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Title: Genetic consequences of multigenerational and landscape colonisation bottlenecks for a neotropical forest pioneer tree, *Vochysia ferruginea*.

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Abstract

Deforestation and abandonment of neotropical agricultural land has led to rapid exploitation by pioneer species. As recolonised populations may be founded by a limited number of individuals, there is significant potential for genetic bottlenecks. Previous studies of pioneer tree dynamics have failed to consider population density interactions (by sampling populations with different densities) and the multigenerational consequences of recolonisation. In this paper we examine the genetic outcomes of a clearance/recolonisation regime for a Costa Rican long-lived pioneer species, *Vochysia ferruginea*, at a series of sites with different densities and across multi-generational cohorts (old growth forest, secondary forest and seedlings) using variation for amplified fragment length polymorphism (AFLPs) and single sequence repeats (SSRs, microsatellites). A clearance/recolonisation phase was found to significantly increase fine-scale genetic structuring (average intensity of spatial genetic structure, Sp [SSR] = 0.0358) compared to old growth forest (Sp = 0.0126), and significantly reduces genetic diversity (Shannon's index [AFLP] = 0.202 and 0.271-0.355 for other forest histories following density correction), which compounds over generations (e.g. at Tirimbina: old growth forest, allelic richness, R_T [SSR] = 8.86; secondary forest R_T = 7.95; seedlings R_T = 4.76). Spatial structuring of closely related individuals suggests that *V. ferruginea* colonises sites via early invaders, which establish patches with half sib relationship. The variability observed between cohorts for genetic differentiation and inbreeding coefficients suggests that the presence of remnant trees can have an important impact on the genetic make up of recolonised populations. One main concern from these results is that if secondary forest blocks harbour reduced genetic diversity and persist in the

landscape, then species like *V. ferruginea* may be forced into a downward spiral of diversity loss if old growth remnants, which harbour higher diversity, are cleared and secondary blocks are used as reforestation sources.

Introduction

Spatial genetic structure in plant populations is primarily brought about by limited propagule dispersal (Epperson, 2000). Most tropical forest canopy trees occur at low density in old growth forest and so long distance seed and/or pollen dispersal are expected (Degen et al. 2001; Ward et al., 2005; Hardesty et al. 2005). As a result, they often show little population genetic structure (Dutech et al., 2002; Latouche-Hallé et al., 2003; Lowe et al., 2003; Ward et al., 2005; Hardesty et al., 2006). However, unlike the majority of tropical canopy trees, pioneer species often occur at locally high densities because of their light-demanding, gap-colonising strategy. In addition, when large scale disturbances occur, such as following hurricanes or forest clearance, pioneer species may dominate forest regeneration, forming large stands (Boucher et al., 1994; Boucher and Mallona, 1997). These dense populations may be founded by the offspring of very few individuals and as a result genetic structure is largely a consequence of seed dispersal during initial recolonisation (Hamrick et al 1993; Dick et al., 2003b; Dutech et al., 2003; Sezen et al., 2005; Jones et al., 2006). Pioneer species thus have the potential to exhibit strong fine-scale genetic structuring within recently recolonised stands. However, several characteristics of pioneer species would also lead us to expect that spatial genetic structuring would be reduced in old growth forest compared to recently established secondary forest. First, random mortality and competition effects over time will reduce

individual densities as stands mature, potentially reducing the number of individuals within spatially structured half-sib communities. Second, high individual densities in secondary forest cause overlapping seed (and pollen) shadows, lowering the probability that subsequent progenies are siblings (Vekemans and Hardy, 2004). Third, since pioneer species require distance dispersal to utilise forest gaps or recolonise cleared areas, genetic structuring within old growth forest is expected to be disrupted over time due to extensive ongoing gene flow (Slatkin, 1985; McCauley, 1991, Alvarez-Buylla and Garay, 1994, White et al., 1999; Dick et al., 2003a; Vekemans and Hardy, 2004).

Colonisation of cleared areas also has the potential to introduce a genetic bottleneck in the demographic cycle of pioneer species due to limited propagule sourcing (Dlugosch and Parker, 2007; Lowe et al., 2005), and the few studies of tropical trees from secondary forest confirm this expectation (*Symphonia globulifera*, Aldrich and Hamrick, 1998; *Swietenia macrophylla*, Cespedes et al., 2003; *Elaeocarpus grandis*, Rosetto et al., 2004). Indeed a study of colonisation in the palm *Iriartea deltoidea* (Sezen et al., 2005) found that although gene flow via seed dispersal was high into regenerated stands, the reproductive dominance of a few old growth parents had strong genetic consequences and genetic diversity was much lower than in old growth forest.

For *Vochysia ferruginea*, a study examining chloroplast and AFLP variation across Costa Rican populations (Cavers et al., 2005b) found that total genomic variation was reduced in secondary forest on recently cleared sites compared to old growth or lightly disturbed forest. However the density of trees in secondary forest was typically much higher than in

old growth forest, and so a question remains as to whether the difference observed in genetic diversity between secondary and old growth forests is a consequence of spatial sampling (small scale vs large scale) or an actual change in population dynamics due to recolonisation. In addition, for many species we have limited knowledge of whether genetic bottlenecks are recoverable over time or whether effects compound over generations. Finally, in most cases we have very limited understanding of the colonisation and seed dispersal dynamics between remnant and recolonised sites, with which to guide landscape scale genetic management and resourcing in neotropical trees.

Here we undertake a study of fine-scale genetic structure, genetic diversity and genetic differentiation across different sites and incorporating different demographic stages (old growth vs recently recolonised populations). We use the neotropical tree, *Vochysia ferruginea* as our case study species since its life history characteristics fit the theoretical framework of a species likely to be genetically impacted by clearance/recolonisation dynamics, the species has been the subject of ecological studies and due to its strong regeneration ability it has potential economic importance in the neotropics. The species, a long-lived pioneer (Flores, 1993), occurs at low density in neotropical old growth forest but colonises disturbed areas, such as abandoned agricultural land, forming dense stands. Its high tolerance of the low nutrient levels and high iron/aluminium concentrations typical of such sites, have made it a candidate for land reclamation projects (Finegan, 1992).

The study has two components. In the first component, we use AFLP markers to examine the diversity and fine-scale genetic structure within four Costa Rican populations of varying exploitation history and density, including one site that has recently regenerated from a cleared area (Florencia), two lightly disturbed sites (Caño Negro and Brasillea) and an old growth forest fragment (Volcán). For these sites we independently examine the impact of site history and population density on genetic diversity, and for the recently recolonised site (Florencia) we infer seed-mediated colonisation dynamics. In the second component we use nuclear microsatellite markers to compare spatial genetic structure, genetic diversity, genetic differentiation and inbreeding across three generational cohorts (old growth forest, neighbouring recolonised secondary forest (colonised with last 50 years) and seedlings resulting from secondary forest) at two additional sites in Costa Rica (Tirimbina and Ladrillera).

Results

Component 1

Spatial genetic structure

Analysis of spatial genetic structure using AFLPs indicated significant positive autocorrelation within the Florencia population at a short spatial scale (up to 10 m) and significant negative autocorrelation at the 30 m class (Fig 2a). Such a pattern suggests that genetically similar individuals are clustered in groups of approximately 20 m in diameter. No significant positive autocorrelation was evident at short spatial scales for the other populations (Fig 2b,c,d), although in Brasillea and Volcán significantly negative autocorrelation was recorded at 100, 130 and 170, and 70 m respectively, and may

indicate larger spatial aggregations in these populations. In Caño Negro, significant spatial structuring was recorded at 2400 m (Fig 2e), although, due to the dispersed nature of the population, it is difficult to assess the significance of this result without further sampling.

Genetic diversity

For all parameters of genetic diversity estimated from AFLP profiles (Table 2), Florencia was the least diverse population. Volcán and Brasillea were the most diverse populations and Caño Negro had intermediate diversity. The order and magnitude of this relationship between populations was retained after a standardising correction was applied to subsample individuals over the same spatial scale (i.e. removing density effects; Table 2).

Spatial aggregation of genetic groups

The fine-scale pattern of genetic structure evident at Florencia was subject to further investigation. Individual pairwise genetic distance estimates were used to construct a Neighbour-Joining dendrogram (Fig 3). Clusters of genetically similar individuals were labelled as groups (1 to 10) and mapped onto the original tree coordinates, along with dbh (Fig 4). The spatial clustering of closely related individuals is evident from this plot and explains the high spatial autocorrelation at fine scale observed within this population.

Component 2

Spatial genetic structure

Based on SSR screens, old growth forest populations from both Tirimbina and Ladrillera

showed the lowest intensity of spatial genetic structure (average Sp = 0.01259, see Table 3). Greater structuring was found in secondary forest populations (average Sp = 0.0358), and seedlings had similar intensities of spatial genetic structure to secondary forest populations (average Sp = 0.0358, see Table 3).

At Tirimbina, significant spatial genetic structuring in the old growth forest was evident only from 10 m to 30 m (see Fig 5). In secondary forest, significant spatial structure was found at 8 m. Significant structure was also observed at 30 m to 50 m. At distances of 90 m and 190 m individuals showed significant negative autocorrelation, i.e. lower genetic relatedness than would be expected in a random distribution.

At Ladrillera, no significant spatial genetic structure was found at the smallest spatial scales (below 50 m) in old growth forest, but significant structure was observed at 50 m and 90 m (Fig 6). In secondary forest, spatial structuring at the smaller scales was strong, with the population showing significant relatedness from 4 m up to 30 m. Spatial structure then decreased, but between 90 m and 190 m, individuals showed significant negative relatedness.

Seedling populations were sampled at a smaller spatial scale than adults. At both sites the relatedness coefficient, r, was higher than in old growth and secondary forest populations, indicating that seedlings in both populations were more genetically similar than adults (see Figs 5 and 6). At Tirimbina weak structure was found at the smallest distance class

of 4 m. At Ladrillera the seedling population showed a peak of genetic structuring at 8 m, and at 70 m individuals were significantly less related than expected.

Genetic diversity

Remnant old growth forest populations had higher diversity (expected heterozygosity, measured using SSRs) and greater allelic richness than 25 and 40 year old secondary forest stands (Table 3). The seedling cohort regenerating in neighbouring pasture showed reduced diversity compared to old growth and secondary forest, and regeneration from a single year showed lower diversity than seedling cohorts accumulated over several years (seedling stands 1 and 2; H_E = 0.47 and 0.74 respectively).

The two sites showed similar patterns in the changes of allelic richness between different populations but varied for heterozygosity (H_E Table 3). At Ladrillera, heterozygosity and allelic richness decreased from old growth forest (0.68 and 5.71 respectively) to secondary forest (0.60 and 4.50 respectively), and to seedling cohorts (0.47 and 3.48 respectively). At Tirimbina, across the same gradient, heterozygosity stayed relatively constant (H_E = 0.72-0.74), whilst allelic richness dropped from old growth forest (8.86), to secondary forest (7.95) to the seedling cohort (4.76). At Tirimbina the greatest diversity was found in old growth fragments (H_E = 0.77), although they exhibited very low allelic richness (1.82).

Population differentiation and inbreeding coefficients

At Tirimbina, the old growth forest was most genetically similar to the old growth fragments (all genetic differentiation, F_{ST}, results estimated from SSR data in Table 4), was weakly differentiated from the secondary forest block (F_{ST} = 0.036), but showed greater differentiation from the seedlings (F_{ST} = 0.068). Secondary forest individuals also showed weak differentiation from the seedling cohort (F_{ST} = 0.041). At Ladrillera, old growth forest was moderately differentiated from the secondary forest (F_{ST} = 0.089), and the seedling population adjacent to it (F_{ST} = 0.069).

Most populations had low or negative values for the population inbreeding coefficient, F_{IS} (see Table 3). An excess of homozygotes was observed in the seedling population at Tirimbina (F_{IS} = 0.11), but not at Ladrillera. There was an excess of heterozygotes observed in the secondary forest populations at Tirimbina (F_{IS} = -0.136) and Ladrillera (F_{IS} = -0.157).

Discussion

Phases of clearance and recolonisation for pioneer trees are expected to have a strong influence on genetic structure and diversity. However studies to date have not fully considered how population density may interact with diversity effects, or the outcome of a multigenerational colonisation sequence on population structure and diversity. In addition, in many cases the contribution of the colonisation process and the role of neighbouring forests to the composition of regenerated stands remains unresolved. Understanding such dynamics for a pioneer tree like *V. ferruginea*, and being able to predict genetic parameters from population history, will be a tremendous advantage for

conservation and forest managers wishing to maximize genetic diversity and potential resilience at a landscape scale.

Spatial genetic structure

Both AFLP and SSR studies found that old growth forest had minimal spatial genetic structure. This observation is expected for a pioneer species, which is mainly a gap colonist in old growth forest due to a combination of successional decline as it is out-competed by shade-tolerant forest trees and effective dispersal from adults at longer distances. The hermaphroditic flowers, although self-compatible (Bawa et al., 1985) are predominantly outcrossing (Davies, 2006), and are visited by hummingbirds and large insects (bees and butterflies). Its mating system therefore allows selfed seed to be set where pollen donors are absent, but also facilitates long distance pollen transfer and therefore the potential for genetic mixing over time.

Even in blocks of relatively disturbed forest (i.e. Caño Negro and Brasillea), there is little difference in fine-scale genetic structure compared to an old growth forest fragment (Volcán). Thus disturbance itself appears to have done little to disrupt populations of *V. ferruginea*, although fire and logging may have opened up gaps within the forest that are suitable for recolonisation by *V. ferruginea*.

The highest level of spatial structuring was found in secondary forest, with individuals in close proximity being more genetically similar than expected under random distribution. Significant positive spatial autocorrelation at the lower distance scales (1-15 m) suggests

a structure of family clusters (Levin and Kerster, 1974; Sokal and Oden, 1978; e.g *Iriartea deltoidea*, Sezen et al., 2005) due to founder effects, with patches of related individuals from limited seed donors. The family clusters were at approximately 20 m in Florencia (AFLP), 8 m in Tirimbina (SSR), and 4-8 m in Ladrillera (SSR).

In old growth forest there was no spatial structure at the shortest spatial scales (Volcán, Ladrillera and little at Tirimbina). This was in contrast to other studies which have shown genetic stucture in gap populations of some species, e.g. episodic recruitment in gaps following an El Niño Southern Oscillation (*Jacaranda copaia*; Jones and Hubbell, 2006; *Cecropia obtusifolia*, Alvarez-Buylla and Garay, 1994).

Genetic diversity

As predicted genetic diversity was negatively impacted by disturbance history and clearance/ recolonisation cycles in *V. ferruginea*. For example, the old growth forest remnant at Volcán harboured the highest level of genetic diversity (all measures), whereas the recently recolonised site, Florencia, had the lowest levels of diversity. The two old growth forest sites with a history of disturbance (Brasillea, and Caño Negro) maintained intermediate levels of diversity. These diversity differences remained evident after a correction was applied to standardise sample area and population density. Also at Tirimbina and Ladrillera sites, secondary forest blocks had reduced genetic diversity and allelic richness as measured by SSR (Tirimbina $H_E = 0.72$, $R_T = 7.95$; Ladrillera $H_E = 0.60$, $R_T = 4.50$) compared to old growth forest (Tirimbina $H_E = 0.75$, $R_T = 8.86$;

Ladrillera $H_E = 0.68$, $R_T = 5.71$), and represent a relative decrease in allelic richness of 10% and 21%, respectively.

Other studies of regeneration by pioneer species have found a resistance to loss of diversity with colonisation. Investigating the response of *Swietenia macrophylla* to regeneration into pastures, Céspedes et al. (2003) found that newly colonised populations had relatively high levels of diversity, presumably due to high levels of gene flow (as found previously, White et al., 1999; Céspedes et al., 2003). However, other studies have found decreases in diversity due to bottlenecking in founder populations similar to this study. For example, Sezen et al. (2005) found that, after colonisation, founder populations of *Iriartea deltoidea* showed significantly reduced diversity.

A further significant decrease in genetic diversity was observed in seedling cohorts using SSR variation (Tirimibina $H_E = 0.74$, $R_T = 4.76$; Ladrillera $H_E = 0.47$, $R_T = 3.48$) relative to both secondary (allelic richness decrease of 40% and 23% respectively) and old growth stands (allelic richness decrease of 46% and 39% respectively). The lower allelic richness in seedling populations is an expected result of the loss of alleles during colonisation by a low number of seed donors, although the effect was greater at Tirimbina than Ladrillera. At Ladrillera, pastureland was cut yearly and most seedlings and saplings were of similar height (ranging from 5 to 390 cm in height with the majority under 100 cm). In contrast, the abandoned plantation at Tirimbina was less regularly cut so more than one generation of seedlings are likely to be present with seedling and sapling height ranging from 12 to 640 cm. The increased loss of seedling diversity

observed at Ladrillera is likely a result of recruitment from a single year and consequent reproductive bias (cohort effect). Regeneration by successive generations may counter diversity loss in founding populations. Indeed, this reduction of dependency on single reproductive events is one of the benefits that trees gain from the high investment of the tree lifeform (Petit & Hampe 2006). In other scenarios, like species invasions, multiple introductions have also been found to augment diversity and increase allelic variation over time (Austerlitz et al., 2000; Dlugosch and Parker, 2007; Wilson et al., 2009; Prentis et al., 2009).

The genetic diversity results also highlight some anomalies. At Tirimbina, the old growth fragments consisted of three small isolated hilltop populations each containing a very small number of adult trees. These remnant trees are geographically widespread, which accounts for the high diversity of this group of adults, whilst the small population number is consistent with the low allelic richness estimate.

Overall the diversity of microsatellite variation for *V. ferruginea* adult populations ranged from 0.60 to 0.75. There is a considerable range of diversity found in other pioneer species, with H_E ranging from 0.37 (*Antirhea borbonica*, Litrico et al., 2005) to 0.81 (*Swietenia macrophylla*, Lemes et al., 2003), and ranging between 0.42 and 0.64 in different populations of *Elaeocarpus grandis* (Rossetto et al., 2004).

Genetic differentiation and family cohort

More detailed study of the family structure and genetic differentiation at recolonised sites gave some insight into the colonisation process and the mechanisms by which spatial genetic structure is established and genetic diversity lost.

Examination of the spatial distribution of genetic clusters at Florencia using AFLPs was particularly informative. Of the trees that were sampled, many belong to groups of genetically similar individuals (Fig 3), which cluster in close proximity at the site (Fig 4, e.g. groups 2, 4, 5, 6, 7 and 9). It appears likely that these clusters represent sibling groups derived from codistributed seed or are the product of seed rain from early colonisers of the site. Indeed some of the trees sampled from this population had reached diameters of more than 50 cm and are therefore likely to have arrived at the site soon after clearance and, given the relatively rapid generation time of this species (15 years), would have had enough time to mature and produce a second generation.

Investigations into population differentiation and inbreeding coefficients for generational cohorts at Tirimbina and Ladrillera using SSRs are also highly informative with respect to the colonisation process. For both sites, secondary forest blocks exhibited an excess of heterozygotes (Ladrillera F_{IS} = -0.157; Tirimbina F_{IS} = -0.136). Since random mating with some selfing can be presumed to be the norm for *V. ferruginea* (for old growth forests F_{IS} = 0.016-0.175, also see Davies, 2006), such heterozygosity excess indicates significant levels of full and half sibs within these secondary forests. Indeed the secondary forest population at Tirimbina was potentially founded by one very large remnant tree that was left in the area after clearing and has since died (B. Finegan pers.

obs.). Ladrillera also has a landscape history of a small number of remnant trees left after clearing. The genetic character of these secondary forest populations suggests that small differences in the colonisation process, such as distance from source population and presence of remnant trees, have important consequences for levels of genetic differentiation, diversity and heterozygosity in the resultant secondary forest.

The range of genetic differentiation estimates for *V. ferruginea* varied between F_{ST} = 0.069-0.112 at Ladrillera and 0.028-0.086 at Tirimbina. These values are within the range of differentiation found in other tropical tree populations, where low differentiation was found for *Dinizia excelsa* (F_{ST} = 0.00167; Dick et al., 2003a) and high levels of differentiation have been found for *Caryocar brasiliense* (F_{ST} = 0.29; Collevatti et al., 2001).

Conclusions and management considerations

The structure of genetic variation in the secondary forests surveyed in this study offers insight into the processes of seed dispersal and colonisation. However, the phenomenon of genetic bottlenecking during colonisation of pioneer species also has major implications for the management of tree genetic resources. One would expect that genetic structure within secondary forest would be disrupted and genetic diversity restored over time by ongoing gene flow, and as the early-colonising cohorts senesce and slower-growing, longer-lived species become established in the canopy (Finegan and Delgado, 2000). However, on the basis of its growth rate and maximum size, we predict that *V. ferruginea* will remain the dominant species in secondary forest for more than 100 years

(Finegan et al 1999a, b). Under this scenario, and for an area like northern Costa Rica where forest cover is greatly reduced and fragmented, seed rain at sites of active forest recolonisation (e.g. logged forest and abandoned pastures) will not come from old-growth forest which harbours high genetic diversity, but from genetically bottlenecked sources in other heavily disturbed or cleared areas. The genetic diversity of this species could therefore be compromised, with adaptive and long term consequences. However as seen at Tirimbina, where multigenerational seedlings exhibited higher diversity than the single generation cohort at Ladrillera, there may be a significant role for pollen-mediated gene flow and mutli-generational seed rain to restore diversity in recolonised stands. Considering the implications of this finding for the management of pioneer trees in increasingly disturbed landscapes of the neotropics, we would recommend further studies of the dynamics of pollen flow from genetically diverse sources as a potential agent of genetic rescue.

Methods

Vochysia ferruginea is widely distributed in the neotropics. It is hermaphroditic and although self compatible in controlled pollinations (Bawa et al., 1985), *V. ferruginea* populations in both old growth and secondary forest have been found to be predominantly outcrossing (Davies, 2006). The fruit of *V. ferruginea* are mainly dispersed by wind and occasionally birds (Flores, 1993). Its tolerance to poor soils and aluminium toxicity mean it can colonise the low fertility conditions typical of areas of land from which tropical forest has been removed for agriculture (Flores, 1993; Herrera

and Finegan, 1997; Herrera et al., 1999). In fact, there is evidence that *V. ferruginea* becomes more abundant in the low nutrient/high acidity soils of abandoned agricultural land than under more natural situations (Herrera and Finegan, 1997; Herrera et al., 1999).

Vochysia ferruginea quickly colonises disturbed areas to become a dominant species of secondary forest below 1000 m.a.s.l. and is occasionally found as a canopy tree in old-growth forests, usually on slopes or less fertile soils (Finegan 1996, Herrera & Finegan 1997). It is one of the fastest growing trees in lowland Mesoamerican moist forests, large as an adult, and potentially long-lived; in a logged forest at Tirimbina, northern Costa Rica, Finegan et al. (1999a,b) recorded median annual dbh increments between 1.5 cm and 2 cm for the population > 10 cm dbh, the largest tree observed at their site reaching 114 cm dbh. *V. ferruginea* is a vigorous long-lived pioneer species that can disperse over wide areas and recolonise cleared sites quickly (Herrera & Finegan 1997). In rural Costa Rica, *V. ferruginea* is commonly allowed to colonise abandoned agricultural land: the species' natural dispersal, fast growth and environmental tolerance enabling colonisation from neighbouring forest, even where the species is present at low density.

Component 1

From each of four *V. ferruginea* populations (Brasillea, Caño Negro, Florencia, Volcán; Fig 1), 45-50 individuals were collected (Table 1), which provides a suitable population size for conducting a spatial genetic analysis using AFLPs (Cavers et al., 2005a; Kremer et al., 2005). Individual trees were sampled for leaf or cambium tissue (Colpaert et al., 2005), which was dried on silica gel. The location of sampled trees was recorded using

polar coordinates and site maps were constructed (Figure 1). Populations were defined as groups of reproductively mature trees within a specified area (which varied between populations depending on density, Table 1).

Genomic DNA was extracted using a modified CTAB protocol (Harris, 1995). The AFLP protocol was as described in Vos et al. (1995), using four Eco+2 / Mse+4 selective primer combinations: E-CG / M-TACT, E-GC / M-CTGC, E-GC / M-CACA, E-CC / M-CACA. AFLP fingerprints were visualised on a LICOR-IR sequencer, with the EcoRI selective primer labelled with an IR fluorescent tag (IRD 700 / 800, MWG Biotech). Reactions were denatured for 5 mins at 95 °C prior to loading, then 1µl of reaction was loaded onto a 6% denaturing polyacrylamide gel (LongRanger™ Gel solution, BMA) prepared according to manufacturers instructions and including treatment with AG®501-X8 resin (Biorad), filtration and degassing prior to polymerisation. To maximize consistency and minimize scoring errors, approximately 5% of individuals were rescored, and gels were run during a single period and scored by a single person. Gels were scored for presence / absence of bands and a binary matrix was prepared.

Genetic/spatial autocorrelation of fine-scale population structure was examined using Tanimoto's distance using the software package Spatial Genetic Software (Degen et al., 2001). For each neighbourhood distance class, the observed Tanimoto distance (D (obs)), and a positive and a negative measure of the 95% confidence intervals (D (-CI) and D (+CI)) were provided. These latter values were based on 1000 resamplings of genotypes

amongst the existing tree coordinates. If D (obs) falls outside of the D (+CI), then there is a significant autocorrelation at that distance class.

Population genetic diversity parameters, Nei and Shannon's diversity indices, percentage of polymorphic loci and effective allele number were calculated using POPGENE (Yeh, 1998). To control for the scale difference between study sites, 10 individuals were resampled at random, without replacement, within a standard 0.25 ha area for each population. The procedure was repeated 10 times for each data set and the mean and standard deviation values for the above statistics calculated.

To investigate the nature of spatial genetic structure at Florencia, the site which exhibited significant fine-scale spatial genetic structuring, pairwise Tanimoto's distance between individuals was used to construct a Neighbour-Joining dendrogram; clusters of samples from the dendrogram were plotted on the site map to examine the spatial organisation of genetically similar cohorts in more detail.

Component 2

In northern Costa Rica´s San Juan – La Selva Biological Corridor (10° 34'N, 84° 06'W), two lowland sites of tropical wet forest containing old growth forest and an area of secondary forest were selected (Tirimbina and Ladrillera; Fig 1). At both sites the secondary forest boundary was adjacent to agricultural land that was either recently abandoned or minimally managed with seedling regeneration found as secondary forest encroaches. The first site was at Tirimbina Biological Reserve (Finegan et al., 1999a) and

consists mainly of old growth forest with small patches of secondary forest that is 25-45 years old. Adjacent to the secondary forest is an area of land that has been cleared and used as a plantation for palms, here there is abundant but patchy regeneration of *V. ferruginea*. Seedling and saplings at this site ranged in size from 12 to 640 cm are likely to be the product of a multigenerational cohort and were sampled randomly for study. At Tirimbina site there are also three isolated fragments within 2km of the old growth/secondary/seedling blocks sampled. All individuals from these 'fragment' populations were sampled (n=12) and included in the analysis for comparative purposes. The second site, Ladrillera S.A., a privately owned cattle farm, (Finegan et al., 1999a) consists of approximately 25 year old secondary forest, with a large old growth forest fragment. In one area where high-density *V. ferruginea* dominated secondary forest is adjacent to sloped pastureland, there is a high incidence of seedling regeneration and a number of remnant *V. ferruginea* trees present. The seedling and saplings at this site ranged in height from 5 to 390 cm, with the majority under 100 cm, and due to the frequent slashing of this pasture are likely the product of a single generational cohort.

To identify spatial genetic structure an ideal sampling strategy was defined as 100 individuals in a homogenous forest block approximating, where possible, a square plot, rather than transect (Cavers et al., 2005a). In each population 100-140 adult trees over 10 cm DBH or seedlings were exhaustively sampled from a defined block area (old growth vs secondary forest blocks vs seedling regeneration) until the target number of individuals was obtained. At Tirimbina, populations were sampled from within old growth and secondary forest. At Ladrillera the whole old growth fragment was sampled

and a secondary forest block 100 m from the old growth forest and adjacent to regenerating *V. ferruginea* in pasture. At Tirimbina and Ladrillera seedlings and saplings were sampled from plantation and pasture respectively. At Ladrillera, 100 seedlings in a continuous population were sampled but in Tirimbina, due to the very high number of seedlings, a random sample was taken. Each adult tree was mapped by measuring distance and angle from GPS points. Cambial tissue was taken from adult trees (Colpaert et al., 2005) and leaf material was collected from seedlings and dried on silica gel.

DNA was extracted from leaf and cambial material using a commercial extraction kit (DNeasy 96 Plant Kit, QIAGEN). All molecular analyses were performed using a suite of microsatellite markers within nuclear genomic DNA. A set of primers had been previously developed for *V. ferruginea* (Lowe et al., 2002) and populations were screened for variation at five of those loci, A1-5, A1-10, A1-15, A1-20 and A1-35. The general PCR protocol was: 1-5 ng DNA, polymerase buffer (1x volume, Promega), 0.2 mM dNTPs, 1.5 mM MgCl2, 0.2 μM of each primer, 2 % dimethyl sulfoxide (DMSO, Anachem), ½ unit Taq polymerase (Promega), and made up to a final reaction volume of 15 μl using dH20. Amplification of PCR products used a touchdown protocol as follows: initial denaturation at 95 °C for 3 minutes; a touchdown program of 95 °C for 15 seconds, 65 °C for 25 seconds, 72 °C for 35 seconds for 11 cycles (where the annealing temperature decreased by 1 °C per cycle); followed by a further 25 cycles with the annealing temperature at 55 °C; and a final extension step at 72 °C for 15 minutes.

Fine-scale spatial genetic structure within mapped populations was analysed using spatial autocorrelation. Relatedness was assessed using a multi-locus pairwise relationship co-efficient (Wang 2002), which jointly estimates two-gene and four-gene coefficients of relatedness between individuals from an outbreeding population (Wang, 2002; Hardy and Vekemans, 2002). Statistics were computed using the program SpaGeDi (Hardy and Vekemans, 2002), with values of the spatial statistic Sp derived by dividing -b-log (the regression slope of pairwise relatedness values on the logarithm of distance) by the mean pairwise relatedness co-efficient at the smallest spatial scale. At each site individual locations (populations) were randomised among all individuals with 1000 permutations.

Allelic richness and unbiased genetic diversity (Nei, 1987) within populations were estimated for each of five microsatellite loci and averaged over all loci, with significance tested using 1000 bootstraps. The inbreeding co-efficient (F_{IS}) was estimated over all loci for all populations, following Weir and Cockerham (1984). All estimates were obtained using the program FSTAT 2.9.3 (Goudet, 2001). Genetic differentiation (F_{ST}) between all pairs of populations was estimated following Weir and Cockerham's (1984) multilocus weighted analysis of variance using the program GENEPOP 3.4 (Raymond and Rousett, 1995). Again, significance was tested using bootstrap sampling with 1000 simulations.

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Table 1: Characteristics of populations and samples collected for analysis using AFLPs (Component 1).

Population	Latitude (N)	Longitude (W)	Sample size, *N*	Altitude (m)	Sample area (ha)	Approx density Trees/ha	Characteristics
Florencia	10°33’	84°48’	47	400	0.4	117.5	Secondary regeneration, site cleared 30 years before study
Volcán	9°20’	83°45’	50	400	2	25	Old growth forest fragment
Brasillea	11°03’	85°36’	48	330	2	24	Disturbed old growth forest
Caño Negro	10°94’	84°70’	45	55	240	0.187	no extraction history , but large fire in 1998

Table 2: Genetic diversity measures based on AFLP variation for each of the four sampled populations in component 1. A correction is applied to standardise the area over which populations were sampled, where n=10 and ten replicates were taken without replacement, and means and standard deviations (in parentheses) are presented.

	Florencia	Volcán	Brasillea	Caño Negro
Total sample	n = 47	n = 50	n = 48	n = 45
% polymorphic loci	41.27	84.1	79.4	79.4
Shannon's diversity index	0.204	0.424	0.399	0.304
Resampled to area of 0.25 ha	n = 10	n = 10	n = 10	n = 10
Effective number of alleles	1.250 (0.018)	1.420 (0.044)	1.391 (0.090)	1.262 (0.018)
Shannon's diversity index	0.202 (0.015)	0.355 (0.020)	0.332 (0.072)	0.271 (0.014)

Table 3: Spatial genetic structure, genetic diversity, allelic richness and F_{IS} in adult and seedling cohorts based on microsatellite variation (component 2).

Site	Population	*N**	*Sp**	*P**	H_E*	R_T*	F_{IS}*
Tirimbina	Fragments	12	0.02187	0.118 (0.006)	0.77	1.82	0.016
	Old growth forest	100	0.01026	0.000 (0.000)	0.75	8.86	-0.026
	Secondary forest	120	0.01888	0.980 (0.006)	0.72	7.95	-0.136
	Seedlings	132	0.03061	0.204 (0.012)	0.74	4.76	0.11
Ladrillera	Old growth	140	0.00565	0.081 (0.011)	0.68	5.71	-0.069
	Secondary	136	0.04225	0.647 (0.019)	0.60	4.50	-0.157
	Seedlings	100	0.03745	1.000 (0.000)	0.47	3.48	-0.082

*__N__, population size; *Sp*, intensity of spatial genetic structure (Hardy et al 2006); *P*, departure from Hardy-Weinberg equilibrium, standard error in brackets; H_E, average genetic diversity over all loci according to Nei (1987); R_T, allelic richness; F_{IS}, deficit of heterozygosity.

Table 4. Genetic differentiation (F_{ST}) for each pair of populations based on microsatellite variation (component 2) using GENEPOP (where F_{ST} is estimated as in Weir and Cockerham 1984).

Site		Tirimbina				Ladrillera	
	Population	Seedlings	Secondary	Old growth	Fragments	Seedlings	Secondary
Tirimbina	Seedlings						
	Secondary	0.041					
	Old growth	0.068	0.036				
	Fragments	0.043	0.086	0.028			
Ladrillera	Seedlings	0.342	0.259	0.262	0.28		
	Secondary	0.25	0.210	0.175	0.207	0.112	
	Old growth	0.162	0.138	0.128	0.141	0.069	0.089

Figure 1: Location of sites and maps of tree distribution for Component 1 plots (Brasillea, Cano Negro, Florencia and Volcan), see Table 1 for forest characteristics. See Table 3 for sample sizes and nature of forest sampled for Component 2 (Ladrillera and Tirimbina).

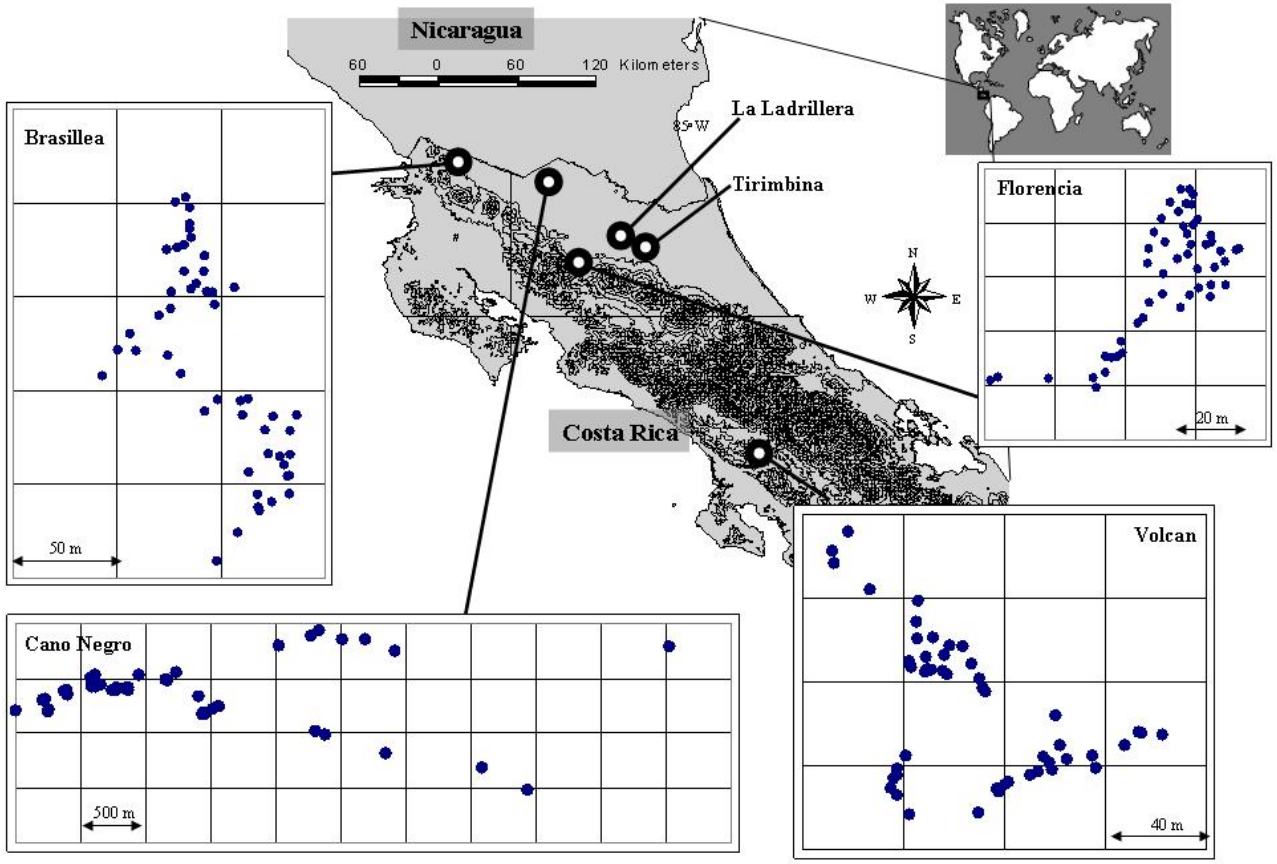
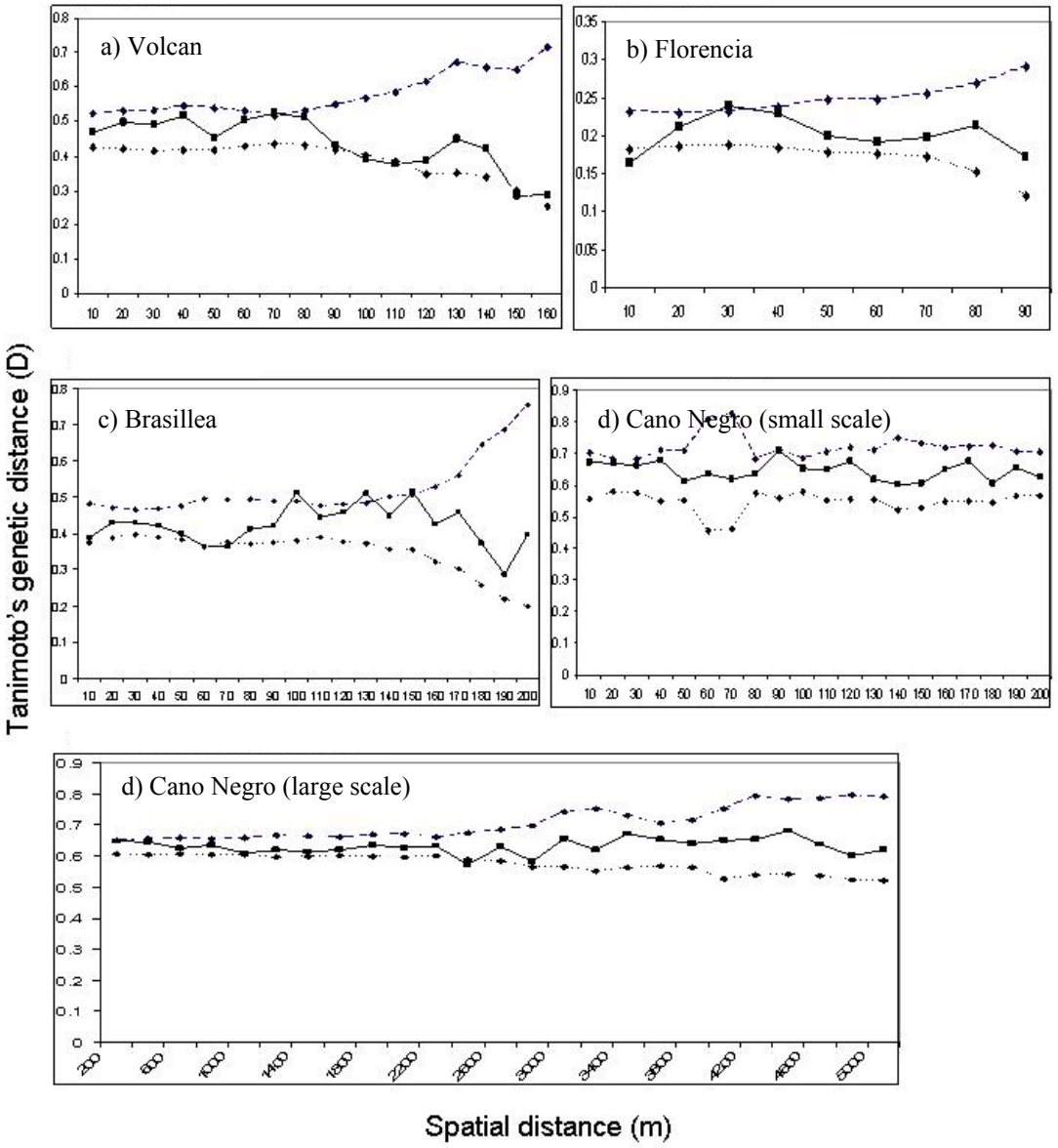


Figure 2: Spatial autocorrelation diagrams for *V. ferruginea* from each of four populations based on AFLPs (component 1). A significant (95%) observed correlation between genetic distance and distance class is indicated if points are plotted outside of the limits of the confidence interval plots. If the observed correlation is below the lower 95% CI line then individuals within that distance class have lower genetic distances between one another (i.e. are more genetically similar) than expected at random and a significant positive spatial structure is observed. If the observed correlation is above the upper 95% CI line then individuals within that distance class have higher genetic distances between one another (i.e. are less genetically similar) than expected at random and a significant negative spatial structure is observed.



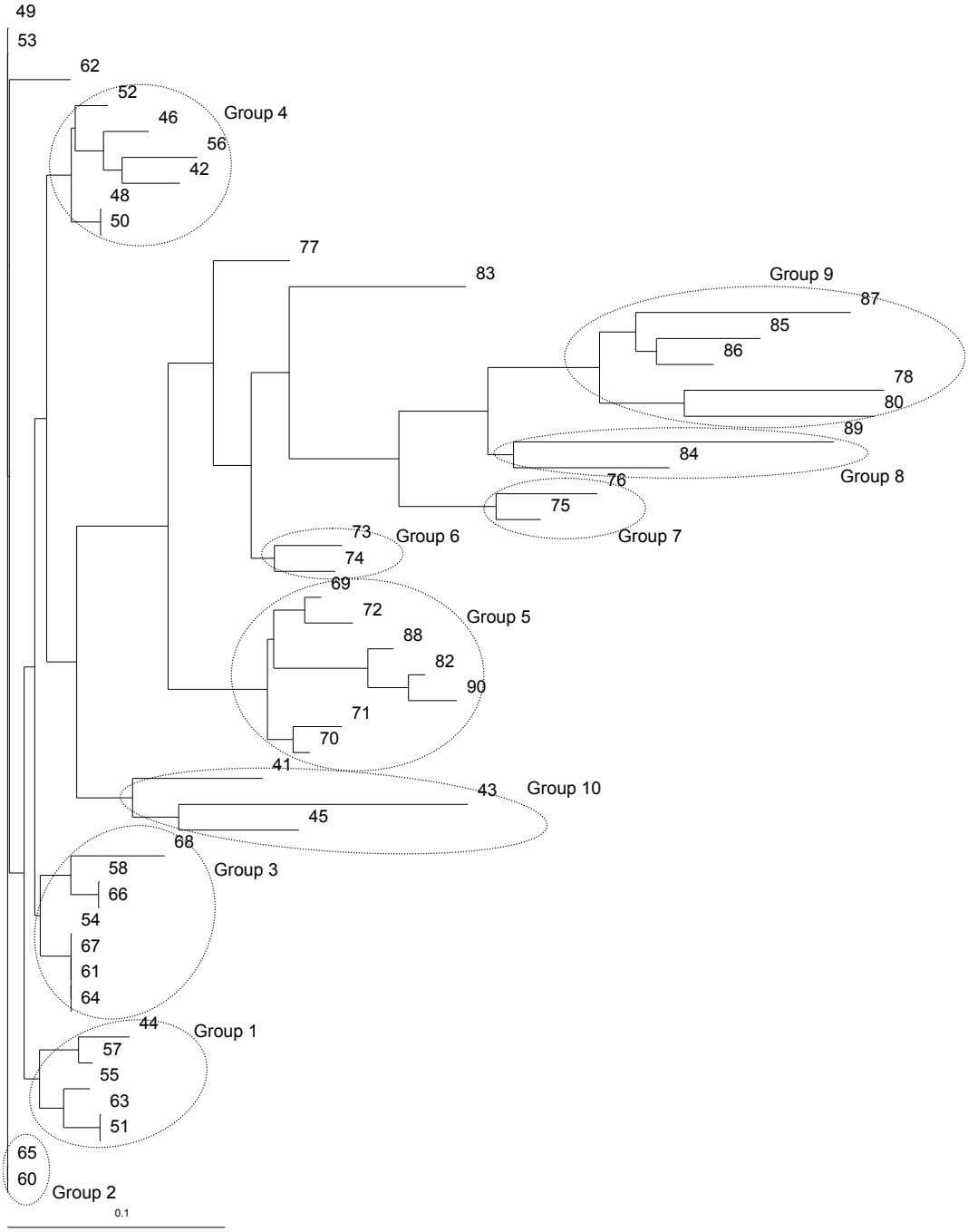


Figure 3: Neighbour joining dendrogram of individual pairwise Tanimoto's distances calculated for AFLP profiles for individuals from Florencia secondary forest. Clusters of genetically similar individuals are indicated, locations of group members are plotted in Figure 4.

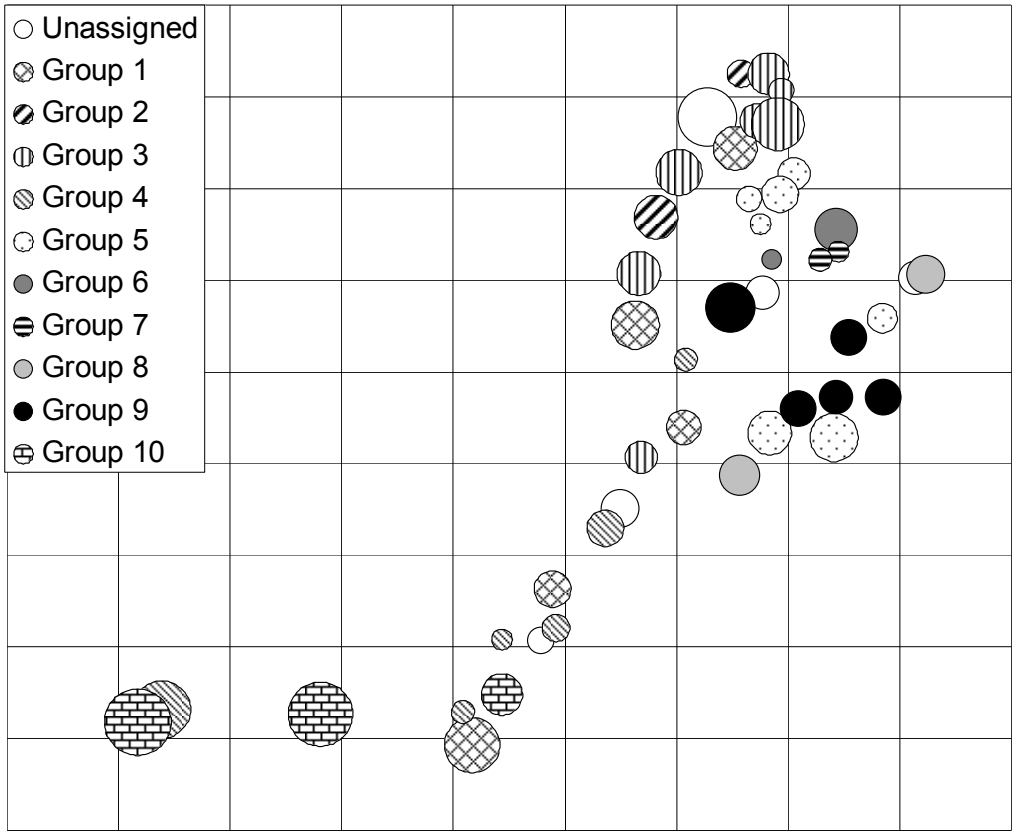


Figure 4: Plot of tree positions within the intensively sampled secondary forest at Florencia. Grid squares represent 10 x 10 m areas. Size of circle indicates diameter at breast height (dbh) of trees, where the maximum recorded dbh was 59 cm. Groups 1 to 10 indicate individuals which clustered together based on genetic distance analysis, Figure 3.

Figure 5: Spatial genetic structure of primary forest (old growth), secondary forest and seedling cohorts at Tirimbina from assessment of the multi-locus pairwise relationship co-efficient, r (Wang 2000), for microsatellite variation (component 2). Dotted lines show the 95% confidence limits. Log scale is used for adults to indicate detail at the smaller distance classes.

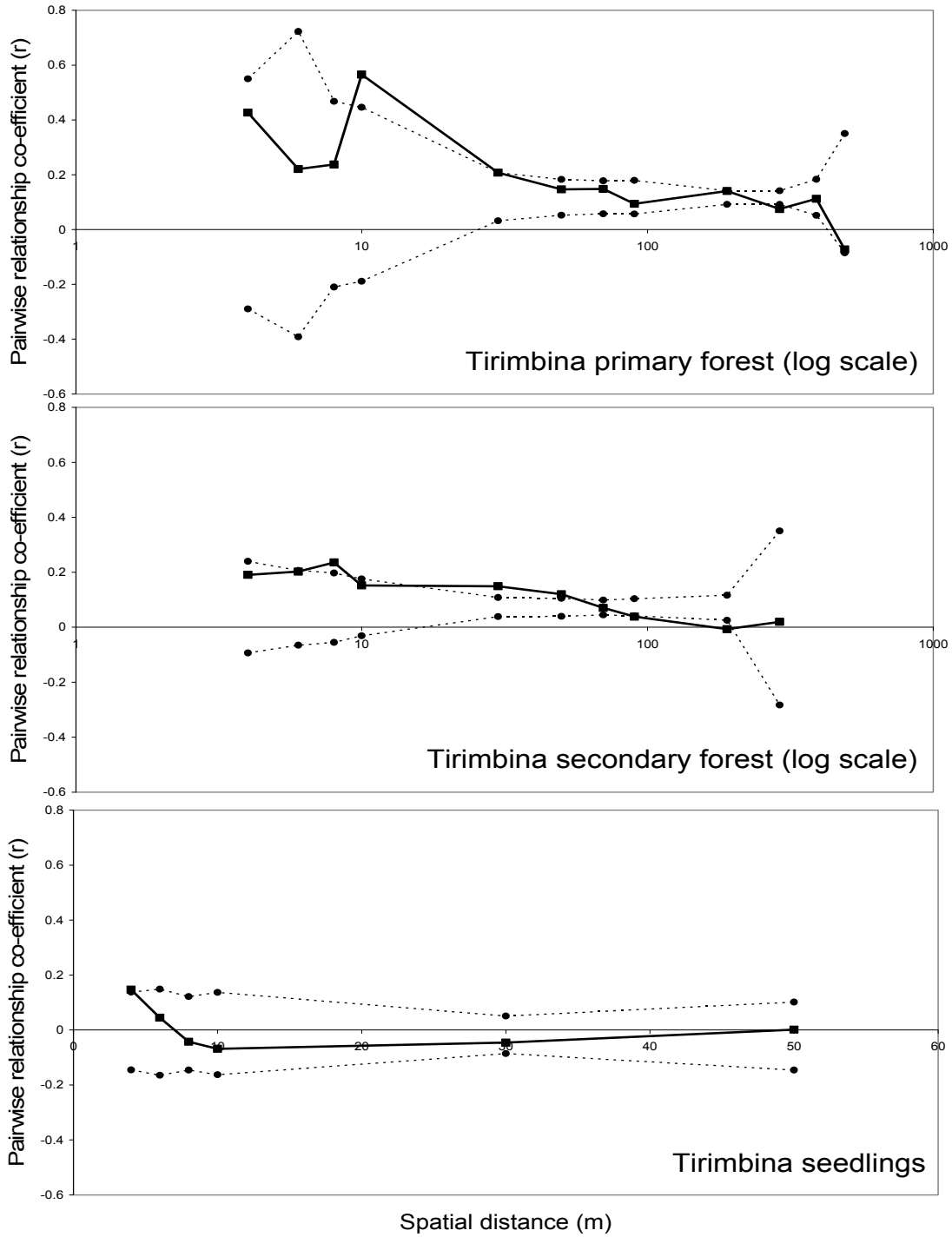


Figure 6: Spatial genetic structure of primary forest (old growth), secondary forest and seedling cohorts at Ladrillera from assessment of the multi-locus pairwise relationship co-efficient, r (Wang 2000), for microsatellite variation (component 2). Dotted lines show the 95% confidence limits. Log scale is used for adults to indicate detail at the smaller distance classes.