed dispersal. We found no directional bias in successful seedling establishment, though pollen movement was significantly biased to the northeast. *Simarouba amara* flowers during the dry season (from February until early April) and the prevailing wind direction on Barro Colorado Island is from the northeast, as trade winds move across Panama from the Atlantic to the Pacific. We hypothesize that insect pollinators tend to orient with the wind current. A similar pattern of pollen movement was seen for *Platypodium elegans*, another canopy tree species on BCI (Hufford et al. unpublished). In contrast, large vertebrate frugivores such as those that disperse the seeds of *S. amara* are unlikely to be influenced by directional wind patterns.

We chose a conservative approach for seedlings with multiple potential mothers and assigned the seedling to the nearest of the possible mothers. In doing so, we potentially underestimate dispersal distances. Fewer than 10% of the assigned seedlings belonged to the nearest reproductive female tree. In addition, we found a nearly 100 m difference in mean dispersal distance for seedlings with a single mother match compared to those with >1 possible mother. Given that inverse modeling techniques find a mean seed dispersal distance of 39 m for *S. amara* (Muller-Landau 2001), we were impressed to find a mean seedling establishment distance of 349 m, with >50% of the seedlings deposited from mothers more than 200 m away from the maternal source tree.

In contrast with inverse modeling techniques that weigh seed distribution heavily by proportionately assigning more seeds to the nearest adult, we found seedlings located next to the nearest possible parent (i.e. reproductive adult) to be the exception rather than the rule. However, inverse models focus on seed arrival rather than successful seedling establishment

(Clark et al. 1999, Muller-Landau 2001), and as such do not take into account that where seeds are most abundant may not necessarily be where they are most likely to germinate and become established. Our findings suggest that Janzen-Connell 'escape' effects influence the distribution patterns of seedlings observed for *S. amara* in the BCI population. Perhaps seedlings are less likely to survive under parent trees due to high predation or pathogens, in spite of an abundance of immature and ripe fruits (and hence seeds) falling beneath reproductive females (BDH unpublished data). Although fruits occurring underneath the canopy of one parent tree may have been deposited there by dispersers, we seldom detected obvious signs of animal handling on intact fruits.

We compared genotypes and allele frequencies to look for any allelic differences between parents and non-parents. For female trees, we detected 5 unique alleles within four individuals. One of these was a unique mother (3 assigned offspring), a second individual was a default by distance paternal parent (for a case of two potential mothers, 1 assigned offspring) and two were not maternal parents. For male reproductive adults, again we detected unique alleles, representing four individuals. Two of these trees were paternal parents, with eight and one assigned progeny. The other two trees were not paternal parents. Hence, we see no pattern to suggest that there is a bias of unique alleles for parentage assignment in our study population.

Interestingly, rarely were the parent-pairs close to one another. We found only three instances of parent pair distances < 100 m, although the mean distance between nearest possible pairs of parents was 54 m. Only in one case was the closest pair of reproductive adults the true parent pair. We expected a higher frequency of short distance pollen movement between reproductive adults because possible parent pairs are located at relatively short distances and because there is an area of apparently high reproductively males and females in a clump of 11

large adults in the FDP, it seems reasonable to expect pollinators to move between adjacent flowering trees, and we collected tissue from seedlings in that area (BDH pers. obs). While it is certainly possible that nearby parent-pairs produced seedlings, in our analyses we were unable to discern any such pairings; perhaps because we did not map, genotype, and match every seedling in the FDP.

A possibility of bias in recruitment distances exists because we did not survey seedlings at random throughout the entire 50 ha of the FDP and because we made a special effort to sample away from any reproductive sized trees if possible. However, the areas we sampled for seedlings >100 m from any reproductive sized adult constituted only a total of 1 ha, and accounted for 14% (N = 44) of the matched seedlings. Of these 44 seedlings, 48% (N=21) had no maternal parent within the 84 ha study area. The other 23 seedlings were assigned to a single maternal parent in 11 cases (25%), and two or three possible maternal parents in seven (16%) and five cases (11%), respectively. Only two of the 44 seedlings occurred >150 m from the nearest reproductive female tree. Also, filtering the data by removing these 44 seedlings does not significantly alter our findings.

Some nearby reproductive adults may have been overrepresented due to proximity to seedlings, but this is to be expected even in randomly sampled data. However, the frequency of long distance dispersal and the relative infrequency of assigning as parents those reproductive adults closest to matched seedlings suggested this was quantitatively unimportant. There was also an assumption on our part that seedlings near the edge of the plot would be more likely to have unknown mothers since the genetic neighborhood for edge seedlings may include adults that occurred outside of the 100 m buffer zone, and were therefore not genotyped. Nonetheless,

we found no evidence to indicate that seedlings in any particular area of the FDP were more or less likely to have assigned parents.

The frequency of long distance dispersal we detected greatly exceeded dispersal distances reported by Godoy and Jordano (2001), in a study somewhat similar to ours. Godoy and Jordano found many seeds arriving under conspecific adults and found that up to 62% of their seeds were delivered within 15 m of the source parent tree. However, they report on seed arrival distances, not distance between maternal trees and established seedlings, and they, too detected a large (17.9%) proportion of immigrant seed rain.

While Godoy and Jordano (2001) suggest that "distance limited spatial aggregation of seed shadow" is typical of fleshy fruited animal dispersed plant species, we found successful long distance recruitment to be common. We detected dispersal distances comparable to, though longer than, successful dispersal distances reported by Sezen et al. (2005), with the tropical palm *Iriartea deltoides*. In contrast to their findings that pollen movement was considerably shorter than distances between maternal trees and established founder trees, we found pollen and seed movement distances to be similar for *S. amara* seedlings in Panama.

Although in instances of forest fragmentation genetic variability may be maintained by long distance pollen flow (e.g. Dick 2001), gene movement via seed appears equally important in this tropical tree species, at least in the intact forest environment where this study took place. Whereas Aldrich and Hamrick (1998) and Sezen et al. (2005) found reproductive dominance by few individuals in their study sites, we found that more than half (54%) of the reproductive females and 41% of the known males contributed progeny in this study and Hufford (2000) reported that more than 75% of the possible fathers sired progeny for *Platypodium elegans* on

the FDP. This difference in genetic dominance in cases of altered landscape compared to intact forest may have a tremendous long term influence on genetic diversity through time.

To our knowledge, few studies have estimated both pollen and seed dispersal directly for a tropical tree. Although Aldrich and Hamrick (1998) and Sezen et al. (2005) both reported pollen and seed dispersal distances, each of these studies focused on a species within a disturbed landscape, either fragmented or second-growth forest, and neither reported testing for directional movement of either seed or pollen. In addition, these two studies took place at spatial scales of 30 ha and 38.5 ha, which is less than half of the area we sampled for adults in our study. Our study provides an important baseline for dispersal studies, measuring realized dispersal distances for a vertebrate dispersed tree species in an intact tropical forest.

In summary, we found that gene movement via seed was comparable to that of pollen flow, in contrast with other studies of tropical trees which generally attribute gene movement to long distance pollen flow (Aldrich and Hamrick 1998, Konuma et al. 2000, Latouche et al. 2003, Hamrick and Loveless 1989, and Nason et al. 1998). Successful seedling establishment 500 m or more from the maternal parent was common, providing strong evidence that long distance dispersal and subsequent seedling establishment is unlikely to be a rare event. Whereas previous studies have focused on seed arrival in traps or have coupled radio-tracking of frugivore dispersers with seed passage trials to estimate dispersal distances, we can now be more precise and garner exact data on seed arrival (sensu Jordano and Godoy 2002) and seedling survival distances from parent trees using molecular techniques. Our findings of frequent long distance dispersal and seedling recruitment suggest that vertebrate dispersers are indeed playing a critical role of active seed dispersal that results in germination and seedling establishment for *S. amara*.

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References

Aldrich, PR and JL Hamrick (1998). Reproductive dominance of pasture trees in a fragmented tropical forest mosaic. Science, 281:103-105.

Ahumada, JA, SP Hubbell, R Condit and RB Foster (2004). Long term survival in a Neotropical forest: the influence of local biotic neighborhood. In Tropical forest diversity and dynamism Findings from a large-scale plot network. E C Losos and EG Leigh, Jr, Eds. University of Chicago Press, Illinois.

Anderson M (1991). Mechanistic models for the seed shadows of wind-dispersed plants. American Naturalist 137(4): 476-497.

Bonin, A, E Bellemain, P Bronken Eidesen, F Pompanon, C Brochmann and P Taberlet (2004). How to track and assess genotyping errors in population genetic studies. 13:3261-3273.

Borda de Agua, L, SP Hubbell and F He. In press. Scaling biodiversity under neutrality. Proceedings from the workshop Scaling Biodiversity, Czech Republic.

Camargo, JLC, IDK Ferraz, and AM Imakawa (2002). Rehabilitation of degraded areas of Central Amazonia using direct sowing of forest tree seeds. Restoration Ecology 10: 636-644.

Clark, J.S., M. Silman, R. Kern, E. Macklin, and J. HilleRisLambers (1999). Seed dispersal near and far: generalized patterns across temperate and tropical forests. Ecology 80: 1475-1494.

Condit, R, PS Ashton, P Baker, SS Bunyavejchewin, S. Gunatilleke, N Gunatilleke, and SP Hubbell (2000). Spatial patterns in the distribution of tropical tree species. Science 288: 1414-1418.

Croat, T (1978). Flora of Barro Colorado Island. Stanford University, California.

Devlin, B and NC Ellstrand (1990). The development and application of a refined method for estimating gene flow from angiosperm paternity analysis. Evolution. 44:248-259.

Dick, CW (2001). Genetic Rescue of Remnant Tropical Trees by an Alien Pollinator. Proceedings of the Royal Society of London Series B-Biological Sciences 268:2391-2396.

Eriksson, O. and A. Jakobsson (1999). Recruitment trade-offs and the evolution of dispersal mechanisms in plants. Evolutionary Ecology 13(3): 411-423.

Godoy, JA, and P Jordano (2001). Seed dispersal by animals: exact identification of source trees with endocarp DNA microsatellites. Molecular Ecology 10: 2275-2283.

Hamilton, MB (1999). Tropical tree gene flow and dispersal. Nature 401:129.

Hamrick, JL and MD Loveless (1989). The genetic structure of tropical tree populations: associations with reproductive biology. Pp 129-146 in JH Bock and YB Linart, eds. The evolutionary ecology of plants. Westview, Boulder, CO.

Hamrick, JL, DA Murawski, and JD Nason (1993). The influence of seed dispersal mechanisms on the genetic structure of tropical tree populations. Vegetatio 107/108:281-297.

Harms, K. E., S. J. Wright, O. Calderon, A. Harnandez, and E. A. Herre (2000). Pervasive density-dependent recruitment enhances seedling diversity in a tropical forest. Nature 404: 493-495.

Hladik, A and CM Hladik (1969). Rapports trophiques entre végétation et primates dans le foret de Barro Colorado (Panama). La Terre et la Vie. 1:25-117.

Holbrook, KM and TB Smith (2000). Seed dispersal and movement patterns in two species of Ceratogymna hornbills in a West African tropical lowland forest. Oecologia. 125: 249-257.

Howe, H (1989). Scatter-and clump dispersal and seedling demography: hypothesis and implications. Oecologia 79: 417-426.

Hubbell, SP (2001). The Unified Neutral theory of biodiversity and biogeography. Princeton University Press.

Hubbell, SP, R Condit, and RB Foster (1990). Presence and absence of density dependence in a Neotropical tree community. Transactions of the Royal Society of London. 330: 269-281.

Hubbell, SP, and RB Foster (1983). Diversity of canopy trees in a Neotropical forest and implications for conservation. In Sutten SL, TC Whitmore and A C Chadwick, eds. Tropical Rain Forest: Ecology and Management. Blackwell Scientific Publications, Oxford.

Hubbell, SP, JA Ahumada, R Condit and R Foster (2001). Local neighborhood effects on long-term survival of individual trees in a Neotropical forest. Ecological Research 16:859-875.

Hufford, KM (2000). Viability selection, inbreeding depression and male reproductive success at three early life stages of the tropical tree, *Pladypodium elegans*. PhD Diss. University of Georgia. Athens, Georgia.

Jones, AG and WR Arden (2003). Methods of parentage analysis in natural populations. Molecular Ecology 12:2511-2523.

Jordano, P, and JA Godoy (2002). Frugivore-generated seed shadows: a landscape view of demographic and genetic effects. Pages 305-321 in D. J. Levey, W. Silva, and M. Galetti, editors. Frugivores and seed dispersal: ecological, evolutionary, and conservation issues. CAB International, Wallingford, UK.

Konuma, A, Y Tsumura, CT Lee, SL Lee and T Okuda (2000). Estimation of gene flow in the tropical-rainforest tree *Neobalanocarpus heimii* (Dipterocarpaceae), inferred from paternity analysis. Molecular Ecology 9: 1843-1852.

Latouche-Hallé C, A Ramboer, E Bandou, H Caron, and A Kremer (2003). Nuclear and chloroplast genetic structure indicate fine scale spatial dynamics in a Neotropical tree population. Heredity 91:181-190

Lewis, MA (1997). Variability, patchiness, and jump dispersal in the spread of an invading population. In Spatial Ecology: the role of space in population dynamics and interspecific interactions, D Tilman and P Kareiva, eds. Princeton University Press, New Jersey.

Marshall, TC, J. Slate, LEB Kruuk, and ZJM Pemberton (1998). Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology 7:639-655.

Meagher, TR (1986). Analysis of paternity within a natural population of *Chamaelerium luteum* I. Identification of the most-likely male parents. American Naturalist. 128: 199-215.

Muller-Landau, HC (2001). Seed dispersal in a tropical forest: Empirical patterns, their origins, and their consequences for community dynamics. PhD Diss. Princeton University. Princeton, New Jersey.

Nathan R and HC Muller-Landau (2000). Spatial patterns of seed dispersal, their determinants and consequences for recruitment. Trends in Ecology and Evolution 15: 278-285.

Nason, JD, EA Herre and JL Hamrick (1996). Paternity analysis of the breeding structure of strangler fig populations: evidence for substantial long-distance wasp dispersal. Journal of Biogeography. 23:501-512.

Ouborg, N. J., Y. Piquot, and J. M. van Groenendael (1999). Population genetics, molecular markers and the study of dispersal in plants. Journal of Ecology 87: 551-568

Petit, RJ, E Pineau, B Demesure, R Bacilieri, A Ducousso, and A Kremer (1997). Chloroplast DNA footprints of postglacial recolonization by oaks. Proceedings of the National Academy of Sciences of USA. 94: 9996-10001.

Ribbens E, JA Silander, and SW Pacala (1994). Seedling recruitment in forests: calibrating models to predict patterns of tree seedling dispersion. Ecology 75:1794-1806.

Ripley, BD (1981). Spatial statistics. Wiley series in probability and mathematical statistics, New York.

Rodriguez von Platen, H, J Geistlinger, G Berlyn, G Kahl, and K Weising (2000). Characterization of novel microsatellite loci isolated from the tropical dioecious tree *Simarouba amara*. Molecular Ecology Primer Notes 9(4): 498-500.

Sezen, UU, RL Chazdon, and KE Holsinger (2005). Genetic consequences of tropical second-growth forest regeneration. Science. 307:891.

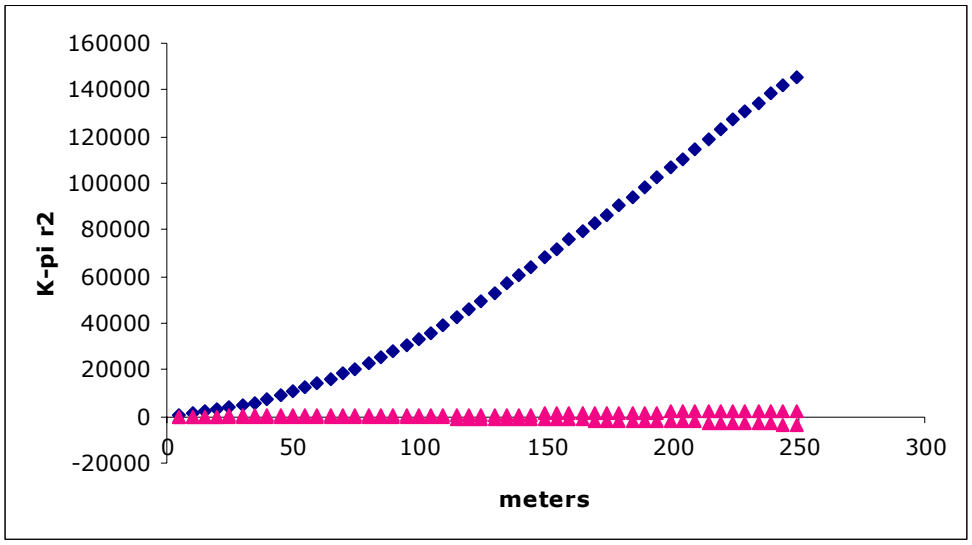
Terborgh, J (1990). Seed and fruit dispersal-commentary. *Reproductive ecology of tropical forest plants* (eds. K.S. Bawa and M. Hadley), pp181-190. The Parthenon Publishing Group, Park Ridge, New Jersey.

Turchin, P (1998). Quantitative analysis of movement: measuring and modeling population redistribution in animals and plants. Sinauer, Sunderland, Massachusetts.

Uriarte, M CD Canham, J Thompson, JK Zimmerman and N Brokaw (2004). Seedling recruitment in a hurricane driven forest: light limitation, density-dependence, and the spatial distribution of parent trees. Journal of Ecology.

Westcott, DA & DL Graham (2000). Patterns of movement and seed dispersal of a tropical frugivore. Oecologia, 122, 249-257.

a)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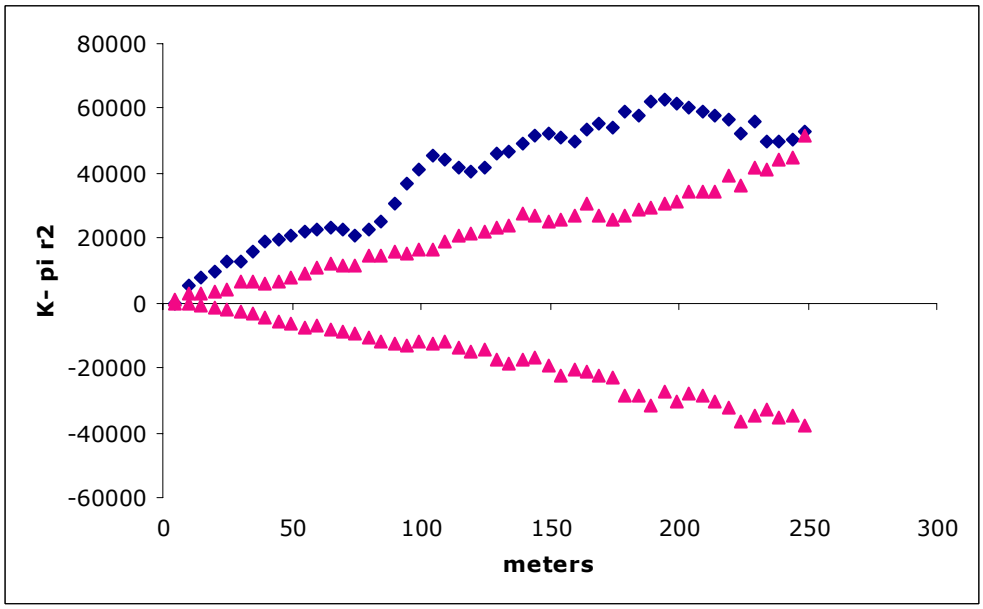
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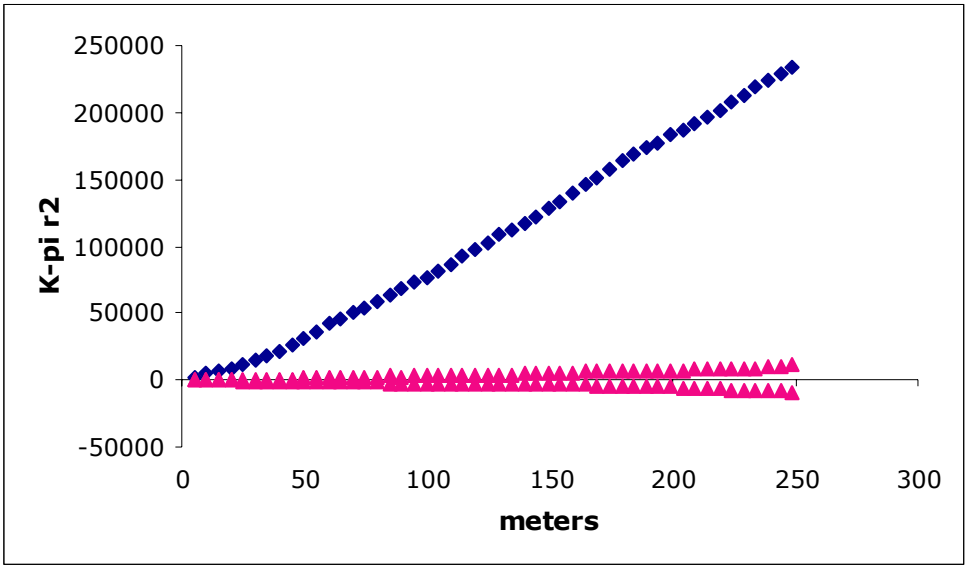

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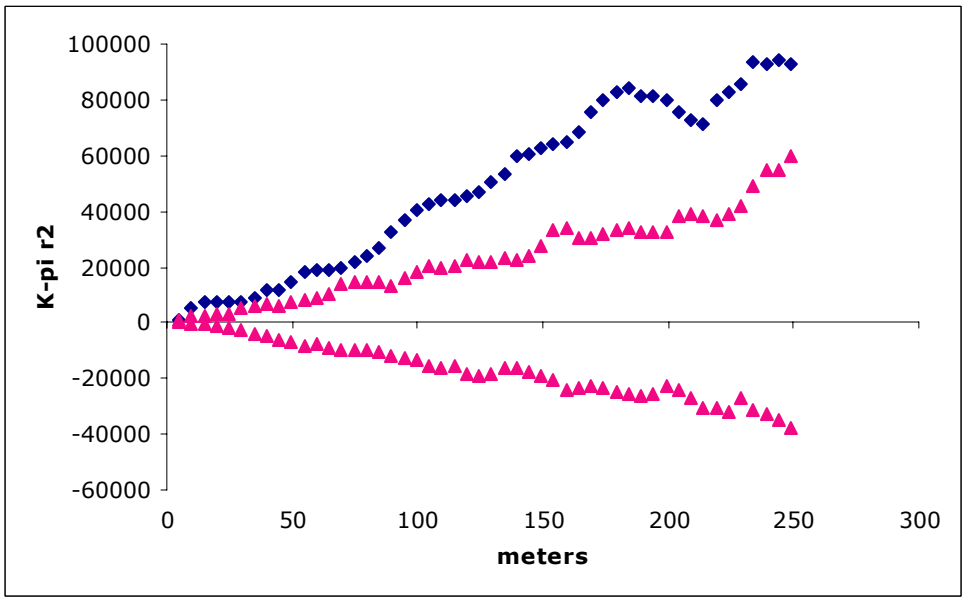
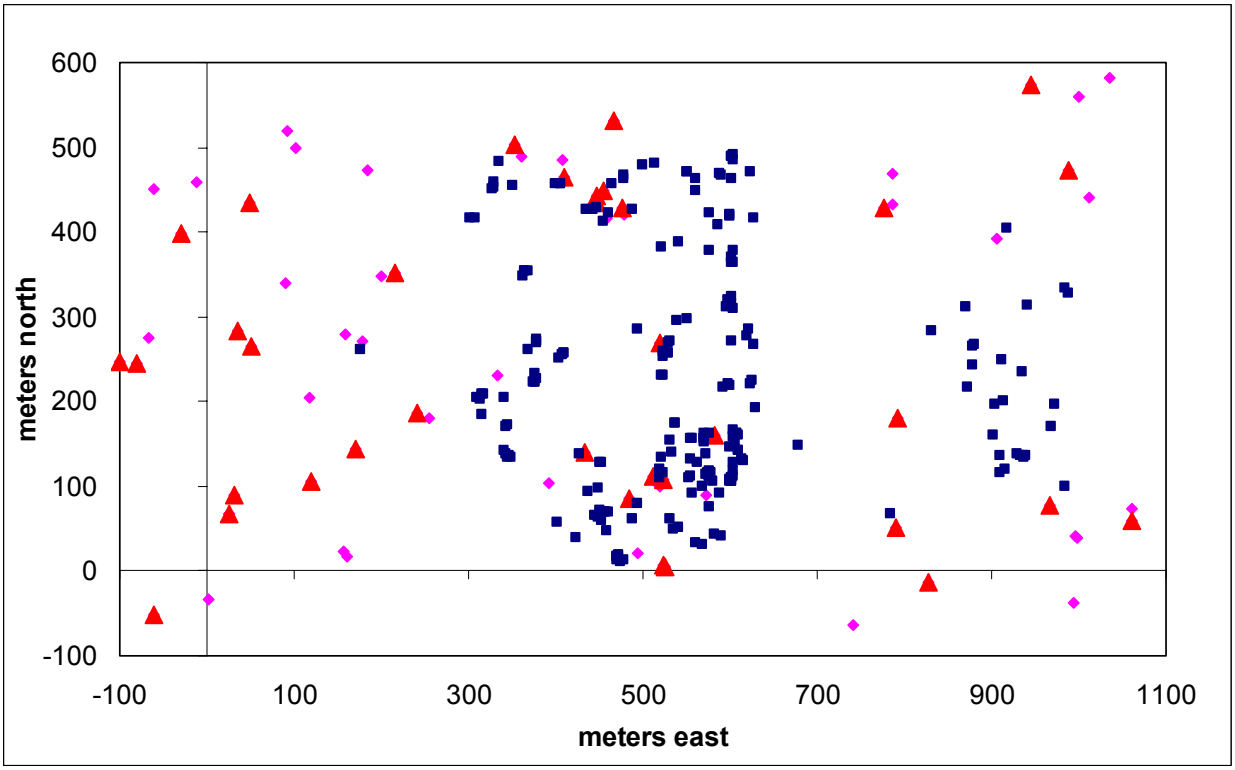
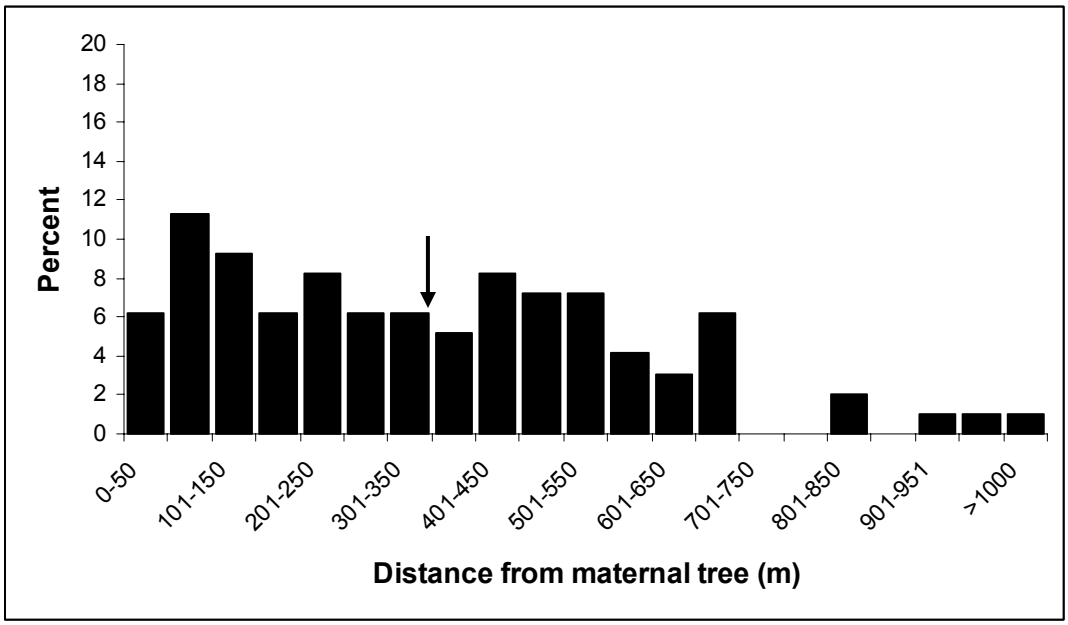


Ripley's K – πr^2 used to test for spatial aggregation. Observed values (diamonds) outside the envelope (500 simulations, triangles) >0 indicate aggregation, whereas values <0 demonstrate a regular distribution for a) the 1230 *S. amara* within the FDP; b) reproductive females (N = 49); c) seedlings (N=306); and d) reproductive males (N=51).



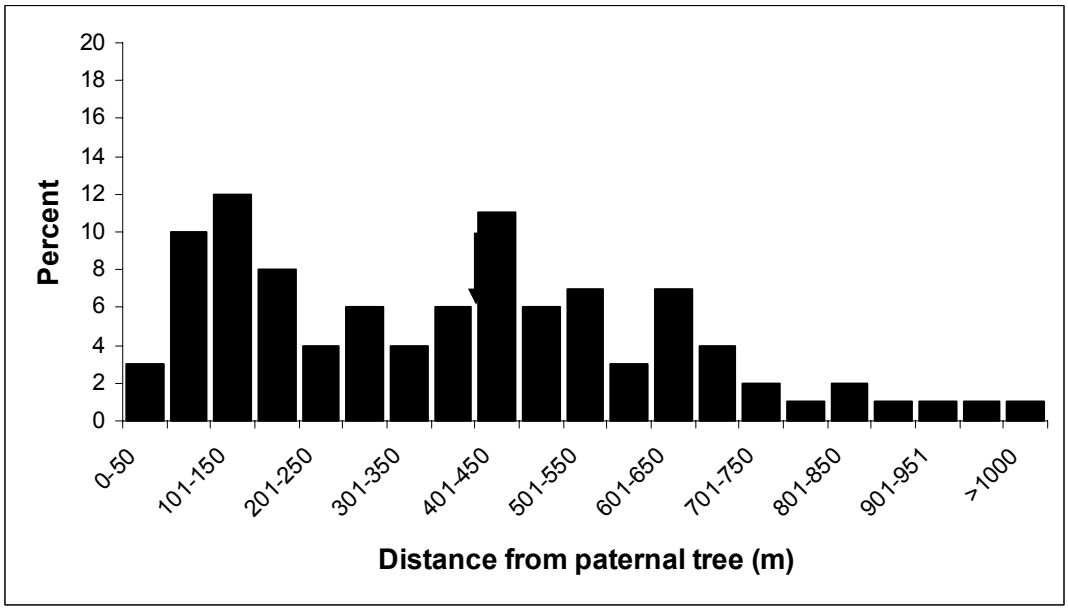
Spatial locations of genotyped seedlings that have maternal matches (squares) or were unmatched to female reproductive trees (diamonds). Actual mothers are shown in triangles.

a)



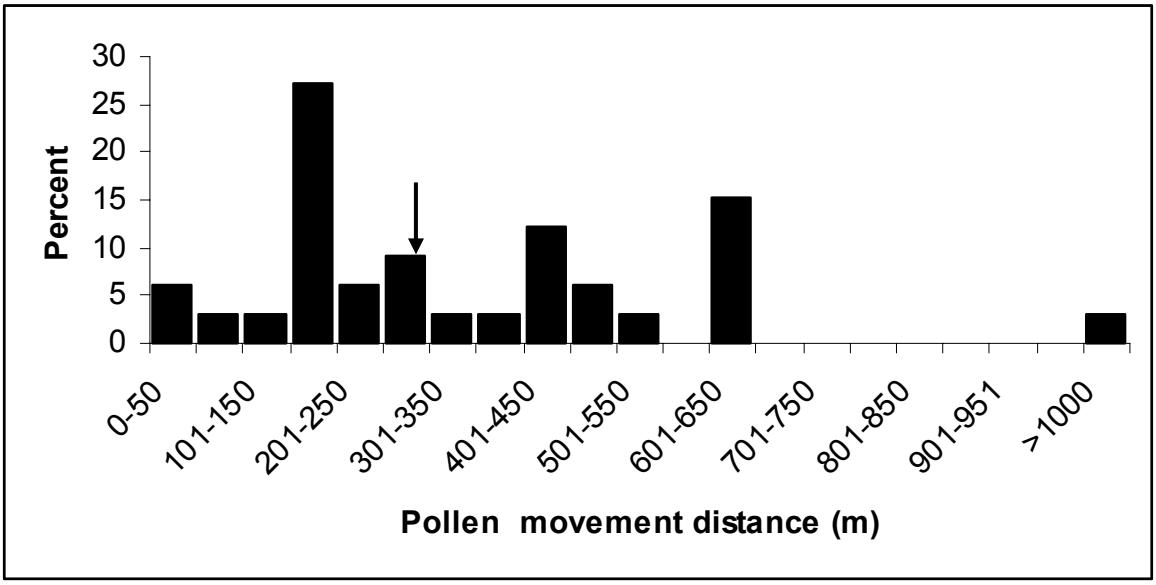
Frequency distribution of *Simarouba amara* seedling distances to maternal parent. Arrow indicates mean distance.

b)



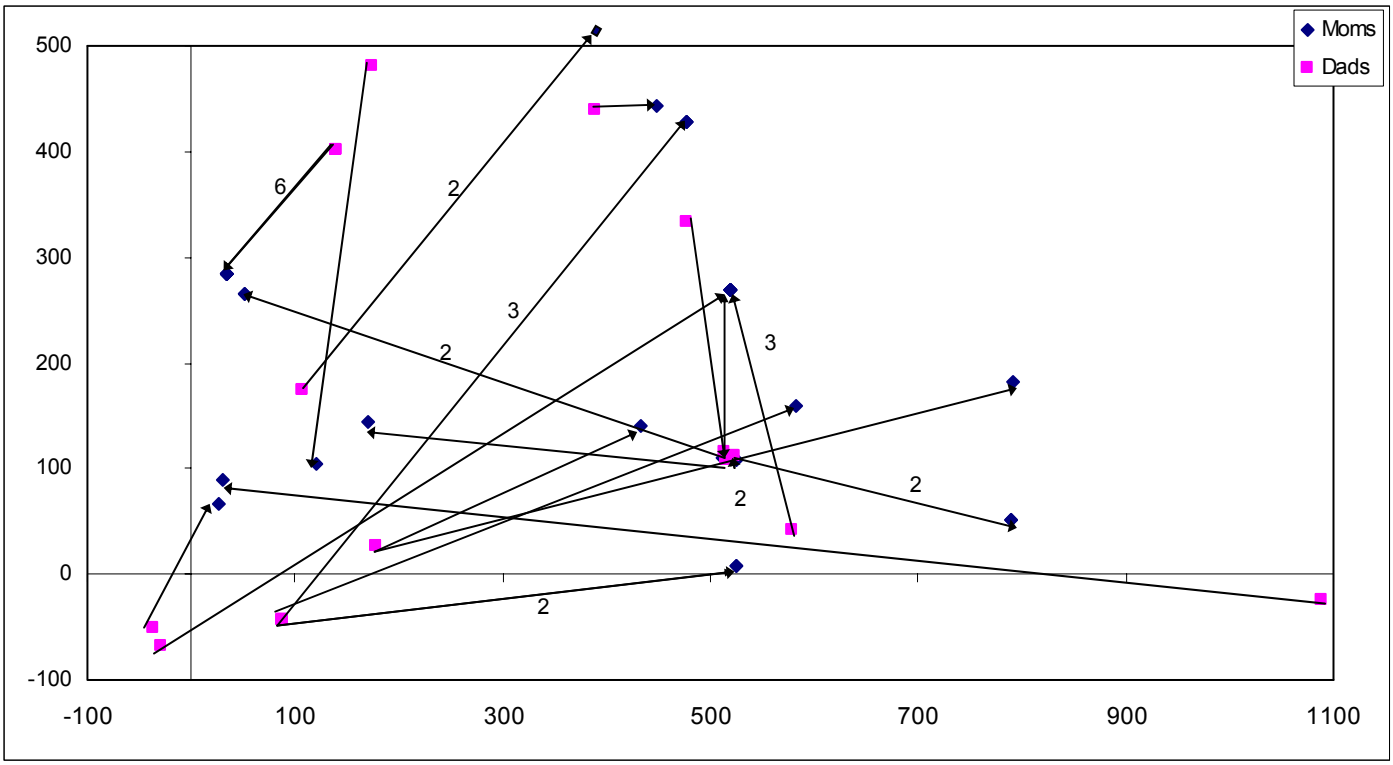
Frequency distribution of net *Simarouba amara* pollen movement (paternal parent to seedling distances).

a)



Frequency distribution of *Simarouba amara* direct pollen movement (paternal to maternal parent).

b)



Direction of pollen flow from paternal to maternal parent. Values next to lines indicate number of pollen flow events from a particular father to mother tree. Lines without numbers represent single pollen movement events. Diamonds represent maternal parents and squares represent paternal parents.

Table 3.1. Number of alleles at each locus (k), Expected Heterozygosity (H_e), and exclusion probabilities for single parent analyses (maternity analysis), and second parent analysis when one parent is known (paternity analysis). Total values represent pooled values for H_o, H_e, and first and second parent exclusion probability.

Locus	k	H_e	1st parent exclusion	2nd parent exclusion
SA02	15	0.553	0.167	0.327
SA05	10	0.733	0.327	0.502
SA06	14	0.254	0.034	0.136
SA27	16	0.835	0.509	0.678
SA29	10	0.502	0.145	0.317
Means	13.0	0.575		
Totals	65		0.772	0.936

CHAPTER 4

Genetic variation in natural populations of a widespread Neotropical tree, Simarouba amara Aubl. (Simaroubaceae) [1]

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[1] Britta Denise Hardesty, Christopher W Dick, James L Hamrick, Bernd Degen, Stephen Hubbell, and Eldredge Bermingham; to be submitted to *Molecular Ecology*

Abstract

Our goal was to assess the population genetic structure of a widespread Neotropical tree species at local, intermediate, and large spatial scales and to test the hypothesis of isolation by distance. We examined genetic variation in 14 natural populations (N=620 individuals) of Simarouba amara, a vertebrate-dispersed dioecious tree species, in Panama, Ecuador, and French Guiana. We assessed genetic diversity and population structure using five variable microsatellite loci. The mean number of alleles per locus was 15.0. Nearly twenty-five percent of all alleles were private alleles, occurred within a single population. Observed heterozygosity for all loci combined across populations ranged from 0.182 - 0.700. These values were always lower than expected heterozygosity across populations (mean = 0.579, range 0.351-0.656), but the differences between observed and expected heterozygosities were not significant. Measures of genetic differentiation (F_{st} and R_{st}) indicated significant differences among all but one population pair and differentiation was moderate across the landscape (F_{st} = 0.22, R_{st} =0.32). The majority (94%) of the genetic variation among populations resulted from differences among geographic regions. Unrooted dendrograms based upon genetic distances provide strong evidence of biogeographic structure across Central and South America for S. amara. Our results support a significant isolation by distance effect, P=0.006, suggesting that populations closer in geographic distance are more genetically similar. The southernmost Panama population, however, is significantly distinct from all other Panama populations, and the coastal Ecuador population is no more related to sites in Amazonian Ecuador than to those in Panama, though it is in closer geographic proximity.

Introduction

Typically, vertebrate dispersed tropical tree species occur in low densities and many of these species are threatened with the loss of genetic diversity due to extinction of local populations, reduced population sizes, and reduction or extirpation of their animal dispersal agents. The potential consequences of the loss of genetic diversity through fragmentation and deforestation have increased the interest in determining how genetic diversity is distributed within and among populations of tropical trees. In recent decades, the genetic variability in natural populations of numerous tropical tree species has been characterized (Chase et al. 1995, Hamrick and Loveless 1989, Hamrick and Murawski 1991, Bawa 1990, Alvarez- Buylla et al. 1996, Lee 2000).

While many studies of tropical trees have focused on the genetic variability within one or several localized plant populations, recently researchers have begun to evaluate genetic variability within and among populations across broader geographic ranges. In the Neotropics, these studies have included species of high economic value whose populations are likely to be impacted by commercial logging such as mahogany, Swietenia macrophylla (Novick et al. 2003, Lemes et al. 2003), and invasive plant species such as Clidemia hirta (DeWalt and Hamrick 2004 and others). Few studies in the Neotropics have focused on natural populations of non-commercially harvested species across a broad geographic range.

Variability in mating systems, life form, dispersal mode, and other life history characteristics affect levels and distribution of genetic diversity within and among populations (Hamrick 1994a). Because tropical tree species exhibit such a wide array of life history traits, generalizations are difficult to make, though with knowledge of pollen and seed dispersal mechanisms, reasonable predictions for the distribution of genetic diversity can be made (see

Hamrick 1994b). The majority of tropical trees studied are outcrossing species and they exhibit high levels of genetic diversity and gene flow at local and regional spatial scales (Loveless 1992, Nason and Hamrick 1997, Alvarez-Buylla et al. 1996). High levels of heterozygosity are generally found within Neotropical tree populations (Bawa 1990, Alvarez-Buylla et al. 1996, and Hamrick 1994a and b), which is consistent with predominantly out-crossing species with long-distance pollen flow (Hall et al. 1994, Chase et al. 1995, Aide and Rivera 1999). In contrast, transcontinental and Mesoamerican studies of mahogany (Lemes et al. 2003 and Novick et al. 2003) and a study of Symphonia globulifera in both Afro- and Neotropical forests (Dick et al. 2003), found high levels of regional population differentiation. These seemingly contradictory observations may indicate that the geographic ranges of Neotropical tree species have historically been divided by vicarious events that have limited gene movement between geographic regions.

The potential for long-distance pollen and seed dispersal may prove critical for maintaining genetic diversity and ensuring viable population sizes for fragmented tropical tree populations. This may be particularly important if relatively few adult trees within a population are responsible for the majority of successful pollination, dispersal, and subsequent establishment events in fragmented or successional forests, as has been reported for Symphonia globulifera (Aldrich and Hamrick 1998) and Iriartea deltoidea (Sezen et al. 2005). The genetic contribution of few individuals to subsequent generations may result in a bottleneck and loss of genetic diversity, if long distance gene flow from outside the population is restricted or does not occur.

The goal of this study was to characterize the population genetic structure of a widespread Neotropical tree species, Simarouba amara, and to examine genetic diversity and

differentiation at three spatial scales: local (individual sites), intermediate, (within regions, i.e. Panama and Ecuador), and among geographic regions. We sampled populations from Panama (9 sites), Ecuador (4 sites) and French Guiana (1 site), (Figure 1). We estimated genetic diversity using microsatellite markers to make comparisons within and among populations to address the following questions: What is the level of genetic diversity within populations and how does this vary among populations of this widespread tree species? Does genetic diversity vary geographically? How closely related are Central and South American populations of S. amara? Does genetic distance between populations depend upon geographic distance or do geographic boundaries play a discernible role in genetic differentiation among populations?

Study species

Simarouba amara Aubl. (Simaroubaceae) is a dioecious, vertebrate-dispersed tree species with a geographic range extending for more than 3300 km southeast from Costa Rica to Panama, coastal and Amazonian Ecuador, and east to French Guiana. Trees grow to ca. 35 m with a maximum reported diameter at breast height (dbh) of 70 cm on Barro Colorado Island (BCI), Panama (Croat 1978). Simarouba amara appears to maintain populations of higher density in the west of its range (Panama) and lower density in the east (Ecuador and French Guiana) as overall species richness increases. Within the two 50-ha Forest Dynamics Plots (FDP) in Panama (BCI) and Ecuador (Yasuni), S. amara occurs at a density of 5.32 and 0.68 trees of > 10 cm dbh per hectare, respectively. At the easternmost site in French Guiana, the density of > 10 cm dbh S. amara trees is 0.37 individuals per hectare.

This tree is a fast-growing intermediate-to-high light requiring species (Clark and Clark 2001) and on BCI presents a floral display for 3-4 months annually (February – May). The

unisexual flowers occur in terminal panicles composed of numerous, small (<1 cm long), pale yellowish flowers. The floral syndrome of S. amara is typical of pollination by generalist small insects such as small bees and moths (Bawa 1990), which have been observed to visit its flowers in Panama. Ripe, fleshy purplish black drupe fruits are produced within 1-3 months after pollination and are dispersed by numerous vertebrates including primates and frugivorous birds. Simarouba amara has been described as a 'locally used' tree for making paper, furniture building, interior construction, plywood, and matches in Central America (Rodriguez 2000). Despite its local usage, there is no evidence that S. amara is heavily planted or harvested across its range.

Materials and Methods

We analyzed five variable microsatellite loci (Rodriguez et al. 2000) across 14 natural populations of S. amara in Central and South America. Study populations were generally located in unlogged forest sites in the 9 Panama populations, though altitude varied among these sites (Figure 1, Table 1). We collected one population from a highly disturbed area in coastal Ecuador, three populations located in undisturbed continuous lowland forest in Amazonian Ecuador, sampled from a single population in the Paracou experimental plot in French Guiana (Figure 1, Table 1). Sample sizes ranged from 18 to 141 individuals (mean = 42.8) per population. Sites were separated by 35-3275 km (mean = 960 km) (Appendix 1). We GPS referenced each population and calculated pairwise geographic distances using a web-based Latitude/Longitude Distance calculator (Michels 1997). We collected leaves from individuals ≥ 10 cm dbh whenever possible. At sites with low population density, smaller individuals were collected as necessary. Wherever possible, tissue samples were taken from individuals located >

40 m apart to reduce the likelihood of selecting from closely related individuals. A separate study of S. amara on BCI detected no significant relatedness for individuals with ≥ 10 cm dbh located more than 20 m from one another, at the scale of 50 ha (Hardesty et al. in press).

We collected leaves by using a sling-shot or rifle to drop leaves from the canopy of tall trees or we collected leaves directly from branches of smaller individuals. Fresh tissue was placed in plastic bags in a cooler with ice while in the field and returned to the laboratory within 24 hours. In the laboratory, samples were either refrigerated and the DNA was extracted within two days, or they were put into labeled vials and flash-frozen in liquid nitrogen. We performed DNA extractions with DNEasy™ kits (Qiagen Corporation) using 40-50 mg of fresh tissue.

We used five dye-labeled trinucleotide primer pairs that amplified consistently and proved variable: SA02 (TET), SA05 (FAM), SA06 (FAM), SA27 (HEX), and SA29 (FAM) (Rodriguez et al. 2000). The PCR (10.0 µl total) contained 250 µM of dNTPs, 25µM $MgCl_2$, 0.0625 µl of Taq polymerase (Qiagen Corporation), and 0.5µl of the forward and reverse primer (10µM stock). All PCRs were performed on an MJ Research PTC-200 thermal cycler, using the following protocol: 5 min at 94˚C; 25 cycles of 45 s at 94˚C, 1 min at 53˚C, and 30 s at 72˚C; ending with 15 min at 72˚C. Amplification products were electrophoresed along with a Rox 400 size standard (Applied Biosystems Incorporated, ABI) on an MJ Research Base Station Automated DNA Analyzer. Forward primers were labeled with the same fluorescent dyes (HEX, FAM, or TET) on loci with non-overlapping size ranges to permit loading of all five loci in a single lane.

Genetic analyses

Microsatellite loci were scored using the program Cartographer (MJR). Control individuals were run on different gels to verify consistency between gel runs. To ensure reliability in scoring gels and avoid genotyping errors (recently reviewed in Bonin et al. 2004), we extracted DNA, performed PCR, and genotyped approximately 15% of all individuals twice. If allele sizes were ambiguous, individuals were run again. We found < 1% error rate in allele assignment for the 620 individuals genotyped.

We used SPaGeDI (Hardy and Vekemans 2002) to estimate allele frequencies and mean observed and expected heterozygosity (Nei 1987). The number of effective alleles and the frequency of null alleles were used to calculate F_{is} according to Weir and Cockerham (1984). Departure from Hardy-Weinberg equilibrium was tested using Haldane's exact test (1954), with exact P-values determined using the Monte Carlo Markov chain method with 1000 iterations. We also used SPaGeDI (Hardy and Vekemans 2002) to test for mutation effect on genetic differentiation for each population pair (1000 permutations).

We describe genetic differentiation among populations including a hierarchy of analyses; among populations within geographic regions, among regions (Panama, coastal Ecuador, Amazonia and French Guiana), and across the geographic range sampled. We present global R_{st}- and F_{st}-statistics due to the advantages and drawbacks of each (see review of microsatellite markers and R and F statistics by Balloux and Lugon-Moulin 2002). R-statistics are generally equivalent to F-statistics, but are based on allele sizes rather than allele identity (Slatkin 1995, Rousset 1996). F_{st} is more accurate for smaller, more recently diverged populations since F_{st} is deflated by the presence of a high mutation rate. For F-statistics, the estimation procedure is based on a nested ANOVA following Weir and Cockerham (1984) (Hardy and Vekemans 2002),

where populations are weighted according to their sample size. In contrast, R_{st} is unaffected by the rate of mutation under a stepwise-mutation model and is more accurate for larger populations with longer divergence times (Balloux and Lugon-Moulin 2002).

We used Phylip 3.573c (Felsenstein 1993) to perform cluster analysis using the UPGMA algorithm for both Nei's standard genetic identity (Nei 1987) and Goldstein's' genetic distance, $\delta\mu^2$ (Goldstein et al. 1995). Consensus trees were constructed from the best topology based on 1000 bootstrap replicates. For Nei's genetic identity and $\delta\mu^2$, differences in sample sizes across populations do not have a discernible effect on the sampling variances (Ruzzante 1998). Goldstein's genetic distance is adapted for loci such as microsatellites that undergo stepwise mutations. We also used the ISOLDE feature of GENEPOP to test the hypothesis that genetic distance between pairs of populations is dependent upon geographic distance using 1000 permutations, based upon $F_{st}/1\text{-}F_{st}$ (Rousset 1997).

Results

We analysed 620 individuals from 14 populations of S. amara (Figure 1) at five highly polymorphic microsatellite loci. Across all populations we observed a mean of 15.0 alleles per locus (range 10-31 alleles) (Table 2), and the effective number of alleles per locus ranged from 2.1 at locus SA02 to 13.7 at locus SA27 (Table 3). Within-population diversity was much lower, however, as the mean number of alleles per locus per population was 6.0 (range 2.4-10.4). No locus pairs demonstrated significant linkage disequilibrium. The mean expected heterozygosity (H_e) was higher than the observed heterozygosity (H_o) at all loci (Table 3) and within all populations (Table 2), and was particularly pronounced at SA06.

Unlike the other populations which showed moderate to high allelic diversity, the allele frequency for the most common allele ranged from fixation at locus SA02 to 0.57 at locus SA27 (3 alleles) within the MON populations. The other three loci contained two alleles (SA29) and four alleles (SA05 and SA06) with an allele frequency for the most common allele $\geq$ 0.63 for these three loci.

Allele size distributions were continuous in three of the five loci: SA05, SA27, and Sa29 over all populations. At locus SA02 and SA06, allelic distributions follow a stepwise distribution, but skip one and four size classes, respectively. Overall, this suggests that the stepwise mutation model is an appropriate model for these loci. At SA06 there is a high probability of null alleles, perhaps evidenced as the missing four size classes. When assessing allele distributions within sites, we found that none of the 13 sites showed stepwise allele distribution at all five loci. Only locus SA06 demonstrated continuous alleles in 9 of 13 populations. For the other four loci, no more than three of all the populations demonstrated a stepwise pattern of allelic distribution.

Among the 14 populations, 19 (25%) of the 75 total alleles detected at all five loci were 'private alleles', occurring in only a single population (Table 2). Private alleles were generally more common at the sites with larger sample sizes, though it is worth noting that the two populations with the highest number of private alleles do not have the highest effective number of alleles. Six private alleles (32% of the total) were present at BCI, followed by 5 at YAS (26%), two each at BDT and SR (11%) and one private allele each at CJ, MP, Tip and FG (5%) (Table 2).

The mean expected heterozygosity pooled across sites was 0.732, and ranged from 0.368 at MON to 0.700 at FTS (mean = 0.579) (Table 2). When tested for significant departure from

Hardy Weinberg equilibrium, 10% of 70 tests demonstrated a significant departure at the 5% confidence level (though valid H-W tests were not possible in 42 cases). Of the fourteen populations, only CC and BDT sites did not exhibit significant heterozygote deficiency. The mean pooled F_{is} was 0.10 across all loci at all populations, with four of the five loci exhibiting positive F_{is} (from 0.048-0.315), indicating a general excess of homozygotes. The exception to this occurred at locus SA02 where F_{is} was -0.011, suggesting a nominal excess of heterozygotes. Only at locus SA06 was F_{is} significant ($x^2 = 673.75$, DF = 132, $P < 0.0001$). On a per-population basis, F_{is} ranged from 0.001 at ESME to 0.195 at SR.

The F_{st} and R_{st} pairwise tests of genetic differentiation indicated significant differences among all 156 population pairs at the 0.05 level, with the exception of Santa Rita and Cerro Jefe, which are separated by 26 km (Figure 2, Appendix 1). The global F_{st} and R_{st} values were 0.225 and 0.449, respectively, across all 14 populations. Within Panama, pairwise F_{st} estimates were similar between eight of the nine populations, ranging from 0.023 to 0.147 (mean = 0.085) and suggest relatively low, though significant genetic differentiation among populations. For Montuouso however, pairwise F_{st} values between this and the other Panama populations ranged from 0.334 to 0.446, indicating that this site is genetically quite distinct from the other eight Panama sites. Including this single population with the other Panama populations nearly doubled the mean F_{st} among Panama sites to 0.151. Montuouso was distinct from the Ecuador sites as well, with pairwise F_{st} and R_{st} values ranging from 0.425 to 0.516 and 0.566 to 0.865, respectively (Appendix 2).

When we pooled values for different regions, we see that Panama and Amazonia share two-thirds of their alleles, although the distribution of alleles appears to be more even in Amazonia (Table 2). Using comparisons of diversity between Panama, the coastal Ecuador

(ESME), Amazonian (TIP, YAS and MP) and French Guiana (FG) sites, the F_{st} values were 0.22 in all cases (Table 4). Between ESME and Amazonia the F_{st} value was also 0.22, and Amazonia showed less differentiation to the French Guiana site (F_{st} = 0.13). The mean F_{st} value among the three Amazonian Ecuador sites was only 0.041, showing that these populations are quite similar to one another. Genetic diversity measures among the four geographic regions (Panama, coastal Ecuador, Amazonia and French Guiana) showed that the majority of the genetic diversity (94%) occurred among the regions.

UPGMA consensus trees using Nei's D and $\delta\mu^2$ distance show that populations on the eastern and western sides of the Andes are genetically divergent, regardless of geographic distance (Figures 3a and b). Topologies were consistent for the more divergent populations, although they differed for the within-Panama populations which are more genetically similar to each other. Nei's D is likely to be more accurate for the closely related Panama populations, whereas $\delta\mu^2$ is more reliable for older, more divergent populations (Takezaki and Nei 1996).

The pairwise F_{st} values between the Esmeralda site in coastal Ecuador and the Panama sites (Appendix 2) indicate that Esmeralda was no more similar to the Amazonian Ecuador sites than to the Panama populations, in spite of being geographically closer to the Amazonia populations. This corresponds to the $\delta\mu^2$ consensus tree, but contrasts somewhat with the consensus tree based upon Nei's D. Overall, we detected a significant (P=0.006, Spearman Rank test, ISOLDE) correlation between geographic and genetic distance whether using Nei's D, $\delta\mu^2$, F_{st}, or R_{st} (Figures 2, 3, Tables 2, 4).

Discussion

Similar to findings in other studies of Neotropical tree populations, we detected high levels of heterozygosity within populations of S. amara, which is consistent with outcrossing tree species with the potential for long-distance pollen flow (Hall et al. 1994, Chase et al. 1995, Aide and Rivera 1999). The generally high level of genetic diversity for S. amara populations (with the exception of MON) suggests that historically this species has been relatively common throughout its range or it has experienced sufficient long-distance gene flow to counteract the effects of drift (Hamrick 1994a). Across the 3200 km sampled range, however, we find that most of the genetic differentiation is partitioned among regions. This leads us to surmise that geographically separated areas have experienced different (and separate) evolutionary histories. While the Panama and Amazonian regions share 76% of their alleles and the number of alleles between these regions is remarkably similar, the difference between Panama and the other regions accounts for 94% of the differentiation observed.

Simarouba amara exhibits comparable gene movement by seed and pollen, at least at the scale of 84 ha. (Hardesty, unpublished data). With seedling establishment exceeding distances of 1 km from a known maternal parent, long distance gene flow may be relatively common. On the scale of a few kilometers, S. amara likely experiences frequent long distance gene movement via both seeds and pollen, at least in an intact forest community (Hardesty, unpublished). Although the remaining forest in Panama and Ecuador is by no means continuous, there remain intermittent forest patches across an increasingly fragmented landscape.

Relatively low levels of within population variability within some sites may be attributed to high levels of within-population relatedness. At some sites the sampling of adults and nearby 'juveniles' was necessary because of the locally low density of S. amara. Moreover, at other

sites it is possible that sampling encompassed adults of similar size which represent a cohort of related individuals. The Montuouso site was genetically distinct from all others; demonstrating low levels of heterozygosity and a low estimate of effective number of alleles compared to the other thirteen populations (see further discussion in following section).

A high prevalence of private alleles occurred across all populations (25% of all alleles occurred only within a single population), with 11 private alleles occurring within the Panama sites and seven in the three Amazonian Ecuador sites. Not surprisingly, the populations with the highest number of private alleles were the two populations with the greatest number of individuals sampled. Within Panama, private alleles were detected in four of the nine populations; BCI, SR, CJ, and the most northwestern site of BDT. Within Ecuador, all three Amazonian sites contained private alleles, in spite of the close proximity to one another and the continuous non-fragmented forest in which these populations were located. Additional sampling within the less-sampled sites may have changed the proportion of private alleles detected. At sites with lower sample sizes, however, additional individuals were simply not available, and over half of all the study sites contained unique alleles. Given the high mutation rate of microsatellite markers and the broad geographic expanse encompassed by this study, it may not be surprising that we detected such a large number of alleles carried within single populations.

Several studies have used microsatellite markers to demonstrate historical distance relationships among populations (Goldstein 1999, Takezaki and Nei 1996, Novick et al. 2004 and others). The significant isolation by distance pattern detected in S. amara is consistent with findings for other Neotropical species including mahogany (Lemes et al. 2003, Novick et al. 2003), Caryocar brasiliense (Collevatti et al. 2001) and Euterpe edulis (Cardoso et al. 2000). For

seven Shorea leprosula populations studied in Malaysia using allozymes, however, geographic proximity provided little insight into the relatedness of populations (Lee 2000).

While we generally predict more similarity among spatially nearby populations, geographic boundaries may also restrict gene exchange between sites. For Cordia alliodora populations across 1000 km in Central America, Chase et al. (1995) found significant regional differences between Atlantic and Pacific populations; and topography was shown to play an important role in the local population structure observed for mahogany in Mesoamerica (Novick et al. 2003). Several of the Panama populations including El Cope, Cerro Campana, Cerro Jefe, and Santa Rita, and Cordillera del Montuouso occur along ridges at higher elevations than the surrounding area. In spite of the short distances between many of the Panama sites, significant pairwise genetic differences are apparent among all but the Santa Rita and Cerro Jefe population pairs. Long distance gene flow and a shared history may account for the similarity between these two nearby sites. The landscape across Panama is heterogeneous, and topographic features may have played a role in the distribution of genetic diversity here.

Within Ecuador, pairwise F_{st} and R_{st} values (Appendix 2) show that the single coastal population (ESME) is somewhat more similar to several Panama populations than it is to the Amazonian sites even though it is geographically closer to Amazonia (Figure 3b). Perhaps oceanic dispersal events have historically been more frequent than gene dispersal events spanning the Andes mountain range. The trans-Andean differentiation is not surprising, given the time since the Andean uprising and the potential for gene movement between Panama and coastal Ecuador, as has been found for another widespread tropical tree species, Symphonia globulifera (Dick et al. 2003). In Amazonian Ecuador where three populations of S. amara exist in a continuous forested habitat (TIP, YAS, and MP), significant but slight genetic differentiation

(F_{st} = 0.041) occurred among sites. These three eastern Andes populations have greater genetic similarity to the geographically distant French Guiana site (FG) (F_{st} = 0.131) than to the geographically closer coastal Ecuador population on the western side of the Andes range (F_{st} = 0.220). In spite of the long distances between the Amazonian and French Guiana populations, the general lack of biogeographic barriers may have allowed gene flow and reduced genetic differentiation within the Amazon basin.

Geographically isolated populations or regions may have experienced long periods of ecological and evolutionary separation. Within Panama, the southernmost population of MON was notably distinct. This site lies on the southern side of the Cordillera del Montuoso, which separates it from the other eight Panama populations. The MON population has a low number of alleles (fewer than half of any of the other sites, ranging from 1-4 alleles per locus), low allelic diversity overall, and high F_{st} values compared with the other Panama sites. It seems likely that Montuoso has suffered from a genetic bottleneck with insufficient subsequent gene flow to counteract this event and add new alleles to the population. Although we have no strong anthropological evidence, anecdotal information (S. Alguilar, pers. comm.) suggests that this area has historically suffered more from pre-Columbian and contemporary human disturbance than some other regions of Panama. Our data are consistent with a pattern of a genetic bottleneck that has resulted in a small population that remained small and isolated for several generations: we not only find fewer rare alleles, but the common alleles in MON are those that are common within the other populations (Nei et al. 1975).

In summary, we detected no discernible relationship between the relative abundance or number of sampled individuals per population and genetic diversity. We found similar levels of genetic diversity for S. amara compared with other tropical species. Regional differences in

genetic differentiation were apparent across the 3200 km range. The relatively low between-population-component of genetic diversity indicated by low F_{st} values suggests that historically, gene flow has been extensive between populations for S. amara at the regional level but has been limited among geographic regions. Although the isolation by distance hypothesis was supported, we also can infer from our data that geographic barriers may restrict gene flow and result in differentiation, regardless of distance; and we found evidence to support the idea that historical gene flow across a large landscape may be extensive in the absence of geographic barriers. The Montuoso site in southern Panama was a notable outlier from all other populations and has likely suffered from genetic isolation. As populations decrease in size and become increasingly fragmented, care must be taken to maintain patches of genetically variable individuals to increase the likelihood of population persistence in the event of environmental change. This may be particularly important in Amazonia and French Guiana where the population density of S. amara appears to be much lower than in Panama. If gene flow among populations is sufficient to counteract the effects of drift and maintain diversity, the loss of genetic diversity within populations would be prevented, or at least slowed. The Montuouso population may well serve as an example of the long-term effects of fragmentation or continued isolation.

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References

Aide, T and E. Rivera (1999). Geographic patterns of genetic diversity in Poulsenia armata (Moraceae): implications for the theory of Pleistocene refugia and the riparian forest. Journal of Biogeography. 25: 695-705.

Aldrich, PR and JL Hamrick (1998). Reproductive dominance of pasture trees in a fragmented tropical forest mosaic. Nature. 281:103-105.

Alvarez-Buylla, ER, A Chaos, D Pinero, and AA Garay (1996). Demograhic genetics of a pioneer tropical tree species: patch dynamics, seed dispersal, and seed banks. Evolution. 50:1155-1166.

Balloux, F and N Lugon-Moulin (2002). The estimation of population differentiation with microsatellite markers. Molecular Ecology 11:156-165.

Bawa, KS (1990). Plant-pollinator interactions in tropical rain forests. Annual Review of Ecology and Systematics. 20: 399-422.

Bonin, A, E Bellemain, P Bronken Eidesen, F Pompanon, C Brochmann and P Taberlet (2004). How to track and assess genotyping errors in population genetic studies. 13:3261-3273.

Cardoso, SRS, NB Eloy, J Provan, MA Cardoso, and PCG Ferreira (2000). Genetic differentiation of Euterpe edulis Mart. Populations estimated by AFLP analysis. Molecular Ecology. 9: 1753- 1760.

Chase, MR, DH Boshier, and KS Bawa (1995). Population genetics of Cordia alliodora (Boraginaceae), a Neotropical tree. 1. Genetic variation in natural populations. American Journal of Botany 82:468-475.

Clark, DA and DB Clark (2001). Getting to the canopy: tree height growth in a Neotropical rainforest. Ecology 82:1460-1472.

Collevatti RG, D Grattapaglia, and JD Hay (2001). Population genetic structure of the endangered tropical tree species Caryocar brasiliense, based on variability at microsatellite loci. Molecular Ecology. 10: 349-356.

Croat, T (1978). Flora of Barro Colorado Island. Stanford University, California.

DeWalt, SJ and JL Hamrick (2004). Genetic variation of introduced Hawaiian and native Costa Rican populations of an invasive tropical shrub, Clidemia hirta (Melastomataceae). American Journal of Botany 91:1155-1162.

Dick, CW, K Abdul-Salim, and E Bermingham (2003). Molecular systematic analysis reveals cryptic tertiary diversification of a widespread tropical rain forest tree. American Naturalist. 162: 691-703.

Felsenstein J (1993). Phylip: phylogenetic inference package, Version 3.57c. University of Washington, Seattle WA.

Goldstein, DB, A Ruiz-Linares, M Feldman, and LL Cavalli-Siorza (1995). Genetic absolute dating based on microsatellites and origin of modern humans. Proceedings of the National Academy of Sciences of the USA. 92: 6720-6727.

Goudet, J (1995). FSTAT (Version 1.3): A computer program to calculate F-Statistics. Journal of Heredity. 86: 485-486.

Haldane, JBS (1954). An exact test for randomness of mating. Journal of Genetics. 52:631-635.

Hall, P, LC Orrell, and KS Bawa (1994). Genetic diversity and mating system in a tropical tree, *Carapa guianensis* (Meliaceae). American Journal of Botany. 81: 1104-1111.

Hamrick, JL (1994a). Distribution of genetic diversity in tropical tree populations: implications for the conservation of genetic resources. In Resolving tropical forest resource concerns through tree improvement, gene conservation and domestication of new species. CC Lambeth and W Dvorack eds. Proceedings of the IUFRO conference, Columbia.

Hamrick, JL (1994b). Genetic diversity and conservation in tropical forests. In Proceedings from the international symposium on genetic conservation and production of tropical forest tree seed. Drysdale, RM, SET John and AC Yapa eds. pp 1-9.

Hamrick, JL and DA Murawski (1991). Levels of allozyme diversity in populations of uncommon tropical tree species. Journal of Tropical Ecology. 7: 395-299.

Hamrick, JL, DA Murawski, and JD Nason (1993). The influence of seed dispersal mechanisms on the genetic structure of tropical tree populations. Vegetatio 107/108:281-297.

Hamrick, JL and MD Loveless (1989). The genetic structure of tropical tree populations: associations with reproductive biology. Pp 129-146 in JH Bock and YB Linart, eds. The evolutionary ecology of plants. Westview, Boulder, CO.

Hardesty, BD, C Dick, A Kremer, S Hubbell and E Bermingham (In press). Spatial genetic structure of Simarouba amara Aubl. (Simaroubaceae), a dioecious, animal-dispersed Neotropical tree, on Barro Colorado Island, Panama. Heredity.

Hardy, OJ and X Vekemans (2002). SPAGeDi: a versatile computer program to analyze spatial genetic structure at the individual or population levels. Molecular Ecology Notes. 2: 618-620.

Lee, SL (2000). Genetic diversity of a tropical tree species, Shorea leprosula Mia. (Dipterocarpaceae), in Malaysia: Implications for conservation of genetic resources and tree improvement. Biotropica. 32: 213-224.

Lemes, MR, R Gribel, J Proctor, and D Grattapaglia (2003). Population genetic structure of mahogany (Swietenia macrophylla King, Meliaceae) across the Brazilian Amazon, based on variation at microsatellite loci: implications for conservation. Molecular Ecology 12:2875-2883.

Loveless, MD (1992). Isozyme variation in tropical trees: patterns of genetic organization. PP 67-94 in WT Adams SH Strauss DL Copes and AR Griffin, eds. Population genetics of forest trees. Kluwer, Dordrecht.

Michels, C (1997). Latitude/Longitude distance. ttp://jan.ucc.nau.edu/~cvm/latlongdist.html

Nason, JD and JH Hamrick (1997). Reproductive and genetic consequences of forest fragmentation: two case studies of Neotropical canopy trees. Journal of Heredity. 88: 264-276.

Nei, M (1987). Molecular Evolutionary Genetics. Columbia University Press, New York.

Nei, M, T Maruvama, and R Chakraborty (1975). The bottleneck effect and genetic variability in populations. Evolution. 129: 1-10.

Novick, RR, CW Dick, MR Lemes, C Navarro, A Caccone and E Bermingham (2003). Genetic structure of Mesoamerican populations of Big-leaf mahogany (Swietenia macrophylla) inferred from microsatellite analysis. Molecular Ecology 12: 2885-2893.

Raymond, M and F Rousset (1995). GENEPOP (Version 3.3): population genetics software for exact tests and ecumenicism. Journal of Heredity. 86: 248-249.

Rodriguez von Platen, H (2000). Genetic diversity of the tropical tree Simarouba amara in fragmented forests in Costa Rica as measured by DNA microsatellites. Dissertation to Yale University School of Forestry and Environmental Studies.

Rodriguez von Platen, H, J Geistlinger, G Berlyn, G Kahl, and K Weising (2000). Characterization of novel microsatellite loci isolated from the tropical dioecious tree Simarouba amara. Molecular Ecology Primer Notes 9(4): 498-500.

Rousset, F (1997). Equilibrium values of measures of population subdivision for stepwise mutation process. Genetics 142: 1357-1362.

Russell, JR, JC Weber, A Booth, W Powell, C Sotelo-Montes and IK Dawson (1998). Genetic variation of Calycophyllum spruceanum in the Peruvian Amazon Basin, revealed by amplified fragment length polymorphism (AFLP) analysis. Molecular Ecology. 199-204.

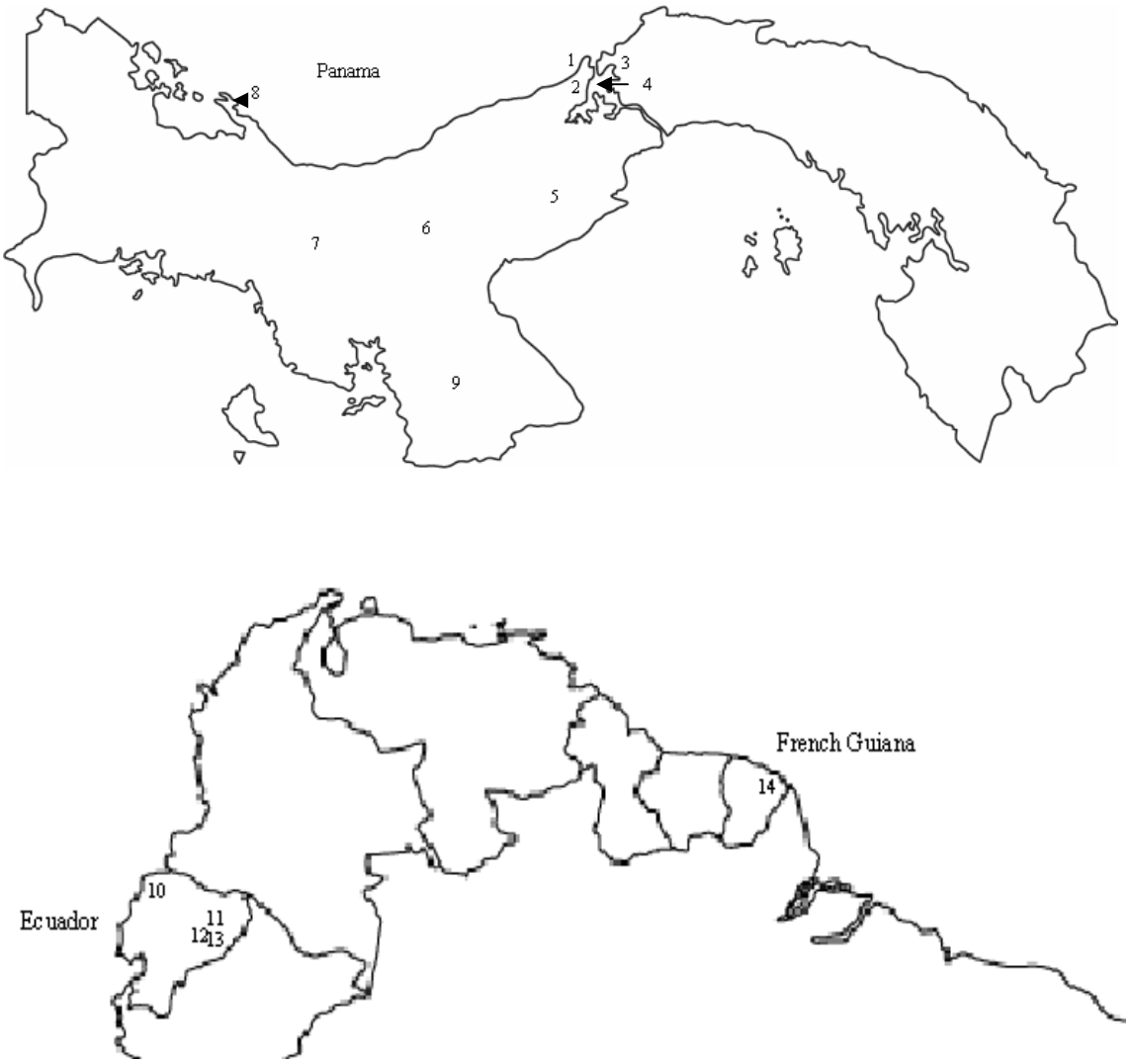
Ruzzante, DE (1998). A comparison of several measures of genetic distance and population structure with microsatellite data: bias and sampling variance. Canadian Journal of Fisheries and Aquatic Sciences. 55: 1-14.

Sezen, UU, RL Chazdon, and KE Holsinger (2005). Genetic consequences of tropical second-growth forest regeneration. Science. 307:891.

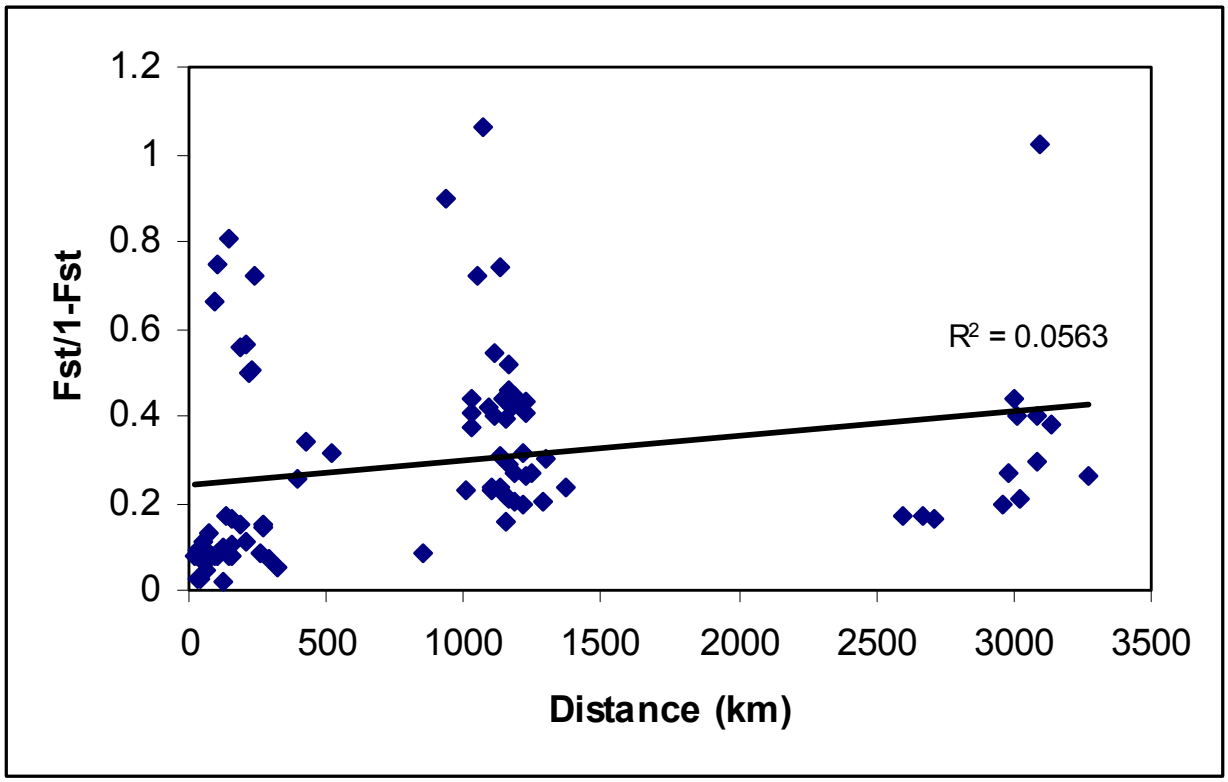
Slatkin, M (1995). Rare alleles as indicators of gene flow. Evolution 39:53-65.

Takezaki, N., and M Nei (1996). Genetic distance and reconstruction of phylogenetic trees from microsatellite DNA. Genetics 144: 389-399.

Weir, BS and CC Cockerham (1984). Estimating f-statistics for the analysis of population structure. Evolution. 38:1358-1370.

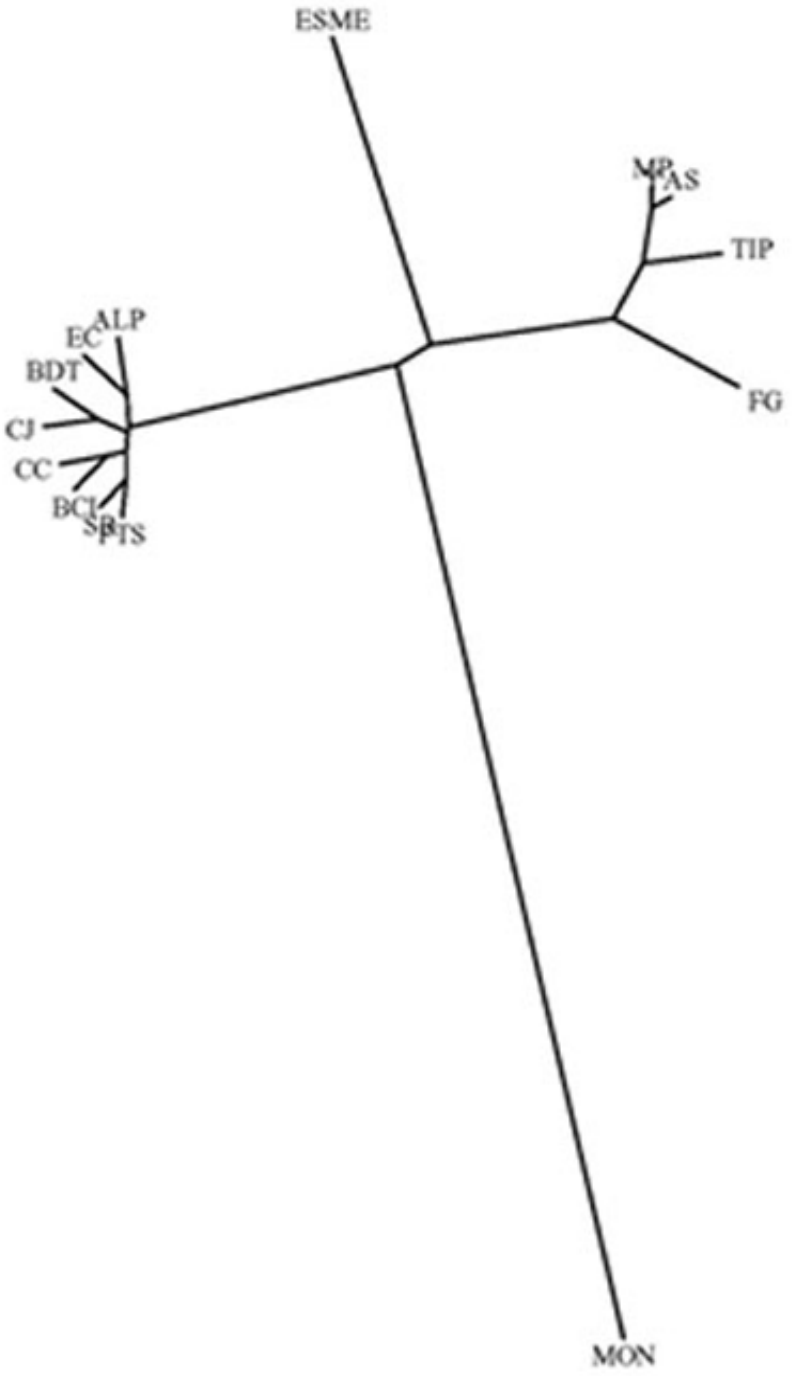


Locations of the 14 Simarouba amara populations sampled for this study.



Relationship between geographic and genetic distance measured by F_{st}/ 1-F_{st} among 14 populations of Simarouba amara.

a)



UPGMA consensus trees for Simarouba amara populations across Central and South America using a) Nei's D and b) $\delta\mu^2$. Branch lengths are proportional for genetic distance.

b)

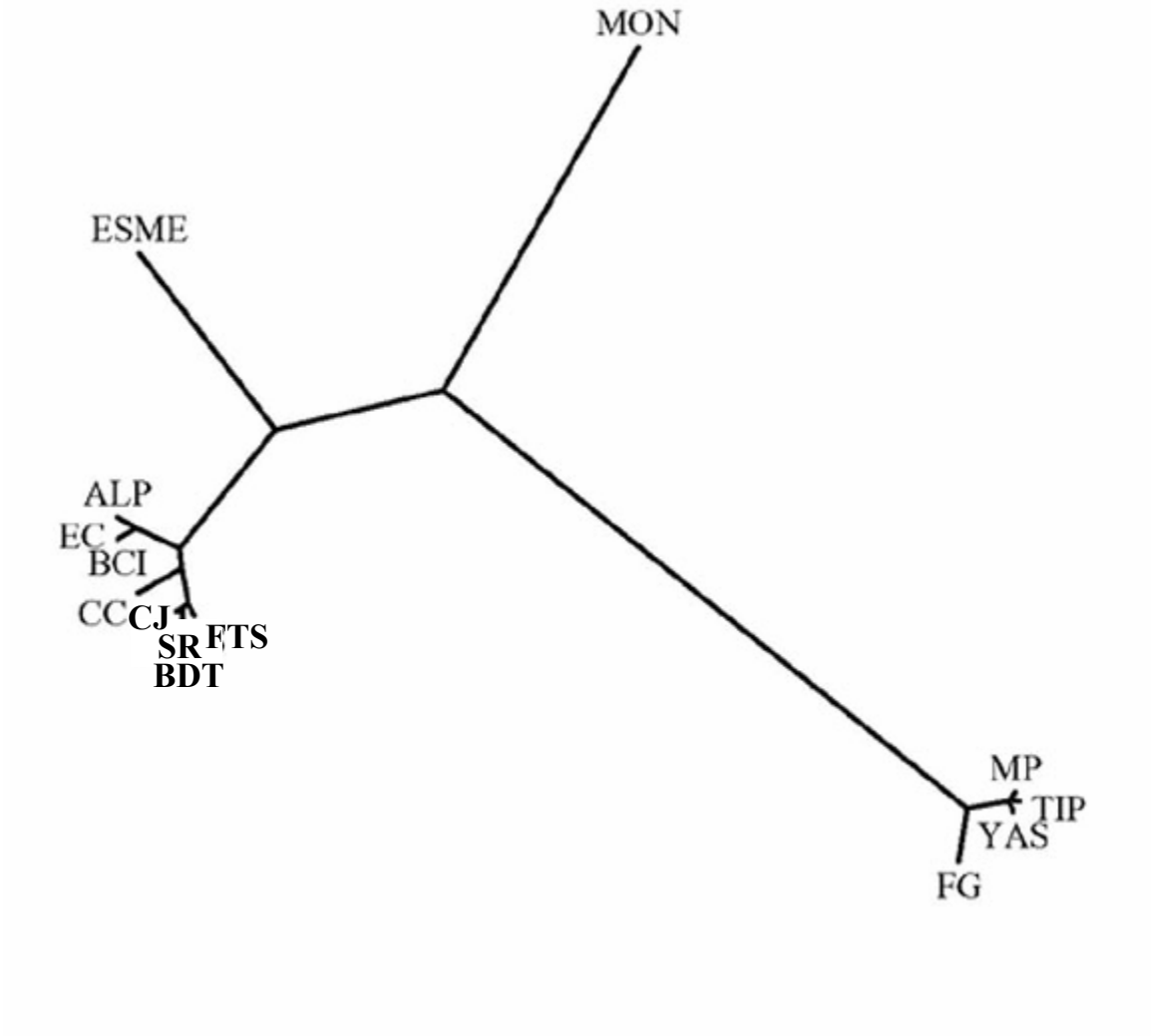


Table 4.1. Country, name and (code) for each sample population, number of population on map (M), number of individuals sampled per population (N), location of each site (Latitude/Longitude) and elevation (Elev.).

Country	Population	M	N	Latitude	Longitude	Elev (m)
Panama	Ft. Sherman (FTS)	1	20	009 21'00" N	079 57'00" W	106 m
Panama	Santa Rita (SR)	2	28	009 15'00" N	079 37'00" W	528 m
Panama	Cerro Jefe (CJ)	3	32	009 15'00" N	079 23'00" W	634 m
Panama	Barro Colorado Is (BCI)	4	100	009 10'00" N	079 50'00" W	25 m
Panama	Cerro Campana (CC)	5	30	008 40'00" N	079 55'00" W	463 m
Panama	El Cope (EC)	6	29	008 39'31" N	080 35'25" W	678 m
Panama	Alto de piedra Santa Fe Veraguas (ALP)	7	30	008 31'19" N	082 17'00" W	768 m
Panama	Bocas del Toro (BdT)	8	30	009 18'45" N	082 17'00" W	171 m
Panama	Cordillera del Montuoso (MON)	9	30	007 43'57" N	080 48'02" W	640 m
Ecuador	Esmereldas (ESME)	10	35	000 36'00" S	080 01'00" W	92 m
Ecuador	Monkey Plot (MP)	13	50	000 41'36" S	076 28'15" W	229 m
Ecuador	Tiputin (TIP)	11	18	000 38'15" S	076 09'39" W	203 m
Ecuador	Yasuni (YAS)	12	141	000 55'00" S	075 24'00" W	214 m
French Guiana	French Guiana (FG)	14	47	005 18'00"N	052 53'00" W	24 m
	ALL		**620**			

Table 4.2. Summary of microsatellite information for each population (Pop), mean number of alleles (A, (range)), number of effective alleles (A_e), ratio of effective alleles:alleles (A_e/A), number of private alleles (Pa), heterozygosity observed (H_o), and expected heterozygosity (H_e). Mean and pooled values are given for each region and over all the regions.

Region	Pop	A	A_e	A_e/A	Pa	H_o	H_e
Panama	FTS	6.2 (4-12)	3.33	0.538	0	0.656	0.700
	SR	6.3 (4-12)	3.29	0.522	2	0.562	0.696
	CJ	6.6 (3-12)	3.16	0.479	1	0.576	0.684
	BCI	8.2 (6-11)	2.29	0.279	6	0.520	0.563
	CC	4.0 (2-6)	2.06	0.514	0	0.509	0.514
	EC	5.0 (2-12)	2.22	0.443	0	0.468	0.549
	ALP	4.6 (2-8)	2.10	0.457	0	0.438	0.524
	BdT	5.4 (2-8)	2.39	0.442	2	0.578	0.581
	MON	2.8 (1-4)	1.58	0.659	0	0.351	0.368
	Mean	**5.46**	**2.49**	**0.481**	**1.2**	**0.517**	**0.575**
	Pooled	**57 (9-19)**	**3.97**	**0.335**	**11**	**--**	**0.655**
Coastal Ecuador	ESME	5.6 (2-7)	2.47	0.441	0	0.594	0.595
Amazonia	MP	8.0 (3-16)	2.79	0.348	1	0.529	0.641
	TIP	4.4 (2-5)	2.21	0.502	1	0.524	0.547
	YAS	10.4 (3-21)	2.53	0.243	5	0.488	0.605
	Mean	**7.6**	**2.51**	**0.364**	**2.3**	**0.514**	**0.598**
	Pooled	**56 (4-24)**	**4.68**	**0.402**	**7**	**--**	**0.617**
French Guiana	FG	6.6 (2-15)	2.14	0.324	1	0.529	0.532
Overall Mean		**15.0**	**2.47**	**0.442**	**1.29**	**0.523**	**0.579**
Overall Pooled		**75 (10-31)**	**5.53**	**0.353**	**18**	**--**	**0.732**

Table 4.3. Summary statistics of all individuals from 14 sampled populations for each locus.

Locus	N	A	A_e	A_e/A	H_o (range)	H_e (range)	global F_{st}	global R_{st}	HW
SA02	613	11	5.13	0.47	0.659 (0.0-0.818)	0.805 (0.405-0.819)	0.188	0.216	**
SA05	601	10	3.45	0.35	0.554 (0.392-0.756)	0.710 (0.393-0.756)	0.179	0.325	**
SA06	617	12	2.06	0.17	0.154 (0.042-0.558)	0.514 (0.042-0.585)	0.499	0.069	**
SA27	598	31	13.70	0.44	0.722 (0.556-0.915)	0.927 (0.554-0.914)	0.123	0.572	**
SA29	596	11	3.39	0.31	0.483 (0.098-0.838)	0.705 (0.098-0.838)	0.229	0.370	**
Mean across loci	Tot 620	15.0	5.54	0.35	0.516	0.732 (0.510-0.929)	0.225 (SE=0.053)	0.449 (SE= 0.168)	

Table 4.4. Regional Pairwise F_{st} values based on Weir and Cockerham (1984).

Site	Panama	Coastal Ecuador	Amazonia	French Guiana
Panama	--			
Coastal Ecuador	0.216	--		
Amazonia	0.215	0.222	--	
French Guiana	0.215	0.227	0.131	--

CHAPTER 5

SUMMARY

Dispersal, the movement of propagules away from the parent, is a fundamental life history stage in plants that is central to forest structure and regeneration. Dispersal alters community dynamics by affecting the distribution and abundance of future generations. For sessile plant species, seed dispersal is one of two mechanisms by which gene flow occurs. The other mechanism is the movement of pollen that fertilizes ovules, resulting in seed production. One of the motivations for this dissertation was to evaluate the potential for long-distance pollen and seed dispersal (and hence, gene movement) in tropical trees, because this may become more important for maintaining genetic diversity in the increasingly fragmented tropical forest landscapes of the future.

My dissertation research focused on the dispersal, recruitment, genetic structure and genetic diversity of a widespread, dioecious tree, Simarouba amara Aubl. (Simaroubaceae). In Chapter 2, I examined the fine scale relatedness among different size classes for 300 randomly selected S. amara individuals within a 50 ha Forest Dynamics Plot in Panama. In this study we found that the spatial scale of significant aggregation is 20 and 40 m for juvenile and adult S. amara individuals, respectively. In contrast, seedlings exhibited fine-scale genetic structure up to 140 m, indicating that neighboring seedlings are more related to each other than to randomly selected individuals from the seedling population as a whole. That there is spatial structure in seedlings but not in larger size classes led us to conclude that nonrandom thinning among related

individuals must be taking place between the seedling and adult life history stages. There is no other plausible explanation for the reduction in relatedness among nearby individuals. Furthermore, our results show that S. amara is not as severely dispersal limited in the 50 ha FDP on Barro Colorado Island (BCI) as might have been expected from seed rain data alone, in the absence of genetic parentage information. Our data show that pollen and seed movement is likely to be quite extensive in this species, ranging to distances in excess of 1 km.

In Chapter 3, we tested these conclusions further by measuring seed and pollen movement directly and quantifying the frequency of long-distance seedling establishment. The genetic study of realized seed dispersal (i.e. seedling recruitment), demonstrated that long-distance seedling establishment was commonplace in the BCI S. amara population. The mean mother-offspring distance exceeded 300 m. Also, seedlings frequently establish at quite large distances away from their half- and full siblings. We observed that distances between half and full sibling seedling recruits were large, (mean = 215.8 m± 137.0 SD, range = 0.81-801.6 m) and were significantly different than the distance between neighboring seedlings (mean = 11.3 m, ±15.8 SD, range = 0.34-132.8 m). Furthermore, established seedlings were seldom located near to their maternal parent. The closest reproductive female parent was the actual parent in fewer than 15% of the established seedlings. This is despite the fact that a large number of seeds are deposited directly beneath maternal parents (Hardesty, unpublished data). However, very few seedlings recruit beneath parent plants. This lack of establishment near parents may be partly due to an unfavorable change in abiotic microsite conditions (reduced light levels). However, the nonrandom genetic thinning found in Chapter 2 indicates that there is also an "escape-from-relatives" phenomenon occurring. Dispersal away from parents and siblings will reduce kin competition and exposure to possible genotype-specific pathogens.

At present, our data cannot distinguish between a competition hypothesis and a predatory/pathogen hypothesis to explain the results. There is another possible explanation of these patterns that could be due to changes in the abiotic environment. For example, light gaps may be less likely to occur in the vicinity of a reproducing maternal adult because the forest in the immediate area is now in a building phase and is less subject to formation of tree fall gaps. In this case, the lowered probability of gap formation in the neighborhood will also produce nonrandom thinning patterns among related seedlings near the parent because the neighborhood is currently too shady for successful seedling establishment of this relative high light requiring species. Thus, if the environment varies in space and time, dispersal away from parents and siblings may provide a selective advantage because it allows for bet-hedging over spatial and temporal uncertainty, even if individual seeds are dispersed to habitats where they have only a very low probability of success (Cohen & Levin, 1991; Howe & Smallwood, 1982).

In this study we also tested for directional bias in pollen movement and seedling establishment, i. e., whether non-random movements might occur in a vertebrate-dispersed tree, as has been found for wind-dispersed species (Augspurger 1983). Our results supported our null hypothesis of isotropy (uniform randomness) for direction between maternal parent and established seedling. This result is not surprising because it is unlikely that the net effect of large vertebrate frugivore movements would result in a directional bias in their movement patterns. Pollen movement, however, was significantly biased directionally and followed the prevailing wind direction. From this result we infer that the insect pollinators for S. amara are orienting along by the wind current. However the direction of pollen movement was *against* the current, suggesting that insect pollinators are actively choosing the direction of movement. We also observed that pollen and seed dispersal distances are similar for S. amara. In contrast with

studies of other Neotropical tree species in which pollen dispersal distances exceed seed dispersal distances often by hundreds or even thousands of meters (Dick 2001, Nason et al. 1998, and others), both pollen and seed were regularly moved several hundred meters within the continuous forest on BCI.

Finally, our results showed that approximately half of all possible reproductive-sized adults contributed to seedling establishment of S. amara over two years on BCI. This result contrasts with observations of genetic dominance by relatively few adult trees in fragmented tropical landscapes (Aldrich and Hamrick 1998, Sezen et al. 2005). This result suggests that continued maintenance of genetic diversity for S. amara on BCI is reasonably assured. When only a few individuals contribute genetically to the subsequent generations, a loss of genetic diversity or a bottleneck effect may occur, if, in addition, long distance gene flow from outside the population is restricted or does not occur.

Chapter 4 presented an analysis of genetic diversity within and among populations of S. amara across a broad geographic range. We tested the hypothesis that populations closer in geographic distance are more genetically similar. As has been observed in other tropical trees species, we found high levels of heterozygosity and genetic diversity within S. amara populations. We found a significant isolation by distance and evidence of biogeographic structure across central and South America. More than 90% of the genetic variation among populations results from differences among geographic regions.

Our results suggest that the once continuous geographic range of S. amara has been subdivided by one or more past vicarious events that limited gene movement among regions, although the data suggest that high levels of gene flow likely occurred within regions. Even within Panama, however, we found significant genetic differences among all but two nearby

populations. Long distance gene flow and a shared history may account for the genetic similarity of these two sites. The Andes mountain range is perhaps the most obvious impediment to gene flow, and likely accounts for the marked genetic differentiation between coastal and Amazonian populations of S. amara. However, we also found that smaller geographic barriers may have possibly restricted gene movement among populations in Panama, explaining the significant differentiation among nearby populations in this region. Despite the importance of seed dispersal in the ecology, evolutionary biology and phylogeography of tropical trees, relatively little is known about seed dispersal for the vast majority of vertebrate-dispersed tree species in tropical forests. This project attempted to "close the dispersal loop" (*sensu* Wang and Smith 2002) for at least one species, by using genetic markers and parentage analysis to link pollen and seed dispersal, seedling establishment, and subsequent adult population structure.. Our results indicate that both past and present and gene movement is extensive in S. amara is extensive on local population scales, but that there is evidence for restricted gene movement on large spatial scales.

Within the 50 ha FDP we found little or no evidence genetic structure in juvenile and adult trees. This suggests that pollen movement and seed shadows of neighboring reproductive trees overlap extensively and that dispersal occurs across relatively long distances. When we measured realized dispersal, we observed that the majority of seedlings established far away from their maternal parent, and we found that gene movement was comparable via both pollen and seed. When we looked at genetic diversity of S. amara across a broad geographic range, we see that despite the occurrence of high levels of heterozygosity within populations, there remain biogeographic and distance boundaries that limit gene movement across regional scales.

References

Aldrich, PR and JL Hamrick (1998). Reproductive dominance of pasture trees in a fragmented tropical forest mosaic. Nature. 281:103-105.

Augspurger, CA (1983) Seed dispersal of the tropical tree, Platypodium elegans, and the escape of its seedlings from fungal pathogens. Journal of Ecology. 71: 759-771.

Cohen, D. & SA Levin (1991) Dispersal in patchy environments: the effects of temporal and spatial structure. Theoretical Population Biology, 39, 63-99.

Dick, CW (2001) Genetic Rescue of Remnant Tropical Trees by an Alien Pollinator. Proceedings of the Royal Society of London Series B-Biological Sciences 268:2391-2396.

Howe, H.F. and J Smallwood (1982) Ecology of seed dispersal. Annual Review of Ecology and Systematics, 13, 201-228.

Nason, JD, EA Herre, and JL Hamrick (1996) Paternity analysis of the breeding structure of strangler fig populations: evidence for substantial long-distance wasp dispersal. 23:501-512.

Sezen, UU, RL Chazdon, and KE Holsinger (2005). Genetic consequences of tropical second-growth forest regeneration. Science. 307:891.

Wang, B and TB Smith (2002) Closing the dispersal loop. TREE. 117: 379-385.

Pairwise Geographic distances (km) among populations and tests of significance (P<0.01) for F_{st} measures of differentiation

	FTS	SR	CJ	BCI	CC	EC	ALP	BdT	MON	ESME	TIP	YAS	MP	FG
FTS	-	*	*	*	*	*	*	*	*	*	*	*	*	*
SR	38	-	NS	*	*	*	*	*	*	*	*	*	*	*
CJ	63	26	-	*	*	*	*	*	*	*	*	*	*	*
BCI	24	26	50	-	*	*	*	*	*	*	*	*	*	*
CC	76	73	87	56	-	*	*	*	*	*	*	*	*	*
EC	104	126	148	101	74	-	*	*	*	*	*	*	*	*
ALP	158	184	208	159	134	60	-	*	*	*	*	*	*	*
BdT	256	293	319	270	270	849	155	-	*	*	*	*	*	*
MON	203	213	230	192	142	106	95	240	-	*	*	*	*	*
ESME	1008	1097	1099	1087	1032	1033	1023	1132	932	-	*	*	*	*
TIP	1183	1161	1153	1169	1110	1137	1148	1287	1054	395	-	*	*	*
YAS	1188	1165	1157	1165	1116	1146	1159	1299	1065	429	35	-	*	*
MP	1249	1224	1215	1225	1179	1211	1228	1371	1134	515	122	90	-	*
FG	3021	2983	2958	3006	3010	3083	3140	3275	3099	3087	2706	2671	2597	-

Pairwise F_{st} values based on Weir and Cockerham (1984), below the diagonal; and Rho-st as defined by Rousset (1996), above the diagonal, for 14 populations of Simarouba amara across Central and South America

	FTS	SR	CJ	BCI	CC	EC	ALP	BdT	MON	ESME	TIP	YAS	MP	FG
FTS	-	0.0091	0.0262	0.0272	0.0900	0.0723	0.0281	0.0509	0.5636	0.2675	0.4394	0.5417	0.5398	0.4598
SR	0.0234	-	-0.0111	0.1022	0.1224	0.0936	0.0735	0.0098	0.4474	0.2103	0.3574	0.4225	0.4821	0.4199
CJ	0.0454	0.0256	-	0.1312	0.1070	0.1504	0.1220	0.0139	0.5128	0.2292	0.3264	0.4036	0.4469	0.3939
BCI	0.0748	0.0741	0.1013	-	0.1458	0.0548	0.0236	0.1499	0.4422	0.3751	0.5108	0.5474	0.5944	0.5388
CC	0.0748	0.0765	0.0738	0.0612	-	0.3015	0.2371	0.1521	0.7463	0.3607	0.3984	0.5197	0.4711	0.3721
EC	0.0793	0.0886	0.0703	0.0729	0.1177	-	0.0017	0.1721	0.3757	0.3052	0.5665	0.6432	0.6526	0.5850
ALP	0.0707	0.1306	0.0991	0.0971	0.1473	0.0763	-	0.1416	0.5146	0.3127	0.5571	0.6592	0.6324	0.5686
BdT	0.0801	0.0693	0.0505	0.1310	0.1269	0.0803	0.1417	-	0.6211	0.2199	0.4031	0.5094	0.5035	0.4342
MON	0.3604	0.3334	0.3343	0.3578	0.4460	0.4287	0.3973	0.4186	-	0.5660	0.7301	0.8646	0.7630	0.7604
ESME	0.1847	0.1893	0.1857	0.2957	0.2733	0.2881	0.3061	0.1927	0.4729	-	0.4845	0.4980	0.5805	0.4916
TIP	0.1711	0.1712	0.1371	0.2967	0.2850	0.2362	0.2823	0.1670	0.4198	0.2037	-	0.0158	0.0225	0.1225
YAS	0.2129	0.2248	0.1772	0.3424	0.3513	0.3063	0.3148	0.2332	0.5158	0.2537	0.0826	-	0.0236	0.0425
MP	0.2099	0.2061	0.1630	0.3008	0.3082	0.2397	0.2905	0.1906	0.4254	0.2381	0.0222	0.0754	-	0.1112
FG	0.1744	0.2101	0.1667	0.3050	0.2842	0.2842	0.2753	0.2070	0.5061	0.2270	0.1416	0.1450	0.1446	-