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Seed Dispersal Limitation in a Neotropical Nutmeg, *Virola flexuosa* (Myristicaceae): an Ecological and Genetic Approach

Kimberly Mae Holbrook
University of Missouri-St. Louis, kholbrook@umsl.edu

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UNIVERSITY OF MISSOURI – ST. LOUIS

Department of Biology

Program in Ecology, Evolution and Systematics

Seed Dispersal Limitation in a Neotropical Nutmeg, *Virola flexuosa* (Myristicaceae): an Ecological and Genetic Approach

by

Kimberly Mae Holbrook
M.A. Biology, San Francisco State University, 1999
B.S. Biology, San Francisco State University, 1994

Dissertation Advisory Committee:

Dr. Bette A. Loiselle, Ph.D. (Advisor)

Dr. Pedro Jordano, Ph.D.

Dr. Patricia G. Parker, Ph.D. (Chair)

Dr. William Connett, Ph.D.

Dr. John G. Blake, Ph.D.

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requirements for the degree of Doctor of Philosophy

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Dissertation Abstract

Seed dispersal contributes significantly to tropical forest maintenance, influencing processes, such as metapopulation dynamics and population persistence. Although several hypotheses have been debated regarding the contribution of seed dispersal to tropical forest diversity, recent work suggests that recruitment and dispersal limitation are major components in determining plant population patterns. This dissertation research broadly addresses hypotheses that seed dispersal behaviour of frugivores influences dispersal limitation of a Neotropical tree, *Virola flexuosa* (Myristicaceae).

To test these hypotheses, I studied how different frugivores influenced the seed dispersal of *V. flexuosa* at two sites in Amazonia Ecuador. General research objectives were to estimate fruit removal by all dispersers, model toucan-generated dispersal curves, and determine effective dispersal distances using molecular markers. By using a combination of ecological and genetic methods, this project contributes to our understanding of how dispersal processes influence spatial distribution patterns of seeds, seedlings, and saplings. Research was conducted at the Tiputini Biodiversity Station (non-hunted) and Yasuní Research Station (hunted), Ecuador. I conducted observations of frugivore behaviour and seed removal at fruiting *Virola* trees. Radio-telemetry and gut retention rates were used to estimate toucan-generated dispersal curves. Microsatellite markers were used to identify relatedness between seedlings and saplings with maternal *V. flexuosa* trees.

Probability models of toucan dispersal suggested the majority of seeds were dispersed away from parent plants; up to 84% of seeds were predicted to fall >100 m from trees. Between-site comparisons revealed that fewer seeds were dispersed from fruiting trees at the hunted site based on seed traps; furthermore, fewer large-bodied dispersers visited trees at that site. Using genetic methods, I also demonstrated significantly reduced dispersal distances at the hunted site compared to the non-hunted site. Consequently, I found that dispersal was limited in a site where large frugivores were hunted.

Local conservation in Amazonia Ecuador faces many severe threats. Of primary concern are an increase in hunting activities and habitat degradation. As these pressures intensify, the influence of seed dispersers on forest regeneration may become increasingly important. This research provides new data on the contribution of frugivores to the process of seed dispersal and the degree to which disturbance alters the ecological function of these seed dispersers.

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Table of Contents

Dissertation Abstract....................ii

Acknowledgments....................iv

Chapter 1 Home Range and Movement Patterns of *Pteroglossus pluricinctus* and *Ramphastos* Toucans in Amazonia Ecuador....................1

Chapter 2 Using Toucan-Generated Dispersal Models to Estimate Seed Dispersal in Amazonia Ecuador....................32

Chapter 3 Seed Dispersal Limitation in a Neotropical Tree, *Virola flexuosa* (Myristicaceae): Does Hunting of Large Vertebrates Affect Seed Removal?....................75

Chapter 4 Eight Polymorphic Microsatellite Loci for a Neotropical Nutmeg, *Virola flexuosa* (Myristicaceae)....................115

Chapter 5 A Large-Scale Genetic Comparison of Seed Dispersal and Recruitment in a Neotropical Nutmeg Tree....................122

Chapter 1

Home Range and Movement Patterns of *Pteroglossus pluricinctus* and *Ramphastos* Toucans in Amazonia Ecuador

Unpublished manuscript: Holbrook, K.M.

Introduction

Movement patterns of frugivores are directly related to seed dispersal patterns (i.e. the spatial dispersion of seeds in the environment) and likely influence forest structure and tree population dynamics (Gibson and Wheelwright 1995, Julliot 1997, Pacheco and Simonetti 2000, Jordano and Godoy 2002, Russo and Augspurger 2004). Recent research suggests that different dispersers, because of variation in movement patterns, have disproportionate effects on seed dispersal. In the Mediterranean, certain frugivores (medium-sized birds and carnivorous mammals) were more likely to contribute to the long-distance dispersal of a tree (Jordano 2007). Conversely, fruiting patterns of plants may influence frugivore movements. In Costa Rica, Levey (1988) and Blake and Loiselle (1991) found that variation in avian frugivore abundance followed the same general pattern of temporal and spatial patterns of fruit availability, providing evidence for resource-tracking. Resource-tracking has also been suggested for hornbills (Bucerotidae) in Cameroon (Whitney and Smith 1998) and toucans (Ramphastidae) in Mexico (Graham 2001a). Seed dispersers, by moving seeds around in the landscape, directly influence processes such as plant colonization, population persistence and population structure (Loiselle et al. 1995, Ouborg et al. 1999, Cain et al. 2000). Furthermore, since large avian frugivores, such as hornbills and toucans, have the ability to travel across different habitat types, including degraded and fragmented forest areas (Graham 2001a, Holbrook et al. 2002), they likely improve chances of gene flow and help maintain genetic diversity of plant populations (Hamilton 1999).

Toucans are highly frugivorous birds that typically inhabit the canopies of lowland and montane humid forests (Hilty and Brown 1986, Ridgely and Greenfield 2001), but also are found in dry forests and human-altered landscapes (Graham 2001a, b). Distributed from southern Mexico to South America, toucans are likely one of the most important seed dispersers throughout their range. Studies have long suggested the general importance of fruit in toucan diet (Snow 1981, Wheelwright et al. 1984) and based on stomach contents, Remsen et al. (1993) documented that approximately 95% of toucan diet was fruit. Moreover, toucans are specialist frugivores and have been observed to effectively disperse up to 57% of seeds from fruiting trees (Howe 1981; Holbrook and Loiselle, unpublished manuscript). Although toucans are conspicuous and well-known throughout the Neotropics, little information is available on their spatial ecology across different habitats and virtually nothing is known about home range characteristics, especially in closed-canopy forest.

The Yasuní Biosphere Reserve, eastern Ecuador is located within the Napo refuge, which may have been the largest and most ecologically important forest refuge during the Pleistocene (Haffer 1969). Today, the area is one of the most diverse places on earth

(Parker et al. 1996, Nabe-Nielsen 2001, Kreft et al. 2004). There are seven species of toucans in the Yasuní Biosphere Reserve (Ridgely and Greenfield 2001), including two large toucans (*Ramphastos*), four araçaris (*Pteroglossus*), and one toucanet (*Selenidera*). *Ramphastos* toucans are reported to nest in cavities in pairs and roost in trees, while *Pteroglossus* toucans maintain cooperative social groups that use cavities all year round for roosting and nesting (Skutch 1958, 1971, Bourne 1975). The three largest species, the white-throated toucan (*R. tucanus*), channel-billed toucan (*R. vitellinus*), and many-banded araçari (*P. pluricinctus*), are well-distributed throughout much of western Amazonia and are especially important seed dispersers in my study area (Holbrook and Loiselle, 2007). Focusing on *P. pluricinctus* and *Ramphastos* toucans, this study is part of a larger project investigating how movement patterns of toucans influence seed dispersal in a lowland tropical forest.

Here, I present home range and movement patterns of *P. pluricinctus* and *Ramphastos* toucans in the Yasuní Biosphere Reserve, Ecuador. I used radio-telemetry to address the following questions. First, what are the movement patterns, including home ranges and daily maximum distance travelled, of *P. pluricinctus* and *Ramphastos* toucans? Second, with an interest in how far seeds are dispersed, what are the distances travelled by toucans in the estimated time it takes to regurgitate or pass a seed? To address these questions, I captured and attached radio-transmitters to *P. pluricinctus* and *Ramphastos* toucans and conducted radio-telemetry at two study sites over four field seasons from 2001-2005. I then discuss these movement patterns in light of what is known about other large-bodied avian frugivores in tropical forests.

Methods

Study sites – I conducted this study in the Yasuní Biosphere Reserve, eastern Ecuador, at the Tiputini Biodiversity Station (TBS; ~0^o 38' S, 76^o 09' W) and Yasuní Research Station (YRS; ~0^o 40' S, 76^o 24' W) (27 km apart). The forest at both sites is classified as tropical wet forest (Holdridge 1967). For details on the flora, fauna, and climate of the study sites, see Valencia et al. (2004) and Holbrook and Loiselle (2007).

Field methods – To estimate toucan home ranges and movement patterns I radio-tracked three species of toucans during two 3-month and two 8-month field seasons (2001-2005). I captured a total of 25 *P. pluricinctus*, two *R. tucanus*, and two *R. vitellinus* at TBS and YRS using canopy nets at fruiting trees and/or nesting sites. Following Holbrook and Smith (2000), I attached radio-transmitters (Holohil Systems Ltd, Carp, Ontario, Canada; 6 g each, ~12-month battery life) ventrally at the base of both central tail feathers. Transmitters had a range of approximately 500-2000 meters, depending on whether the observer was on the ground or in the canopy. Body mass of tagged birds ranged from 195-684 g, resulting in the transmitter weighing less than 3% of the bird's weight. Transmitter attachment did not appear to affect bird movements.

Toucan locations were measured by triangulation using receivers and hand-held 2-element Yagi antennas (Telonics Inc., Mesa, AZ, USA) following White and Garrott (1990) and Kenward (2001). Tracking stations were located on existing canopy towers, temporary canopy platforms, and ground positions throughout the study areas to allow for minimal error in location data. Station positions were determined using a global positioning system (Garmin GPS 12XL). Three observers, using two-way radios, collected simultaneous bearings approximately two days per week at each site over a period of 3-8 months during each of four field seasons. Tracking periods lasted 4-6 daylight hours with individual birds located every 15 minutes. Tracking sessions alternated between morning and afternoon to account for daily variation in movement. In addition, I dedicated several tracking days to following individually tagged birds in order to collect more detailed movement and location data (e.g. tree to tree movements, cavity roost locations). These detailed movement data were used to supplement data collected through triangulation for calculation of home ranges. Bird locations were estimated through triangulation using the program LOAS 2.03 (Ecological Software Solutions).

Sample size and time between locations – Minimum sample size for estimating kernel home range (KHR) is suggested to be between 30 and 50 observations per animal (White and Garrott 1990, Kernohan et al. 2001, Millspaugh and Marzluff 2001). Thus, I used only those individuals with more than 40 locations for calculations of average home range size. The mean number of locations collected per day per bird was 6.1 (median, 6.0; SD, 4.1; range, 1-25) and time between successful locations ranged from 15-130 minutes, with the majority of observations recorded 15 minutes apart. Time between observations (15 min) was chosen, as part of a larger study on seed dispersal, to have a measure of distances travelled by toucans in the minimum time to regurgitate a medium-sized seed (15.0 x 11.8 cm) (Holbrook and Loiselle 2007). Successive locations recorded 15 minutes apart are potentially autocorrelated (Swihart and Slade 1985a, 1985b);

however, since movement is essentially a non-independent phenomenon, elimination of autocorrelated observations may reduce sample size unrealistically, underestimate movement rates, or lessen the biological relevance of home range estimates (Rooney et al. 1998, De Solla et al. 1999, Otis and White 1999, Millspaugh and Marzluff 2001). I chose to include all locations in an effort to capture as much movement data as possible in the interest of relating travel distances to seed dispersal behavior. Finally, because I captured so few *Ramphastos*, I combined movement data from *R. tucanus* and *R. vitellinus* to calculate the mean home range for the genus *Ramphastos*.

Home range estimation – Toucan locations were entered in a GIS database as x, y coordinates and KHRs were estimated in the Animal Movement Extension (Hooge and Eichenlaub 2000) in the program ArcView GIS 3.2 (ESRI, Inc.). Kernel methods allow for non-parametric estimation of the utilization distribution (van Winkle 1975), which is the distribution of an animal's position in space. Kernel home range assigns a probability of area use based on the number and spatial arrangement of locations and describes the relative amount of time that an animal spends in a given area (e.g. 95% kernel is where you have a 95% chance of finding the animal at any given time) (Worton 1989). I used a fixed kernel with a smoothing parameter (controls the width of individual kernels and determines the amount of smoothing applied to the data; Kernohan et al. 2001) estimated by least square cross-validation, which has been shown to provide area estimates with high accuracy and little bias (Worton 1989, Seaman and Powell 1996). Two kernel estimates were calculated: the 95% kernel and a core area consisting of the 50% kernel. The 95% kernel excludes locations with obvious errors by removing 5% of outliers.

Home ranges were estimated and compared between species using data collected throughout all field seasons.

Sexing toucans – Since *P. pluricinctus* are not sexually dimorphic, all individuals were sexed using a standard polymerase chain reaction (PCR) using P2 and P8 primers (Griffiths et al. 1998). I extracted DNA from blood samples through a standard phenol-chloroform extraction method (Sambrook et al. 1989). Extraction was followed by a clean-up step with dialysis in 1X TNE^2 (10 mM Tris-HCL, 10 Mm NaCl, 2 Mm EDTA). Following the PCR, a digestion enzyme (Hae III) was added to the samples and they were allowed to incubate at 39° C overnight. Samples were run on a 0.8% agarose gel for one hour and stained using ethidium bromide. Males and females are differentiated by the presence of one band for females and two bands for males.

Statistical analyses – I compared KHR (both 50 and 95% kernel) estimates between *Ramphastos* spp. (hereafter *Ramphastos*) and *P. pluricinctus* using one-way analysis of variance (ANOVA; home range as dependent variable with species as factor). Comparisons between species were conducted using all individuals for which more than 40 locations were collected. Several *P. pluricinctus* with sufficient location data were from the same social or family group. Individuals in these social groups typically travelled together and were observed to follow one another as they flew from one tree to another. Thus, to avoid pseudoreplication, I chose a single bird with the greatest number of locations from each of those groups for a second comparison. One *Ramphastos* (no. 15) was radio-tracked over two different field seasons resulting in a greater combined sample size in both days and locations for that individual. I included this bird in two separate analyses: the first using only the field season with the greater number of locations and the second combining data collected over both field seasons. Differences in the maximum travel distance recorded in a single tracking period (maximum daily distance as the dependent variable and species as factor) were compared with ANOVA. Maximum distances were log-transformed to achieve conditions of normality. To determine whether potential seed dispersal distances varied between species, I tested differences in distance travelled in 15 minutes (minimum seed retention time; Holbrook and Loiselle 2007), 30 minutes (mean retention time: *P. pluricinctus*, 28 min and *Ramphastos*, 34 min; Holbrook and Loiselle 2007), and in all time observations, using a Mann-Whitney test. All analyses were performed using SPSS 11.0 (SPSS 2001).

Results

I trapped and radio-tagged 25 *P. pluricinctus*, two *R. tucanus*, and two *R. vitellinus*. Six individuals lost their transmitters the day after tagging, thus data are presented for 20 *P. pluricinctus*, two *R. tucanus*, and one *R. vitellinus* (Table 1). Among these radio-tracked birds, two individuals were juvenile *P. pluricinctus*. As the nesting season for *P. pluricinctus* and *Ramphastos* is from November to February, I collected movement data on individuals both during and outside the nesting period. Several individuals had nestlings for a portion of the telemetry period (including *Ramphastos* 15, 25, 26 and *Pteroglossus* 1, 3, 5, 7, 29, 30) for which movements might be expected to be somewhat restricted. In most cases, cavities (for roost and nest sites) were located within the 50%

core use area, similar to the roosting locations of the keel-billed toucan (*R. sulfuratus*) (Graham 2001b). A total of 1013 and 480 locations were collected for *P. pluricinctus* for *Ramphastos*, respectively. Individual tracking periods ranged from four to 25 days per individual. The end of each bird's tracking period represented a dropped transmitter.

Home range estimation – Including all radio-tracked individuals, 50% KHR varied from 3 to 53 ha, while 95% KHR ranged from 21 to 395 ha (Table 1). The 50% KHR (i.e. core areas) of individuals with more than 40 locations, which include twelve *P. pluricinctus* (ten at TBS and two at YRS) and three *Ramphastos* (two at TBS and one at YRS), are shown in Figure 1. Core home ranges varied from 11 to 48 ha and from 12 to 25 ha for *P. pluricinctus* and *Ramphastos*, respectively. *Pteroglossus pluricinctus* individuals from the same social or family group have largely overlapping home ranges (Fig. 1). Also, three *P. pluricinctus* individuals from one social group (1, 3, and 5) used two core areas, rather than a single area as reported for all other groups. The two areas represent regions of intense use; while the group cavity was located in one of the two core areas, the other potentially encompassed a preferred foraging region, at least over the time period the birds were tracked. *Ramphastos* 15 was followed over two study periods and had a 95% KHR of 64 ha in 2003 and 121 ha in 2004; combining both years, the home range was 97 ha (see below for further discussion).

Mean home range sizes are presented for all radio-tagged *Ramphastos*, for the 12 *P. pluricinctus* with more than 40 location points, and for seven individual *P. pluricinctus* from independent social groups (Table 2). Discussion of home range statistics are generally for the seven independent *P. pluricinctus* individuals, unless otherwise stated. Mean home ranges (SD) for 95% KHR were 191 (64) ha and 86 (25) ha for *P. pluricinctus* and *Ramphastos*, respectively (Table 2). *Pteroglossus pluricinctus* had significantly greater 95% KHR than did *Ramphastos* in all comparisons (all individuals with > 40 locations: $F_{1,13} = 6.12$, $P = 0.028$; independent social group: $F_{1,13} = 5.36$, $P = 0.049$; 2-yr combined data for bird 15: $F_{1,13} = 5.15$, $P = 0.041$). Although mean core home ranges were also larger for *P. pluricinctus* than *Ramphastos* (26.7 vs. 19.9), this difference was not significant (Table 2). Maximum travel distances recorded in a single tracking period were 3,665 and 3,027 m for *P. pluricinctus* and *Ramphastos*, respectively. Both of these observations were recorded 30 minutes apart, a time that represents the mean seed retention time. *Pteroglossus pluricinctus* moved farther in a single tracking day on average than did *Ramphastos* (all individuals with > 40 locations: $F_{1,167} = 5.969$, $P = 0.016$; independent social group: $F_{1,113} = 5.786$, $P = 0.018$; 2-yr combined data for bird 15: $F_{1,123} = 8.406$, $P = 0.004$) (Table 2).

Home range sizes might be sensitive to the number of days, locations, and reproductive status of individuals. For example, home ranges for *P. pluricinctus* individuals with nestlings (birds 3, 7, 30; mean 50% and 95% KHR were 22.8 and 172.8 ha, respectively), at least for a portion of the tracking period, tended to be smaller than individuals tracked during the non-breeding season (birds 28, 49, 13; mean 50% and 95% KHR were 31.7 and 208.0 ha, respectively). Also, *Ramphastos* 15 was followed over two study periods and despite slightly fewer number of locations had a core home range that was 50% larger in 2003 (23 ha) than in 2004 (16 ha) and a 95% KHR that was twice as large

(2003, 121 ha and 2004, 64 ha). The radio-tracking period for *Ramphastos* 15 in 2003 was conducted just after the pair fledged a single chick, while the tracking period in 2004 was conducted entirely during the nestling stage, suggesting that home range sizes are smaller during nesting periods. Kernel home range size was not related to number of locations for either 50% or 95% KHR ($r < 0.26$, $P > 0.20$; $n = 24$; includes all birds), especially when only those individuals with more than 40 locations are included ($r < 0.06$, $P > 0.68$, $n = 16$; includes bird 15 from both field seasons). Kernel home range size for both 50% or 95% KHR was related to number of days when including all radio-tracked individuals ($r > 0.41$, $P < 0.05$, $n = 24$); but not for individuals with more than 40 locations ($r < 0.47$, $P > 0.07$, $n = 16$). As might be expected, 50% KHR size were highly correlated with 95% KHR ($r = 0.874$, $P < 0.001$, $n = 24$).

Dispersal distances – Distances that toucans travelled within 15-30 minutes represented potential seed dispersal distances away from fruiting trees, at least based on medium-sized seeds typical of Myristicaceae (Howe and Kerckhove 1981, Holbrook and Loiselle 2007). Thus, to compare how far most seeds are likely to be moved from their source by *Ramphastos* and *P. pluricinctus*, I calculated mean travel distances collected in various time frames (Fig. 2). Distances travelled in 15 minutes were 449 and 269 m for *P. pluricinctus* and *Ramphastos*, respectively. For observations collected 30 minutes apart, distances were 528 m for *P. pluricinctus* and 348 m for *Ramphastos*. *Pteroglossus pluricinctus* were more likely to move a greater distance in 15 or 30 minutes, although 30 minute comparisons were not statistically significant (Fig. 2). Individual distances moved between successive locations were quite variable and the smaller sample size of

Ramphastos observations in the 30 minute time category likely contributes to increased variation and failure to detect statistical significance. I also calculated the average distance travelled between *all* successive locations, which represented observations collected 15, 30, 45, 60, 75, 90, and 130 minutes apart (Fig. 2). For example, although toucan locations were attempted every 15 minutes, on occasion individual birds were not detected by one or more researchers and/or the triangulation error was too great to accept. As in previous results, *P. pluricinctus* travelled significantly greater distances between any two recorded locations in succession than did *Ramphastos* toucans.

Discussion

In the American tropics, toucans are one of the most important groups of seed dispersers and though very distinctive and symbolic of their Neotropical forests, virtually nothing is known of their movement patterns and use of space (but see Graham 2001b). Here, in the first telemetry study on South American species, I found large home ranges and long average movement distances. Home range estimates (95% KHR) of individually-tracked toucans varied from 55 to 395 ha with mean home ranges of 86 to 191 ha for *Ramphastos* and *P. pluricinctus*, respectively. Similar to results reported here for *Ramphastos*, a study examining the movement patterns and spatial ecology of *R. sulfuratus* in Mexico found average minimum convex polygon (MCP) home range to be 97 ha (range 61-144 ha) (Graham 2001b). In Peru, Terborgh et al. (1990) reported territory sizes of ≤ 40 and 50 ha for *R. vitellinus* and *R. tucanus*, respectively; however, this study did not use radio-telemetry and is likely an underestimate of total home range.

Toucans have much smaller home ranges than those reported for larger-sized (ca. 1000-3000 g) hornbills (925-28,000 ha) (Poonswad and Tsuji 1994, Suryadi et al. 1998, Holbrook and Smith 2000). In the current study, no tagged birds were determined to leave the study area and several banded individuals were re-sighted throughout the 4-year study period, suggesting that though birds may be tracking resources at a daily or local level, large-scale seasonal resource tracking is rare or non-existent. Fruit abundance in Yasuní National Park, Ecuador was variable and high fruit availability generally coincided with the wettest months (Di Fiore 2003); however, the difference in monthly fruit abundance in Yasuní is small compared to the high variation in fruit availability reported in Africa and Asia (Leighton and Leighton 1983, Whitney and Smith 1998). Differences in movement patterns between these two ecologically and morphologically similar groups of frugivores may be partially explained by a higher degree of spatio-temporal patchiness of fruit in the Paleotropics (Fleming et al. 1987). Fleming et al. (1987) speculate that large-scale nomadic or migratory movements of frugivores may be more common in Asian and African forests and though there are still few empirical studies, recent research supports this idea.

Tropical tree species have highly aggregated distributions (Condit et al. 2000) and marked seasonality in fruiting phenology (Frankie et al. 1974, Opler et al. 1980, Terborgh 1986, van Schaik 1986, Whitney and Smith 1998). As a consequence, fruit availability in tropical forests can show high variation both spatially and temporally (Fleming et al. 1987, Jordano 1992, van Schaik et al. 1993) and several studies have demonstrated resource-tracking by vertebrates through daily and/or seasonal foraging activities (Levey 1988, Loiselle and Blake 1991, Jordano 1992, van Schaik et al. 1993). In Borneo, Indonesia and Cameroon, hornbill abundances were positively correlated with changes in fruit availability, suggesting that hornbills track fruit resources (Leighton and Leighton 1983, Whitney and Smith 1998). Furthermore, Leighton and Leighton (1983) and Holbrook et al. (2002) found birds to emigrate from study areas (in some cases travelling up to several hundred kilometers) when fruit resources became scarce. Although somewhat smaller, resplendent quetzals (*Pharomachrus mocinno*) were found to travel up to 37 km in search of food resources in Costa Rica (Powell and Bjork 1994) and three-wattled bellbirds (*Procnias tricarunculata*) (also in Costa Rica) migrated along an elevational gradient between sites 200 km apart (Powell and Bjork 2004). Thus, the very large home ranges and distances moved may be associated with species that track resources on a large-scale. Local resource-tracking was suggested for toucans in Mexico, where below a certain distance threshold (between forest remnants), fruit abundance influenced toucan movements (Graham 2001a).

Home ranges and movement patterns varied between *P. pluricinctus* and *Ramphastos* toucans, and although sample size for *Ramphastos* is small, my results are similar to home range characteristics reported for *R. sulfuratus* (Graham 2001b). That the smaller *P. pluricinctus* had a home range nearly twice as large as the larger *Ramphastos* toucans is somewhat counter-intuitive. One might expect home ranges to be smaller for toucans using cavities year-round for roosting (e.g. *P. pluricinctus*) since the constraint of returning to the same roost site/cavity should limit home range size and movement patterns. On the other hand, resource fluctuations on a local scale could impose a larger home range (at least over shorter time periods) due to the inability to take advantage (by changing roost site) of small-scale variation in food resource availability. If this is true, *P. pluricinctus* would travel more widely tracking local changes in fruit availability, while returning each night to the roost cavity. *Ramphastos* roost in trees and consequently are not restricted to particular areas in the forest by night-time roost (Skutch 1971, Bourne 1975, personal observation). Thus, in contrast to perceived roosting constraints for *P. pluricinctus*, *Ramphastos* toucans may alter roost sites regularly based on where preferred fruit sources are located. Another contributing factor to the variation in *P. pluricinctus* and *Ramphastos* home ranges may be differences in foraging behavior, resulting from competition at highly preferred food sources. A study in Costa Rica, examining the feeding assemblages of birds, found that the largest species of toucan (*R. sulfuratus*) actively defended preferred food resources by chasing out other frugivores (Howe 1981). In my study area, *Ramphastos* were often observed to defend fruiting trees by chasing out other individuals, including *P. pluricinctus* (personal observation). Also, mean visit length to fruiting nutmeg trees (*Virola flexuosa*; Myristicaceae) by *Ramphastos* was significantly longer (6.9 min) than visit length by *P. pluricinctus* (4.0 min) (Holbrook and Loiselle, unpublished manuscript). It is unknown whether shorter foraging times (at least in *V. flexuosa*) for *P. pluricinctus* is related to interspecific competition, but regardless it may lead to a wider foraging strategy for *P. pluricinctus*.

How frugivores move through space has a direct impact on seed dispersal patterns (Jordano and Godoy 2002, Russo and Augspurger 2004). Feeding on the fruits of up to

60 tree species (Wheelwright et al. 1984, Galetti 2000, Guix et al. 2001; K.M. Holbrook unpublished data), toucans are especially important in dispersing seeds of Neotropical plants, and, given their ability to move between different habitats, they may play a key role in forest regeneration following disturbance. Average distances travelled by toucans within 15 and 30 minutes were relatively long, suggesting the capability to move seeds long distances from their source. If a particular seed disperser consistently travels greater distances, then that disperser is more likely to contribute to long-distance dispersal and consequently impact the distribution of seed shadow tails (Holbrook and Smith 2000, Westcott and Graham 2000, Westcott et al. 2005, Holbrook and Loiselle 2007). Long-distance dispersal is important in maintaining gene flow both within and between plant populations. In much of the world's tropical forests, human activities have greatly modified the forested landscape, resulting in significant impacts on population sizes of a number of birds, especially large-bodied avian frugivores (Kattan 1994, Renjifo 1999, 2001). Further, with the steady increase of habitat fragmentation, frugivore movements in many instances are thwarted (Bierregaard and Lovejoy 1989, Cordeiro and Howe 2001, Wright and Duber 2001, Cordeiro and Howe 2003), which likely leads to reduced long-distance dispersal. Large-bodied canopy frugivores, like toucans, are known to cross open areas and thus, may be partially resistant to landscape changes up to a certain point. Yet, these same birds are also targeted by hunters for their meat, and human activities that alter forested landscapes (e.g. oil development, logging, and agriculture) almost inevitably lead to increased hunting pressure (Redford 1992, Peres 2000). Consequently, understanding movement patterns of large-bodied frugivores under different degrees of disturbance is key to understand the impacts on their role as seed

dispersers. This study provides a look at one end of the disturbance gradient – that is, movements of toucans in a largely intact, expansive forest.

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Figure Legends

Figure 1**.** Location estimates and core home ranges (50% kernel home range) of 12 *P. pluricinctus* (n = 877 locations) and three *Ramphastos* (n = 480 locations) at (a) Tiputini Biodiversity Station and (b) Yasuní Research Station, Ecuador. Numbers refer to individuals in Table 1. Locations were estimated by triangulation over four field seasons (2001-2005). Seven *Pteroglossus* family groups from different cavities are represented by the following birds: (1, 3, 5); (7); (13, 19); (22); (28); (29, 30); and (49, 84). Birds 7, 22, and 28 were the only individuals tagged from their respective family groups.

Figure 2. Distance moved by *P. pluricinctus* and *Ramphastos* for 15 min, 30 min, and all time observations. Sample sizes (above bars) do not include locations collected outside telemetry sessions and are less 5% of outliers. Data are presented as means (+ 1 SE), and asterisks indicate statistical significance determined by Mann-Whitney tests ($P < 0.001$).

Table 1. Estimated home range for *P. pluricinctus, R. vitellinus,* and *R. tucanus* showing 50% and 95% Kernel Home Range (KHR). The 50 % KHR represents the core area of use and 95 % KHR is the area where there is a 95% probability of finding the individual. A total of 25 *P. pluricinctus* and four *Ramphastos* were radio-tagged; data are presented for 20 *P. pluricinctus* and three *Ramphastos*, respectively, for which there are sufficient data. Birds radio-tracked at Yasuní Research Station are 20, 29, 30, and 15; the remainders are from Tiputini Biodiversity Station. Sex determined using DNA-based sex identification (Griffiths et al. 1998); M = male, F = female, and U = unknown.

Bird	Species	Sex (wt g)	No. locations [a]	No. days detected	KHR (ha) 50%	KHR (ha) 95%	Tracking period
28	*P. pluricinctus*	U (235)	55	15	28	179	22 Jul - 22 Aug 01
49	*P. pluricinctus*	U (195)	109	19	48	286	5 Jul - 22 Aug 01
84	*P. pluricinctus*	U (255)	101	20	28	276	5 Jul - 22 Aug 01
1	*P. pluricinctus*	M (289)	63	20	21	223	30 Nov 02 - 21 Mar 03
3	*P. pluricinctus*	M (262)	105	25	39	266	2 Dec 02 - 14 Mar 03
5	*P. pluricinctus*	M (270)	61	14	53	395	28 Dec 02 - 6 Mar 03
7	*P. pluricinctus*	M (225)	89	18	11	141	26 Jan - 12 June 03
17	*P. pluricinctus*	M (244)	13	6	29	189	16 Apr - 15 May 05
13	*P. pluricinctus*	U (228)	68	9	19	159	18 Apr - 18 May 03
19	*P. pluricinctus*	M (239)	64	9	21	171	18 Apr - 18 May 03
9	*P. pluricinctus*	M (203)	11	10	12	68	9 Dec 03 - 11 Feb 04
12	*P. pluricinctus*	M (210)	19	4	3	21	11 Jan - 26 Jan 04
14[b]	*P. pluricinctus*	M (215)	13	3	16	121	13 Feb - 28 Feb 04
21	*P. pluricinctus*	M (220)	21	18	7	62	16 Mar - 30 Apr 04
22	*P. pluricinctus*	M (225)	60	12	23	196	31 Mar - 6 May 04
10	*P. pluricinctus*	M (222)	11	9	42	185	31 Mar - 1 May 04
29	*P. pluricinctus*	M (206)	41	8	19	118	24 Jan - 12 May 04
30	*P. pluricinctus*	M (197)	61	10	19	111	24 Jan - 26 Mar 04
20	*P. pluricinctus*	M (200)	30	4	4	50	29 Mar - 14 May 04
24	*P. pluricinctus*	U (217)	18	5	12	100	10 Jan - 24 Jan 05
15a[c]	*R. tucanus*	F (684)	88	12	23	121	1 Apr - 25 May 03
15b			97	12	16	64	27 Dec 03 - 8 Mar 04
25	*R. tucanus*	M (640)	185	20	25	135	29 Jan - 27 Mar 05
26	*R. vitellinus*	F (392)	110	13	12	55	30 Jan - 10 Mar 05

[a] Locations are supplemented with detailed on-foot tracking data and visual observations of individuals collected during other field activities.

[b] *P. pluricinctus* 14 (originally caught in 2003 and tagged 7) was recaptured and radio tracked again in 2004.

[c] *Ramphastos tucanus* 15 was captured in early 2003 and radio tracked over two separate field seasons (15a and 15b).

Table 2. Mean home ranges for *P. pluricinctus* and *Ramphastos* showing 50% and 95% kernel home range (KHR). Values for *P. pluricinctus* are presented for all 12 individuals with sufficient data and for seven individuals from individual family groups.

Movement parameter	*P. pluricinctus* ($n = 12$)	*P. pluricinctus*[a] ($n = 7$)	*Ramphastos*[b] ($n = 3$)	*F* ratio[c]
50% KHR (ha)				
Mean (SD)	27.4 (12.9)	26.7 (12.8)	19.9 (4.1)	1.589
Median (range)	21.9 (11-53)	22.8 (11-48)	23.5 (12-25)	
95% KHR (ha)				
Mean (SD)	209.9 (83.1)	191.0 (64.1)	85.8 (25.2)	5.364*
Median (range)	187.3 (111-395)	178.8 (141-286)	64.5 (55-135)	
Maximum distance moved (m)				
Mean (SD)	1149.0 (901)	1110 (871)	760 (678)	5.786*
Median (range)	856.0 (78-3665)	846 (159-3642)	557 (71-3027)	
Number of days tracked				
Mean (SD)	15 (5)	15 (6)	15 (3)	
Median (range)	15 (8-25)	15 (9-25)	13 (12-20)	
Number of locations				
Mean (SD)	73 (22)	78 (23)	131 (27)	
Median (range)	64 (41-109)	68 (55-109)	110 (97-185)	

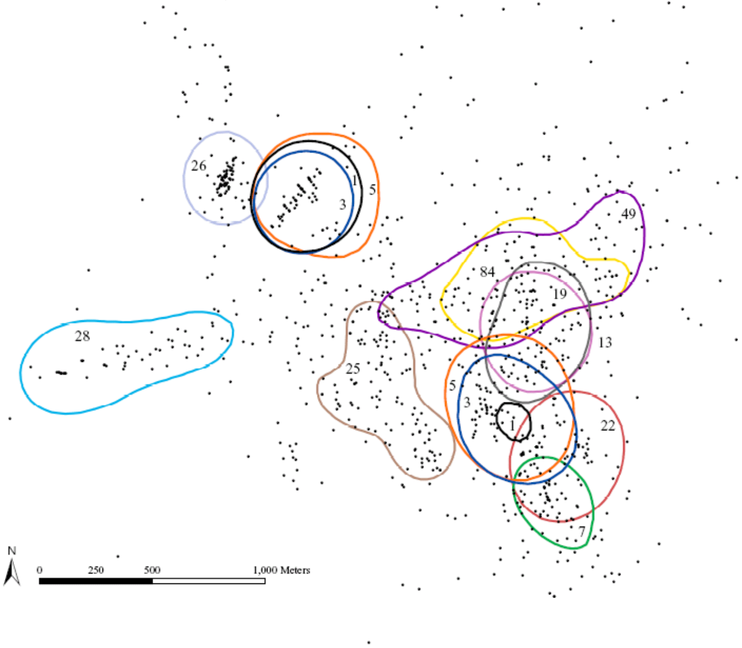
[a] Represent individuals with the greatest number of locations from seven separate social or family groups which used different cavities.

[b] Used movement data from bird 15b because of greater number of locations.

[c] ANOVA result of test between seven independent *Pteroglossus* and *Ramphastos*. * $P < 0.05$; other comparisons were also significant (see text).

Figure 1

(a) Tiputini Biodiversity Station



(b) Yasuní Research Station

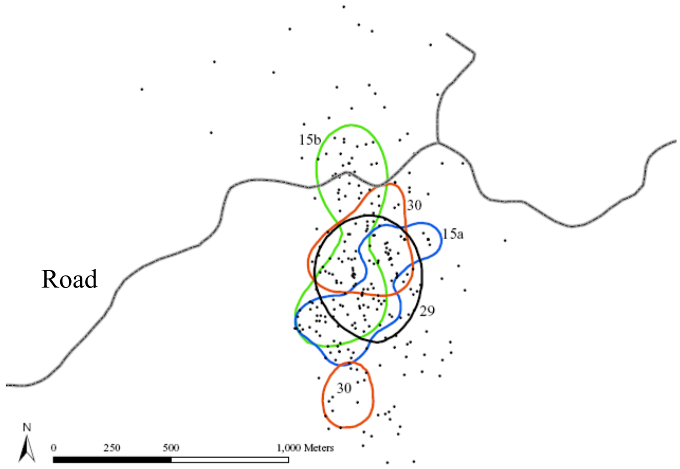
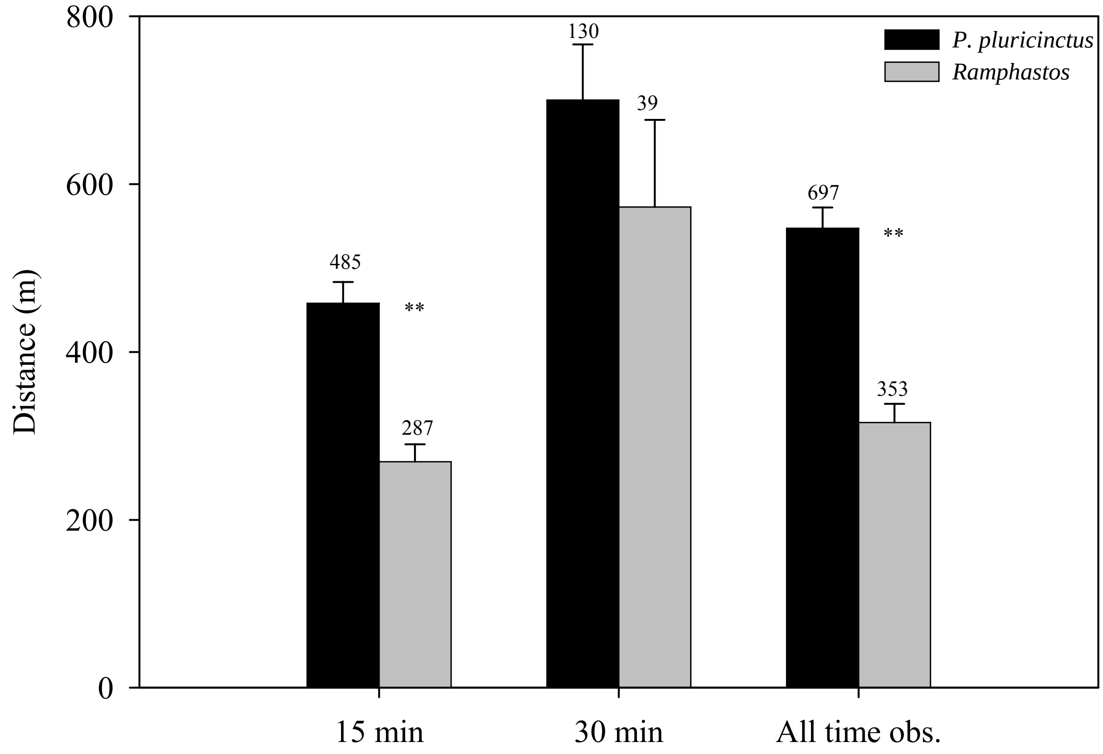


Figure 2


800
600
400
200
0
Distance (m)
15 min
30 min
All time obs.
P. pluricinctus
Ramphastos
485
287
**
130
39
697
353
**

Chapter 2

Using Toucan-Generated Dispersal Models to Estimate Seed Dispersal in Amazonia Ecuador

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Introduction

Animal-mediated seed dispersal plays a significant role in plant recruitment and, thus, helps determine tropical forest composition (Gautier-Hion *et al.*, 1985; Willson, 1992; Howe, 1993; Finegan, 1996; Hamann and Eberhard, 1999). Although the majority of tropical trees depend on vertebrates to move their seeds (Gentry, 1982; Howe and Smallwood, 1982; Willson and Crome, 1989; Jordano, 1992; Howe and Westley, 1997), few studies have directly measured the effectiveness of seed dispersers. In a Central American forest, Howe and Vande Kerckhove (1981) examined foraging behaviour and ranked frugivore species by their propensity to move seeds away from maternal *Virola nobilis* (*Myristicaceae*) trees. They found that chestnut-mandibled toucans (*Ramphastos swainsonii*, Ramphastidae), which swallowed fruits whole and moved relatively quickly away from fruiting trees, were the most effective at removing seeds and, thus, were likely to have had the greatest impact on plant fitness from among the assemblage of frugivores that fed on *Virola* fruits. For *V. nobilis* seeds, escaping distance- and density-dependent mortality below the maternal tree was critical to plant recruitment (Howe and Vande Kerckhove, 1981). Certain environments may be particularly favourable for seed and seedling recruitment, and any behaviours of animals that result in seeds being deposited in these more favourable environments clearly benefit plant fitness and impact disperser effectiveness. Wenny and Levey (1998) found that seedling survival was significantly higher for seeds dispersed by male three-wattled bellbirds (*Procnias tricarunculata*, Cotingidae) than for seeds dispersed by four other species of birds due to the favourable environmental conditions found below bellbird perch sites. Thus, bellbird behaviour and movement following fruit consumption directly influenced plant recruitment patterns. An important step in understanding the role of frugivores in dispersing seeds of tropical trees is studying their seed dispersal ecology and movement patterns on different temporal and spatial scales. Here we combine observations on the foraging and movement behaviour of toucans with fruit crop data to predict spatial patterns of seed dispersal of a Neotropical tree.

A seed disperser's effectiveness, measured as its contribution to plant fitness, is defined by qualitative and quantitative components of the dispersal it provides to a particular plant species (Schupp, 1993). For example, the distance a seed is moved from the plant, which is a function of animal movement behaviour, is one of the qualitative components of effectiveness, whereas the average number of seeds removed per visit, a function of animal feeding behaviour, is a quantitative component. One way to measure the effectiveness of a disperser is to estimate its contribution to a particular tree's seed shadow, which is defined as the spatial dispersion of seeds relative to a parent plant (Janzen, 1970). Seed dispersal and resultant seed shadows may influence key processes, such as colonization, population persistence (Ouborg *et al.*, 1999; Cain *et al.,* 2000), and plant population structure (Loiselle *et al.,* 1995). Movement patterns and feeding behaviours of frugivores directly impact seed shadows (Janzen *et al.,* 1976; Fleming and Heithaus, 1981; Murray, 1988; Fragoso, 1997; Sun *et al.,* 1997; Holbrook and Smith, 2000). Frugivores that remain for long periods in fruiting trees will drop most seeds beneath a parent tree, thereby influencing the seed shadow differently than frugivores that visit trees for short periods and deposit most seeds at sites away from the parent. These differences can result in spatial variability in seed shadows, which can have consequences for seed and seedling survival and population demographics (Kwit *et al.,* Chapter 19 this volume). Further, because some frugivores, such as hornbills and toucans, travel across different habitat types including degraded and fragmented forest areas (Graham, 2001a, b; Holbrook *et al.,* 2002) they are likely to facilitate gene flow and help maintain genetic diversity of plant populations. Jordano and Godoy (2000) found high levels of genetic diversity in *Prunus mahaleb* (*Rosaceae*), a bird-dispersed species, suggesting that long-distance dispersal by frugivorous birds contributes to within-population genetic diversity.

Theoretically, seed shadows are expected to show a distance-density effect, in which the number of seeds declines with increasing distance away from a parent, regardless of whether the dispersal system involves animals (Janzen, 1970; Fleming and Heithaus, 1981). A dispersal kernel describes the frequency distribution of dispersal distances and is differentiated from the seed shadow, which is the product of the dispersal curve and the total number of seeds dispersed (Levin *et al.,* 2003). Dispersal kernels have typically been described as having a leptokurtic distribution with a peak in seed density at the parent plant (Harper, 1977; Levin, 1979). Such theoretical distributions have been supported by empirical data. Studies that examine seed shadows from the plant perspective traditionally use seed traps to predict dispersal functions for plants dispersed by different vectors (typically wind or animal). These studies have revealed that dispersal curves largely match theoretical expectations and are best fit by distributions that show distance-density effects, such as exponential, lognormal, Gaussian, Student *t*, Weibull, and inverse power functions (Hoppes, 1988; Willson, 1993; Laman, 1996; Clark *et al.,* 1999, 2005; Jones *et al.,* 2005). Studies that measure seed shadows from an animal perspective, however, have described non-leptokurtic dispersal curves, and in many cases, show distributions that are not exponential in shape and thus that do not match theoretical expectations (Murray, 1988; Sun *et al.,* 1997; Holbrook and Smith, 2000; Westcott and Graham, 2000; Wehncke *et al.,* 2003; Westcott *et al.,* 2005). These examples suggest a great diversity in seed dispersal patterns and potential difficulty in attributing any one form of dispersal function to a dispersal system.

Why does this apparent conflict in dispersal kernels exist between distributions generated from animal- and plant-perspectives? Discrepancies might simply result from the species investigators select for study. Studies that rely on seed traps might tend to select plants that produce large crop sizes with many small seeds, while those that focus on animals might tend to select plants that produce relatively small crop sizes with nutritious, high-quality, and often, large-seeded fruits (Sun *et al*., 1997; Holbrook and Smith, 2000; Westcott *et al.*, 2005). Dispersal functions for these two kinds of plants might be expected to differ regardless of the method employed to measure seed shadows. Alternatively, assumptions underlying data strictly dependent on seed traps may not always hold, and seed movement patterns might be underestimated. Recent genetic studies demonstrate that dispersal distances are often underestimated and/or that the assumption that the parent is the nearest reproductive adult to a deposited seed or seedling is frequently incorrect (Abe *et al.,* 2005; Jones *et al.,* 2005; Hardesty *et al.,* 2006). Jones *et al.* (2005) found dispersal of *Jacaranda copaia* (*Bignoniaceae*), a wind-dispersed tropical tree, to be more complex than can be described by a simple, single-distribution dispersal kernel. Furthermore, fitted models potentially underestimated the long-distance dispersal component of *J. copaia* (Jones *et al.,* 2005). Hardesty *et al.* (2006) suggest that even though it may appear that the majority of seeds are dropped beneath a reproductive tree, one must be cautious about assigning parentage; their genetic data clearly showed that germinated seedlings were seldom produced by the nearest or even nearby reproductive adults. Model simulations found that movement patterns of frugivorous birds contributed to asymmetry in fruit-removal, and that at least in populations where fruiting plants were aggregated, dispersed seeds belonged to the nearest neighbour (Morales and Carlo 2006; Carlo *et al.,* Chapter 16 this volume). Finally, Steele *et al.* (Chapter 14 this volume) demonstrate that dispersal of *Quercus alba* (*Fagaceae*) is more extensive than behavioural studies would suggest.

Efforts to describe the seed shadows of vertebrate-dispersed plant species from the frugivore perspective have focused on measuring dispersal distances and seed passage times of frugivores (Murray, 1988; Sun *et al.,* 1997; Holbrook and Smith, 2000; Westcott and Graham, 2000; Wehncke *et al.,* 2003; Westcott *et al.,* 2005). Dispersal kernels estimated from the frugivore perspective allow a better understanding of the direct contribution of particular seed dispersers to observed seed deposition patterns. Frugivore-generated dispersal kernels also allow estimation of the dispersal tail, which can reflect rare, but important long-distance dispersal events (Cain *et al.,* 2000; Holbrook and Smith, 2000).

Here we estimate dispersal kernels for *Virola flexuosa* (*Myristicaceae*) that have been generated by toucans. Although some studies have focused on seed dispersal by toucans (Howe *et al.,* 1985; Wenny, 2000), none have examined toucan species-specific dispersal kernels. Furthermore, there are no studies that integrate frugivore-generated dispersal kernels estimated from movement data and seed passage times with foraging behaviour of the disperser and fecundity of the plant. We combine our frequency distribution of dispersal distances with information on fruit removal by toucans and crop size of individual trees to present a spatially explicit model that predicts the pattern of seed dispersal for *V. flexuosa* within a large-scale plot at the individual tree and population level. We refer to the frequency distribution of dispersal distances generated by toucans as dispersal kernels and the spatially explicit models of seed dispersion patterns as seed shadows, recognizing that the latter are not complete seed shadows. Specific objectives are to: 1) estimate seed dispersal distances for a Neotropical tree, *V. flexuosa*, based solely on toucan movements and seed retention times, and, 2) present a spatially explicit model, which more realistically 'outlines' the dispersion patterns generated by toucans.

Methods

Study site and species

This study was conducted at the Tiputini Biodiversity Station (Tiputini; ~0^o 38' S, 76^o 09' W) and Yasuní Research Station (Yasuní; ~0^o 40' S, 76^o 24' W) in the Orellana Province, Ecuador from 2001-2005. Ecuador is extremely biodiverse hosting 6% of the world's vascular plant species (> 16,000; Jørgensen and Leòn-Yánez, 1999) and 15% of the world's bird species (> 1500; Parker *et al.,* 1996). Both stations (approximately 27 km apart), located in the Yasuní Biosphere Reserve (1.5 million ha) in equatorial western Amazonia, are floristically similar with elevations ranging from ca. 200-245 m. The reserve represents the largest protected area of mature forest in the Amazon region of Ecuador. The vegetation is evergreen lowland rain forest and the area receives > 3000 mm of rain each year (Jørgensen and Leòn-Yánez, 1999) with the majority of rainfall occurring from October-November and May-July (J. Guerra, 2005, unpublished results; Valencia *et al.,* 2004). The presence of canopy towers at Tiputini and Yasuní, access to office and laboratory space, herbarium collections, and existing trail systems at both sites provided an excellent setting for this study. Additionally, Tiputini has two 100-ha plots (see Loiselle *et al.,* 2007) and Yasuní has a 50-ha Forest Dynamics Plot (FDP) (see Valencia *et al.,* 2004). Research was conducted primarily in the FDP at Yasuní and within 50 ha of one of the 100-ha plots at Tiputini; both study plots are located in mature terra firme forest. All stems > 10 cm diameter at breast height (dbh) in the FDP at Yasuní have been mapped and identified (Valencia *et al.,* 2004). In addition, we searched for and mapped all adult *V. flexuosa* in a 100 m strip (34 ha) surrounding the existing FDP. At Tiputini, we located and mapped all adult individuals of *V. flexuosa* within 84 ha, including the 50-ha plot mentioned above and the surrounding 34 ha. Data on toucan foraging and movement behaviour were collected at both Yasuní and Tiputini. Data on the fecundity of individual trees were collected only at Tiputini and subsequent dispersal models are presented for Tiputini only. A total of seven female, 14 male, and four unknown *V. flexuosa* adults are shown in the plot at Tiputini (Fig. 13.1). Unknown individuals were of reproductive size (> 30 cm dbh), but did not produce flowers or fruit during the study period.

This study focuses on toucans (Ramphastidae) because they are important members of seed disperser communities and are found throughout the Neotropics. Moreover, although toucans are large conspicuous members of the canopy and subcanopy bird community, their ecology has been relatively little studied. The many-banded araçari (*Pteroglossus pluricinctus*), white-throated toucan (*Ramphastos tucanus*), and channel-billed toucan (*R. vitellinus*) are ideal study species because they are abundant in Amazonia and present an opportunity to compare seed dispersal by toucans that differ in size and likely differ in diet, movement patterns, and seed dispersal ecology. *Virola flexuosa* A.C. Sm. (*Myristicaceae*) is a dioecious, shade tolerant species widespread throughout South America (Lambright, 1981). In eastern Ecuador, *V. flexuosa* is regularly consumed by toucans (Holbrook, 2006) and is likely important in toucan diets, as are other species in the genus (Howe *et al.,* 1985; Galetti, 2000). For example, Galetti (2000) found 11-38% of all foraging observations by *Ramphastos* toucans were concentrated in *Virola*. Howe *et al.* (1985) found that dispersers of *V. nobilis* were restricted to a small, specialised group of frugivores, of which toucans were shown to disperse the majority of seeds away from parent trees. *Virola flexuosa* is relatively easy to identify in the field in both fertile and sterile condition and the fruit is distinctive with a bright red aril enclosed by a capsule, which dehisces during early and mid-morning hours (K.M. Holbrook, 2002, personal observation).

Estimating seed shadows, field methods

Fruit removal – Tree watches were conducted throughout the fruiting season (November to March, 2002-2004) to determine the relative role toucans play in dispersing the seeds of *V. flexuosa*. A total of 13 focal female trees at Tiputini and Yasuní were observed from 0600-1000 h with a minimum of eight replications per tree. All visiting frugivores were identified during tree watch observations. We recorded the number of visits, total number of fruits eaten and removed per visit, and time spent foraging for each visit. In addition, for each frugivore species, we recorded the number of seeds ingested, regurgitated, and dropped or knocked down from the canopy. Fruit removal was defined as the number of seeds swallowed and taken away from the fruiting tree.

Crop size **–** Seed traps (1 m^2) made of PVC tube and mosquito screen were placed at randomly selected points between the tree bole and the edge of the crown of each focal tree, to estimate crop size. We used enough traps (4-6) to cover approximately 5% of the area below each crown. Each fruit consists of a capsule enclosing an arillate seed, with the arillate seed the unit of dispersal. Following Howe and Vande Kerckhove (1981), capsules collected in the traps provided an estimate of the total crop matured. Specifically, we counted the number of capsules collected in seed traps and divided by the proportion of the canopy area sampled by traps. To estimate crop size, we used seed trap data from nine female trees at Tiputini. Seven trees were located within the 84-ha plot area (Fig. 13.1) and two trees were located 1.9 and 3.4 km from the centre of the 50-ha plot.

Seed retention – We determined seed retention times with captive toucans and use these data in combination with movement data to estimate toucan-generated dispersal kernels (see below). Seed passage trials were conducted both in the field in Ecuador and with captive toucans at the Saint Louis Zoo, USA. For field trials, four individuals of *P. pluricinctus* were held captive for up to two days in cages constructed of flexible nylon mesh (1 x 1 x 2 m) (Santana *et al.,* 1986). We marked seeds from ripe *V. flexuosa* fruits with short strands of cotton thread for easy discrimination between individual seeds and to differentiate any that may have remained in the gut from feeding in the wild. Seeds were placed in small cubes of papaya (*Papaya carica*, *Caricaceae*) or a supplemental, gel-like bird food designed for frugivores (PurinaMills; J. Dempsey, St. Louis, MO, 2003, personal communication) and were provided from 0600-1700 h. In addition to papaya or bird gel with seeds, we provided *ad libitum* locally available fruit, including papaya, watermelon (*Citrullus lanatus*, *Cucurbitaceae*), and cantaloupe (*C. melo*, *Cucurbitaceae*). Each bird was observed continuously between 0600-1800 h from a blind near the cage. At the Saint Louis Zoo, trials were conducted with four *Ramphastos* individuals (one *R. tucanus,* one *R. vitellinus,* and two *R. toco*). We placed seeds of *V. flexuosa* (collected and frozen in the field) into fruit pieces (papaya and grapes [*Vitis vinifera, Vitaceae*]) that were provided from 0800-1430 h. We observed birds continuously from outside their cage between 0800-1700 h. In all trials, seeds were noted immediately after regurgitation or defecation and retention times calculated. We tested for differences in seed retention times between species using a Mann-Whitney *U* test. Retention data from all three species of *Ramphastos* were used in estimation of seed shadow models for the genus *Ramphastos*.

Movement patterns – To determine toucan movement patterns, we radio-tracked three species of toucans during two 3-month and two 8-month field seasons (2001-2005) following methods employed in Cameroon by Holbrook and Smith (2000). We captured a total of 25 *P. pluricinctus*, two *R. tucanus*, and two *R. vitellinus* at Tiputini and Yasuní using canopy nets at fruiting trees and/or nesting sites. We attached radio transmitters (6 g each; Holohil Systems Ltd, Carp, Ontario, Canada) at the base of the central tail feathers. Body mass of tagged birds ranged from 195-684 g, resulting in the transmitter weighing less than 3% of the bird's mass. Transmitter attachment did not appear to affect bird movements. Toucan locations were measured by triangulation using receivers and hand-held 2-element Yagi antennas (White and Garrott, 1990; Kenward, 2001). Tracking stations were located on permanent canopy towers, temporary canopy platforms, and ground positions allowing for minimal error in location data. Station positions were determined using a Global Positioning System. Three observers, using two-way radios, collected simultaneous bearings approximately two days per week at each site over a period of 3-8 months during each of four field seasons. Tracking periods lasted 4-6 daylight hours with individual birds located every 15 minutes. We chose a 15 minute interval period to ensure we could collect bird locations within the time frame of seed passage or regurgitation, which were expected to be longer than 15 minutes (Sun *et al.,* 1997; Holbrook and Smith, 2000). In addition, we dedicated several tracking days to following individually-tagged birds in order to collect detailed movement and location data, such as tree to tree movements and cavity roost locations. These detailed movements complemented location data collected through triangulation and were used to supplement data collected for calculation of toucan-generated dispersal kernels. Bird locations were estimated through triangulation using the program LOAS 2.03 (Ecological Software Solutions). Because of small sample size for *Ramphastos*, we combined movement data from both species to estimate seed shadows for the genus *Ramphastos*; our expectation was that home ranges for the two species would be similar based on similar territory sizes reported in Terborgh *et al.* (1990).

Estimating seed shadows, dispersal models

Probability of seed deposition – Following Murray (1988) and Holbrook and Smith (2000), we estimated toucan-generated dispersal kernels ('seed shadows' in Murray, 1988 and Holbrook and Smith, 2000) using seed retention times and movement data from radio telemetry. Location accuracy using radio telemetry may be reduced in tropical forests, due to wet habitats, thick vegetation, and uneven topography (Zimmerman and Powell, 1995; Millspaugh and Marzluff, 2001). Because of this, we used conservative distance categories of 100 m increments to better represent the precision of our telemetry data. We chose time categories (e.g. 15, 30, 60, and 90 min) for the model based on seed retention trials. Within each of these time categories, distances (e.g. 100 m, 200 m, 300 m) moved by toucans were grouped and we calculated the probability of movements made within each distance category, within each time category. These are summed across each time category to give a final probability for each distance category. The final calculation is:

$$p_d = \sum_t (a_{dt} \bullet b_t) \tag{1}$$

where *p* = probability of a seed being deposited at a particular distance category (*d*) from the parent tree, *a* = probability of a bird being within a particular distance category (*d*) in time interval (*t*), and *b* = probability of a seed being passed in that time interval. We then plotted *p* against distance to give a probability of seed deposition at various distances. In each case, the first location of the day was considered the 'origin' or parent tree in the model.

Spatially explicit models – Probabilities of seed deposition for each toucan species (Equation 1) were combined with *V. flexuosa* fruit removal data to more realistically estimate seed shadows of *V. flexuosa*. Using a spatially explicit model, all adult female trees (located by x, y coordinates and entered in a GIS database) in the 84-ha plot at Tiputini serve as the origin for calculations in the model. Thus, seed dispersal predictions were limited to the scale of the 84-ha plot, which was chosen because we expected this area to encompass the major proportion of a toucan home range, where most seeds would likely be dispersed.

For model estimates, we divided the 84-ha plot into 5 x 5 m cells, each assigned a value for the distance to each adult female tree in the plot. Using *reclassify* in Spatial Analyst (ArcGIS 9.1, ESRI, Inc), a probability of dispersal for each toucan species (p, Equation 1) was then assigned to each cell based on the distance from each female tree that cell falls into (i.e. < 100, 100-200, etc.). The estimated dispersal distances that seeds are dispersed were based on the toucan models and were assumed to be the same for each female tree. We then multiplied each set of probabilities by the number of seeds dispersed away from each tree based on observation data (these vary depending on crop size) to provide tree-specific seed shadows. A population-level seed shadow was then generated by adding individual tree shadows on the 84-ha plot. The equation for the final summed model is written as:

$$N_x = \sum_m (p_{xm} \bullet r_m) \tag{2}$$

where N = the number of seeds predicted to fall at a particular location (x, individual 5 x 5 m cells within the 84-ha plot), p = the probability of seed deposition at varying distances (x) from each female tree (m), and r = number of fruit removed at each female tree. The numbers of seeds dispersed to each location are reported as the proportion of total *Virola* seeds estimated to be dispersed by toucans in the landscape. This method allows us to draw the ‘individual tree seed shadows’ that together define the population model. We examine differences in the final summed seed shadows generated by *Ramphastos* and *Pteroglossus* toucans using GEODIST.BAS, a QuickBASIC 4.5 program that tests for differences between two spatial distributions, based on a modified Cramér-von Mises non-parametric test (Syrjala, 1996). This non-parametric test evaluates the differences between the spatial distributions of two populations. Specifically, we compared the spatial distribution of seed deposition by *Ramphastos* with the seed deposition by *P. pluricinctus* by generating 200 random points within the study plot. The minimum distance selected between points was 5 m to account for our 5 x 5 m cell size. Each point was associated with x, y coordinates and a 'value' corresponding to the relative number of seeds dispersed by *Ramphastos* or *P. pluricinctus*. We tested for differences in the resulting spatial distribution of seeds predicted to be dispersed by *Ramphastos* and *P. pluricinctus* at these 200 random points using 1000 permutations to generate the Cramér-von Mises test statistic. A significant test statistic indicates that the seed deposition pattern produced by *Ramphastos* differs from that produced by *P. pluricinctus*.

Results

Fruit removal and seed retention

More than 400 hours of observations at 13 individual fruiting trees indicate that *Ramphastos* and *P. pluricinctus* represent 64.3% of visits and remove more than 52.0% of dispersed seeds (Table 13.1). To calculate the number of seeds dispersed from individual *Virola* trees, the proportion of seeds removed was multiplied by crop size, which varied widely from 183 to 39,100 fruits (mean ± SD, 14,408 ± 15,526).

Multiplying by crop size allowed us to better estimate removal over the entire fruiting period (mean ± SD, 78 ± 25 days), rather than restricting removal to times when foraging behaviour data were collected. The resulting mean (SD; range) number of seeds dispersed per tree was 1689 (2022; 22-3779) for *P. pluricinctus* and 5503 (6589; 73-15,576) for *Ramphastos*. The level of variation in crop size observed here is not unusual for tropical trees (Howe and Vande Kerckhove, 1981; Russo, 2003; Clark *et al.,* 2005). Seed retention experiments revealed little difference between *Ramphastos* and *Pteroglossus* toucans (Fig. 13.2), with the average seed retention time approximately 30 minutes. Most seeds ingested by toucans were regurgitated (97% for *P. pluricinctus* and 91% for *Ramphastos*); some in as little as between 5-10 minutes ($n = 19$) and others between 60-90 minutes ($n = 14$; $N\text{tot} = 141$). A small percentage (5%; $n = 7$) were retained in the gut between 100-140 minutes by *Ramphastos*, suggesting a trend for longer retention times by the larger *Ramphastos* species.

Movement patterns

We tracked radio-tagged individuals for between three to 25 days with the number of locations ranging from 11-185. Because birds were radio-tracked using a triangulation method, we were not able to collect continuous movement data on each bird. Rather, data represent locations collected in 15 minute intervals. *Ramphastos* and *P. pluricinctus* had significantly different mean (SD) home ranges of 86 (25) ha and 191 (64) ha for *Ramphastos* and *P. pluricinctus*, respectively ($F_{1,13} = 5.36$, $P = 0.049$) (see Holbrook, 2006). These home range estimates were based on individuals with more than 40 locations recorded. However, movements from all radio-tracked individuals were used for estimating dispersal kernels. Distances travelled per movement bout ranged from 0 to > 2000 m (Fig. 13.3). Time calculated between locations varied from 15–165 and 15-135 minutes for *Ramphastos* and *P. pluricinctus*, respectively. These distributions are strongly leptokurtic with most movements being < 300 m. A greater proportion of *Ramphastos* (28%) versus *P. pluricinctus* (16%) movements were within 100 m, which is reflected in the overall shape of dispersal kernels. The longest recorded movements (both recorded within 30 minutes) were 3027 and 3665 m for *Ramphastos* and *P. pluricinctus*, respectively.

Supplementary tracking data, in which we directly followed tagged birds for up to 5 h, was collected on more than 20 different days. During these tracking periods we never observed toucans to travel from one fruiting *V. flexuosa* to another in the same tracking period, suggesting that dispersal to conspecific individuals is rare.

Dispersal models

Dispersal kernels predict that *Ramphastos* and *P. pluricinctus* disperse 72% and 84% of seeds > 100 m away from parent trees, respectively (Fig. 13.4). The shapes of dispersal kernels differed significantly with the peak of the distribution occurring at < 100 m for *Ramphastos*, but between 100-200 m for *P. pluricinctus*. The dispersal kernel generated by *Ramphastos* appears to be an exponential distribution, with the peak located within 100 m of the source, while the kernel for *P. pluricinctus* suggests a chi-square distribution. Both dispersal kernels exhibit very long thin tails, suggesting long-distance dispersal, similar to those reported for the southern cassowary (*Casuarius casuarius*, Casuariidae) in Australia (Westcott *et al.,* 2005) and African hornbills (*Ceratogymna atrata* and *C. cylindricus*, Bucerotidae) in Cameroon (Holbrook and Smith, 2000).

The spatially explicit models of seed shadows generated from our combined data on plant fecundity, fruit removal rates, and dispersal kernels are presented Figure 13.5. Using these population-level seed shadows, we predicted that some areas in the landscape were more likely to receive seed fall than others. The areas with the highest density of seed rain occurred around trees with the highest fecundities and in areas where individual seed shadows overlapped (Fig. 13.5). These regions of high seed deposition also likely represent areas of seed mixing, where dispersed seeds represent a genetic mix of adult trees in the study area. Our estimates indicate that seed mixing occurred throughout the study area, with each location receiving different probabilities of seed fall from different adults depending on how toucans moved through space and the fecundity of individual trees. Note that the estimated percentage of seeds dispersed by *Ramphastos* or *P. pluricinctus* represents the proportion of the total seeds dispersed by that species. Most reproductive *V. flexuosa* do not produce fruit every year and there appears to be a general trend towards fruit production every second or third year (K.M. Holbrook, 2006, unpublished results). Consequently, we can not evaluate whether the differences in fruit production among maternal trees observed during the course of this study will hold over time. Therefore, we restrict the spatial depiction to this time period and do not make any assumption about future years.

DISCUSSION

Due in large part to the great diversity of animal dispersers in tropical forests, understanding and predicting seed dispersal patterns have been difficult. In this paper, we integrate animal behaviour and seed dispersal processes across temporal and spatial scales. In doing so, we have improved upon a two-dimensional dispersal kernel by incorporating aspects of disperser behaviour and crop size of individual female trees (Fig. 13.5). The resulting spatially explicit model of seed dispersion is more realistic in depicting patterns of seed deposition than using just frugivore-generated dispersal kernels. We predicted *V. flexuosa* seeds would land in a patchy fashion on our study site with greater numbers of seeds being deposited in overlapping areas between fruiting trees. At the population level, trees with the highest fecundities clearly drive much of the seed deposition pattern. We observed the highest density of seed fall in the south-eastern portion of the study plot where the more fecund trees were located at the time (Fig. 13.5). Our results have demonstrated that patterns of behaviour, including disperser movements, seed retention times, foraging behaviour, in addition to plant parameters such as crop size, can significantly impact the shape and scale of dispersal kernels, and patchy nature of seed shadows.

While we have attempted to incorporate several of the many factors contributing to generating a seed shadow, we recognise that others remain to be incorporated. For example, our models do not detail specific repeated movements of toucans to dispersal foci, such as roost sites, nest cavities, or other fruiting trees. A closer examination of movement behaviour may indicate that toucans exhibit measurable non-random movement (see Kwit *et al*., Chapter 19 this volume). In addition, seed dispersal studies require integrating processes across a wide range of scales. For example, other species contribute to a total seed shadow for *V. flexuosa*, which may change the scale at which a seed shadow is investigated. Although, 84 hectares represents a large proportion of a toucan home range, it may not be large enough to encompass movement patterns of all dispersers of *V. flexuosa*. In addition, our seed shadow estimates do not incorporate seed rain from adult trees outside the 84-ha area. Further, individual plant recruitment operates at a much finer scale and our study does not address environmental heterogeneity on the study plot at this scale.

Shape and scale of dispersal

The shape and scale of dispersal kernels can be affected by a number of attributes of the process. For example, in simulation models, Morales and Carlo (2006) found the degree of plant aggregation to influence scale, while shape was mostly dominated by frugivore density; overall, mean dispersal distances were reduced as the spatial aggregation of plants increased. In contrast, Westcott and Graham (2000) found that gut passage rate determined the overall shape of the dispersal kernel for an understorey flycatcher (*Mionectes oleaginous*, Tyrannidae), while movements determined scale.

How long seeds are retained by fruit consumers potentially has strong impacts on animal-mediated seed shadows (Westcott and Graham, 2000; Morales and Carlo, 2006; Carlo *et al*., Chapter 16 this volume). For example, seeds with longer retention times tend to be dispersed farther away from the parent tree and may also be deposited in a greater variety of locations and habitats (Sun *et al.*, 1997). In our retention trials, nearly all *V. flexuosa* seeds were regurgitated within 60 minutes. However, a small proportion of seeds was defaecated and had passage times exceeding 100 minutes. In addition, there was a trend for the larger *Ramphastos* to have longer retention than the smaller *P. pluricinctus*. This trend, for larger species to have longer seed retention was found in African hornbills (Holbrook and Smith, 2000) and a range of small Neotropical frugivores (Levey, 1986). Avoiding ballast is largely driven by the size of the bird and is likely to shorten the scale of a dispersal kernel. *Pteroglossus pluricinctus* would be more likely to regurgitate large seeds than *Ramphastos* to avoid ballast due to its smaller size (see Levey, 1986). Despite these potential differences the toucans investigated here demonstrated statistically similar patterns of seed retention.

Due to similar retention times of seeds, differences in dispersal kernels generated by *P. pluricinctus* and *Ramphastos* were largely the result of different movement patterns. Home range use of *P. pluricinctus*, which forages in family (or single-species) flocks, was nearly twice as large as that of *Ramphastos*. This suggests that *P. pluricinctus* has the ability to move seeds a greater distance than *Ramphastos*. Within 30 minutes (mean seed retention time), the average distance moved by *P. pluricinctus* and *Ramphastos* was 528 m and 348 m, respectively. These dispersal distances are similar to those recently reported for *C. casuarius*, where the average dispersal distance from the parent plant was 337 m (Westcott *et al.*, 2005).

Although many dispersal studies suggest that dispersal kernels peak at the parent plant or fit a negative exponential distribution (Portnoy and Willson, 1993) or some combination of distributions (Clark *et al.*, 1999; Nathan and Muller-Landau, 2000; Clark *et al.*, 2005; Jones *et al.*, 2005), many vertebrate-generated dispersal kernels do not exhibit this pattern (Murray, 1988; Sun *et al.*, 1997; Holbrook and Smith, 2000; Westcott and Graham, 2000; Wehncke *et al.*, 2003; Westcott *et al.*, 2005; but see Dennis and Westcott, Chapter 9 this volume). We found that *P. pluricinctus* dispersal kernels, like many other vertebrate-generated kernels, were not highly leptokurtic and most seeds *were not* dispersed directly underneath or within metres of a parent tree (Fig. 13.4). Instead, a large proportion of seeds were moved a considerable distance. *Ramphastos*, on the other hand, did exhibit a roughly negative exponential curve. Our observations at fruiting trees suggest that *P. pluricinctus* arrived, foraged for several minutes, and departed without returning to the tree in that observation block (4 h). *Ramphastos* often arrived to forage for several minutes, departed to adjacent trees, and returned. This behaviour was recorded with some frequency and appears to be reflected in radio-tracking data, further supporting the observed differences between species. Both curves, however, had distributions with long thin tails indicating some seeds would be dispersed long distances.

Accurately measuring the frequency and scale of long-distance seed dispersal is well understood to be a difficult process (Clark *et al.*, 1999; Cain *et al.*, 2000; Nathan and Casagrandi, 2004). In Panama, Jones *et al.* (2005) found considerable uncertainty in the tail of the distribution after fitting several dispersal models to seed dispersal data of a wind-dispersed tropical tree. Nevertheless, seed dispersal studies are challenged to provide measures of long-distance dispersal because of its likely importance in influencing recruitment processes and determining plant population structure. Long-distance dispersal likely allows for greater gene flow and genetic mixing (Schupp, 1993; Hamilton, 1999, Shilton *et al.*, 1999). In addition, longer dispersal distances make it possible for the offspring of a single parent to sample a larger area, thus reducing kin competition and effecting bet-hedging over a greater number and variety of environments (Muller-Landau and Hardesty, 2005). At the population level, longer dispersal distances reduce dispersal limitation (Clark *et al.*, 1998; Nathan and Muller-Landau, 2000). Finally, long-distance dispersal may facilitate arrival of rare species and colonization of new sites (Holbrook and Smith, 2000; Holbrook *et al.*, 2002).

The effectiveness of toucan dispersal

Toucans appear to be very effective dispersers for *V. flexuosa* at Tiputini. Our results indicate that *Ramphastos* and *P. pluricinctus* toucans effectively disperse seeds beyond the canopy of fruiting trees, with *P. pluricinctus* dispersing seeds farther than *Ramphastos* (Fig. 13.4). Despite fairly short seed retention, which could lead to many seeds being deposited beneath a parent, visits to fruiting trees were short and movement rates were such that seeds were brought well beyond the parent canopy. Dispersal to fruiting conspecifics is unlikely as the chance of a toucan travelling from one conspecific to another is limited by: 1) *V. flexuosa* being rare with a mean 3.6 adult female trees per 50 ha; 2) toucan *core* home ranges were from 19-28 ha, incorporating only two trees; and 3) the timing of peak fruiting for individual trees was frequently offset (K.M. Holbrook, 2006, unpublished data). Recent studies on Barro Colorado Island, Panama, found that negative density-dependent recruitment contributed to an increase in diversity when comparing seeds to seedling recruits, confirming the importance of distance and/or density-dependent mortality (Harms *et al.*, 2000). The extensive seed shadows reported here suggest toucans may decrease density-dependent seed and/or seedling mortality by transporting seeds away from parent plants (Janzen, 1970; Connell, 1971) and creating large areas of low density seeds (Fig. 13.5).

Unlike in many theoretical dispersal kernels, most *V. flexuosa* seeds escape the immediate vicinity of the maternal tree. But what is an effective dispersal distance? Howe *et al*. (1985) found a 44-fold increase in survival of seeds moved greater than 45 m from parent *Virola* trees. Although, we do not have seed survival data on *V. flexuosa*, seedling transects conducted at fruiting trees found greater numbers of larger-sized seedlings (> 30 cm) beyond 40 m, while smaller-sized seedlings (< 30 cm) were found only up to 40 m from trees (K.M. Holbrook, 2006, unpublished data); a pattern that may reflect differential survivorship in seedlings. Therefore, seeds dispersed beyond 100 or 200 m are likely to be well beyond the distance at which seed and/or seedling survival increase significantly.

The dispersal kernels we estimated for toucans highlight the frequency of long-distance dispersal. Seeds dispersed by toucans may be transported to locations several kilometres away. Although the mean dispersal distance for *P. pluricinctus* was 560 m and 338 m for *Ramphastos*, we recorded individual movements up to 3027 m and 3665 m for *Ramphastos* and *P. pluricinctus*, respectively. A toucan can potentially fly several kilometres within the average retention time of a *V. flexuosa* seed, suggesting they are capable of dispersing seeds very long distances, particularly individuals that travel outside their core home range.

The importance of toucans as seed dispersers is underscored by their primarily frugivorous diet (Remsen *et al.*, 1993; Galetti, 2000), likely gentle treatment of seeds (Whitney *et al.*, 1998 and references therein), foraging behaviour at fruiting trees, and relatively large home ranges (Holbrook, 2006; this study). Furthermore, toucans readily cross open habitat and secondary forest (Graham, 2001a, b), suggesting their importance in facilitating gene flow and forest regeneration. Toucans are also considered to be scatter-dispersers (Howe, 1989), which is likely to be beneficial to plant fitness by lowering mortality caused by density-dependent processes and distributing seeds to more environments, and, thus, enhancing recruitment probabilities.

Conclusion

As reviewed by Harper (1977) and Wang and Smith (2002), many factors interact to determine the density and dispersion patterns of plant populations (see also Russo *et al.*, Chapter 23 this volume). Seed dispersal is only the first step in the process. Ultimately, the spatial and temporal distribution of 'suitable sites' determines seedling establishment patterns (Schupp, Chapter 20 this volume). Although estimating dispersal kernels from a frugivore perspective is limited by the ability to track frugivores throughout a landscape, these models allow us to more accurately quantify seed and seedling shadows and have great potential to be combined with genetic-based models. In the future we intend to address questions of dispersal limitation using both ecologically-based and genetic-based models of seed dispersal. This combination will further our understanding of how frugivore behaviour and dynamics influence plant recruitment and population structure.

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FIGURE LEGENDS

Figure 13.1. Tiputini plot showing all adult *V. flexuosa* located within the 84-ha study area. The inner 50-ha plot is the site of our original study; the plot was expanded to include all adult *V. flexuosa* within a 100 m strip surrounding 50-ha plot.

Figure 13.2. Seed retention times of *V. flexuosa* by *P. pluricinctus* and *Ramphastos* sp. (*R. tucanus, R. toco, R. vitellinus*). Trials were conducted with four individuals in each group. Mean (SD) retention times were 28 (20) and 34 (32) min for *P. pluricinctus* and *Ramphastos*, respectively. Comparisons between *P. pluricinctus* and *Ramphastos* (grouped due to equivalent size) showed no significant differences ($U = 2544$, $P = 0.486$; Mann-Whitney). Mean body mass for *P. pluricinctus* and *Ramphastos* were 288 and 566 g, respectively.

Figure 13.3. Distances travelled per movement bout for *P. pluricinctus* and *Ramphastos.* These included all recorded movements between temporally consecutive locations. Although most observations were collected within the 15-min time category (*P. pluricinctus*, $n = 401$, $N\text{tot} = 1225$; *Ramphastos*, $n = 287$, $N\text{tot} = 915$), many locations were collected at longer intervals due to an occasional loss of transmitter signal during telemetry sessions.

Figure 13.4. Estimated dispersal kernels for *V. flexuosa* produced by *P. pluricinctus* and *Ramphastos* toucans. These kernels represent two dimensional models at the individual

tree level. Differences between dispersal kernels were significant (Kolmogorov-Smirnov; $Z = 1.651$; $P = 0.009$).

Figure 13.5. Probability of seed deposition of *V. flexuosa* by a) *P. pluricinctus* and b) *Ramphastos*. Seed shadows represent data from toucan-generated model, fruit removal, and crop size of *V. flexuosa*. Differences between distributions are significant (Cramér-von Mises; $\psi = 2.461$; $P = 0.001$). Only female trees are shown.

Table 13.1. Visitation and seed removal of *Virola flexuosa* by *Ramphastos* and *Pteroglossus*. *Ramphastos* includes *R. tucanus*, *R. vitellinus*, and *Pteroglossus* includes *P. pluricinctus*

Bird species	% total visits (*n*)	Mean ± SD # seeds removed per visit (*n*)	% total seeds removed (*n*)
Ramphastos	47.6 (234)	1.6 ± 1.9 (115)	39.8 (115)
Pteroglossus	16.7 (82)	1.4 ± 1.2 (8)	12.2 (8)

Figure 13.1

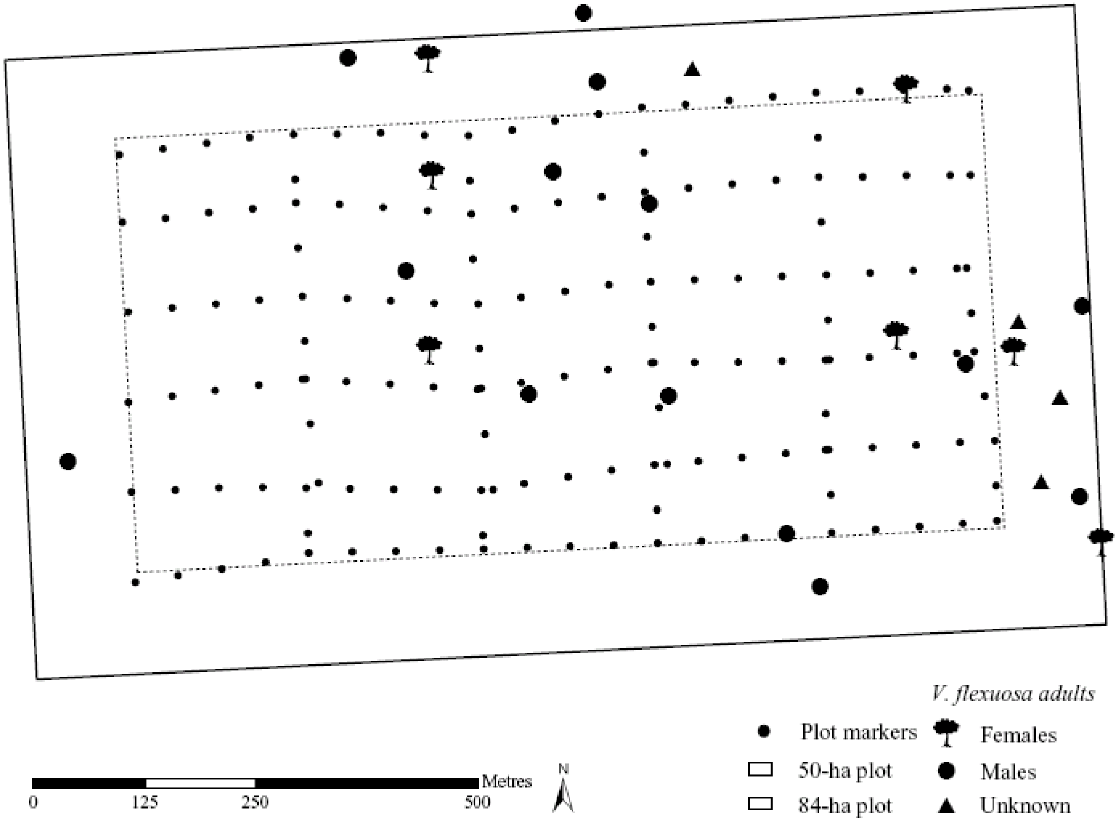


Figure 13.2

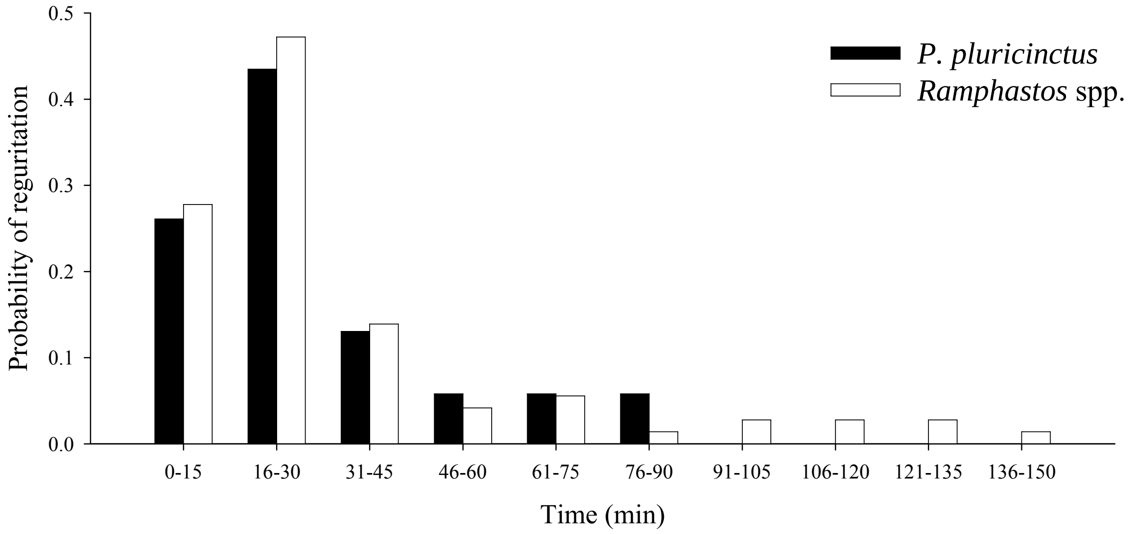


Figure 13.3

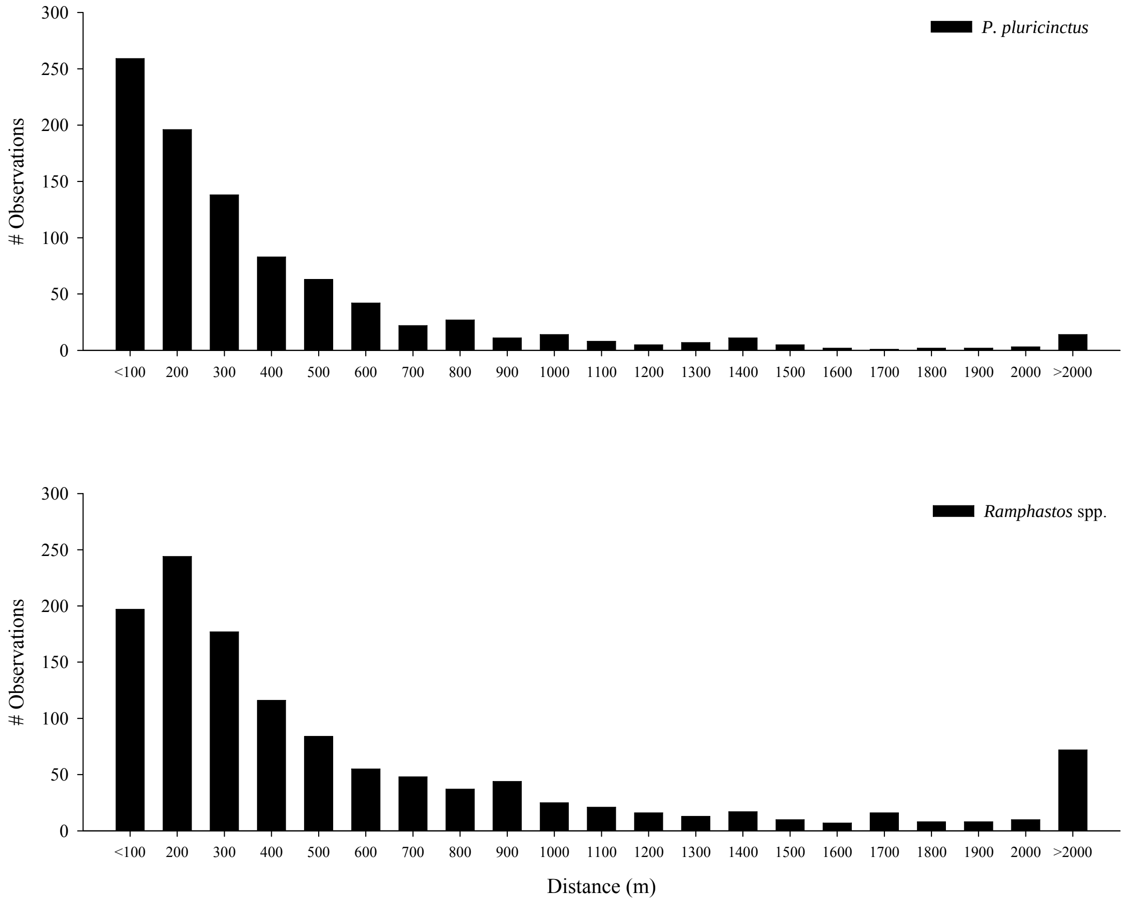

P. pluricinctus
Observations
300
250
200
150
100
50
0
<100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
2000
>2000
Ramphastos spp.
Observations
300
250
200
150
100
50
0
<100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
2000
>2000
Distance (m)

Figure 13.4

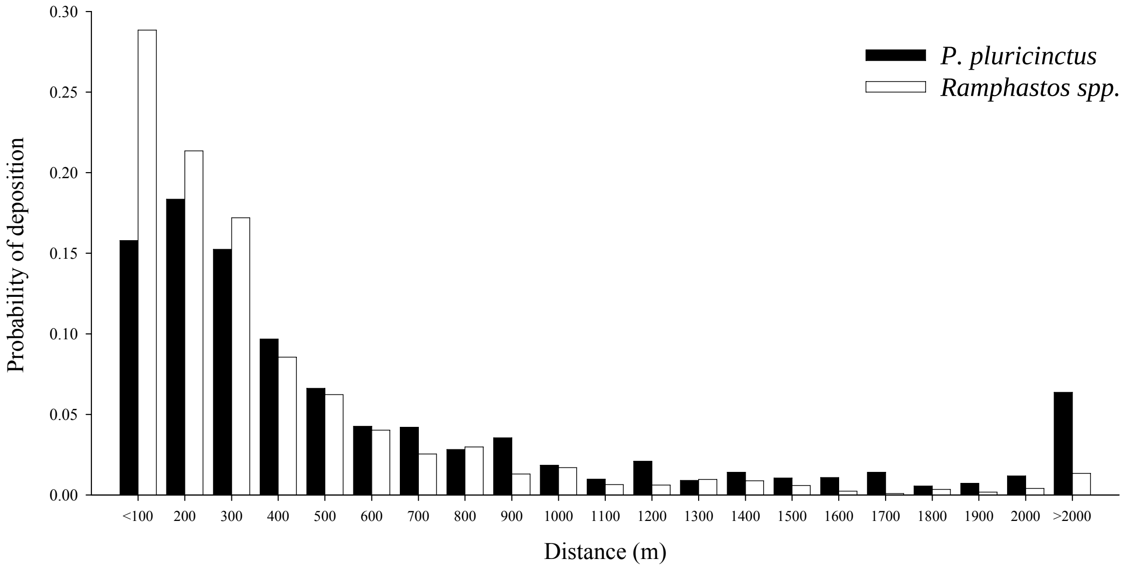
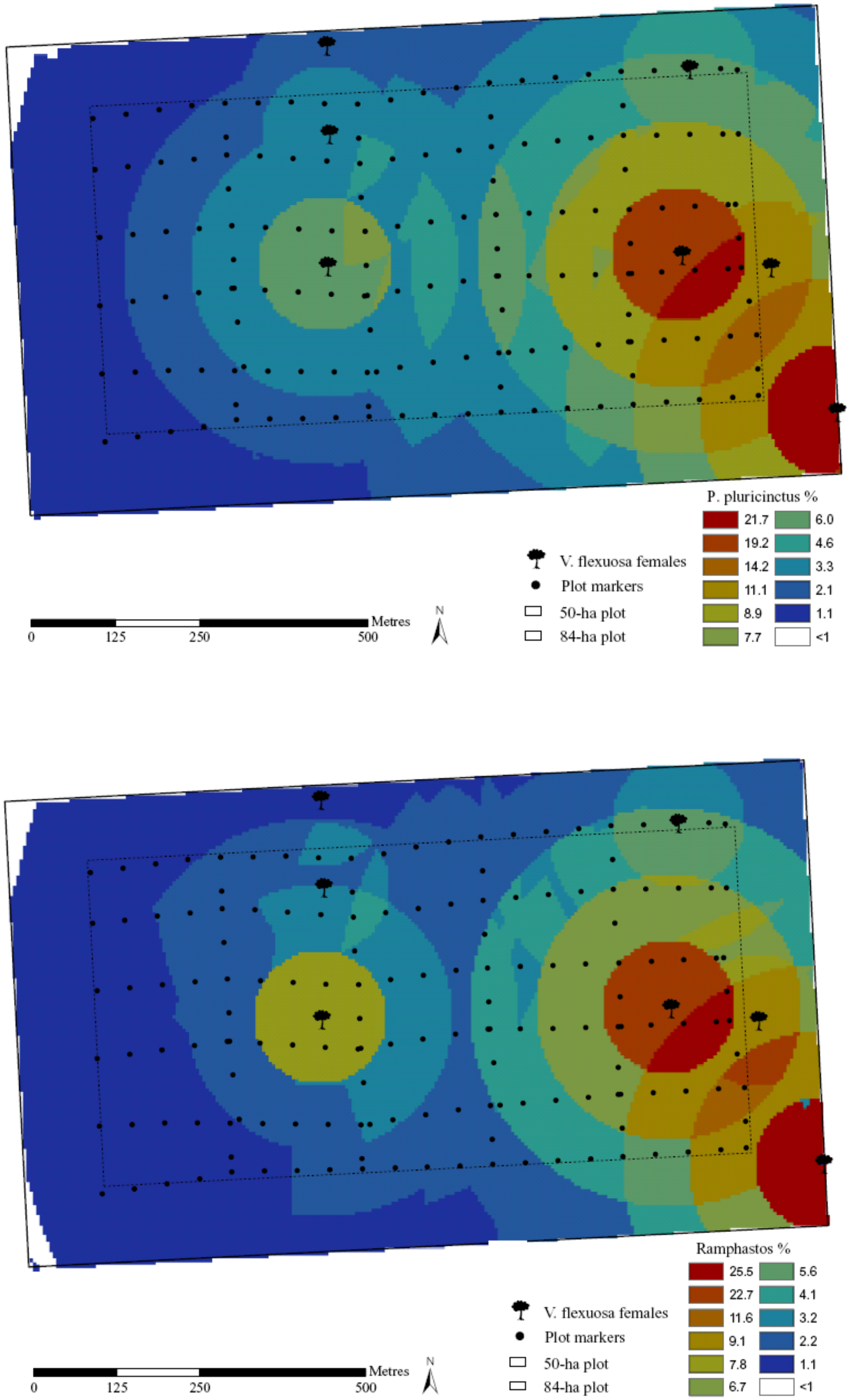

0.30
0.25
0.20
0.15
0.10
0.05
0.00
Probability of deposition
P. pluricinctus
Ramphastos spp.
<100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
2000
>2000
Distance (m)

Figure 13.5


P. pluricinctus %
21.7
19.2
14.2
11.1
8.9
7.7
6.0
4.6
3.3
2.1
1.1
<1
V. flexuosa females
Plot markers
50-ha plot
84-ha plot
0
125
250
500
Metres
N
Ramphastos %
25.5
22.7
11.6
9.1
7.8
6.7
5.6
4.1
3.2
2.2
1.1
<1
V. flexuosa females
Plot markers
50-ha plot
84-ha plot
0
125
250
500
Metres
N

Chapter 3

Seed Dispersal Limitation in a Neotropical Tree, *Virola flexuosa* (Myristicaceae): Does Hunting of Large Vertebrates Affect Seed Removal?

Unpublished manuscript: Holbrook, K.M. and B.A. Loiselle

Abstract

A seed disperser's effectiveness, measured as its contribution to plant fitness, has both qualitative and quantitative components. Quantitatively-restricted seed dispersal occurs when the quantity of seeds dispersed is limited by disperser abundances or behavior. To understand how different avian frugivores impact dispersal, we studied the assemblage of frugivores that feed on *Virola flexuosa* (Myristicaceae) over a two-year period at two sites differing in hunting pressure in Amazonia Ecuador. Here, we focus on the effectiveness of toucans (Ramphastidae) as dispersers and test the hypothesis that removal of *V. flexuosa* seeds, influenced by differential visits of large-bodied frugivores, will differ between hunted and non-hunted sites. All visiting frugivores were identified and fruit handling behavior, fruit removal rates and time spent foraging were quantified. Seventeen bird and three primate species were recorded foraging in *V. flexuosa* trees. Toucans and primates were the most important avian dispersers comprising nearly 85% of visits with six toucan species recorded in 74% of visits. For all visiting frugivores, mean visit length was 7.1 ± 9.1 minutes and mean number of seeds consumed per visit was 2.4 ± 3.1. *Ramphastos* toucans and *Pteroglossus pluricinctus* were responsible for 38.2% and 11.9% of all seeds removed, respectively. A proportionately larger number of seeds were removed from fruiting trees at the non-hunted site (TBS, 89.4%) than at the hunted site (YRS, 66.8%). In addition, there were more frugivore visits at TBS than YRS. We found that species more likely to swallow seeds, and, thus more likely to disperse seeds away from parent trees made significantly more visits to trees at TBS than did species that drop seeds. Moreover, species more likely to be hunted had higher visitation rates at TBS than YRS. Examining the dispersers of the Neotropical tree *V. flexuosa*, we found differences in the frugivore assemblage and the overall number of seeds dispersed from individual trees between two structurally similar forest sites. We suggest a quantitative effect on dispersal of seeds at the Yasuní Research Station, potentially resulting from a reduction in seed dispersers.

Introduction

The role of seed dispersal in plant population and community level-dynamics was first addressed in detail by Janzen (1970) and Connell (1971) who hypothesized that many tropical tree species benefit by having their seeds dispersed away from the direct vicinity of the parent. This has more recently been referred to as the escape hypothesis, in which distance- and or density-dependent mortality patterns maintain high tree species diversity (Howe and Smallwood 1982). Recent work linking seed dispersal to tropical forest diversity and structure has focused on the failure of a species to arrive and establish in all sites favorable for its growth and survival (Hamilton 1999, Hubbell et al. 1999, Harms et al. 2000, Nathan and Muller-Landau 2000, Schupp et al. 2002, Terborgh et al. 2002, Volkov et al. 2005). A recent overview of recruitment limitation and the maintenance of tropical forest diversity suggests that limitation results from three broad classes of mechanisms: source limitation, establishment limitation, and dissemination or dispersal limitation (Jordano and Godoy 2002, Schupp et al. 2002). Schupp et al. (2002) discuss three processes related to dispersal limitation: quantitatively-restricted, distance-restricted, and spatially-contagious seed dispersal. These processes are all influenced by frugivore foraging and are thought to limit the number, dispersal distance, and spatial distribution of seeds over the landscape (Jordano and Godoy 2002). Here, we focus on the quantitatively-restricted component of dispersal limitation, to examine the effectiveness of dispersers in removing seeds from a Neotropical tree, *Virola flexuosa*.

A seed disperser's effectiveness, measured as its contribution to plant fitness, has both qualitative and quantitative components (Schupp 1993) (see Table 1). For example, the distance a seed is moved from the plant is a qualitative component and the average number of seeds removed per visit is a quantitative component. Quantitatively-restricted seed dispersal occurs when, independent of seed production, the quantity of seeds dispersed away from the parent tree is limited by disperser activity or behaviour (Schupp et al. 2002). Thus, if hunting or other anthropogenic activities alter disperser abundances or behaviour, the chance of seeds being dispersed may decline due to lowered numbers of dispersers or because altered habitats are no longer conducive to animal movement. In this case, one would expect seeds to be clumped under parent trees.

Hunting of frugivores, in an otherwise structurally undisturbed forest, is expected to lower abundances of large-bodied frugivores (e.g. primates, Atelidae; toucans, Ramphastidae; and guans, Cracidae), and result in fewer visits to fruiting trees by large seed dispersers, allowing us to test the null hypothesis that equal numbers of seeds are dispersed from parent trees at a hunted site and a non-hunted site. Toucans (especially the larger *Ramphastos* species), guans, and primates are hunted for food by local people throughout Amazonia. For example, in the Ecuadorian Amazon, ateline primates, toucans, and guans have been hunted extensively at a site in the Yasuní Biosphere Reserve, where as much as 34% and 39% of biomass of hunted mammals and birds, respectively, were removed in an 11-month period (Mena et al. 2000). Recent work in the Yasuní Biosphere Reserve found substantial hunting pressures at three permanent Huaorani settlements (Franzen 2005), where up to 79% of species harvested were those previously considered vulnerable to hunting (including Cracidae and Atelidae) (Peres 1994, Alvard et al. 1997, Bodmer et al. 1997, Mena et al. 2000). Based on potential hunting impacts on primate and bird abundances (with especially high pressure on fruit-eating vertebrates), we expect that relatively fewer large frugivores will visit fruiting trees at a hunted site than non-hunted site due to lower numbers of primates, toucans, and guans. Consequently, relatively fewer seeds will be removed from trees at a hunted site.

In an effort to understand how different seed dispersers affect dispersal, we studied the assemblage of frugivores that feed on *Virola flexuosa* (Myristicaceae) over a two-year period at two sites differing in hunting pressure in Amazonia Ecuador. First, we examine the role of toucans, primates, and other avian frugivores as seed dispersers. Then, we combined observations at fruiting trees with data collected in fruit traps to test the hypothesis that the quantity of seeds removed from fruiting trees by large frugivores differs between sites demonstrating a potential for quantitatively-restricted seed dispersal. Research objectives specifically were to: 1) describe the frugivore assemblage of *V. flexuosa*, a typically bird-dispersed tropical tree; 2) examine the effectiveness of different frugivores in dispersing the seeds of *V. flexuosa*; and 3) test the hypothesis that removal of *V. flexuosa* seeds, influenced by differential visits of large-bodied frugivores, will differ between hunted and non-hunted sites. We predict that fewer seeds will be removed from fruiting trees at the hunted site because of reduced numbers of visits by large-bodied frugivores, which are expected to be the more effective seed dispersers of *Virola* species (Howe 1981, Howe and Vande Kerckhove 1981, Russo 2003). Reduction in effective dispersers will result in relatively more seeds falling below the crown and, thus, quantitatively-restricted seed dispersal becomes relatively more important as a mechanism contributing to dispersal limitation at our hunted site.

Methods

Study Sites – Research was conducted at the Tiputini Biodiversity Station (TBS; ~0^o 38' S, 76^o 09' W) and Yasuní Research Station (YRS; ~0^o 40' S, 76^o 24' W) in the Orellana Province, Ecuador. The two sites are located within the Yasuní Biosphere Reserve (~1.5 million ha), which forms the largest protected area in Ecuador. The area, part of western Amazonia, is one of the most biologically diverse areas in the world (Pitman et al. 2001) with approximately 1104 tree species in a 50-ha plot (Valencia et al. 2004). The vegetation is described as evergreen lowland rain forest (Sierra 1999) and habitat types include terra firme forest, várzea (frequently inundated floodplain forest), and small patches of palm swamp forest (Nabe-Nielsen 2001). Station areas receive approximately 2800 mm of rainfall per year with mean monthly temperatures 25-27°C (Valencia et al. 2004; Queenborough 2005; J. Guerra, unpublished data); elevation ranges from approximately 200-245 m above sea level. Tiputini Biodiversity Station and YRS are located 27 km apart and both are adjacent to the Tiputini River, a tributary of the Napo River. Access to TBS is by river only and, consequently, it is relatively isolated from human activities and experiences no hunting by indigenous Huaorani. In contrast, YRS is located along an oil access road and hunting by local Huaorani occurs near the research station and inside the 50-ha study plot (Franzen 2005, Queenborough 2005). At YRS, hunting pressures steadily increased between 1994 and 1998 causing several terrestrial avian frugivores to become extremely rare at that site (English 1998). In the last four years, hunting activities have increased more dramatically (K.M. Holbrook, personal observation and A. DiFiore, personal communication) due to a 2001 establishment of a Huaorani community (Tiimpuca) less than three km from the research station and study area. At Tiimpuca, Franzen (2005, 2006) estimated yearly harvests by local Huaorani to be 276-360 primates (including *Lagothrix lagothricha*, *Ateles belzebuth*, and *Alouatta seniculus*) and 264-345 birds (including *Pipile pipile*, *Penelope jacquacu*, *Ramphastos tucanus*, and *R. vitellinus*). These estimates are based on data collected in 2002 and it is likely the number of animals (if not already over-hunted) harvested in recent years has increased. More recently, a study of primate densities at both TBS and YRS found ateline primate densities at YRS nearly half those at TBS (A. Derby, personal communication). Further, there has been continued colonization along the roads leading into the park and increased boat traffic along the Tiputini River within the last six years by both Huaorani and Quichua (K.M. Holbrook, personal observation).

Research at TBS occurred primarily within an 84-ha plot (see Holbrook and Loiselle 2007 and Loiselle et al. 2007). At YRS, research was conducted in a 50-ha Forest Dynamics Plot (see Valencia et al. 2004) and within a 34-ha strip surrounding the plot. Both sites are dominated by terra firme forest and do not differ significantly in tree species diversity and composition (based on analysis of several 1-ha plot surveys by Pitman et al. [2001]). A total of 18 female *V. flexuosa* adults were found and mapped within the two study areas (TBS, 11; YRS, 7); however, only nine trees at TBS and four at YRS produced fruit during the study period. For the purposes of examining dispersal effectiveness and quantitatively-restricted seed dispersal, each individual tree is considered the experimental unit, resulting in nine and four replicates at TBS and YRS, respectively.

Study Species – *Virola flexuosa* A.C.Sm. (Myristicaceae) is a widespread dioecious species that occurs throughout South America (Lambright 1981). Most individuals at TBS and YRS produce flowers from July to September and fruit from October to March (Queenborough 2005). *Virola flexuosa* is a large-seeded species and, thus, expected to be disproportionately affected by the depletion of large-bodied frugivores. The fruit is distinctive, with a bright red aril enclosed by a woody capsule, which dehisces during early and mid-morning hours (K.M. Holbrook, personal observation). Pollinators are likely beetles (Curculionidae, Nitidulidae, and Staphylinidae), which are known to pollinate paleotropical species (Armstrong and Irvine 1989). Dispersers of Myristicaceae are suggested primarily to be ateline primates (Atelidae), toucans (Ramphastidae), guans (Cracidae), and motmots (Momotidae) (Howe 1983, van Roosmalen et al. 1996, Russo 2003, Queenborough 2005).

Foraging behaviour and assemblage at Virola – We conducted observations at fruiting trees during two field seasons (2002-2004) to determine the relative role toucans play in dispersing the fruit of *V. flexuosa*. Nine focal female trees at TBS and four focal trees at YRS were observed from 0600-1000 hrs with a minimum of eight replications per tree. Although the majority of frugivore activity was concentrated in the early morning hours, focal trees were also observed for visiting frugivores throughout the fruiting season between 1000-1800 hrs when seeds from traps were collected. All visiting frugivores were identified and fruit handling behaviour, fruit removal rates, and amount of time spent foraging were quantified. For each individual that was visible in the tree crown, we recorded the number of seeds eaten, regurgitated, knocked down, or carried away. Fruit removal is defined as the number of seeds swallowed and taken away. Seeds carried away in the bill were treated as seeds removed. Time spent foraging is the total number of minutes (from arrival to departure) an individual spends in the fruiting tree and includes foraging, preening, and other behaviours. Important variables in terms of dispersal are the number of visits, time spent foraging, fruit handling, and total number of fruits eaten and removed by frugivores per visit.

For statistical analyses, visiting frugivores were initially put into three groups: toucans, primates, and other birds (including families Cracidae, Momotidae, and Turdidae). These three groups represented 94.5% of total visitors. Species outside these three groups individually represented < 1% of foraging visits and were not included in statistical analyses. Further statistical tests were conducted among toucan genera (*Ramphastos*, *Pteroglossus*, and *Selenidera*). Differences in amount of time spent foraging by individually observed frugivores (time spent foraging as the dependent variable and species as treatment) were compared with one-way ANOVA tests. Time spent foraging was log-transformed to achieve conditions of normality. We used one-way ANOVA to test fruit handling among foraging species (seeds consumed or seeds dropped as the dependent variables and species as treatment). Sample sizes reflect only instances when the animal was visible and foraging behaviour could be quantified; thus, sample sizes are smaller than those in calculations of time spent foraging and total number of visits. The data for fruit handling analyses generally did not meet assumptions of normality and homogeneity of variance required for ANOVA, and standard transformations did not result in normality or homogeneity. Therefore, relationships between treatments (disperser groups/species) and fruit handling were tested using non-parametric tests (Kruskal Wallis). If the latter test was significant, pairwise comparisons of groups were further tested using Mann-Whitney tests. In all cases, the outcomes of the non-parametric results agreed with the outcomes of parametric ANOVA, and only parametric results are presented here. Treatment means were compared a posteriori using a Tukey post hoc test. All analyses were performed using SPSS 11.0 (SPSS 2001). Values reported are mean ± SD unless otherwise noted.

Crop size and seed removal – Seed traps (1 m ^{2}) made of PVC tubing and mosquito netting were placed underneath the canopy of each focal tree to allow estimates of crop size and fruit removal from the canopy. We positioned four to six traps, depending on tree crown size, that covered approximately 5% of the area below the crown. Traps were placed at randomly selected points between the tree bole and the edge of the crown along each of four transects radiating out at 90° intervals (i.e. 0°, 90°, 180°, and 270°). *Virola* fruits consist of a capsule and one arillate seed, with the arillate seed the unit of dispersal (Howe and Vande Kerckhove 1981). Because capsules are not ingested, they provide an adequate estimation of fruit production and seed removal. Seeds handled by frugivores (i.e. consumed and defecated or regurgitated intact) can be identified by the lack of an aril. Fallen seeds (i.e. not handled) that still maintained their aril were not considered the result of a dispersal event. Seed traps were checked every 7 to 10 days. To estimate crop size, we counted the total number of capsules collected in seed traps and divided by the proportion of the canopy area sampled by traps. To estimate the number and proportion of seeds removed from the tree we first determined the proportion of seeds removed from seed trap data as: (the total number of capsules – number of seeds with arils)/total number of capsules; we then multiplied this by the estimated crop size to provide an estimate of absolute number of seeds removed from a tree. This result represents the absolute number of capsules from which a seed was removed. To determine the removal efficiency for each species, the total number of visits recorded for that species was multiplied by the average number of fruits consumed per visit, resulting in the total number of seeds removed (reported relative to other dispersers). For absolute removal by any given disperser, removal efficiency is multiplied by the mean proportion of seeds removed, based on seed traps.

Site comparisons – To determine whether differences in traits of *V. flexuosa* exist between TBS and YRS, we compared crop size, diameter at breast height (dbh), and crown area with two-tailed *t*-tests. A non-significant result will permit us to test whether differences in frugivore visitation between sites are due to extrinsic factors (e.g. differences in frugivore abundance). To determine whether frugivores respond to crop size in a similar way in both sites, we used a linear regression to test the relationship between number of visitors and number of species, and crop size.

To test the hypothesis that seed dispersal is restricted quantitatively at YRS we examined the differences in seed removal based on fruit and seed capture in traps as well as whether there were any differences in the number of frugivores visiting fruiting trees at each site. We used chi-square tests to determine differences between sites in number of visits per 100 h for toucans, primates, and other birds. We also tested site differences in handling behaviour (swallowing versus dropping seeds) between dispersers and site differences in visitation rates between hunted and non-hunted species (see Franzen 2005). We tested for between-site differences in the total number of visitors and species observed (based on direct observation at trees) and seed removal (seed trap studies) using Mann-Whitney tests; in these tests individual trees at each site were the experimental unit or replicate.

Results

Virola flexuosa *plant traits* –There were no significant differences in crop size, crown area, or dbh between trees at TBS and YRS (Table 2). Seed length was 15.04 ± 0.81 (n = 116), seed width 11.77 ± 1.08 (n = 116), seed mass 1.07 ± 0.16 (n = 99), aril mass 0.58 ± 0.18 (n = 72), and aril:seed ratio 0.58 ± 0.12 (n = 72); seed statistics are from seeds collected at TBS only, therefore comparisons between sites are not possible. Diameter at breast height was positively correlated with crop size (Pearson $r = 0.699$, $P = 0.016$, n = 13) and crown area (Pearson $r = 0.688$, $P = 0.005$, $n = 13$).

Foraging assemblage, visit frequency, and visit length – Seventeen bird and three primate species were recorded foraging in *V. flexuosa* trees (see Table 3 for complete list). A total of 529 visits were recorded during 401 hours of observations (both sites combined). Toucans and primates were the two most important groups of visitors (Fig. 1) and comprised approximately 85% of all visits. The remaining 15% of visits were birds from the families Cracidae, Momotidae, Turdidae, Cotingidae, Capitonidae, Cuculidae, Trogonidae, and Tyrannidae. Six species of toucans comprised 74% of visits with *R. tucanus* (63.8 vists/100 h) and *P. pluricinctus* (27.4 visits/100 h) most frequent (Fig. 1). Among primates, *A. belzebuth* and *L. lagothricha* were the most common, accounting for 8.5 and 6.3 visits/100 hours, respectively. The number of visiting frugivores and, to a lesser extent, number of species recorded during tree observations increased with crop size (Fig. 2).

Visit length ranged from a low of 1.3 ± 0.5 min for *Querula purpurata* to a high of 27.8 ± 26.7 min for *Penelope jacquacu* (Fig. 3). Visit lengths were significantly longer for primates (15.9 ± 14.0 min) than for toucans (6.3 ± 7.7 min) and all other avian visitors (6.4 ± 10.0 min) ($F_{2,522} = 21.25$, $P < 0.0001$; Tukey tests, $P < 0.0001$). Visit lengths also differed significantly among toucan genera ($F_{2,397} = 15.05$, $P < 0.001$; Tukey tests, $P < 0.001$), with *Pteroglossus* having shorter visit times than both *Ramphastos* and *Selenidera*.

Fruit handling and seed removal – Species differed in how they handled *Virola* fruits (Table 3). Smaller visitors (e.g. thrushes, barbets, and cotingas) tended to peck at the fruit to obtain the aril rather than swallow the seed. Larger species (e.g. toucans, guans, and primates) were legitimate dispersers in that the majority of seeds (60-85%) were consumed and carried away from fruiting trees (Table 3). Primates dropped more seeds per visit (2.1 ± 3.8) than did toucans (0.3 ± 0.5) or other avian dispersers (0.7 ± 0.8) ($F_{2,190} = 16.48$, $P < 0.0001$; Tukey tests, $P < 0.0001$). Among toucans, *Ramphastos* and *Pteroglossus* (excluding *P. inscriptus* because of small sample size), showed no differences in number of seeds dropped, but *Selenidera* dropped more seeds than either of the larger *Ramphastos* and *Pteroglossus* ($F_{2,145} = 11.43$, $P < 0.0001$; Tukey tests, $P = 0.002$). Primates consumed more seeds per visit than did toucans or other avian visitors (primates, 6.4 ± 7.4; toucans, 1.5 ± 1.8; other birds, 0.9 ± 0.9; $F_{2,190} = 28.01$, $P < 0.0001$; Tukey tests, $P < 0.0001$); there were no differences among toucan genera ($F_{2,145} = 0.871$, $P = 0.421$) (Tables 3 and 4). Four species (*R. tucanus*, 32%; *A. belzebuth*, 16%; *L.*

lagothricha, 14%; and *P. pluricinctus*, 12%) were responsible for removing an estimated 74% of seeds averaged over all sampled trees (Table 4).

Site comparisons – Seed trap data across all trees indicated that 81.6% of seeds were removed from the parent crown. A proportionately larger number of seeds were removed from fruiting trees at TBS (89.4%; non-hunted site) than at YRS (66.8%; hunted site) (Mann-Whitney test; $U = 3.0$, $P = 0.02$) (Fig. 4). In addition, there were more frugivore visits at TBS than YRS. Comparing site and disperser group (toucans, primates, and other birds) showed a greater number of visits at the non-hunted site ($X^2 = 6.533$, $P = 0.038$; Fig. 5a). We found that species more likely to swallow seeds (> 50% of seeds observed swallowed, Table 3), and, thus more likely to disperse seeds away from parent trees made significantly more visits to trees at TBS than did species that drop seeds ($X^2 = 6.166$, $P = 0.013$; Fig. 5b). Moreover, species more likely to be hunted had higher visitation rates at TBS than YRS ($X^2 = 6.098$, $P = 0.014$; Fig. 5b). The mean numbers of individuals (TBS, 43.7 ± 46.7 and YRS, 34.0 ± 40.8) and species (TBS, 7 ± 2.9 and YRS, 5 ± 3.5) observed visiting *V. flexuosa* trees were higher at TBS than at YRS, but were not statistically different.

Discussion

Virola flexuosa is not unique among tropical trees in attracting vertebrates to consume and disperse its seeds. This species relies entirely on vertebrate seed dispersers to move seeds beyond the canopy and is it likely that dispersal away is necessary for seed and seedling survival (Howe et al. 1985). Howe (1984) stated that “animal-mediated seed dispersal is certain to be critical for the demographic recruitment of many or most tropical forest species”. Several other empirical studies have also shown greater mortality near to than far from conspecific adults (Augspurger 1983, Clark and Clark 1984, Howe et al. 1985, Schupp 1988, Schupp and Frost 1989, Condit et al. 1992). Loss of seed dispersers may lead to dispersal limitation simply because fewer animals are present to consume and disperse seeds. For example, sites where many vertebrates have been removed by hunting, the ‘empty’ forests of Redford (1992) may no longer contain one of the most important players in promoting healthy tropical ecosystems, the seed dispersers. Examining the dispersers of the Neotropical tree *V. flexuosa*, we found differences in the frugivore assemblage and the overall number of seeds dispersed from individual trees between two structurally similar forest sites. We suggest a quantitative effect on dispersal of seeds at the Yasuní Research Station, potentially resulting from a reduction in seed dispersers.

Dispersal effectiveness – The disperser assemblage at *V. flexuosa*, as for other *Virola* species, is primarily made up of larger frugivores (primates, toucans, and guans) that are more likely to swallow seeds unharmed and, thus, serve as legitimate dispersers (species more likely to consume fruits whole and remove the seeds from the canopy undamaged; Levey 1986, 1987). The overall proportion of *V. flexuosa* seeds dropped in all observations combined was 23%. From a plant’s perspective, seeds that are swallowed are likely to be deposited farther from the parent tree than seeds that are dropped during fruit handling (Levey 1987). Levey (1986, 1987) determined that, in general, toucans, cotingas, barbets, trogons, thrushes, and flycatchers are gulpers, whereas tanagers, honeycreepers, and finches are mashers, and further suggested that arils may be an adaptation to reduce seed dropping by mashers (Levey 1987). Our 'droppers' typically swallow fruits and seeds whole; however, the larger *V. flexuosa* seed size almost certainly makes handling more difficult (and ingestion more 'costly' due to ballast; Levey 1987) and, inevitably, they drop many of the seeds. We found that among toucans, the smallest species had a greater tendency to drop seeds (*S. reinwardtii*, 54% and *P. inscriptus*, 50%) than did larger species (15-23%). Among primates, spider (*A. belzebuth*) and howler (*A. seniculus*) monkeys were the better dispersers, swallowing more than 80% of seeds. Woolly monkeys (*L. lagothricha*), on the other hand, were observed to drop or more frequently to remove or strip the aril and throw the seeds to the ground 39% of the time.

Quantitative effectiveness of seed dispersers is measured by the product of the number of visits and the seeds removed per visit. In our system, toucans were the most important dispersers of *V. flexuosa*, largely because they were more frequent visitors relative to other dispersers. Howe and Vande Kerckove (1981) and Russo (2003) found that toucans made 48-49% and 26% of visits, respectively, whereas toucans comprised nearly 75% of visits in our study. Although primates consume more seeds on any given visit, the number of visits by toucans far outweighs this effect. Contribution to total dispersal of *V. flexuosa* was estimated from the total number of visits, mean number of fruits removed per visit, and the overall probability of seeds dispersed away from trees (less seed fall underneath the canopy). In this study system, with 20 visiting frugivores, an average of 82% of the fruit crop is removed. Six species of toucans disperse 57% of seeds, while three species of primates were responsible for 33% of seed removal, and other smaller avian frugivores dispersed the remaining 10%. These dispersal proportions are similar to studies in Panama where an estimated 51-65% of seeds were removed by toucans (Howe 1981, Howe and Vande Kerckhove 1981, Ratiarison 2003). However, in Peru, Russo (2003) found that toucans removed only 8% of seeds; whereas 83% of seeds were removed by spider monkeys.

Qualitative effectiveness includes the quality of seed treatment and seed deposition (Table 1). Although we do not directly address these components in this paper, the quantitatively more important dispersers (toucans and primates) of *V. flexuosa* are probably also providing relatively high quality dispersal. Toucans swallow seeds whole and either regurgitate or pass them intact (McKey 1975, Snow 1981, Galetti 2000, K.M. Holbrook, personal observation). Ateline primates (especially *Ateles* spp.) swallow *Virola* seeds whole and defecate them intact (Stevenson 2000, Russo et al. 2005, Suarez 2006). Woolly monkeys are less efficient seed dispersers, occasionally spitting out or throwing down seeds (Dew 2005; K.M. Holbrook, personal observation). Effects on germination by *Ramphastos* and *Pteroglossus* toucans are unknown, but it is likely they have positive or neutral effects, given research conducted with hornbills (Whitney et al. 1998, Cordeiro et al. 2004) and other birds, including toucanets (*Selenidera*) (Wenny 2000). Ateline primates have been shown to have positive or neutral effects on germination (Chapman 1989, Stevenson 2000, Righini et al. 2004). Quality of seed deposition is more difficult to measure given the difficulty of following dispersers and measuring subsequent seed and seedling survival. Several studies have shown that toucans and ateline primates consistently remove seeds from tree canopies (Howe 1981, Howe and Vande Kerckhove 1981, Stevenson 2000, Russo and Augspurger 2004, Holbrook and Loiselle 2007). Primates are more likely to deposit seeds in clumped patterns however, which may result in increased predation and competition at germination and recruitment stages (Howe 1989, Russo and Augspurger 2004); thus, seeds dispersed by toucans may have a greater chance of survival.

Dispersal limitation – Quantitatively-restricted seed dispersal, a process that contributes to dispersal limitation, occurs when, independent of seed production, the quantity of seeds dispersed away from fruiting trees is limited by disperser activity and/or behaviour (Schupp et al. 2002). This model predicts that many seeds fall or are dropped beneath their parents undispersed. We found a 25% difference in the number of seeds removed from fruiting trees (67 vs. 89%) between hunted and non-hunted sites based on seed trap studies. This percentage is closely matched with a significant reduction (24%) in the total number of visiting frugivores at TBS versus YRS, based on foraging observations conducted at fruiting trees. We also show that species harvested by local hunters are 30% more common at fruiting trees at our non-hunted site. These differences in frugivore visits were not due to differences in seed production (i.e. crop size does not differ between sites). The numbers of visiting frugivores (both individuals and species) at the individual tree level, although higher at TBS than at YRS, were not statistically different. Variation in the proportion of seeds removed at YRS was higher than at TBS, and one tree in particular, had very high visitation compared to the other fruiting trees at YRS.

This tree was the farthest from the road and potentially attracted more animals because of its relative isolation from human activities concentrated near the road.

In a review on the consequences of hunting in tropical forests, Wright (2003) suggested that hunting may alter plant species composition and diversity if the harvest of vertebrates disrupts ecological mechanisms or differentially affects mutualists (e.g. seed dispersers). The reduction in frugivore abundances may alter seed dispersal, seed predation, and seedling recruitment for tropical plants (Wright et al. 2000). A study which directly measured the impacts of hunting on seed dispersal in Panama found that ecologically-effective seed dispersal distances were greatest for protected sites and shortest for heavily poached sites and sites with substantial habitat fragmentation (Wright and Duber 2001). Widespread loss of dispersers may eventually result in local extinction of tree species that require dispersal by large-bodied frugivores (Corlett and Turner 1997, Hamann and Curio 1999, Cardoso da Silva and Tabarelli 2000). For example, in northeast Brazil, where large-bodied frugivores including cracids and toucans are threatened by hunting, Cardoso da Silva and Tabarelli (2000) found that at least 31.6% of vertebrate-dispersed trees depend on large-gaped (> 15 mm) birds for seed dispersal. Furthermore, they suggested that where key vertebrate dispersers have already been extirpated, seed removal at several tree species is very limited. As our research spans only two years, we are unable to report whether our findings of reduced seed dispersal at YRS are indicative of a continuing trend. Also, we recognize that our results are specific only to a single hunted and non-hunted site due to logistic constraints that precluded site replication. Nevertheless, our results are consistent with other studies that show a significantly negative impact of seed removal under conditions of hunting (Redford 1992, Hamann and Curio 1999, Cardoso da Silva and Tabarelli 2000, Wright 2003).

The assumption that hunting of large frugivores will lead to reduced abundances of effective seed dispersers relies on no or inadequate substitution in ecological roles by smaller or non-hunted frugivores. In Central Africa, Gautier-Hion et al. (1985) and Gautier-Hion (1990) suggest that hornbills (Bucerotidae) and primates (Cercopithecinae), because they may disperse similar suites of plant species, potentially replace each other in dispersal services. Poulsen et al. (2002), however, tested for compensation between hornbills and primates and found that, because dietary overlap between the two groups was small, it was highly probable that neither group is able to replace the seed dispersal services provided by the other. Another study suggested that the potential for compensation varied with hunting pressure and the range of species selected by hunters (Wright 2003); in more heavily hunted forests, where all candidates for dispersal are depleted, the likelihood of compensation becomes much reduced. Moreover, closely related species are expected to share traits that influence sensitivity to hunting and, thus, the potential for compensatory change may be limited because hunters tend to have similar impacts on closely related species (Wright 2003). Our results suggest some degree of compensation occurs in terms of the number of visits by smaller frugivores at *V. flexuosa*; visitation rates of non-hunted species were higher by 53% at the hunted site (Fig. 5b). Unfortunately these species overall make very few visits and are not highly effective dispersers, dropping the majority of seeds below tree canopies. In addition, as *V. flexuosa* is a large-seeded species and because all of the larger frugivores are listed as hunted, we do not expect any effective compensation to occur, at least in the short-term. A consequence of quantitatively-restricted seed dispersal, which may be occurring at YRS, is that seeds reach fewer recruitment sites than expected based on population-level seed production (Schupp et al. 2002). Understanding the degree to which dispersal limitation is occurring in tropical forests becomes increasingly important given the role of vertebrate seed dispersal and the acceleration of hunting activities throughout much of the Amazon.

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Figure Legends

Figure 1. Number of observed visits to *V. flexuosa* at Tiputini Biodiversity Station and Yasuní Research Station, Ecuador. Data are based on a total of 529 visits recorded in 401 h of direct observation at 13 fruiting trees.

Figure 2. Relationship between visitation and crop size of *V. flexuosa* (Tiputini trees, black circles; Yasuní trees, grey circles). (a) The number of observed visitors ($R^2 = 0.461$, $P = 0.011$) and (b) number of species, ($R^2 = 0.427$, $P = 0.015$).

Figure 3. Mean (SD) visit length of frugivores to *V. flexuosa* at Tiputini Biodiversity Station and Yasuní Research Station, Ecuador. Data are based on a total of 529 visits recorded in 401 h of observations at 13 fruiting trees.

Figure 4. The proportion of seeds removed (mean + 1 SE) from the canopy of *Virola flexuosa* at Tiputini and Yasuní based on seed trap data.

Figure 5. Visitation frequency by frugivores at *Virola flexuosa* at non-hunted (Tiputini, black bars) and hunted sites (Yasuní, white bars). (a) Differences between sites among observed frugivore groups. (b) Site differences in visitors from: (1) species more likely to swallow fruits (*R. tucanus, R. vitellinus, P. azara, P. pluricinctus*, primates, guans, and *B. martii*) than to drop fruits (*P. inscriptus, S. reinwardtii, T. lawrencii, C. auratus, C. cayana, L. vociferans, Q. purpurata, P. cayana, T. cayana,* and *Trogon* spp.) and (2)

species hunted by local Huaorani (toucans, primates, guans, *B. martii*, *C. auratus*, and *Trogon* spp.; Franzen 2005) and species not harvested (*T. lawrencii, C. cayana, L. vociferans, Q. purpurata*, *P. cayana,* and *T. cayana*).

Table 1. A hierarchical outline of the major components of disperser effectiveness (from Schupp 1993).

- I. Quantity of seed dispersal
  - A. Number of visits
    - 1. abundance of disperser
    - 2. diet
    - 3. reliability of visitation
  - B. Number of seeds dispersed per visit
    - 1. number of seeds handled per visit
    - 2. probability of dispersing a handled seed
- II. Quality of seed dispersal
  - A. Quality of treatment
    - 1. destroy or pass seeds intact
    - 2. alter percent or rate of germination
  - B. Quality of deposition
    - 1. movement patterns
      - a. habitat and microsite selection
      - b. rate and directionality of movement
    - 2. deposition patterns
      - a. rate and pattern of deposition
      - b. seed (diet) mixing

Table 2. Summary of *V. flexuosa* traits for all study trees including site comparisons. Mean ± SE are given for all data with sample size in parentheses.

	TBS/YRS (13)	TBS (9)	YRS (4)	Independent *t* test†
DBH (cm)	56.5 ± 2.3	55.6 ± 3.0	58.4 ± 3.9	-0.546 ^{NS}
Crop size (No. fruits)	14,408 ± 4009	15,671 ± 5101	11,881 ± 7018	0.437^{NS}
Crown area (m^2)	155.8 ± 22.0	130.6 ± 18.4	206.1 ± 51.2	-1.726^{NS}

TBS, Tiputini Biodiversity Station; YRS, Yasuní Research Station; DBH, diameter at breast height.
†Results of independent *t*-test (df = 11) for between-site differences; ^{NS} not significant.

Table 3. Fate of fruits and seeds of *V. flexuosa* handled by frugivores comprising > 1% of foraging visits†; *N* = number of observations where direct foraging data were collected. Totals for each group are given in bold.

Species	Body mass‡ (g)	*N*	Swallowed§ (%)	Dropped* (%)
I. Ramphastidae (toucans)				
Pteroglossus azara	138	5	82	18
P. inscriptus	126	2	50	50
P. pluricinctus	225	8	80	20
Ramphastos tucanus	662	92	85	15
Ramphastos vitellinus	364	23	77	23
Selenidera reinwardtii	140	12	46	54
Toucans		**142**	**81**	**19**
II. Atelidae (primates)				
Alouatta seniculus	3,600-11,100	3	82	18
Ateles belzebuth	5,900-10,400	14	80	20
Lagothrix lagothricha	3,600-10,200	5	61	39
Primates		**22**	**75**	**25**
III. Other birds				
Cracidae (guans)				
Penelope jacquacu	1280	4	80	20
Pipile pipile	1200	4	60	40
Momotidae (motmots)				
Baryphthengus martii	156	9	60	40
Turdidae (thrushes)				
Turdus lawrencii	65	6	13	87
Other birds		**23**	**55**	**45**
COMBINED (I-III)		**187**	**77**	**23**

† Frugivores that individually represent < 1 % of visits are *Capito auratus* (Capitonidae), *Cotinga cayana*, *Lipaugus vociferans*, *Querula purpurata* (Cotingidae), *Piaya cayana* (Cuculidae), *Trogon* spp. (Trogonidae), and *Tityra cayana* (Tyrannidae); all were observed to drop > 50% of seeds and with body mass < 100g. ‡ Body masses of birds are from J. G. Blake (unpublished data), K.M. Holbrook (unpublished data), and Terborgh et al. (1990); primate body masses are from Emmons (1990). § Includes seeds swallowed and seeds carried away. * Includes seeds dropped or thrown down during handling and seeds where the aril was removed, but did not drop.

Table 4. Visitation and seed removal by dispersers of *Virola flexuosa*. Absolute removal is the estimated percentage of fruits removed from each fruiting tree. Removal efficiency is the percentage of total crop removed relative to other dispersers. Data represent species comprising > 1% of foraging visits†. Totals for each group are given in bold.

Species	% total visits (*N*)	Mean (SD, *N*‡) number seeds consumed per visit	Absolute removal (%)	Removal efficiency (%)
I. Ramphastidae (toucans)				
Pteroglossus azara	4.1 (20)	1.8 (2.0, 5)	3.3	3.9
P. inscriptus	0.3 (2)	0.5 (0.7, 2)	0.1	0.1
P. pluricinctus	16.7 (82)	1.4 (1.2, 8)	10.3	11.9
Ramphastos tucanus	37.6 (185)	1.6 (2.1, 92)	27.6	32.0
Ramphastos vitellinus	10.0 (49)	1.2 (1.3, 23)	5.4	6.2
Selenidera reinwardtii	5.4 (27)	0.8 (0.8, 12)	2.5	2.9
Toucans %	**74.1**		**49.2**	**57.0**
II. Atelidae (primates)				
Alouatta seniculus	0.6 (3)	10.7 (5.0, 3)	3.0	3.4
Ateles belzebuth	5.5 (27)	5.4 (7.5, 14)	13.6	15.7
Lagothrix lagothricha	4.1 (20)	6.6 (8.6, 5)	12.2	14.2
Primates %	**10.2**		**28.8**	**33.3**
III. Other birds				
Cracidae (guans)				
Penelope jacquacu	1.0 (5)	2.0 (0.8, 4)	0.9	1.1
Pipile pipile	3.5 (17)	0.7 (0.5, 4)	1.2	1.4
Momotidae (motmots)				
Baryphthengus martii	4.7 (23)	1.0 (0.9, 9)	1.4	1.6
Turdidae (thrushes)				
Turdus lawrencii	1.0 (5)	0.2 (0.4, 6)	0.1	0.1
Other birds %	**10.2**		**3.6**	**4.2**
COMBINED (I-III)	**94.5**		**81.6** §	**94.5***

† Frugivores that individually represent < 1 % of visits are *Capito auratus* (Capitonidae), *Cotinga cayana*, *Lipaugus vociferans*, *Querula purpurata* (Cotingidae), *Piaya cayana* (Cuculidae), *Trogon* spp. (Trogonidae), and *Tityra cayana* (Tyrannidae). ‡ Sample size is lower than for % of total visits as it reflects only those observations where the individual was visible throughout the entire visit and foraging behaviour was quantified. § Represents proportion of seeds removed over all 13 trees. * Remaining 5.5% is estimated removed by frugivores listed in table footnote.

Figure 1

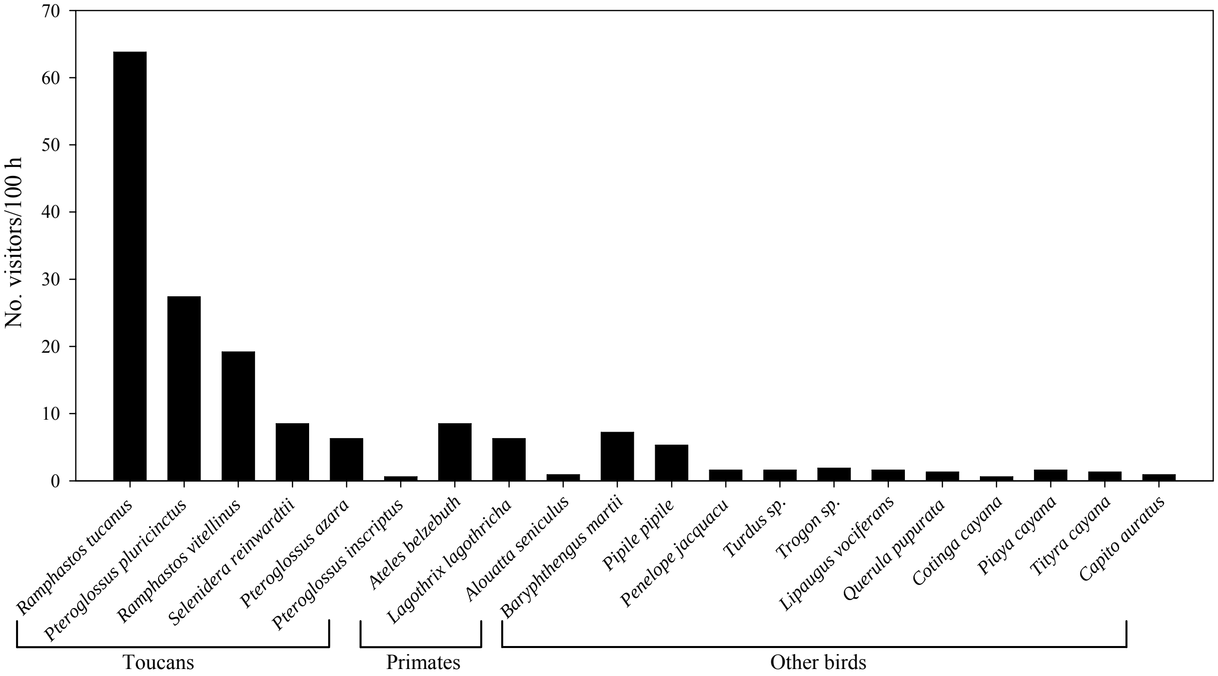

70
60
50
40
30
20
10
0
No. visitors/100 h
Ramphastos tucanus
Pteroglossus pluricinctus
Ramphastos vitellinus
Selenidera reinwardtii
Pteroglossus azara
Pteroglossus inscriptus
Ateles belzebuth
Lagothrix lagothricha
Alouatta seniculus
Baryphthengus martii
Pipile pipile
Penelope jacquacu
Turdus sp.
Trogon sp.
Lipaugus vociferans
Querula pupurata
Cotinga cayana
Piaya cayana
Tityra cayana
Capito auratus
Toucans
Primates
Other birds

Figure 2

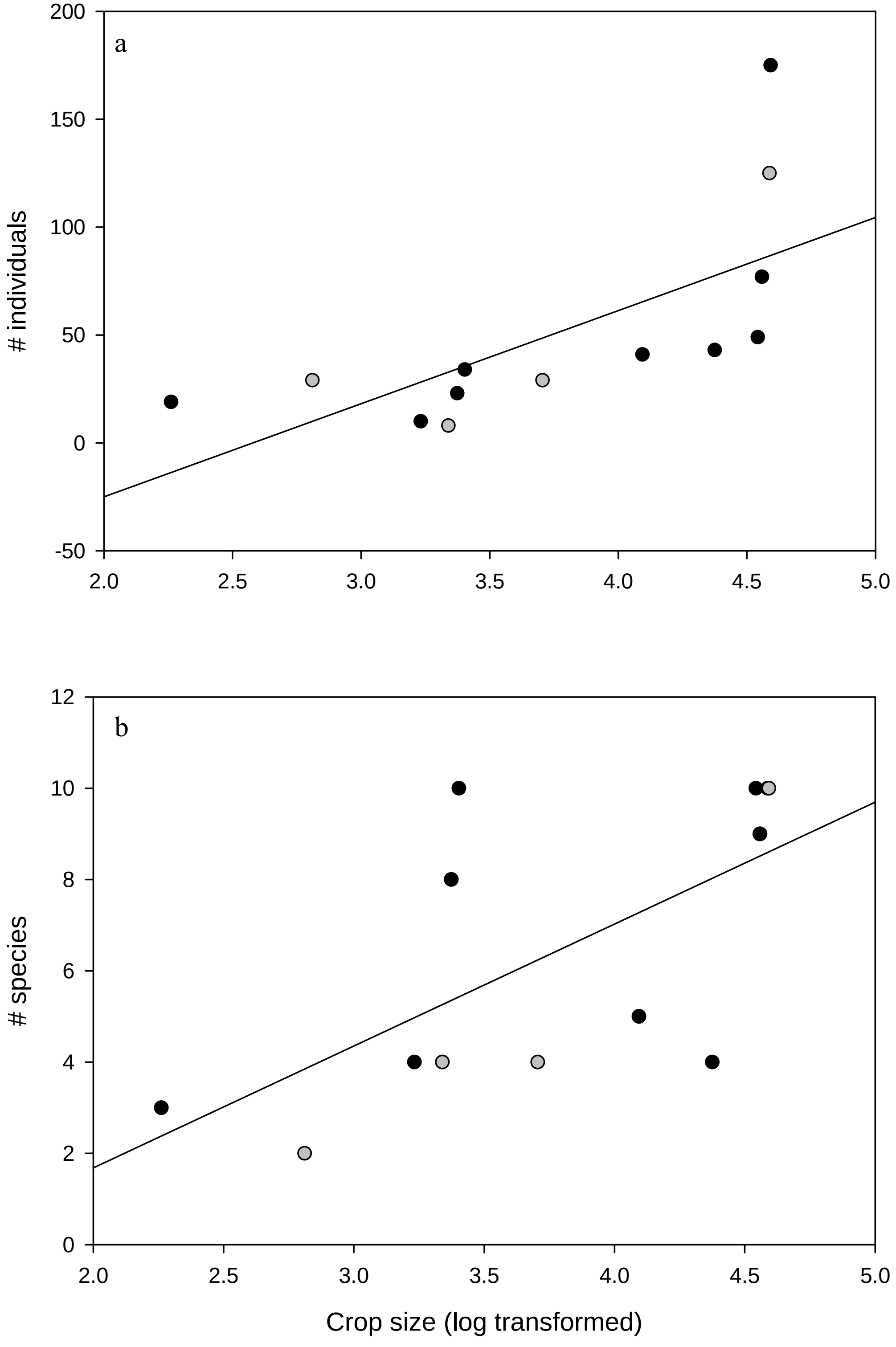

a
individuals
200
150
100
50
0
-50
2.0
2.5
3.0
3.5
4.0
4.5
5.0
b
species
12
10
8
6
4
2
0
Crop size (log transformed)

Figure 3

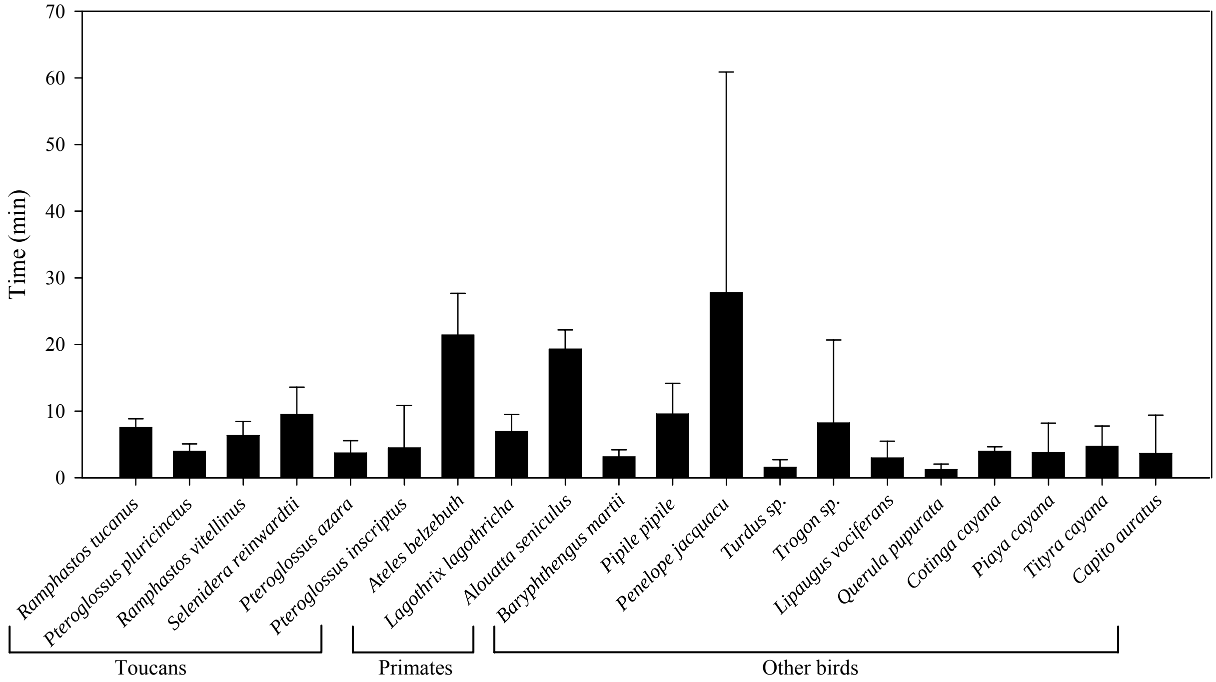

70
60
50
40
30
20
10
0
Time (min)
Ramphastos tucanus
Pteroglossus pluricinctus
Ramphastos vitellinus
Selenidera reinwardtii
Pteroglossus azara
Pteroglossus inscriptus
Ateles belzebuth
Lagothrix lagothricha
Alouatta seniculus
Baryphthengus martii
Pipile pipile
Penelope jacquacu
Turdus sp.
Trogon sp.
Lipaugus vociferans
Querula pupurata
Cotinga cayana
Piaya cayana
Tityra cayana
Capito auratus
Toucans
Primates
Other birds

Figure 4

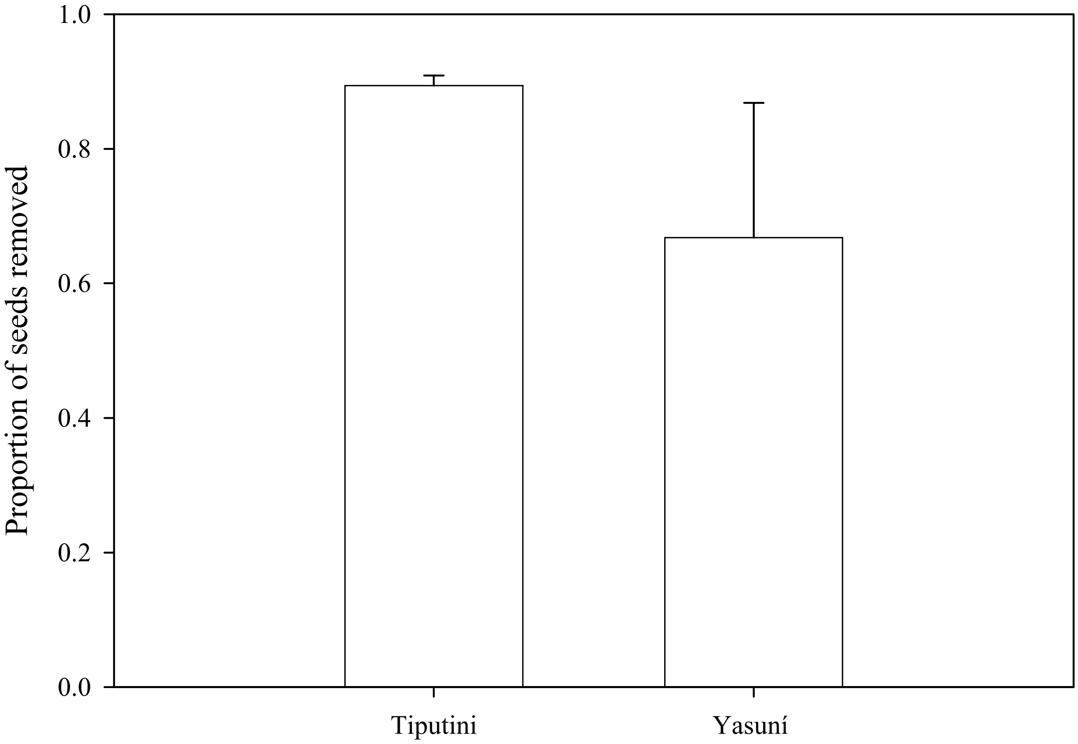
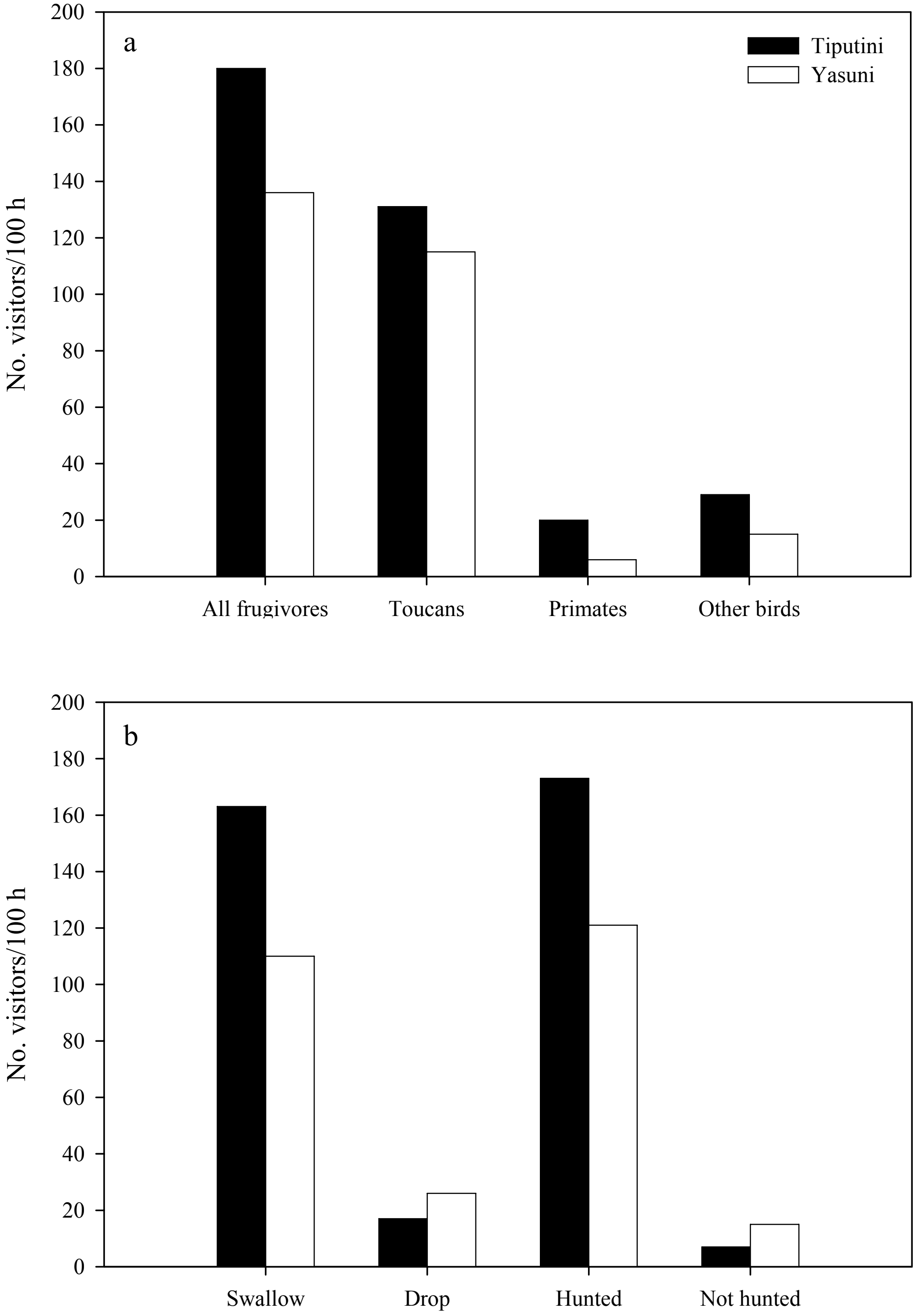

1.0
0.8
0.6
0.4
0.2
0.0
Proportion of seeds removed
Tiputini
Yasuní

Figure 5


a
Tiputini
Yasuni
No. visitors/100 h
0
20
40
60
80
100
120
140
160
180
200
All frugivores
Toucans
Primates
Other birds
b
No. visitors/100 h
0
20
40
60
80
100
120
140
160
180
200
Swallow
Drop
Hunted
Not hunted

Chapter 4

Eight Polymorphic Microsatellite Loci for a Neotropical Nutmeg, *Virola Flexuosa* (Myristicaceae)

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Molecular Ecology Notes: *in press*.

Abstract

Eight highly polymorphic microsatellite loci were developed for *Virola flexuosa* from a $(CA)_n$-enriched genomic library for population and seed dispersal studies in eastern Ecuador. Loci show a high level of variation with the number of alleles ranging from 13 to 27. Observed and expected heterozygosities were from 0.313 to 0.896 and 0.552 to 0.937, respectively. The high levels of polymorphism and exclusionary power of the developed markers will likely prove very useful in direct measurement of seed dispersal.

The majority of tropical trees are dispersed by vertebrates with an estimated 70-90% of species dispersed by animals (Jordano 1992). Interactions between dispersers and plants have been well studied, yet we still know very little about the direct impacts dispersers have upon tropical tree communities. Microsatellite markers allow researchers to examine questions of population structure, as well as link seeds or seedlings with parent plants in seed dispersal studies, thus providing exact dispersal distances and a more precise way to measure seed shadows (Godoy & Jordano 2001; Hardesty *et al.* 2006). We developed eight microsatellite loci from a Neotropical nutmeg, *Virola flexuosa*, for use in seed dispersal studies at the Tiputini Biodiversity Station (TBS) and the Yasuní Research Station (YRS) in Amazonia Ecuador.

Virola flexuosa is a widespread dioecious, canopy tree species throughout lowland forests of South America (Lambright 1981). All *Virola* species are vertebrate-dispersed with several species having served as models for seed dispersal studies (Howe & Kerckhove 1981; Russo 2003). *Virola* and related genera have many anthropogenic uses including medicinal, food and timber. By developing molecular markers for *V. flexuosa,* we hope to encourage further molecular studies in this important genus.

We developed an enriched $(CA)_n$ microsatellite library using protocols modified and optimized by the ICBR Genetic Analysis and Education Core laboratories (Moraga-Amador *et al*. 2002). For the development of the microsatellite library, genomic DNA was extracted from leaf samples of a single individual using a standard cetyltrimethyl ammonium bromide (CTAB) extraction method (Doyle & Doyle 1990). Genomic DNA was restricted with *Sau*3AI enzyme and fragments larger than 400 bp were captured by size fractionation using ChromaSpin 400 + TE columns (Clontech Laboratories). Fractionated DNA was ligated to *Sau*3AI linkers. Excess linkers were removed with the ChromaSpin column, and then PCR amplified using the free linker oligo.

Fragments containing $(CA)_n$ repeats were selected by hybridization to a biotinylated probe [5'-$(CA)_{15}$TATAAGATA-Biotin], captured with Vectrex Avidin D (Vector Laboratories), and PCR amplified with the linker oligo to create a pool of fragments enriched for $(CA)_n$ repeats. To detect success of the enrichment technique, the library was tested via serial dilutions and a chemiluminescent detection system using the above biotinylated probe. The microsatellite-enriched DNA fragments (PCR products from second amplification) were then ligated into a plasmid vector (pCR 2.1 TOPO® vector, Invitrogen) and transformed into *E. coli* (One Shot^{TM} INVαF′ cells, Invitrogen). Colony lifts were screened using the $(CA)_{15}$ probe and a chemiluminescent detection system. Sixty colonies with strong hybridization signals were sequenced on an ABI 377 Prism automated sequencer (Applied Biosystems) at the ICBR DNA Sequencing Core (University of Florida).

Primer pairs complimentary to microsatellite-flanking regions were designed for 29 of the 60 repeat regions using OLIGO (Molecular Biology Insights). For primer testing, DNA was isolated from a subset of leaf tissues collected at TBS and YRS using a DNeasy Plant Extraction Kit (Qiagen). Extractions were further diluted 1:50 with deionized water for use in PCR reactions. The 10 μL-reaction mixtures contained 1.5 μL template DNA, 1.0 μL 10X NH_4 buffer (160 mM $(NH_4)_2$ SO_4, 670 mM Tris-HCL, pH 8.8, and 0.1% Tween-20), 40-100 μM each dNTP, 2.0-5.0 mM $MgCl_2$, 0.5 μM each primer, 0.15 units of *Taq* (Bioline), and 0.5 μL bovine serum albumen (10mg/μL). All PCRs began with 94 °C (4 min), followed by a primer-specific number of cycles at 94 °C (30 sec), primer-specific annealing temperature (30 sec), 72 °C (45 sec), and a final extension at 72 °C (7 min). The number of PCR cycles, annealing temperatures, dNTP and $MgCl_2$ concentrations are presented in Table 1.

Each primer pair was screened for polymorphism with DNA of eight individuals from each site by visualizing the PCR products on 1.5% agarose or 7.5% denaturing polyacrylamide gels. Among 29 primer pairs designed, eight produced consistent amplification of polymorphic loci. Following primer optimization, DNA was used from 48 individuals from both sites for a total of 96 individuals. After PCR amplification, up to four loci were combined, resolved on an ABI 3100 capillary sequencer, and scored using GeneMapper (V4.01) software (Applied Biosystems). The number of alleles ranged from 13 to 27 (Table 1) with a mean 17.9 alleles per locus. Cervus (V2.0) (Marshall *et al.* 1998) was used to calculate expected and observed heterozygosities and null allele frequencies. Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium were tested using GENEPOP (V3.4) (Raymond & Rousset 1995). Mean observed heterozygosity was 0.634. Exclusionary power was 0.998 for first parent and 0.999 for second parent. *F*-statistics following Weir & Cockerham (1984) show significant deviation from HWE in three of eight loci in both populations (Table 1). All departures from equilibrium indicate heterozygote deficit consistent with the presence of null alleles. No evidence for linkage disequilibrium ($P < 0.01$) was found between loci.

The high levels of polymorphism and high exclusionary power of the developed microsatellites will be extremely useful in our seed dispersal studies at TBS and YRS, Ecuador, where dispersal distances can be directly measured by assigning maternity to adult *V. flexuosa* female trees and identifying seed and seedling offspring.

Acknowledgments

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Special thanks to J.E. Guevara for his expertise and hard work in identification and collection of trees. We thank the University of Missouri-St. Louis, especially Drs. E. Kellogg and P. Parker for access to the E. Desmond Lee Molecular laboratories. Kelly Halbert was especially helpful in the Parker lab with support and technical advice. In Ecuador, we thank the Ministry of Environment, Pontifica Universidad Católica del Ecuador, and Universidad San Francisco de Quito.

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Moraga-Amador D, Norton S, Clark AM (2002) Molecular markers: Tools for developing microsatellites. ICBR, University of Florida, Gainesville

Raymond M, Rousset F (1995) GENEPOP Version 1.2: population genetics software for exact tests and ecumeniscism. *Journal of Heredity* **86**, 248-249.

Russo SE (2003) Responses of dispersal agents to tree and fruit traits in *Virola calophylla* (Myristicaceae): implications for selection. *Oecologia* **136**, 80-87.

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

Table 1. Characteristics of eight polymorphic microsatellite loci isolated from *Virola flexuosa* populations at Tiputini Biodiversity Station (TBS) and Yasuní Research Station (YRS), Ecuador. *F*-statistics are estimated for both populations based on global tests using GENEPOP (Raymond & Rousset 1995).

Locus (GenBank ID)	Primer sequences (5'-3')	Repeat motif	Size range	T (°C)	No. of cycles	Mg++ (mM)	*n*	*k*	H_O TBS	H_E TBS	F_{IS} TBS	H_O YRS	H_E YRS	F_{IS} YRS	F_{IS}	F_{ST}	F_{IT}
VF J12 (DQ902660)	F: TACCACAGCGATAAGTCTAACA R: CTGTGTGATTACCCAACT TTCT	$(TG)_{16}$	199-230	56	35	5.0	96	15	0.854	0.907	0.059	0.771	0.868	0.113	0.087	0.019	0.104
VF BE2 (DQ902661)	F: GGCATGTGTAGCAAGAGGTAAA R: GGATCAGTTGGTAGGGACATTG	$(CA)_{14}$	176-230	58	35	4.0	96	19	0.854	0.864	0.011	0.813	0.901	0.099	0.057	0.001	0.058
VF G14 (DQ902662)	F: CACTTCAGTTTTATTTGTCGCA R: CACTACGCCATTCCAACTAAGA	$(CA)_{16}$	190-228	55	35	5.0	96	15	0.896	0.888	-0.009	0.688	0.838	0.181	0.084	0.020	0.102
VF 18 (DQ902663)	F: TTGTTGTGCGATGTATGG R: GCCTTTGTTTCCTTATCTT	$(TG)_{20}$	238-287	50	35	4.0	96	23	0.875	0.900	0.028	0.896	0.924	0.031	0.029	0.027	0.056
VF 5 (DQ902664)	F: TGTATGGGATTAGAGGTT R: GACTGTTTTTCTTACCTT	$(GT)_{16}$ $(GA)_{15}$	198-259	62/42*	20/25*	2.0	96	27	0.625	0.937	0.336†	0.729	0.946	0.231†	0.288†	0.011	0.296
VF I3 (DQ902665)	F: ACTCACTATAGGGCGAATTGGG R: TGGTGTTTCTCTGCGTGTTTGT	$(CT)_{12}$ $(CA)_{21}$	208-309	60/40*	20/25*	2.0	96	18	0.583	0.552	-0.057	0.375	0.388	0.033	-0.012	0.016	0.004
VF BA1 (DQ902666)	F: GGACGGTTGAGATGTGGAATAG F: TGAAATGCTTGTTGGGTCTATC	$(GT)_{17}$	264-297	67/47*	20/25*	2.0	96	13	0.313	0.866	0.641†	0.313	0.659	0.529†	0.592†	0.058	0.615
VF 1I (DQ902667)	F: CGTCTGTTTAGTCCTTTGGTAT R: CCATCACTATCCCTTATCACAA	$(TG)_{13}$	285-310	67/47*	20/25*	5.0	96	13	0.333	0.582	0.430†	0.229	0.833	0.727†	0.599†	0.087	0.634

Forward primers were 5'-fluorescently-labelled 6-FAM, NED, VIC, and PET (Applied Biosystems); (T), annealing temperature of primer pairs; (*n*), number of individuals successfully genotyped; (*k*), number of alleles observed; (H_O), observed heterozygosity; (H_E), expected heterozygosity. *Indicates loci where a 'touchdown' cycle was used; initial T decreases 1.0 °C every cycle for the first set of cycles; final T is maintained throughout second set of cycles; dNTP concentrations were 40 µM for reactions with VF5, VFI3, and VFBA1 primer pairs and 100 µM for remaining reactions. †Indicates significant deviation from Hardy-Weinberg equilibrium ($P < 0.01$).

Chapter 5

A Large-Scale Genetic Comparison of Seed Dispersal and Recruitment in a Neotropical Nutmeg Tree

Unpublished manuscript: Holbrook, K.M. and B.A. Loiselle

Introduction

Vertebrate seed dispersers play a central role in the biology and distribution of tropical plants by dispersing an estimated 60-90% of tropical seeds (Howe and Smallwood 1982, Jordano 1992, Willson 1992). Despite the prevalence of animal-mediated dispersal, many gaps still exist in our understanding of seed dispersal and its contribution to population and community dynamics. From an evolutionary perspective, the consequences of seed dispersal on plant fitness have been well studied, with indications that gut treatment of seeds, how far seeds are moved from parent plants, and the site of deposition all play major roles in whether seeds will survive and be incorporated into the next generation of reproductive adults (Dow and Ashley 1996, Wenny and Levey 1998, Jordano and Schupp 2000, Wenny 2000, Bleher and Bohning-Gaese 2001). From an ecological perspective, the role of dispersal in shaping plant communities and its contribution to forest structure has been the focus of much discussion. Janzen (1970) and Connell (1971) were among the first to suggest that seed dispersal away from parent plants helps reduce mortality from density- and distance-dependent processes, resulting in the maintenance of high tree species diversity. Recent work linking seed dispersal to tropical forest diversity and structure has focused on the failure of a species to arrive and establish in all sites favorable for its growth and survival (i.e. recruitment limitation; sensu Schupp et al. 2002) (Clark et al. 1999, Hamilton 1999, Hubbell et al. 1999, Harms et al. 2000, Nathan and Muller-Landau 2000, Dalling et al. 2002, Schupp et al. 2002, Terborgh et al. 2002, Wright 2002). Thus, while there is little debate on the importance of moving one's seeds away from parent trees, we know little of the consequences of altering seed dispersal processes (Wright et al. 2000). Here, we demonstrate the frequency of long-distance dispersal and potential consequence of hunting activities in a vertebrate-dispersed tropical tree.

Plants employ many strategies in dispersing their seeds, with the capability and mechanism to move seeds influencing key processes, such as colonization probabilities, population structure and persistence (Alvarez-Buylla et al. 1996, Ouborg et al. 1999, Cain et al. 2000). For example, Jordano and Godoy (2000) found high levels of genetic diversity in *Prunus mahaleb*, a bird-dispersed species, suggesting that long-distance dispersal by frugivorous birds may contribute to within-population genetic diversity. Long-distance dispersal (LLD) is fundamental to the spread of plant population and species-level persistence (Clark et al. 1998, Cain et al. 2000, Levin et al. 2003) and may be particularly important in heterogeneous environments (Bolker and Pacala 1999). In general, LDD enables the offspring of a single parent to escape host-specific pathogens and competition with related individuals. Long-distance dispersal likely allows for greater gene flow and genetic mixing (Schupp 1993, Hamilton 1999, Shilton et al. 1999). In addition, longer dispersal distances make it possible for the offspring of a single parent to sample a larger area, thus reducing kin competition and effecting bet-hedging over a greater number and variety of environments (Muller-Landau and Hardesty 2005). Finally, LDD may facilitate arrival of rare species and colonization to gaps or more suitable germination sites (Holbrook and Smith 2000, Holbrook et al. 2002). Despite the importance of long-distance dispersal to plant recruitment, it has traditionally been difficult to measure directly due to the challenges of tracking seeds from their source.

To gain a better understanding of seed dispersal curves from the plant perspective, investigators have often relied on seed traps, which have been deployed in a variety of experimental designs, ranging from placement around individual trees to random arrangement within 50-ha plots (Laman 1996, Hubbell et al. 1999, Clark et al. 2001, Terborgh et al. 2002). Seed trap data have been used to develop empirically-derived and, more recently, theoretical models (Chave et al. 2002) of seed dispersal patterns. Seed trap studies have several potential limitations. In these models, the assumption typically used is that the dispersal distance of any given seed in the trap is to the nearest reproductive adult, an assumption which may greatly underestimate dispersal distances. Additionally, overlapping seed shadows of spatially clumped plants may obscure distribution patterns of the seed source (Anderson 1991, Eriksson and Jakobsson 1999). Finally, the use of seed traps to measure LDD is especially difficult in tropical trees where the majority of species are rare and community-wide seed rain is composed of few, but common species (Hubbell et al. 1999). Some researchers have used fluorescent microspheres to track seeds (Levey and Sargent 2000) or placed magnets or small metal pieces on seeds or fruits to locate them later using a metal detector or magnetic locator

(Sork 1984, Alverson and Diaz 1989, Mack 1995); however, these methods are not efficient over large scales in closed-canopy tropical forests. Another method used to quantify dispersal at larger scales is to measure the movements of dispersers and combine those with gut retention times to estimate disperser-generated dispersal curves, thus measuring the unique contribution of a disperser to the overall pattern of seed rain (Murray 1988, Sun et al. 1997, Holbrook and Smith 2000, Westcott and Graham 2000, Wehncke et al. 2003, Westcott et al. 2005, Holbrook and Loiselle 2007). The advantage to this method is that dispersal curves allow a better estimation of LDD; yet, exact dispersal distances remain unknown, presenting a similar problem as seed trap studies. Vertebrate-generated dispersal models also generally focus on one or a few dispersers and, consequently, capture only a portion of the entire seed shadow.

Recently, molecular techniques to assess patterns of seed dispersal have become increasingly popular (Dow and Ashley 1996, Godoy and Jordano 2001, Jordano and Godoy 2002, Jones et al. 2005, Hardesty et al. 2006). Use of genetic markers, such as microsatellites, allow one to link a seed (Godoy and Jordano 2001, Jones et al. 2005) or seedling (Bacles et al. 2006, Hardesty et al. 2006) with the parent plant, thus providing exact dispersal distances and a more efficient and precise way to measure dispersal and recruitment curves. Still, few studies have examined seed dispersal on a large enough scale to encompass the dispersal capabilities of the dispersers. In an effort to capture the majority of dispersal events, Hardesty et al. (2006) surveyed seedlings from a 50-ha plot in Panama to measure the recruitment curve of *Simarouba amara*. In this vertebrate-dispersed tree, LDD was frequent with 74% of assigned seedlings located more than 100 m from maternal trees and 16% of all genotyped seedlings unassigned and thus assumed to be dispersed from outside the 50-ha plot. Wind-dispersed species also present a problem with respect to scale, especially when trying to measure the tail of the dispersal curve. In quantifying LDD in *Jacaranda copaia*, a Central American wind-dispersed species, Jones et al. (2005) found average dispersal distances to range from 40 to 59 m over a two-year study period. Using data collected in seed traps within a 50-ha plot, these authors suggest that fitted models likely underestimate LDD due to under-sampling of area at large distances. These large-scale molecular analyses are rare and comparative studies are lacking.

In this study, we investigated effective dispersal distances of *Virola flexuosa* (Myristicaceae) in two lowland tropical forest plots, focusing on the role of LDD. We measure recruitment curves using molecular markers, where the exact distance between genotyped adults to genotyped seedlings and saplings are determined by matching multilocus genotypes between offspring and putative parent trees. Our two sites are similar floristically, however, one site has experienced increasing hunting pressure by indigenous Huaorani since 1994 (Franzen 2006, Queensborough 2006, Holbrook and Loiselle, unpublished manuscript). Previously, we found differences between the two study sites in the number of dispersers visiting fruiting *V. flexuosa* and the overall numbers of seeds removed from fruiting trees (Holbrook and Loiselle, unpublished manuscript) suggesting a reduced quantitative effect on seed dispersal at the hunted site. Here, focusing on the *distance* that seeds are dispersed, we show significantly shorter dispersal distances at the hunted site.

Our overall objective is to examine the impact of animal-mediated dispersal on recruitment patterns of a Neotropical nutmeg, *Virola flexuosa*, with a special interest in how dispersal distances may differ between sites that vary in anthropogenic effects. Research objectives are to: 1) determine effective seed dispersal distances of *V. flexuosa* using microsatellites, 2) estimate the frequency of LDD relative to local dispersal at two sites, and 3) examine spatial patterns of distribution (geographically and genetically) of seedlings and saplings in our two study plots. Our seed dispersal curves here summarize the frequency distribution of dispersal distances travelled by seeds from the origin. Following Hardesty et al. (2006), we define effective dispersal as the combined net effects of dispersal, post-dispersal survival, and germination of seeds, with establishment of seedlings. How LDD is defined is necessarily scale-dependent and will vary depending on the study organism (Nathan 2005). We employ Nathan's (2005) definition of absolute dispersal, which assigns LDD events as dispersal beyond a certain threshold distance. Our defined threshold is the average pair-wise distance between reproductive adults (~450 m) and although somewhat arbitrary, it represents a biologically relevant process. The boundary of the area in which adults of a local population interact and reproduce is commonly used in the literature to define LDD (Kinlan et al. 2005, Nathan 2005). The average distance between *V. flexuosa* adults is also just beyond the core home range (20-27 ha) of *Ramphastos* and *Pteroglossus* toucans (Ramphastidae), which were found to remove nearly 60% of *V. flexuosa* seeds (Holbrook and Loiselle, unpublished manuscript); suggesting that seeds dispersed beyond this threshold may represent more rare LDD events.

Methods

Study sites – Our research was carried out at the Tiputini Biodiversity Station (TBS; ~0° 38' S, 76° 09' W) and Yasuní Research Station (YRS; ~0° 40' S, 76° 24' W) in the Orellana Province, Ecuador. The two sites, located within the Yasuní Biosphere Reserve, are dominated by terra firme forest and do not differ significantly in tree species diversity and composition (based on several 1-ha plot surveys by Pitman et al. [2001]). The Yasuní Biosphere Reserve (~1.5 million ha) forms the largest protected area in Ecuador. Description of the flora, fauna, and climate of the study sites can be found in Valencia et al (2004a) and Holbrook and Loiselle (2007). Tiputini Biodiversity Station and YRS have two 100-ha plots (see Loiselle et al. 2007) and a 50-ha Forest Dynamics Plot (FDP) (see Valencia et al 2004a), respectively. Research was conducted within 50 ha of one of the 100-ha plots at TBS and in the FDP at YRS; both plots are located in mature terra firme forest. An additional 34 ha (100 m strip) surrounding both 50-ha plots was searched, where all adult *V. flexuosa* were identified and mapped. Tiputini Biodiversity Station is relatively isolated from human activities and experiences no hunting. In contrast, YRS is located along an oil access road and hunting by indigenous Huaorani occurs near the research station and inside the 50-ha study plot (Queenborough 2005, Franzen 2006).

Study species – *Virola flexuosa* A.C.Sm. (Myristicaceae) is a dioecious tree species that occurs throughout South America (Lambright 1981). Most individuals at TBS and YRS produce flowers from July to September and fruit from October to March (Queenborough 2005). *Virola flexuosa* is a large-seeded species, and the fruit is distinctive, with a bright red aril enclosed by a woody capsule. Pollinators are likely beetles (Curculionidae, Nitidulidae, and Staphylinidae), which are known to pollinate paleotropical species (Armstrong and Irvine 1989). Dispersers of *V. flexuosa* are primarily toucans (Ramphastidae), ateline primates (Atelidae), guans (Cracidae), and motmots (Momotidae) (Holbrook and Loiselle, unpublished manuscript).

Virola sampling and collection – We collected leaf tissue from 470 *V. flexuosa* individuals from two 84-ha plots (i.e. 50-ha plot plus 34-ha buffer) at TBS (27 adults, 118 saplings, and 142 seedlings) and YRS (13 adults, 82 saplings, and 88 seedlings). Sampling at TBS reflects an exhaustive search of *V. flexuosa* adults (84-ha plot) and saplings (50-ha plot). At YRS, all stems (including *V. flexuosa*) > 10 cm diameter at breast height (dbh) in the 50-ha FDP had previously been identified, tagged, and mapped through the Center for Tropical Forest Science program (Valencia et al. 2004a, 2004b). In addition, in the western half of the FDP, all stems > 1 cm dbh have been identified and tagged. To sample the remaining 25 ha, we conducted a comprehensive search for stems > 1 cm dbh. Seedlings (< 1 cm dbh) in both 50-ha plots were sampled through systematic searching of the entire plot area. We did not sample every seedling, but searching effort and area in both plots was equal, therefore, we feel the seedling sample is a good representation of relative numbers and should reflect any clumping pattern due to dispersal. Finally, at each site, the 84-ha plot includes all adults and encompasses the core 50-ha plot where potential offspring were collected. At TBS, we collected leaf tissue from three additional trees located outside the 84-ha study area. These outside trees were part of another study focusing on foraging behaviour of frugivores; however, we include dispersal results from seedling matches to these trees as further evidence of the frequency of long-distance seed dispersal. Locations of all individuals were mapped to the nearest one meter and recorded in a GIS database. Leaf samples were collected by hand from smaller individuals and by shaking medium-sized saplings, or by using a sling-shot to shoot leaves out of larger juveniles and adult trees. Fresh leaf tissue was dried directly after collection on silica gel and transported to the University of Missouri-St. Louis for DNA extractions and genotyping.

Information on diameter and height were recorded for all collected individuals. Reproductive adults are defined as > 30 cm dbh (Queenborough 2005), saplings from 1-10 cm dbh and > 1 m height, and seedlings < 1 m height. Reproductive status of adults was determined by presence of flowers and fruit. One tree at TBS was designated as unknown as there was no recorded reproductive effort during the study period. All female trees located within the 84-ha area were considered as maternal candidates in parentage analysis.

DNA extractions and genotyping – We extracted DNA from 466 individual plants collected in the study plots. Extractions of up to 30 mg of dried leaf tissue were performed using a DNeasy Plant Extraction Kit (Qiagen) and were further diluted 1:50 with deionized water for use in polymerase chain reaction (PCR). We developed a species-specific microsatellite library resulting in eight polymorphic loci; information on library development, primer sequences, and PCR and thermocycler conditions are presented in Holbrook et al. (2007). In the current study, we used the four most polymorphic microsatellite loci (VFJ12, VFG14, VFBE2, and VF18) and genotyped a total of 431 individuals. These loci were chosen based on high levels of heterozygosity, the large number of alleles per locus, and a lack of linkage disequilibrium. We had no duplicate parental genotypes and exclusion probabilities were high at 0.984 for first parent (when neither parent is known) and 0.998 for second parent (when one parent is already known); thus, the four loci were sufficient for our analyses (Table 1). Amplified fragments were resolved using an ABI 3100 Genetic Analyzer (Applied Biosystems) and scored using GeneMapper (V4.01) (Applied Biosystems). To ensure reliability in our matching parent-offspring genotypes, we repeated DNA extraction, PCR, and fragment analysis twice for all adults. Further, we repeated all PCR and genotyping reactions of offspring at least twice, requiring a perfect repeat to the multilocus profile before continuing analyses.

Parentage analysis – Parentage of *V. flexuosa* was assigned by simple exclusion based on matching multilocus genotypes of adults with individually sampled seedlings and saplings using Cervus 2.0 (Marshall et al. 1998). Cervus assigns parentage using a likelihood-based approach where offspring are assigned to candidate mothers (or fathers) if the likelihood ratio is large relative to the likelihood of an alternative parent. Confidence levels are assigned based on the distribution of the differences in the likelihood ratio between the two-most likely parents. We analyzed all offspring with candidate mothers only, and with parameters set to no known parent. Our high exclusion probabilities (98.4% for first-parent analyses) should readily distinguish among the limited set of candidate maternal trees. We assigned offspring based on two criteria: strict, which required a 95% confidence in excluding all but one possible parent, and relaxed, which required an 80% exclusion probability. Genotyping error was set to 0.016-0.075 based on estimates of null allele frequencies reported in Cervus. Laboratory-based error is likely very low as we repeated all genotyping reactions at least twice. For seedling analyses, we assume that no parent trees died prior to sampling. These genetic relatedness data are used to estimate effective dispersal distances of *V. flexuosa* at our two study sites. We present separate dispersal curves for individuals assigned at 95% and 80% confidence. All other analyses and reported results are for individuals genotyped at 95% confidence, unless otherwise noted.

From the 84-ha plots, we genotyped 13 adults at YRS (6 females and 7 males) and 27 adults at TBS (11 females, 16 males, and one unknown). A total of 240 and 148 potential offspring in two 50-ha plots were genotyped at TBS and YRS, respectively (Fig. 1). At TBS, we were able to assign 104 offspring to candidate female trees. Forty-four (34%) seedlings and 92 (82%) saplings were not maternally matched. At YRS, we assigned 48 offspring and found no matches for 36 (47%) seedlings and 64 (89%) saplings. Although, sampling effort was similar for the two sites, we found many fewer seedlings at YRS than at TBS. At YRS, there are also fewer reproductive adult trees, thus the smaller number of seedlings found could be due to fewer adults contributing to *V. flexuosa* seed rain. To address this disparity, we tested whether the numbers of offspring in half-sib families were different between plots using a Mann-Whitney test. Also, using categorical statistics we compared the ratio of related (i.e. half-sibs) seedlings to non-related seedlings (i.e. not matched to maternal trees in plot) at each site to minimize potential problems associated with fruit crop size effects.

Effective dispersal – Effective dispersal curves were calculated for seedlings matched to maternal parents with 95% and 80% confidence. To compare dispersal curves among sites for seedlings, we summed the number of seedlings found in 50-m distance classes away from maternal trees. Differences between dispersal curves at TBS and YRS were tested using a Kolmogorov-Smirnov non-parametric test. In addition, we compared mean dispersal distances at each site using a one-way analysis of variance (ANOVA) test with dispersal distance as the dependent variable and site as the treatment.

Long-distance dispersal – To examine the frequency of LDD events relative to local dispersal, we compared the number of seedlings dispersed locally with those dispersed long distances between sites. Long-distance dispersal at each site is defined as the distance greater than the average distance between reproductive adult trees (TBS, 478 m and YRS, 440 m). Locally-dispersed seedlings are those found within 478 m and 440 m of the mother tree at TBS and YRS, respectively. As part of another study, we genotyped three adult female trees outside the 84-ha plot at TBS (located 1.3, 1.9, and 3.4 km from the center of the 50-ha plot). Analyses of seedlings and saplings in the study plot at TBS revealed 18 matches to these outside trees. Reported effective dispersal and mean dispersal distances above do not include these 18 offspring.

Geographic spatial structure – To determine whether different age classes (adults, saplings, and seedlings) are spatially clustered, we used second-order analyses (statistics based on the co-occurrence of pairs of points) in the computer program PASSAGE (Rosenberg 2001). PASSAGE uses Ripley's K function to describe the extent to which there is spatial dependence in arrangement of points (Ripley 1979). Specifically, the function measures the average number of points found within a set distance (d) from each point (e.g. location of seedling) and divides by the number of points per area (λ). For example, each seedling within the plot has a circle of a set radius centered on it; counts of points within each circle are averaged to determine the value of K for that distance. If points are randomly distributed throughout the plot, the expected number of points in a circle of radius d, is $\pi d^2\lambda$; thus for a Poisson distribution of points, the theoretical expectation of $\hat{K}(d)$ is $\pi d^2\lambda$. Values of $\hat{K}(d)$ greater than zero indicate clumping, while values less than zero indicate regular spacing (Dale 1999). We used an edge-correction estimate of $\hat{K}(d)$ as described by Russo and Augsburger (2004).

Genetic spatial structure – We tested the spatial genetic structure of *V. flexuosa* adults, saplings, and seedlings at each site using Moran's Index (Sokal and Oden 1978) in the program Spatial Genetic Software (SGS version 1.0 c; Degen et al. 2001). Moran's I values were calculated for each of 30 continuous distance classes at 20-m intervals from 0-20 m to 580-600 m for saplings and seedlings (seedling distance class at YRS was set at 40-m because of lower sample size). Adult distance classes were set at 40- and 60-m intervals at TBS and YRS, respectively (due to lower sample sizes). For each distance category, the observed values were compared with a null distribution (from permutation test, 1000 times) and 95% confidence intervals (CI) were constructed. Positive values outside the CI suggest that individuals at that distance class are more related than expected and values below the CI represent individuals less related than expected.

Results

Parentage analysis – Offspring assignments are summarized in Table 2. Seedlings in the 50-ha plots that we were unable to assign a mother tree are assumed to have been dispersed into the study plot from beyond the 84-ha perimeter. Dispersal distances for these individuals are conservatively assigned to the closest point of the plot perimeter. At the 95% confidence level, we were able to assign 43% (66% of seedlings; 18% of saplings) and 32% (53% of seedlings; 11% of saplings) of offspring to maternal trees at TBS and YRS, respectively (Table 2). Consequently, 34% and 47% of seedlings at TBS and YRS, respectively, were considered to have been dispersed from trees outside the 50-ha plot area. Despite contrasting sample sizes, the numbers of seedlings assigned per female tree (TBS, 11; YRS, 6) were not different between sites ($U = 22.50$, $P = 0.289$), suggesting that sampled seedlings are representative of seedling populations at each site. Also, there were no significant differences between sites in the ratio of related to non-related seedlings ($X^2 = 3.377$; $P = 0.08$), thus it is likely that any differences between sites were not due to differences in fruit production.

Effective dispersal – Seed dispersal distances for all matched offspring, for seedlings only, and for unmatched seedlings are shown in Figure 2. Mean (SE) effective dispersal distances for seedlings assigned within the 84-ha plots were 193.3 (17.8) m (median, 121.5 and range, 3.3-704.4) at TBS and 102.1 (16.4) m (median, 61.7 and range, 3.4-419.5) at YRS. Dispersal distances at TBS, were nearly double those at YRS ($F_{1,118}$ = 10.823, P = .001), indicating that more LDD events occurred at TBS than at YRS. Effective dispersal curves for seedling assignments at 95% and 80% confidence at TBS and YRS are shown in Figure 3. The greatest numbers of maternally assigned seedlings at TBS were found between 50 and 100 meters, whereas at YRS, most seedlings were within 50 meters of maternal trees. Also, the overall shape of the two curves appears quite different; at YRS the shape is distinctively leptokurtic, while at TBS, the curve is flattened with a much longer tail (especially pronounced for seedlings assigned at 80%), again suggesting more frequent LDD at TBS (Fig. 3a,b). Differences between dispersal curves at TBS and YRS were significant for seedlings assigned at the 80% confidence (Kolmogorov-Smirnov; Z = 1.697; P = 0.006), but not for seedlings at the 95% confidence (P = 0.375).

Long-distance dispersal – Using the average distance between reproductive adults as a measure for LDD, 7.5% of dispersal events were long-distance at TBS, while at YRS, there were no LDD events (95% confidence). Examining the less conservative genotyping data (80% confidence), we found 13.2% and 2.4% of dispersal events were LDD at TBS and YRS, respectively. Long-distance dispersal events were more frequent than local events at TBS than at YRS (80%; X^2 = 3.805, P = 0.05), but only marginally significant for the 95% exclusion assignments (X^2 = 3.158, P = 0.06). Six seedlings and 12 saplings in the 50-ha plot at TBS were matched to female trees outside the 84-ha plot, suggesting that seeds of *V. flexuosa* may be more regularly dispersed long distances than reported in the literature. Mean (SE) dispersal distances for these individuals matched to trees outside the plot were 2,331.1 (294.4) m for saplings and 3,176.9 (455.2) m for seedlings.

Spatial structure – Spatial clustering of *V. flexuosa* seedlings and saplings was evident in both sites (Fig. 4). Spatial aggregation increased strongly as geographic scale increased and there appears to be little difference between sites in the spatial scale of variation for all age classes. The minimum distance between individual adult trees was 35 and 21 m for TBS and YRS, respectively; Figure 4 (adults) indicates spatial clumping increases beyond this distance. Our sample size at YRS was small (13 trees), resulting in a somewhat jagged distribution; however, the aggregation pattern is still evident.

The spatial genetic structure of *V. flexuosa* seedlings and saplings at TBS and YRS were analyzed using Moran's Index (*I*). The average *I* correlograms are shown in Figure 5, together with 95% confidence intervals (CI). Adults and saplings at TBS and YRS show little spatial genetic structuring (Fig. 5a-d), though saplings at TBS were spatially autocorrelated at 40, 80, 380 m (i.e. *I* values fall outside CI limits); such results, especially at 380 m, might represent spurious result because of low sample size (Fig. 5c). Positive *I* values outside confidence intervals indicate significant spatial autocorrelation and were detected in both seedling populations (Fig. 5e,f) at close distances. However, relatedness values for seedlings dispersed up to 100 m at TBS are lower than relatedness values of seedlings within the same distance at YRS, suggesting that dispersal limitation at YRS is higher. In addition, seedlings at longer distances at YRS (> 480 m, Fig. 5f) appear to be less related than expected, which may further indicate limited dispersal. Aside from the differences between sites, the genetic spatial autocorrelation (from 0-100 m) pattern seen at both YRS and TBS could potentially result from short-distance or non-random (spatially-contagious) seed dispersal.

Discussion

Vertebrate seed dispersers directly influence the number of seeds removed from trees and how far those seeds are transported. In a comparative approach, we demonstrate not only the prevalence of long-distance dispersal in a tropical tree, but also that the distances seeds are dispersed are significantly reduced at a site where large-bodied frugivores are hunted (Franzen 2006; A. Derby, personal communication; K.M. Holbrook, personal observation). Mean effective dispersal distances for *V. flexuosa* were longer at the non-hunted site (TBS) than the hunted site (YRS) and effective dispersal curves had different shapes, indicating more frequent LDD events at TBS. Long-distance seed dispersal, which is important for the persistence of plant populations (Cain et al. 2000, see Nathan 2006 for a recent discussion on LDD in plants), was recorded 100 times more frequently at TBS than at YRS (for seedlings assigned at 95% confidence). Evidences for quantitatively-restricted seed dispersal at YRS (Holbrook and Loiselle, unpublished manuscript) support our findings here on distance-restricted dispersal.

Dispersal limitation – Seed dispersal and resulting seedling recruitment can have long-lasting consequences on the spatial and temporal dynamics of plant populations (Howe and Smallwood 1982, Schupp and Fuentes 1995). Empirical data from long-term seed trap studies and seedling censuses on Barro Colorado Island, Panama provide evidence that recruitment limitation may act as a major factor determining local species richness and tropical forest composition (Hubbell et al. 1999). An overview of recruitment limitation and the maintenance of tropical forest diversity suggests that recruitment limitation results from three broad classes of mechanisms: source limitation, establishment limitation, and dissemination or dispersal limitation (Jordano and Godoy 2002, Schupp et al. 2002). Dispersal limitation, the focus of this study, is probably the major demographic effect that frugivores can have on plant populations (Jordano and Godoy 2002). Distance-restricted dispersal, a process related to dispersal limitation, specifically limits the distance that seeds are dispersed from their source (Jordano and Godoy 2002, Schupp et al. 2002).

Even with the use of genetic markers, direct measurement of seed dispersal in tropical forests is neither straightforward nor easy. The most efficient way to collect seeds for dispersal analysis is to use seed traps (Godoy and Jordano 2001, Jones et al. 2005). However, most tropical trees are not common enough to make this sampling strategy feasible (S.J. Wright, personal communication). For example, in their ten-year study, Hubbell et al. (1999) found an average of 88% of species failed to deliver even one seed to any given trap. Our approach, by genotyping seedlings and saplings and assigning them to their maternal source, allows us to examine the spatial relationships between parent trees and their established offspring. Though we are not able to make conclusions of direct dispersal of seeds, we use effective dispersal (a measure of dispersal and subsequent recruitment) as a proxy for seed dispersal. Given our comparative approach, this strategy allows us to examine differences in dispersal limitation processes in a tropical tree, which otherwise would not have been possible.

Floristic similarity between sites is important in order to test and consequently attribute differences in effective dispersal to frugivore effects. Many variables, in addition to dispersal itself, are important in determining plant distributions, such as: fecundity, pre-dispersal seed predation, post-dispersal seed and seedling predation, competition, and microsite environments suitable for germination, growth and survival (Howe and Smallwood 1982, Jordano 1992, Schupp 1993). As part of another study, we determined that fecundity did not differ between sites (Holbrook and Loiselle, unpublished manuscript). Although measurement of the remaining variables was beyond the scope of this project, we expect their influences to be similar at both TBS and YRS, given similarities in the flora, climate, and geography (Pitman 2000, Pitman et al. 2001).

***Effective and long-distance dispersal in* Virola flexuosa –** We found a clear pattern of distance-restricted seed dispersal at YRS. Mean effective dispersal distances for seedlings at TBS were 193 m (median, 121); while at YRS, distances were 102 m (median, 62), results which indicate that dispersers are moving seeds shorter distances at YRS than at TBS. This is likely due to reduced abundance of large vertebrates, such as ateline primates and large toucans that move seeds greater distances (see below).

Effective dispersal curves also demonstrate differences between sites. At TBS, fewer seedlings were found within 50 m of mother trees than at YRS. Dispersal curves were strongly leptokurtic for recruited seedlings at YRS, while at TBS the curve suggests a chi-square distribution. The effective dispersal curve at TBS (especially for seedlings assigned with 80% confidence) is distinctly flatter and with higher frequency of LDD events than the dispersal curves at YRS.

There were no LDD (defined as average distance between reproductive adults) events at YRS for seedlings assigned at 95% confidence. For seedling assignments at 80% exclusion, there were a few events at YRS; however LDD at TBS was still nearly six times more likely than at YRS. How LDD is defined varies. For example, Hardesty et al. (2006) described long-distance seedling establishment as greater than 100 m for *Simarouba amara*. If we consider LDD as events beyond 100 m, then 61% of effective dispersal would be considered LDD at TBS, while at YRS only 37% of seedlings were found farther than 100 m from their maternal match. Moreover, at YRS, 42% of assigned seedlings were less than 50 meters from the source tree. Regardless of LDD definitions, our results (61 and 76% of seedlings at 95 and 80% confidence, respectively) are comparable with Hardesty et al (2006) where 74% of assigned seedlings (using 80% confidence in assigning seedlings) established beyond 100 m. We were surprised to find that several offspring (six seedlings and 12 saplings) in the 50-ha plot were maternally assigned to three trees well outside our 84-ha study area at TBS. These 18 individuals represented 7.5% of all genotyped individuals in the TBS plot (seedlings, 2.5% and saplings, 5%). Thus, at a minimum, 2.5% of effective dispersal into the plot came from female trees an average 3,177 m away. Unfortunately we do not have additional trees genotyped outside the 84-ha plot at TBS nor any trees outside the 84-ha plot at YRS; however, if we are to fully understand the frequency of longer dispersal events for tropical vertebrate-dispersed trees, we must necessarily expand the scale of studies.

The most important dispersers of *V. flexuosa* are toucans (Ramphastidae), ateline primates (Atelidae), and guans (Cracidae) (Holbrook and Loiselle, unpublished manuscript). Toucans, primates, and guans were found to remove 57, 33, and 3% of seeds, respectively. Of this assemblage, primates, *Ramphastos* toucans, and guans were found to be vulnerable to hunting at YRS (Franzen 2006). Toucans are very capable dispersers, able to fly several kilometers within measured seed retention times and, thus, are likely candidates for contributing to LDD of *V. flexuosa* (Holbrook and Loiselle 2007). Ateline primates are also effective seed dispersers. Woolly monkeys (*Lagothrix lagothricha*) were recorded to disperse seeds a mean distance of 355 m (Stevenson 2000) and Suarez's (2006) study that found spider monkeys travel an average 3,311 m in a single day, which combined with gut passage from 2.5 to 18 hours (Milton 1981, Russo and Augspurger 2004) suggests ample opportunity to disperse seeds long distances. Little is known of the movement patterns and dispersal capabilities of guans. Other less quantitatively important dispersers of *V. flexuosa* are motmots (Momotidae), barbets (Capitonidae), cotingas (Cotingidae), trogons (Trogonidae), thrushes (Turdidae), and flycatchers (Tyrannidae) (Holbrook and Loiselle, unpublished manuscript); these species are more likely to disperse seeds locally and are not threatened by hunting at YRS (Franzen 2006).

***Spatial structure in* Virola flexuosa –** Like most tropical trees (Condit et al. 2000), *Virola flexuosa* is distributed in a non-random manner. Spatial aggregation is undoubtedly the result of the combined effects of disperser behaviour (spatially-contagious seed dispersal; Schupp et al. 2002), post-dispersal predation, and differences encountered by seeds at various deposition sites. Sampling at the same scale as our study (50-ha), Hardesty et al. (2005) found spatial aggregation of seedlings and adults of *S. amara* at all scales, suggesting that these patterns may reflect disperser movement patterns. In Peru, Russo and Augspurger (2004) found spatial clumping at all distances for *Virola calophylla* adults and up to 150 m for seedlings and saplings (which was similar to the dimensions of sleeping sites of spider monkeys). Unlike our results, spatial dispersion of *V. calophylla* seedlings and saplings were more likely to be random at larger spatial scales (at least up to 200 m). This lack of spatial clustering at larger scales was attributed to thinning due to density- and distance-dependent mortality in later life stages; however these results are based on transect sampling and observed spatial clumping may change if seedlings and saplings were sampled at a larger plot scale.

If genetic relatedness decreases with increasing spatial scale, dispersal limitation may be playing a role, which may be particularly important in populations with low densities of reproductive adults (Loiselle et al. 1995, Schnabel et al. 1998). In contrast, widespread dispersal (i.e. no dispersal limitation) may result in no observed spatial structure. Patterns of genetic spatial structure of *V. flexuosa* are similar between sites for adults and saplings. Overall, genetic relatedness was low and there was no spatial autocorrelation.

We found the spatial scale of genetic autocorrelation to decline with increasing age, which may be attributed to demographic thinning occurring between seed deposition, seedling establishment, and recruitment later in life history stages (Hardesty et al. 2005). For seedlings, a similar pattern in spatial genetic structure was found at the two sites; however, because Moran's Index is a measure of relatedness between adjacent individuals, seedlings at YRS were more related to each other (0.25 and 0.5 Moran's *I* values are equivalent to half-sib and full-sib status, respectively; Asuka et al. 2005) than seedlings at the same spatial scale at TBS. Therefore, the higher values for all comparisons in the 0-100 m distance classes for seedlings at YRS, suggest relatively higher seedling relatedness at 100 m compared to TBS. It should be noted that relatedness values also reflect male contribution (i.e. gene flow through pollen movement); however, given that adult structure between the two sites is similar, the differences we see in seedlings is likely largely attributable to seed dispersal processes rather than pollen flow. These data support the differences we found in effective dispersal distances.

Conservation implications – The direct influences of anthropogenic activities on seed dispersal processes are little known. Hamilton (1999) showed that fragmentation, through altering the movement of seeds, modifies gene flow and historical patterns of genetic subdivision; further, because gene flow through seeds is responsible for two-thirds of the total genetic-neighborhood size, it is essential for estimating the size of tropical tree breeding populations. Pacheco and Simonetti (2000) found reduced gene flow in sites where spider monkeys were absent, suggesting the disappearance of large-bodied seed dispersers, such as primates, may result in population changes of a Mimosoid tree, *Inga ingoides*. In light of on-going rapid and pervasive changes to tropical forests, there is an emerging need to understand processes, such as seed dispersal, that influence forest regeneration. Here, we show strong distance-related effects in the effective seed dispersal of a Neotropical tree primarily dispersed by large vertebrate frugivores. These differences suggest that loss of important seed dispersers may have demographic and genetic consequences for tropical trees.

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Figure Legends

Figure 1. Locations of genotyped *V. flexuosa* offspring (stars), female (closed circles), male (open circles), and unknown (open triangle) reproductive trees in the 84 ha study plots at (a) Tiputini Biodiversity Station (TBS) and (b) Yasuní Research Station (YRS). Solid and dotted lines mark 50-ha and 84-ha plots, respectively. Average ± SE distance between reproductive adults at TBS was 478 ± 16.9 and 440.4 ± 19.8 at YRS.

Figure 2. Effective dispersal distances (mean + 1 SE) at TBS and YRS for: all offspring (seedlings and saplings) matched to a female tree in the 84-ha plots; matched seedlings only; and offspring for which there was no parental match. Distances calculated for offspring with no match are from seedling or sapling location to the nearest plot perimeter marker, thus are underestimates of true dispersal distance.

Figure 3. Effective dispersal curves for *V. flexuosa* at TBS and YRS. Data represent assigned seedlings at (a) 95% and (b) 80% confidence level. Dispersed seedlings occurring at distances greater than dotted lines are the result of long-distance dispersal (LDD) events. The longest distance for a 95% assigned seedling at YRS was 419 m, which is ~20 m short of LDD defined at YRS. Arrows above bars indicate mean effective dispersal distance.

Figure 4. Spatial aggregation pattern of *V. flexuosa* at (a) TBS and (b) YRS by plotting the Ripley's *K* statistic versus distance. All sampled individuals in two 50-ha plots were

included in analyses. Ripley's *K* values at zero represent complete spatial randomness; as values increase, spatial clustering increases. Note differences in y-scale.

Figure 5. Correlogram of genetic spatial autocorrelation using Moran's Index (*I*) for *V. flexuosa* adults (a, b), saplings (c, d), and seedlings (e, f) at TBS and YRS. Adult distance classes (a, b) were set at longer intervals (TBS, 40-m and YRS, 60-m) due to lower sample sizes. Moran's *I* was calculated for each of 30 continuous distance classes for all age groups except YRS adults because of small sample size. Dashed lines represent upper and lower 95% confidence intervals (CI). Observed values that exceed the upper CI represent increased relatedness at that spatial scale, while values less than the lower CI, decreased relatedness.

Table 1. Number of alleles at each locus and exclusion probabilities for first and second parent assignments for *V. flexuosa*.

Locus	No. alleles	First parent exclusion	Second parent exclusion
VFJ12	17	0.644	0.784
VFG14	16	0.582	0.737
VFBE2	20	0.618	0.764
VF18	27	0.722	0.838
Mean	20	-	-
Total	80	0.984	0.998

Table 2. Summary of offspring assignments for Tiputini Biodiversity Station (TBS) and Yasuní Research Station (YRS). Maternal assignments are for individuals matched at 95% confidence (80% in parentheses) and are for assignments to all genotyped adults including three adult trees located outside the 84-ha plot at TBS*.

	Tiputini Biodiversity Station			Yasuní Research Station		
	Maternal assignments	No maternal assignments	TBS total	Maternal assignments	No maternal assignments	YRS total
No. seedlings	84 (97)	44 (31)	128	40 (42)	36 (34)	76
No. saplings	20 (64)	92 (48)	112	8 (10)	64 (62)	72
Subtotals	104 (161)	136 (79)	240	48 (52)	100 (96)	148
% of samples	43 (67)	57 (33)		32 (35)	68 (65)	

*18 seedlings and saplings were assigned to one of three trees outside plot.

Figure 1

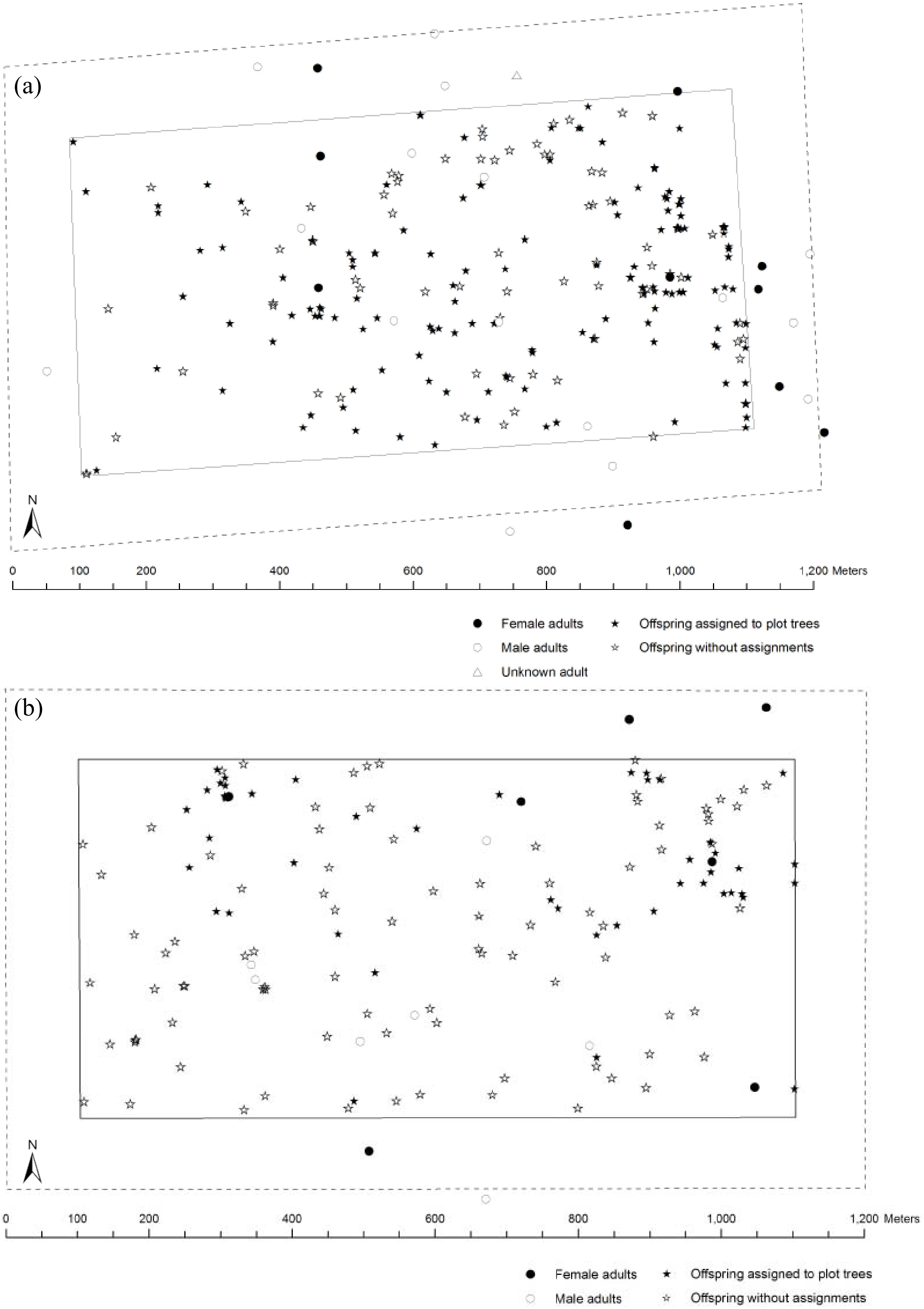

(a)
N
0
100
200
400
600
800
1,000
1,200 Meters
● Female adults
○ Male adults
△ Unknown adult
★ Offspring assigned to plot trees
☆ Offspring without assignments
(b)
N
0
100
200
400
600
800
1,000
1,200 Meters
● Female adults
○ Male adults
★ Offspring assigned to plot trees
☆ Offspring without assignments

Figure 2

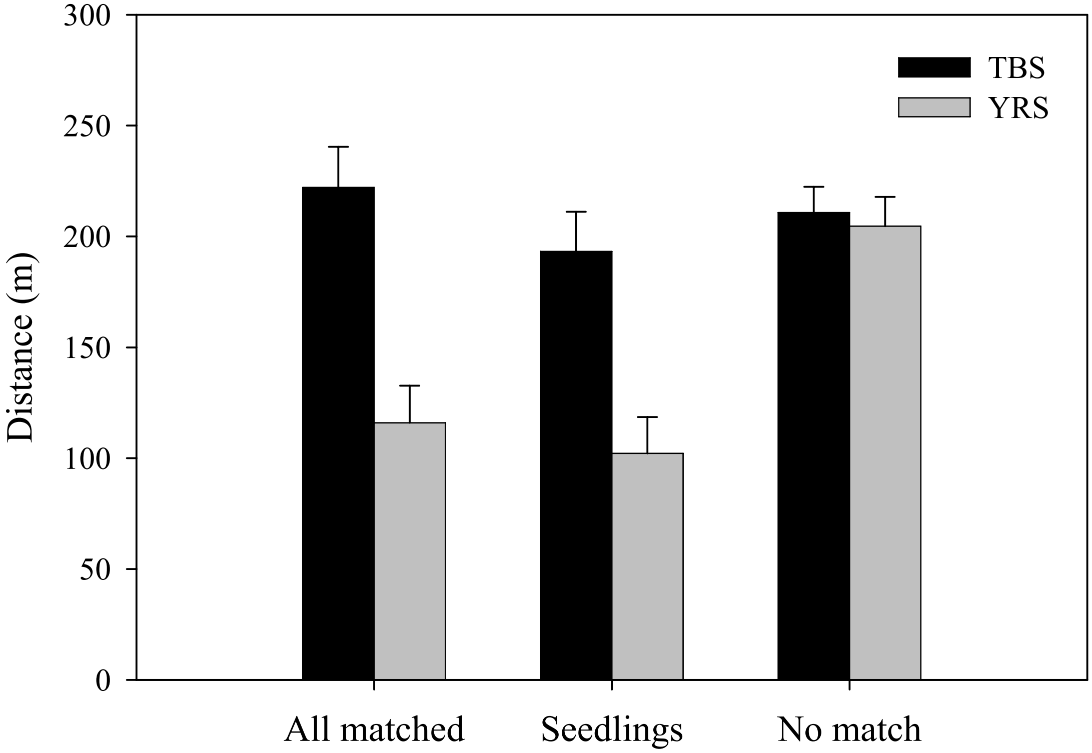

300
250
200
150
100
50
0
Distance (m)
TBS
YRS
All matched
Seedlings
No match

Figure 3

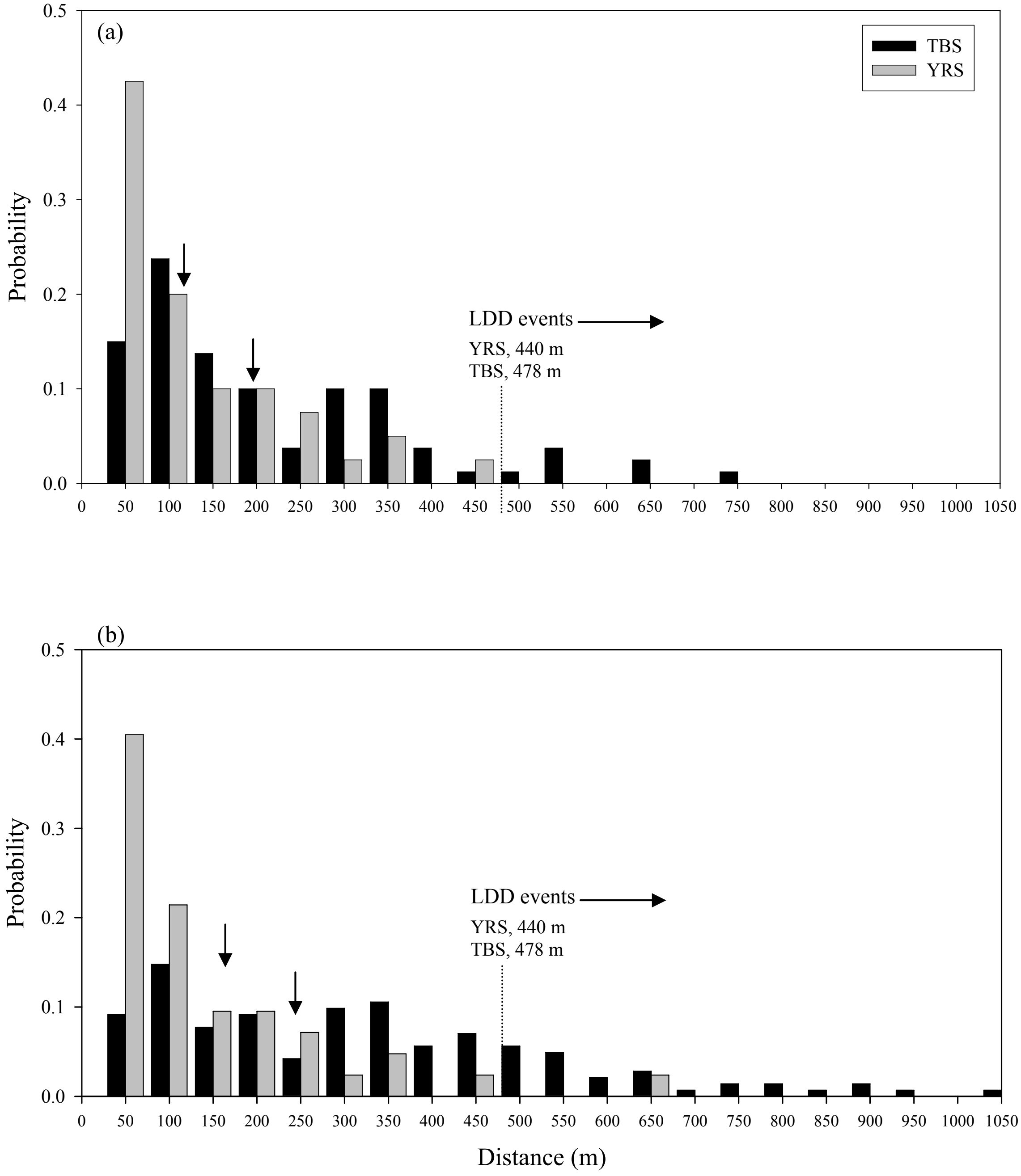

(a)
TBS
YRS
Probability
0.0
0.1
0.2
0.3
0.4
0.5
LDD events
YRS, 440 m
TBS, 478 m
0
50
100
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900
950
1000
1050
(b)
Probability
0.0
0.1
0.2
0.3
0.4
0.5
LDD events
YRS, 440 m
TBS, 478 m
0
50
100
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900
950
1000
1050
Distance (m)

Figure 4

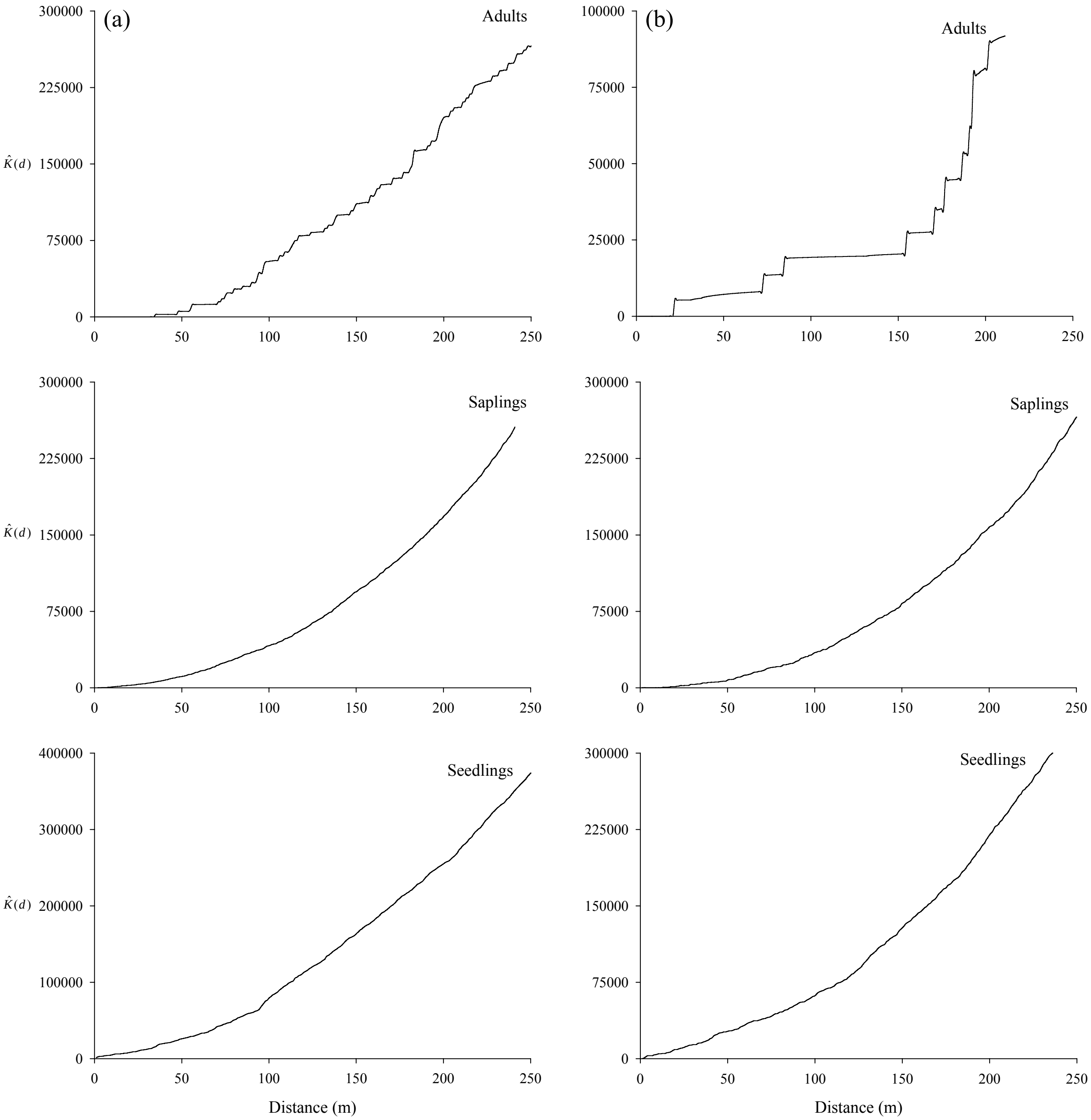
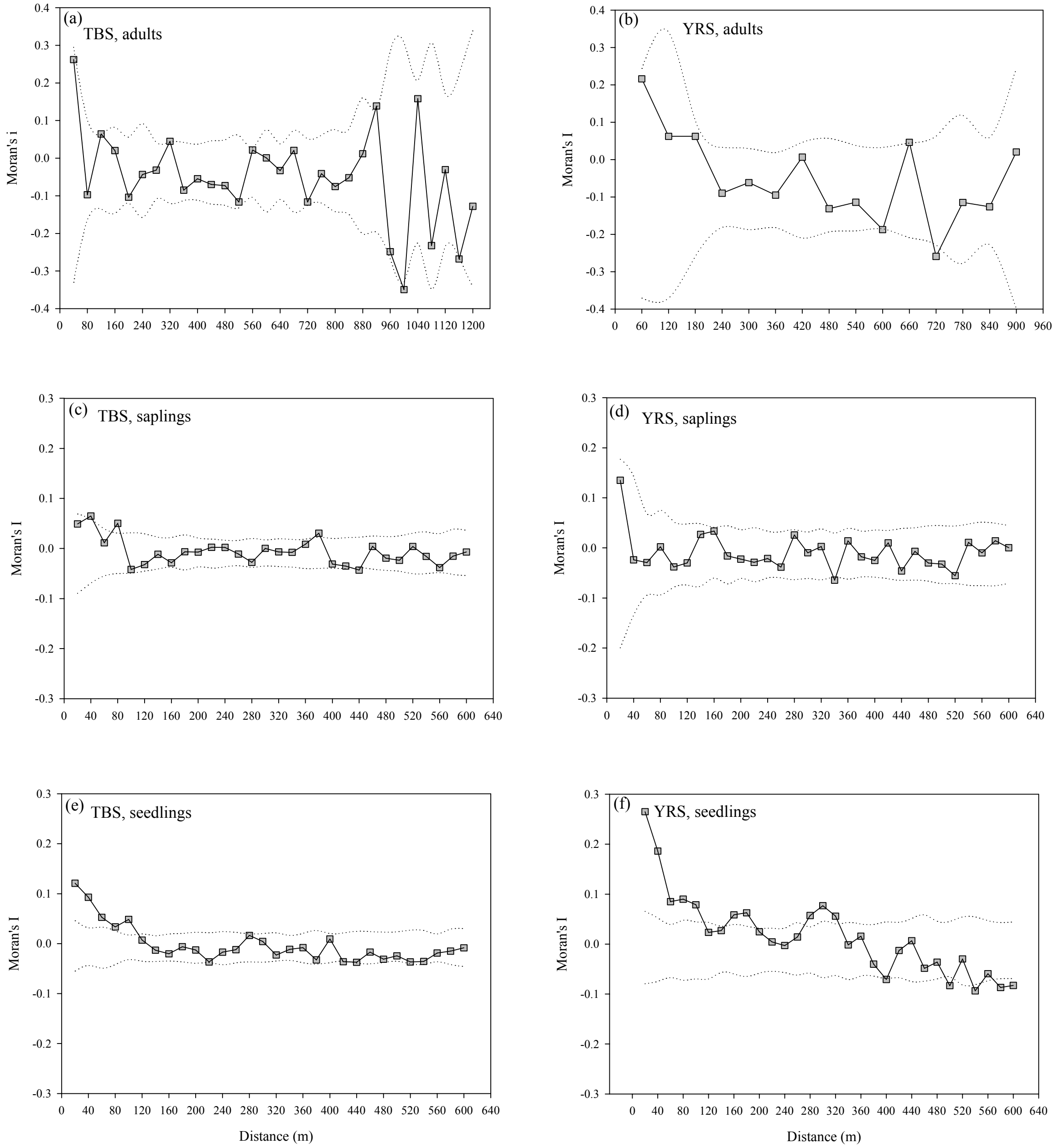

(a)
(b)
Adults
Adults
Saplings
Saplings
Seedlings
Seedlings
$\hat{K}(d)$
$\hat{K}(d)$
$\hat{K}(d)$
300000
225000
150000
75000
0
100000
75000
50000
25000
0
300000
225000
150000
75000
0
400000
300000
200000
100000
0
0
50
100
150
200
250
Distance (m)
Distance (m)

Figure 5


(a) TBS, adults
Moran's i
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
0
80
160
240
320
400
480
560
640
720
800
880
960
1040
1120
1200
(b) YRS, adults
Moran's I
0
60
120
180
240
300
360
420
480
540
600
660
720
780
840
900
960
(c) TBS, saplings
Moran's I
(d) YRS, saplings
Moran's I
(e) TBS, seedlings
Moran's I
(f) YRS, seedlings
Moran's I
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
0
40
80
120
160
200
240
280
320
360
400
440
480
520
560
600
640
Distance (m)
Distance (m)