Forest fragmentation influences on the genetic structure and diversity of understory tree *Symphonia globulifera*: an interdisciplinary approach

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Authorization to Submit Dissertation

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Abstract

Tropical wet forests are unique ecosystems with complex vertical structure and species with unique adaptations and species interactions. Human development and land cover change dynamics are important factors contributing to the loss, fragmentation and modifications of tropical wet forest landscape across the globe. This dissertation evaluated the impacts of forest fragmentation and land use intensification in the northeastern Caribbean lowlands of Costa Rica. I describe the social and economic implications of pineapple expansion, specifically the concentration of land, labor and financial resources, quantifying pineapple cultivation's spatial characteristics, and assessing the effects of pineapple expansion on surrounding forest ecosystems, on the agricultural matrix and on biodiversity conservation. Also within this fragmented landscape we identified the potential pollinators for *Symphonia globulifera* an understory tree species. In addition we compare genetic diversity and genetic structure across three life stages (adults, saplings, seedlings) using 324 *S. globulifera* samples per cohort and 9 nuclear DNA microsatellite loci. Our results indicate that pineapple production concentrates land, labor, and financial resources, which has a homogenizing effect on the agricultural economy in the study region. This constrains farm-based livelihoods, with larger implications for food security and agricultural diversity. Landscape ecology analyses further reveal how pineapple production simplifies and homogenizes the agricultural matrix between forest patches, which is likely to have a negative effect on biodiversity. In this study the species of flower visitors with more interactions with flowers and which display a behavior of potential pollinators for *S. globulifera* are the stingless bee *Tetragonisca angustula* and the hummingbird *Phaethornis longirostris*, during visits they come in contact with anther and stigma which can allow pollen transfer between conspecific adults resulting in effective pollination. This study provides evidence that forest fragmentation and land use change have significantly reduced genetic diversity, increased inbreeding and reduced gene flow for *S. globulifera* understory populations.

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Chapter 1: Coupled social and ecological outcomes of agricultural intensification in Costa Rica and the future of biodiversity conservation in tropical agricultural regions.*

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Abstract

Tropical ecosystem conversion to agriculture has caused widespread habitat loss and created fragmented landscapes composed of remnant forest patches embedded in a matrix of agricultural land uses. Non- traditional agricultural export (NTAE) crops such as pineapple are rapidly replacing multiuse landscapes characterized by a diverse matrix of pasture and smallholder crops with intensive, large-scale, monoculture plantations. Using an interdisciplinary approach, we conduct a case study to examine the coupled social and ecological implications of agricultural intensification in this region, with larger application to regions experiencing similar patterns of agricultural intensification. Guided by frameworks from both political and landscape ecology, we: (1) describe the social and economic implications of pineapple expansion, specifically the concentration of land, labor and financial resources, (2) quantify pineapple cultivation's spatial characteristics, and (3) assess the effects of pineapple expansion on surrounding forest ecosystems, on the agricultural matrix and on biodiversity conservation. Our results indicate that pineapple production concentrates land, labor, and financial resources, which has a homogenizing effect on the agricultural economy in the study region. This constrains farm-based livelihoods, with larger implications for food security and agricultural diversity. Landscape ecology analyses further reveal how pineapple production simplifies and homogenizes the agricultural matrix between forest patches, which is likely to have a negative effect on biodiversity. To offset the effects of pineapple expansion on social and environmental systems, we recommend developing landscape level land use planning capacity. Furthermore, agricultural and conservation policy reform is needed to promote landscape heterogeneity and economic diversity within the agricultural sector. Our interdisciplinary research provides a detailed examination of the social and ecological impacts of agricultural intensification in a tropical landscape, and offers recommendations for improvement relevant not only to our study region but to the many other tropical landscapes currently undergoing non-traditional agricultural export driven agricultural intensification.

Introduction

Tropical forests cover less than 23% of the earth's terrestrial surface, but contain over 50% of its biodiversity and provide essential ecosystem services to the entire globe (Mace et al., 2005). As human populations continue to grow, the demand for food has driven an increase in croplands from an estimated 400 to 1,800 million hectares (ha) globally (Lambin et al., 2003). Recently, much of this growth has occurred in tropical regions (Gibbs et al., 2010). The conversion of tropical ecosystems to agriculture has caused widespread habitat loss and created fragmented landscapes composed of remnant forest patches embedded in a matrix of agricultural land uses. In recent years, a new pattern has emerged whereby pasture and smallholder cropping systems are rapidly being replaced by monoculture plantation agriculture (Brannstorm, 2009; Meyfroidt et al. 2014). Impacts of the expansion of agricultural intensification[1] on social and ecological systems are not well understood, but preliminary studies suggest that intensive plantation agriculture may drive demographic and economic change in local human communities (Hecht et al., 2005; Brannstrom, 2009) and affect the structure and function of remnant forest (Tscharntke et al., 2012) and landscapes (Fahrig et al. 2011).

A primary driver of the expansion of agricultural intensification in the tropics is the increased production of non-traditional agricultural export (NTAE[2]) crops (Thrupp, 1995; Morton et al., 2006; MEA, 2007; Galford et al., 2010). From a policy standpoint, NTAE crop production is viewed as an opportunity for raising farm incomes in developing countries in the tropics, which have the attraction of low labor costs and an extended growing season (Thrupp, 1995). Tropical countries therefore now dominate global NTAE production (FAO, 2011), and NTAE crops have become a major driver of economic globalization by closely linking tropical agricultural producers to consumers in temperate locations.

While NTAEs have the potential to positively affect rural economic conditions and livelihoods, their effects on biodiversity conservation are largely negative. NTAEs are generally produced on a large scale, to accommodate greater mechanization and to maximize profits. These increases in productivity ultimately stimulate more demand for land, rather than incentivizing individuals and firms to spare land for conservation (Lambin and Meyfroidt, 2011). Therefore, NTAE production can result in simultaneous agricultural intensification and expansion, a process which homogenizes the agricultural matrix, reduces total forest cover in

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[1] We define intensification as a multifaceted process where one or more of the following takes place: the unit of production increases per unit of land area (i.e., yield/hectare), cultivated land is under production for a longer period of time (i.e., less fallow), labor use is intensified (person-days/hectare), and inputs (fertilizer, pesticides, technology, capital) per hectare increase.

[2] NTAE crops are those that have not previously been central in a country's export profile, such as fresh tropical fruit or off-season temperate fruit, ornamental foliage, oil palm or biofuels.

the landscape, and increases the isolation of native plant and animal species in remnant forest patches (Rudel et al., 2009a; Rudel et al., 2009b). This sequence of events challenges the linearity of the 'intensification-land sparing' hypothesis (Matson and Vitousek, 2006) This hypothesis states that agricultural intensification increases production efficiencies and creates jobs, and therefore may decrease the need for additional deforestation for agricultural expansion, reducing pressure on surrounding ecosystems (Matson and Vitousek, 2006; Grau and Aide, 2008). However, the type of producer that benefits from intensifying a production system and the social, economic and ecological consequences of intensification differ substantially by the type and scale of the production system (Tomich et al., 2001). This context dependence underscores the importance of evaluating the socio-ecological impacts of NTAE-driven agricultural intensification in the tropics.

Although the ecological impacts are not well understood, intensively managed monoculture plantations with high agrochemical inputs can exacerbate biodiversity loss (Tilman et al., 2002; Ormerod et al., 2003; Jackson et al., 2012; Karp et al., 2012), impede native species' movement across the landscape (Vaughan et al., 2007), increase habitat fragmentation (Morton et al., 2006), and degrade soil and water quality (Hyden et al., 1993; Polidoro et al., 2008). However, it may be possible to retain the economic benefits derived from intensive plantation agriculture's productivity increases while reducing negative impacts on surrounding ecosystems. For example, practices such as retaining live fences, scattered trees, and riparian corridors amongst agricultural fields can enhance some components of biodiversity in agricultural landscapes (Harvey et al., 2006; Chazdon et al., 2009a). In some cases, these changes also lead to higher yields or economic returns, indicating that complementary goals of maintaining ecological integrity and agricultural production may be possible (Harvey and Villalobos, 2007; Robson and Berkes, 2011; van Vliet et al., 2012).

To identify policy and management options that allow for continued rural development and increases in agricultural productivity while mitigating impacts on tropical ecosystems, we need a better understanding of the relationships between NTAE production, agricultural intensification, and biodiversity conservation (Harvey et al., 2006). Such complex problems require an integrated, interdisciplinary approach that recognizes the interdependence of social, economic, and ecological processes inherent in the system (Eigenbrode et al., 2007; Ostrom, 2007; Botey et al., 2014). Here, we utilize such an approach. We first employ a political ecology (PE) analysis to examine the socio-economic implications of intensification from the perspective of local actors in the San Juan-La Selva (SJLS) region in Costa Rica, a rapidly developing agricultural zone where important conservation areas also exist. We then utilize landscape ecology (LE) to quantify and discuss the ecological implications of the composition and configuration of the dominant land cover types in the SJLS region with a special focus on pineapple, the dominant NTAE.

Our ultimate goal is to describe the social and ecological impacts of intensification in this system that are also relevant to other tropical regions where agricultural intensification is now occurring due to NTAE production. Our specific objectives are to: 1) describe the social and economic implications of pineapple expansion, specifically the distribution and concentration of land, labor and financial resources, 2) quantify the spatial characteristics of pineapple cultivation as a landscape component, and 3) assess pineapple expansion's effects on forest ecosystems and on the potential contributions of the agricultural matrix to biodiversity conservation. We conclude by exploring the policy implications of our integrated findings.

Theory

Integrating political ecology and landscape ecology

From this PE perspective natural resource access, use, and control cannot be understood without critically examining how land, labor, and financial resources are distributed in a given period and location (Blaikie and Brookfield, 1987; Turner and Robbins, 2008; Peet et al., 2011). We draw from PE by utilizing stakeholder testimony to develop a qualitative chain of explanation to link sociopolitical drivers of change to local environmental and social outcomes and to assess the tradeoffs and consequences of agricultural intensification among different actors (Robbins, 2004; Turner and Robbins, 2008).

The field of landscape ecology integrates methods from ecology and geography to address questions about the effect of landscape patterns on ecological processes (Turner, 2005). One focus of LE is determining how the composition and spatial configuration of land uses and cover types affect the amount of biodiversity the landscape can support, and the associated amount of ecosystem services that are provided to humans (Turner, 2010; Fahrig et al., 2011; Wu, 2013). Previous studies indicate that some agricultural land use types are frequently used by native species for foraging, breeding, or simply as stepping stones to reach the next habitat patches (Kupfer et al., 2006; Fischer and Lindenmayer, 2007; Harvey and Villalobos, 2007; Chazdon et al., 2009a; Gilbert-Norton et al., 2010; Vilchez et al., 2014). Landscapes that are more heterogeneous, both in composition and configuration, are more likely to include these land use types, and therefore more likely to provide habitat and habitat connectivity for a variety of species than more homogenous landscapes (Daily et al., 2003; Fischer and Lindenmayer, 2007; Milder et al., 2010; Fahrig et al., 2011).

Combined, PE and LE offer a holistic understanding of human-modified landscapes and link ecology to the social and political implications of environmental change. A PE perspective demonstrates how political, economic, and social dynamics operating across multiple scales produce spatially explicit social and environmental change. The LE analysis quantifies the extent and ecological implications of that environmental change across the landscape. PE and LE thus inform each other and illuminate novel opportunities for sustainable agricultural production and biodiversity conservation in agricultural frontiers.

Materials and methods

Study region

The study region (616,615 ha), was delimited by available remote sensing imagery and the Nicaraguan border (Fig. 1). It includes the landscapes within and surrounding the San Juan-La Selva (SJLS) biological corridor in northeastern Costa Rica (centered at 10.61°N, 84.13°W, Fig. 1). This region has a mean annual temperature of 26.5°C and annual precipitation ranging from 3000 - 4500 mm (Grieve et al., 1990; McDade et al., 1994), and lies within a wet tropical forest life zone (*sensu* Holdridge et al., 1975). Old- and second-growth forest remnants currently cover an important proportion of the land area (Morse et al., 2009; Fagan et al., 2013; Section 4 in this paper), retaining high tree species diversity and showing quick regeneration rates (Guariguata et al., 1997; Schedlbauer et al., 2007; Chazdon et al., 2009b; Norden et al., 2009; Sesnie et al., 2009; Bouroncle and Finegan, 2011). Soil types are generally acidic (pH ~4.5), primarily Inceptisols and Ultisols (Sollins et al., 1994). The terrain is composed of low hills and mountain slopes that range from 0-2,696 m in elevation with steep ravines in upper elevation areas, while lowland areas are characterized by alluvial terraces and flood plains that range from 0-400 m in elevation (Sesnie et al., 2009). These soil types and the lowland terrain are well suited for the cultivation of crops, like pineapple, that require well-drained acidic soils. The most common pineapple variety planted in the SJLS region, MD2, grows well in soils with 4.5 to 5.5 pH and slopes < 15% (Barrientos and Porras, 2010).

The land use and land cover change history in the SJLS region reflects a recent pattern in the tropics where intensive agriculture followed initial human colonization and associated deforestation (Lambin et al., 2003). The opening of the SJLS region in the 1970s and 1980s drove massive deforestation; redistributive land reform led to the eventual dominance of smallholder farms and pasturelands (Butterfield, 1994; Schelhas and Sánchez-Azofeifa, 2006). In the late 1980s, the policies driving this land rush officially ended, replaced by policies simultaneously encouraging forest conservation and NTAE expansion (Schelhas and Sánchez-Azofeifa, 2006).

One of these policies, the 1996 Forestry Law of Costa Rica, instituted a national ban on primary forest clearing; this theoretically “froze” remaining forest patches on the landscape (Watson et al., 1998; Morse et al., 2009). The Law also established an incentive system of payments for ecosystem services to encourage landowners to protect primary forest, allow forest regeneration and plant trees (Evans, 1999). To further protect the remaining forest in the region, a committee established the SJLS biological corridor initiative in 2001. The boundaries of the 246,608 ha corridor were delimited to include areas that retained significant primary forest cover and spanned the gap between Indio Maíz Biological Reserve in Nicaragua and Braulio Carrillo National Park in Costa Rica. Together, these protected areas and the SJLS biological corridor form an important link in the larger Mesoamerican Biological Corridor, an initiative begun in 1997 to facilitate regional ecological connectivity from Mexico to Panama while also promoting sustainable development and improving Mesoamericans' quality of life (IEG, 2011).

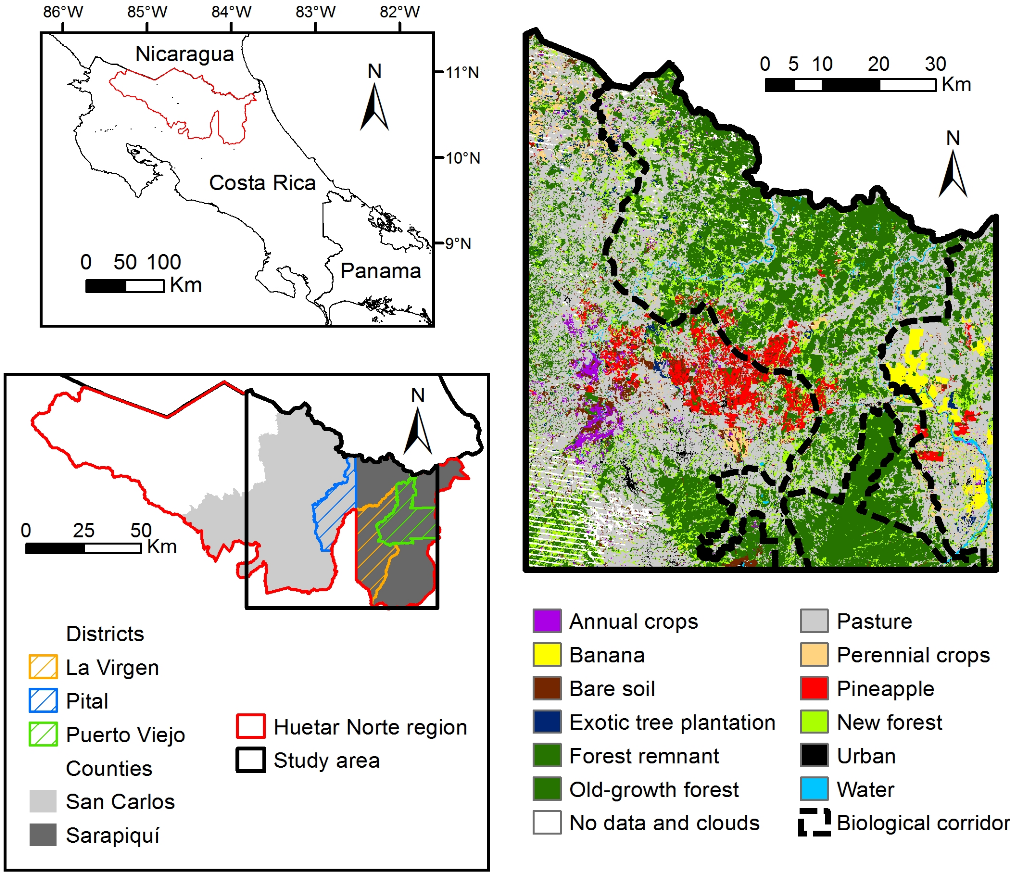

Figure 1.1 The San Juan-La Selva biological corridor and surrounding areas are located in northeastern Costa Rica. High resolution Rapid Eye imagery from 2011 was used to identify 12 major land cover types. New forest land cover type includes secondary growth and native tree plantations.

The primary policy change driving NTAE expansion during the same time period was Costa Rica's participation in Structural Adjustment Programs (SAPs). During SAP reforms Costa Rica restructured its agricultural policies away from protectionist, state-supported production of smallholder food crops toward a liberalized, globalized model promoting

NTAE production and direct foreign investment (Edelman, 1999). The SAPs and more recent free-trade agreements with the European Union, the United States and now China continue to drive the expansion of NTAEs such as pineapple, citrus, and melon (Thrupp, 1995; Vagernon et al., 2009), and the decline of in-country production of food crops (Edelman, 1999). Pineapple expansion, similar to the early banana expansion in the 1990s south of the SJLS biological corridor (Vandermeer and Perfecto, 2005), influenced social and demographic changes in communities of the SJLS region. Employment opportunities at these plantations drew migrants from both Costa Rica and Nicaragua. As a result, Sarapiquí County, which covers most of the SJLS biological corridor (Fig. 1), has the fourth highest population of Nicaraguan immigrants in Costa Rica and the second highest population growth rate of all counties in Costa Rica (INEC, 2011). The growth of economic opportunities has led to some gains in economic welfare, such as increased television and car ownership (Table 1). However, farm ownership has not increased substantially, and other analyses demonstrate the population of farmers who own and work their own farm has decreased along with the population earning their primary income from the agricultural sector (Rodriguez and Avendaño 2005).

Table 1.1 Basic indicators of economic welfare, population composition, and population size in districts that cover the area of the SJLS biological corridor, 1984 and 2011.*

	1984			2011		
Districts	Puerto Viejo	La Virgen	Pital	Puerto Viejo	La Virgen	Pital
Television Ownership	19	8	75	4,469	2,676	3,823
Car Ownership	11	20	51	871	727	1,159
Farm Ownership	336	456	513	442	345	646
Domestic Wood or Charcoal Use	607	822	1,015	455	417	348
Nicaraguan Immigrants	341	193	181	5,249	1,701	4,114
Population (Total)	4,107	4,451	6,614	20,174	10,706	17,325

*All values are numbers of individuals. Puerto Viejo and La Virgen are in Sarapiquí County, while Pital is in San Carlos County. These 3 districts cover most of the area of the SJLS biological corridor (see Fig. 1). CCP Census Data (http://ccp.ucr.ac.cr/) are presented as number of individuals.

The study region is a critical conservation area resilient forest dynamics despite population growth and a modernizing agricultural landscape (Letcher and Chazdon, 2009; Norden et al., 2009; Schedlbauer et al., 2007; Bouroncle and Finegan, 2011; Fagan et al., 2013). These factors make the SJLS region an appropriate site to assess the effects of NTAE-based agricultural intensification on rural economies and biodiversity conservation, and to explore the tradeoffs between parallel agricultural growth and conservation objectives.

Political ecology analysis

From September 2011 to May 2013 we conducted thirty-five semi-structured interviews applying the comprehensive approach (Kaufmann, 2011; Sibelet et al., 2013). Participants in our sample were selected to include a wide range of individuals and organizations involved in land use decisions and policy in the study region, including farmers' organizations, large landholders, conservation organizations and regional and national agricultural government officials. Interviews lasted 1-2 hours and were conducted in both Spanish and English. Large landholders were purposively sampled across the study region and represented the range of land cover types in the SJLS biological corridor, from forested tourism reserves to pineapple plantations. All interviewees were asked to describe the factors and policies that influence land use or their business operation decisions in particular, to describe the scale and operation of their farming system or business, and to reflect on social-environmental change in this region. The interviews were digitally voice-recorded, fully transcribed and then coded in ATLAS Ti for themes drawn from PE related to land, labor and financial resource distribution, and perceptions of agricultural and environmental change and vulnerability. In addition to the interviews, we reviewed census data, peer-reviewed publications, and gray literature in both Spanish and English. Where district-level (Puerto Viejo, La Virgen and Pital) data were unavailable, county level data were used (San Carlos and Sarapiquí counties, Fig. 1). Where county-level data were unavailable, data were derived from analyses of the entire Huetar Norte region, which includes San Carlos and Sarapiquí counties as well as the counties of Guatuso, Los Chiles and Upala (Fig. 1.1).

Landscape ecology analysis

Several historical land cover maps are available for the SJLS region (Sánchez et al., 2001, Morse et al., 2009, Fagan et al., 2013). Recently, Fagan et al. (2013) used Landsat (30 m resolution) imagery to produce land cover maps for 1986, 1996, 2001, 2005, and 2011. In this study we used 2011 RapidEye multispectral satellite imagery (5 m resolution) and extensive ground truth points to produce the most high-resolution land cover map to date of the region.

Low cloud-cover RapidEye images were chosen from a 2010-2011 library of images. For each image, we calculated ten spectral indices based on the red edge band (Schuster et al., 2011) and a texture band based on a 7x7 pixel window from the Normalized Difference Red Edge Index (Appendix A). All layers were stacked to obtain a 17-band image, which was then classified in ENVI 4.7 (Exelis, Inc., McLean, VA, USA) using a support vector machine classification algorithm. Training data were obtained from 3,000 ground truth points gathered from sources across the region by Sesnie et al. (2000), and validated in 2010-2011 (Fagan et al., 2013. We classified 12 dominant land cover types (Fig. 1). *Old-growth forests* represents forest that for the past 100 years has not been clear cut or impacted by a major human intervention; although this forest may have been impacted by selective logging, understory clearing or hunting, the resultant composition and structure is not distinctive from original primary forest with its canopy emergent trees, canopy palms, lianas and native understory species (Sensie et al. 2009). *Forest remnants* corresponds to forest patches that are smaller than 2 ha in total size. *New forests* include both secondary growth, including all stages of natural regeneration, and native tree plantations (Guariguata et al., 1997). *Exotic tree plantations* mainly include species such as *Tectona grandis* and *Gmelina arborea.* Agricultural land cover types are *pasture*, *banana*, *pineapple*, *perennial crops* [e.g. peach palm (*Bactris gasipaes*), black pepper (*Piper nigrum*)] and *annual crops. Urban areas*, *water*, and *bare soil* are the remaining land cover types. Several forest classes exhibited spectral overlap, thus to improve classification we first classified all forest within the RapidEye images into a single category, and then subdivided this category into distinct forest types from the Landsat -based map developed by Fagan et al. (2013). Overall accuracy for the 2011 land cover maps is 94%, with different values for each land cover category. Accuracy was assessed using an independent set of 513 ground-truth points gathered in 2011; this data set was not used for image classification purposes.

To assess landscape composition and measure the effects of agricultural land uses on forest fragmentation, we selected a set of metrics related to area, contrast and aggregation available in the FRAGSTATS spatial statistics program (V.4.2, University of Massachusetts, Amherst, Massachusetts, USA) (Appendix C, Table C1). Metrics were selected based on their universality and consistency as independent components of landscape structure at the class and landscape level as identified by Cushman et al. (2008) and McGarigal et al. (2012). We then calculated all metrics within and outside the biological corridor separately (Fig. 1.1).

Additionally, we conducted an analysis in Arc Map 10.1 (ESRI 2011) to compare the amount of fine-scale landscape elements such as single trees, live fences, and riparian corridors, that are present in pineapple plantations versus other agricultural land cover types. These fine-scale habitat features cannot be identified using lower-resolution (30 m Landsat) imagery; the availability of high-resolution (5 m RapidEye) maps provides a new opportunity to assess the contributions of these fine-scale features to forest connectivity and to determine which land cover types are most likely to retain these features (Boyle et al., 2014). To quantify the fine-scale landscape features in each land cover type in the SJLS region, we used a tree cover map based on 5 m RapidEye and the zonal statistics tool in ArcMap 10.1. Considering single trees and groups of trees with a size < 0.5 ha, we calculated the mean percentage area covered by trees for the entire area of each individual land cover type: pineapple, annual crops, perennial crops, banana and pasture.

To understand the potential growth boundaries of pineapple, we calculated the percentage of the SJLS biological corridor and surrounding landscape that is suitable for its cultivation. We used the following criteria to identify optimal land for pineapple cultivation: a) slope of less than 15%, b) characterized by Inceptisol or Histosol soils, and c) occurring within 3 km of a well-developed (i.e., paved or well-maintained dirt) road (Enríquez, 1994; Pitácuar, 2010). Slope, soil type and distance from an improved road were obtained or calculated using layers from the Atlas of Costa Rica (ITCR, 2008). Although these are agro-ecological criteria for pineapple production, their use is supported by an economic analysis conducted in the SJLS biological corridor that verified pineapple production is the most profitable land use and consistently occurs closest to major road networks when compared to other crops, pasture and forest (Pitácuar, 2010).

Results and discussion

Our findings link spatial patterns of land use in the study region to historical and current economic policy, and reveal the impacts of pineapple expansion on both social and ecological systems. Our LE analyses indicate that the study region (Fig. 1) is dominated by pasturelands (39%), old-growth forest (34%) and new forest (9.1%) (Table 2). Pineapple plantations and patches of bare soil (likely including land in preparation for agricultural uses) respectively cover 3.6% and 2.9% of the landscape. The rest of the landscape is occupied by other types of agricultural lands, tree plantations, urban areas and small (< 2 ha) patches of remnant forest; each of these land cover types represents between 2.1% and 0.72% of the landscape (Table 2).

Table 1. 2 FRAGSTATS analysis results summarizing area and subdivision metrics for all land cover classes in the San Juan-La Selva region. Metric units are given in parenthesis, and a detailed definition of each metric is available in Appendix C, Table C1. Land cover categories are listed from highest to lowest according to their total area in the landscape.

	Area				Subdivision		Isolation	
Land cover type	CA (ha)	PLAND (%)	LPI (%)	AREA (ha)	SPLIT	NP	PROX	ENN (m)
Pasture	244,959	39.7	12.3	57	45	4,299	337,372.10	84
Old-growth forest	210,022	34.0	6.7	50	105	4,185	28,891.80	120.9
New forest*	56,448	9.1	0.1	6	160,503	10,120	113.4	141.6
Pineapple	22,139	3.6	0.9	33	7,017	672	25,759.70	241.9
Bare soil	17,968	2.9	0.1	6	248,864	3,290	127.5	273
Perennial crop	13,259	2.1	0.1	6	337,451	2,291	259.6	238.7
Banana	8,919	1.4	0.6	29	21,397	312	1,571.60	968.2
Annual crop	7,815	1.3	0.1	5	268,389	1,462	625.7	379.2
Exotic tree plantation	6,609	1.1	0.04	4	1,551,421	1,528	43.4	455.8
Urban	4,565	0.7	0.1	5	1,298,114	980	246	329.3
Forest remnant	4,424	0.7	0.001	1	56,602,757	3,088	5.5	429.3

CA: Total area, PLAND: percentage of landscape, LPI: Largest Patch Index, AREA: Mean patch size, SPLIT: Splitting Index, NP: Number of Patches, PROX: Proximity Index, ENN: Mean Euclidean Nearest-Neighbor Distance. *This land cover type includes secondary growth and native tree plantations.

Pineapple expansion and intensification as a social, economic and ecological process

As illustrated in Fig. 2, pineapple was almost non-existent in the landscape in 1986, around the time of the SAP reforms, but increased markedly by 1996 and showed the greatest expansion from 2001-2011. This pattern of expansion was not limited to the SJLS region;

from 2006 to 2010 the land area across Costa Rica used for pineapple cultivation doubled from 22,400 ha to 45,000 ha while the crop export value increased 55% (Barquero, 2011). By 2011, pineapple had become the second most important agricultural export for Costa Rica (worth $666 million in 2010) and had created 27,000 direct jobs and 110,000 indirect jobs in production, harvesting, and processing (Barquero, 2011). Nicaraguan immigrants are the principal labor force for the majority of these unskilled jobs, where wages range from $1.20 to $2.00 per hour (Acuña-González, 2009). Although field interviews confirmed these wages are comparatively better than in less regulated sectors of the agricultural economy (i.e., cassava) and migration for work is the primarily pull to this region, the work in pineapple plantations is more physically demanding, results in higher exposure to pesticides, and can have lower job and wage security (ILRF, 2008; Acuña-González, 2009; Shaver 2014). Nearly 50% (22,138.9 ha) of the total national land area in pineapple lies within our study region. Fagan et al. (2013) found that pineapple production in the SJLS region from 2001-2011 was largely not replacing old-growth forest, but was instead expanding primarily into lands previously used for pasture or annual and perennial crops such as cassava, peach palm, and ornamental plants, as well as young regenerating forests, which experienced high rates of clearing during this time period.

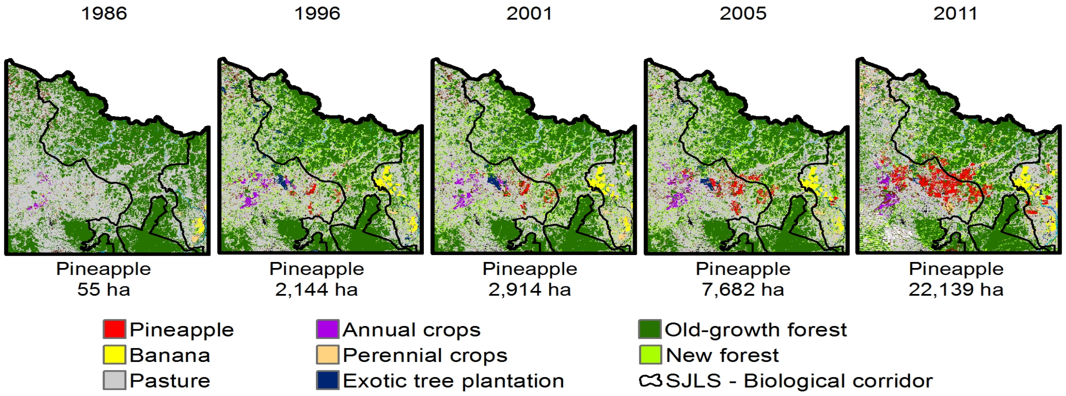

Figure 1.2 The expansion of pineapple in the San Juan-La Selva biological corridor and surrounding landscape, 1986-2011. The 1986, 1996, 2001 and 2005 maps are from Fagan et al. (2013), and the 2011 map was produced for the current study. The legend shows major land use types and forest cover types. The "new forest" class includes secondary growth and native tree plantations

In the SJLS region, pineapple plantations currently occupy a higher percentage of total land than traditional agricultural production systems including annual and perennial crops (Table 2). Although pineapple plantations cover less than 4% of the total study region, they usually occupy large patches, second in size only to pasture and forest patches (Table 2). Of total land dedicated to pineapple plantations in the study region, 78% occurs outside the SJLS biological corridor and 22% lies within (Table 3). Outside the corridor, pineapple patches are 10 ha larger on average and more aggregated than those found within. Pineapple's more aggregated spatial configuration relative to other crops (Table 2 and 3) illustrates how pineapple homogenizes the agricultural matrix, converting smaller farm parcels into large-scale plantations.

Table 1. 3 Comparison of the spatial characteristics of dominant land cover types both within (245,008 ha) and outside (371,607 ha) of the San Juan-La Selva biological corridor. Metrics units are given in parenthesis. Core area and contrast metrics are given only for old-growth forest.

	Metric	Old-growth forest	New forest*	Pasture	Pineapple
Within	PLAND (%)	47.2	11.3	32.0	2.0
	LPI (%)	13.7	0.1	3.0	0.9
	AREA (ha)	76	6	37	26
	SPLIT	33	44,863	269	9,892
	PROX	47,516	129	22,451	9,659
	ENN (m)	94	123	87	407
	CORE (ha)	62			
	TECI (%)	57			
Outside	PLAND (%)	25.3	7.7	44.7	4.6
	LPI (%)	7.6	0.1	13.3	1.1
	AREA (ha)	30	5	59	33
	SPLIT	146	142,119	36	4,552
	PROX	10,596	92	253,769	20,329
	ENN (m)	128	156	80	197
	CORE (ha)	22			
	TECI (%)	63			

AREA: Mean patch size, CORE: Mean core area per patch, ENN: Mean Euclidean Nearest-Neighbor Distance, LPI: Largest Patch Index, PLAND: percentage of landscape, PROX: Mean Proximity Index, TECI: total edge contrast index: mean edge contrast index, SPLIT: Splitting Index. *This land cover type includes secondary growth and native tree plantations.

Our pineapple suitability analysis suggests that this trend of homogenization is likely to spread across more of the landscape, especially if road development continues at its current pace. We found that in the entire study region, 26.2% of the land is highly suitable for pineapple cultivation and an additional 15.7% is moderately suitable (Fig. 3). Considering only land within the corridor, currently 2% is under pineapple cultivation (Table 4). However, 17.1% is highly suitable for future pineapple cultivation and an additional 16.6% is moderately suitable. Both our suitability analysis and current economic trends (Fold and Gough, 2008; Vagneron et al., 2009) suggest future pineapple production will likely expand both within and outside of the corridor.

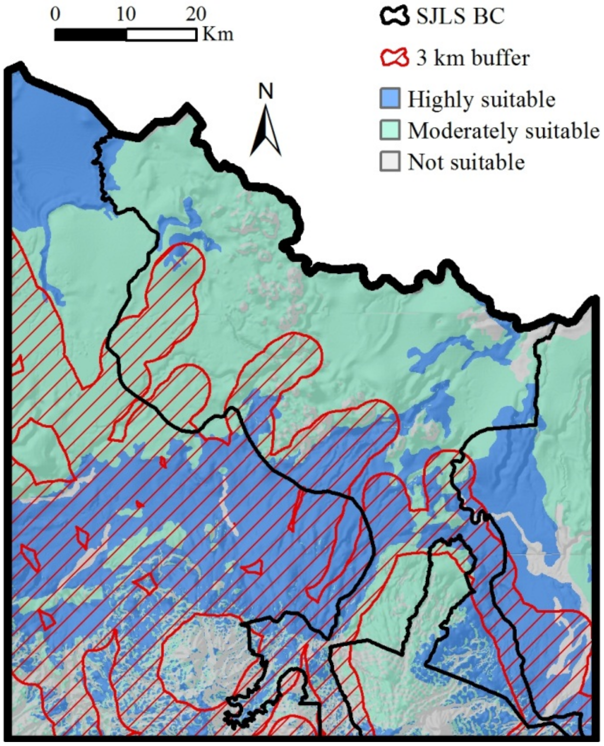

Figure 1.3 Pineapple suitability analysis. Suitable areas for pineapple cultivation were identified according to soil type and slope. Because the probability of pineapple cultivation increases with accessibility to roads, a 3 km buffer (red hatch) around principal roads is also shown.

In addition to changing the composition and configuration of land cover types, pineapple is also driving a social economic shift within the agricultural sector away from smallholder crops and toward intensive, large-scale, agribusiness-dominated production systems (Table 4). The NTAE sector's social and economic organization is related to cost advantages associated with larger scale operations that favor agribusinesses and inhibit smallholder participation (Table 4). For example, in the Huetar Norte region, the average investment to begin planting pineapple is $9,900/ha (Villegas et al., 2007). In an area where the median monthly income of agricultural households is $625, this investment capital requirement is prohibitive for most households (Progama Estado de la Nación, 2010). When the last pineapple census was conducted in 2004, pineapple farms in the Huetar Norte region with less than 10 ha accounted for only 12.9% of the land in pineapple production, while farms larger than 100 ha accounted for 76.8% (MAG census, 2005). These large farms range in size from 200-1,200 ha, with an average of 492 ha under cultivation (Villegas et al., 2007). In our FRAGSTATS analysis (Table 3), the largest patch of pineapple outside the SJLS biological corridor was 5,466 ha and the largest within the corridor was 2,308 ha; this suggests individual pineapple plantations are large and tend to border each other to form contiguous mega-patches of pineapple across the landscape.

The high investment costs and market structure of the pineapple sector also favor large-scale plantations over small pineapple farms and limit smallholder participation in this sector. The pineapple variety MD2 is densely planted, and the proportion of labor done by hand requires a large, year-round hired labor force. Conventional pineapple cultivation relies on high agrochemical and infrastructure investments (Table 1.4), an expense most small farmers cannot afford (Piñero and Díaz Ríos, 2007).

Table 1. 4 Comparison of different production system variables demonstrating that intensification occurs across multiple components of a production system and shifts the socio-economic organization of agricultural production.*

Component of the production system	Smallholder farm	Extensive cattle ranch	Agribusiness pineapple plantation
Labor type and intensity	Family labor	Family and hired labor: 0.001person-days/ha.	Hired labor: 0.5 person-days/ha.
Cost of production	Varies; most costly product is pepper at $2500/ha	Low	High (average $9,900/ha for international export) up to $22,000/ha for organic production**
Use of inputs	Varies	Low	High (average of 1000 kg/ha/yr of fertilizer); uses machinery, continuous production
Land cover type	Diversified, often including subsistence food crops	Pasture, sometimes with remnant trees and live fences Density: 1 to 3 cow/ha.	Monoculture Density: 72,000 plants/ha
Average size	.9 -6 ha	35 ha	492 ha
Market destination	Sold at national farmers' markets, to packing plants or to intermediaries at farm gate	Sold at regional auctions for international export or for national consumption	Exported internationally to major supermarket chains via direct contracts
Principal reason for land use	Low investment, easy market accessibility, low technical/labor requirements.	Easy market accessibility, low labor requirements, culture	Price, international demand

*Interviews 2011-2013. Smallholder data: Saenz-Segura et al. (2007); MAG (2005). Pineapple data: FAO (2007). Cattle data: Hollman (2008). All data are. for the Huetar Norte region (see Fig. 1).

** Organic production is more costly than conventional production due to increased labor and production costs (e.g. manual weeding/pest management, covering fields in plastic), limited availability of research on optimal production techniques and plant varieties, and lower yields per hectare.

Large agribusiness is vertically integrated in this sector (i.e., it dominates all stages of production and market distribution) (Lee et al., 2013), or fulfills contracts for a larger company, typically Dole or Del Monte, who together control 85% of all pineapple exported from Costa Rica (Vagneron et al., 2009; Blacio et al., 2010; Amanor, 2012). This market structure favors economies of scale and is high risk for households who are easily outcompeted by larger companies (Piñero and Díaz Ríos, 2007; Lee et al., 2013).

Local government officials in the SJLS biological corridor are aware of how large agribusinesses dominate pineapple production and of how untenable pineapple is as a primary rural development strategy for small farmers. A Ministry of Agriculture representative remarked, "With MD2, there was an explosion of big producers…some small and medium farmers also got involved who were in other crops, were in livestock, tubers or palm and they got into pineapple. Why? Because in 2003-2008, it was profitable. There were good prices, costs were good, but with the 2008 crisis which erupted in the U.S…. followed [by] Europe in the years 2010-2012…we were in a bad situation, and people moved away from the activity, especially smallholders."

Several times interviewees described land conversion to pineapple as a duel process of concentrating land and reducing smallholder land ownership. A prominent farmer and rancher's organization leader explained "Many farmers who produced not only cattle but also tubers, very few of them changed their activities to grow pineapple because those that had 50 hectares or less -in pineapple that is very little-, so many of them sold their land to [pineapple] companies and have left the activity [farming]." For example, one of the larger pineapple plantations in the region covers 1,500 ha, 43% of which is rented land from neighboring farms. This trend of 'land grabbing' has been documented in pineapple in Ghana (Amanor,

2012) as well as for other NTAEs like oil palm in southern Costa Rica (Piñero and Díaz Ríos, 2007). Although, this may provide immediate rent-based income for smallholders or income in the short term from the sale of their land, often small farmers struggle to transfer into another profession due to low education and professional experience. These losses of control either in land use decision-making or in land ownership are often detrimental in the long term as they can lead to land degradation and foster insecurity in the rural poor through dependency on wages and commodity booms that are typically temporary and unsustainable ecologically and economically (Amanor, 2012).

In reflecting on the social and environmental change caused by the expansion of pineapple, different stakeholders have distinct interpretations of how pineapple expansion plays into the larger vision of rural development. Stakeholders interested in sustainable development for both local farmers and local biodiversity often expressed concern about the economic and ecological vulnerability to pineapple expansion. As one representative of the SJLS biological corridor initiative said "I have a very encompassing vision of sustainability and I see that the pineapple scheme is not what is going to make the country advance in the theme of sustainable development or for the local people. We are betting on an export product that in any given moment the market changes, at an international level, the next day it is going to be Philippines or Ecuador or Hawaii... If the prices fall, the farmers here will be left in complete ruin because they are not owners of their farms, many times they sell or rent, lose control of the production, they lose control of their land and they all have big loans for machines, fertilizers and costly technology packages. It is a very big risk and for [forest] connectivity it is fatal."

In contrast, a pineapple company manager saw this expansion increasing employment and therefore development in an economically marginalized region. He explained, “Always, this type of company [agribusiness] brings development. For example, with 400 ha someone can handle more or less 300 cows. To handle 300 cows, they have to employ about three people. Pineapple needs one person per half hectare. That is to say, yes it brings development.” One of the largest forest landowners in the region reiterated this idea that pineapple companies develop the region and facilitate economic growth: “the town was here, but it was a very small town. There was no economic activity to speak of, I mean, a lot of people were just living off their land…when these pineapple guys came here, they improved a lot of stuff. They had the money to improve roads, they had the money to talk to politicians and bring infrastructure in here, I mean, you see now in this area, a lot of nice pick-ups driving around-- those are people that sold land for a good price here, so a lot of stuff has changed here.” These diverging descriptions demonstrate that people living and working in this landscape have conflicting ideas about a desirable path to development in this region and the long and short-term benefits of pineapple.

This rural development model, with its emphasis on large-scale production of pineapple and exclusion of smallholders, demonstrates the tradeoffs between national economic objectives for export growth and job creation and regional issues of equity, household food security and rural poverty alleviation (Tomich et al., 2001).

On a global scale, large agribusiness prevalence and smallholder exclusion do not always characterize NTAE crop production. For example, prior to 2000, the majority of the fresh pineapple imported to the European Union (E.U.) came from West African countries, where smallholder production and smallholder integration into the value chain predominated

(Fold and Gough, 2008). The primary reason pineapple production in Costa Rica has not followed a similar pattern is Del Monte's dominance in its market, which until 2003 held the exclusive patent to the MD2 pineapple variety. This monopoly excluded initial smallholder participation in the production boom and consolidated the pineapple value chain into the hands of large agribusinesses (Fold and Gough, 2008). MD2's recent introduction in Ghana is driving a shift from smallholder to agribusiness production systems, resulting in growth in land ownership concentration, agricultural wage labor dependence for livelihoods, and prohibitive production costs for smallholders (Fold and Gough, 2008; Amanor, 2012). As these aspects of the 'Costa Rican' model of pineapple production continue to be replicated globally in other NTAE crops, other regions may also experience similar changes to socio-economic characteristics and landscape composition.

Impacts of pineapple expansion on forest and future biodiversity conservation in the agricultural matrix

Given the proportion of original forest cover remaining, the study landscape can be categorized as fragmented (Table 2; McIntyre and Hobbs, 1999). However, results from FRAGSTATS analysis indicate the remaining old-growth forest is not highly subdivided, as the aggregation metrics SPLIT, PROX, ENN and LPI show (Table 3); the largest old-growth forest patch covers almost 7% of the total study region (Table 2). In accordance with the original criteria selected to establish the SJLS biological corridor, our results show that more than half of the total old-growth forest cover within the SJLS region is located within the corridor limits, and in contrast to the landscape outside the SJLS biological corridor, forest remnants within the corridor are considerably larger and less isolated (Table 3).

These results confirm the findings of Morse et al. (2009) and Fagan et al. (2013) that showed the 1996 Forestry Law and the system of payment for ecosystem services have been successful in promoting conservation of old-growth forest in this landscape. The matrix between these forest patches continues to change, though, and the assessment of how these changes affect remaining forest should become a priority.

Previous studies document that forest directly adjacent to agricultural land uses suffers from “edge effects”, which drive changes in forest microclimate, tree mortality, and in the abundance and distribution of animal species; the severity of edge effects vary depending on the type of adjacent land use (Fischer and Lindenmayer, 2007; Schedlbauer et al., 2007; Bouroncle and Finegan, 2011; Laurance et al. 2011). FRAGSTATS metrics such as core area (CORE), which describes the patch area free of edge effects, and edge contrast indices (TECI), which describe the proportion of forest edge in maximum contrast (Table A1), are useful metrics for assessing the impact of edge effects. TECI is based on the dissimilarity in vegetation structure between two adjacent land cover types; for example, new forest and old-growth forest would have low contrast values, whereas pineapple and old-growth forest would have high contrast values. When higher contrast land covers, such as bare soil, pineapple, or pasture are adjacent to forest, it reduces the core area of the forest patch that is free of edge effects (CORE) (Table 3). In the SJLS biological corridor there is a high incidence of old-growth forest patches that border high contrast land covers like pasture or pineapple and are thus vulnerable to strong edge effects (Table 3).

Euclidian distance to the nearest patch of the same type (ENN) and the proximity index metric (PROX) are also useful for assessing how old-growth forest patches are affected by the agricultural matrix (Table 5). A low value of the proximity metric indicates that the patch is more isolated and has more forest fragmentation in its surroundings (Whitcomb et al., 1981). Our results reveal that old-growth forest patches sharing a border with pineapple have higher ENN values and lower PROX values than similar patches bordered by pasture (Table 5), meaning that the patches surrounded by pineapple are dramatically more isolated. Interestingly, old-growth forest patches that share a boundary with pineapple have a larger mean area than those surrounded by pasture (Table 5). This is due to differences in production strategies between pasture and pineapple. Pastures often retain small old-growth forest patches, groups of trees, and riparian areas, which serve to provide water and shade for livestock. Furthermore, Fagan et al. (2013) found that between 2001 and 2011, pasture was three times more likely to revert to natural secondary regeneration than were croplands, including pineapple. In contrast, pineapple plantations seek to maximize continuous planted area, and therefore retain the old-growth forest patches protected by law but eliminate single trees or groups of trees within the production area, which can be important for connectivity. The isolating effect of pineapple on forest patches is a concern that conservation interests in the corridor identified. One reserve owner noted, "We have a small [forested] area that depends on the larger [protected] areas to have a diversity of organisms…we want to generate connectivity so that we do not become converted into an island surrounded by pineapple."

Table 1. 5 Mean patch area and isolation metrics for forested land cover classes.

	All patches			Share boundary with pasture (n=3,559)			Share boundary with pineapple (n = 402)		
	AREA (ha)	PROX	ENN (m)	AREA (ha)	PROX	ENN (m)	AREA (ha)	PROX	ENN (m)
Old-growth forest	57	28,892	121	29	31,459	115	44	3,202	176
New forest*		113	142						
Forest remnant		6	429						

* This land cover type includes secondary growth and native tree plantations. AREA: Mean patch size, PROX: Proximity Index, ENN: Mean Euclidean Nearest-Neighbor Distance.

Although the new forests land cover type occupies more than 55,000 ha in the landscape, the high number of patches (NP) of small mean size (AREA) with low mean proximity values (PROX) to other similar patches indicates that this type of vegetation cover is subdivided and isolated (Tables 1.2, 1.3). The new forest land cover type is equally distributed outside and within the SJLS biological corridor, but within the corridor, patches are less subdivided and represent a higher percentage of the total land area (Table 1.3). Within this land cover type, later stages of secondary growth are known to have different species composition but similar vegetation structure and tree species richness to old-growth forest, (Finegan 1996; Guarigauata and Ostertag 2001), and provide habitat for species of conservation concern (Fischer et al., 2006). Using high-resolution imagery allowed us to detect small (< 2 ha) old-growth and new forest patches not detected in previous studies using Landsat imagery (Fagan et al., 2013). These small forest patches grouped within the forest remnant land cover type represent a very low percentage of the landscape, but potentially serve as stepping-stones to enhance forest connectivity (Harvey et al., 2005; Hanson et al., 2007). For example, Hanson et al. (2008) found long-distance gene flow can be maintained among separated populations of canopy tree species through the connectivity stepping-stones of isolated trees or small forest patches provide.

Results of our analysis of fine-scale landscape features indicate that, among all land covers types analyzed, pineapple has the lowest percentage of tree cover per unit area, with the exception of banana plantations (Fig. 1.4). The greatest differences in tree cover were observed between pineapple and perennial crops, such as peach palm or fruit trees and pasture, which have twice the percentage of tree cover (3.9-4.7%) than pineapple plantations. Another important difference between pineapple versus pasture or perennial crops is the spatial distribution of tree cover. In pasture and crops, single trees and small groups of trees are retained within the land use rather than just at the edges, as in pineapple (Fig. 1.4). A pineapple producer explained the practice of maintaining only legally mandated tree cover within the plantations. There is a river that cuts across the plantation, and as he said, "I have to leave 60 meters or 30 meters on each side [of the river] and that makes lot[s] of hectares. Over there- there is a spring and with a spring you have to leave 1,000 meters around it. So that's how they form patches of forest. There are patches all over but when you combine them it's a lot of forested land." The practice of retaining forest cover only along riparian corridors is evident in Fig. 4, where it can be seen that trees in pineapple plantations (a) are confined to depressions or river corridors within the plots, leaving most of the plantation void of tree cover. In contrast, trees in pasture (b) are usually dispersed across a large area, creating patches of low and high tree density and maintaining heterogeneity within this land use.

Land Use	Tree cover (%)	STD
Banana	0.4	0.6
Pineapple	2.1	1.1
Annual crops	2.7	1.6
Perennial crops	3.9	1.9
Pasture	4.7	2.1

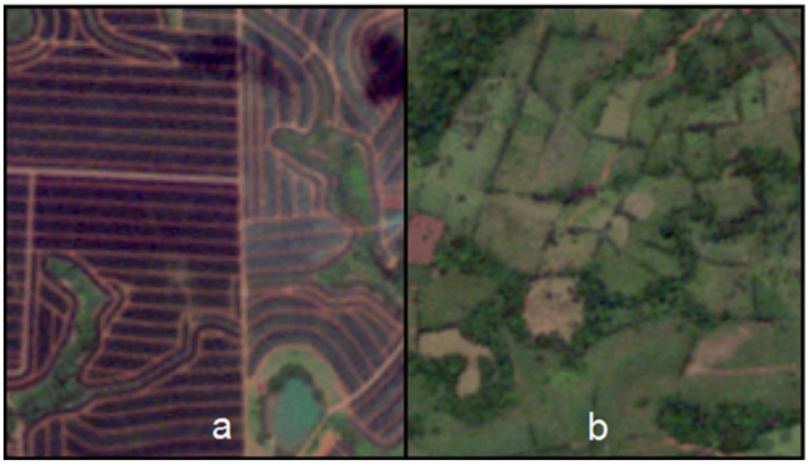

Figure 1.4 Mean percentage of area covered by fine-scale forest features such as single trees, groups of trees and live fences, in the dominant agricultural land cover categories: (a) Pineapple, (b) Pasture. Pictures correspond to 5 m resolution RapidEye imagery. STD is standard deviation.

Land cover types characterized by having either more scattered trees and live fences (Perfecto et al., 2003;Vaughan et al., 2007; Harvey et al., 2008), or vegetation structure that is more similar to natural forest cover (Brotons et al., 2003; DeClerck et al., 2010; Prevedello and Vieira, 2010; Eycott et al., 2012; Vilchez et al., 2014), are more likely to be used by wildlife for foraging, breeding, or as stepping stones to reach other habitat patches (Kupfer et al., 2006; Fischer and Lindenmayer, 2007; Harvey and Villalobos, 2007; Chazdon et al., 2009a; Gilbert-Norton et al., 2010; Vilchez et al., 2014). The reduced tree cover within pineapple plantations and the pronounced difference in vegetation structure between pineapple and natural forest suggest that pineapple likely reduces habitat availability and connectivity when compared to other land cover types such as pasture or annual and perennial crops.

The SJLS region retains a significant proportion of old-growth forest cover, but our analyses show conversion of smallholder crops and pasturelands to pineapple plantations affects forest cover, leading to loss of total tree cover and of landscape heterogeneity. Furthermore, our pineapple suitability analysis suggests that if road development and favorable market conditions continue, pineapple plantations will further spread into the SJLS biological corridor. These findings emphasize the importance of developing effective policies to mitigate current and future impacts of pineapple expansion on the linked social and ecological systems in the study region.

Current policy on pineapple at a landscape scale

Policy discussions about the future of pineapple in Costa Rica have been occurring at the national level through the National Pineapple Platform (Plataforma Nacional de Piña - PNP), which is a two-year participatory dialogue hosted by the United Nations Development Program, the Ministry of Agriculture and the Ministry of the Environment. Participants in this dialogue have developed an action plan for 2013-2017 (http://www.pnp.cr/plan.php), focusing mostly on actions to improve practices at the farm level. However, according to leaders in the SJLS biological corridor initiative, “There are management standards but they are focused completely on the plantation; there is no vision of the landscape.”

Municipalities are also important players in forming policies to regulate pineapple. They have legal power to develop a territorial land use-zoning plan called a “plan regulador” which can direct where pineapple expands and limit its growth if desired. This plan is the best mechanism municipalities have to effectively partition public and private land and exclude certain land uses or developments, but most rural municipalities do not have current or well-developed plans (Pérez Peláez and Alvarado Salas, 2003). “Sometimes, there are not sufficient resources to do studies, because of this they [municipalities] get behind a bit…so until they do the studies, they cannot determine legally, under their land use zoning plan, what is the zone for this [X] land use,” explained a representative of the National Environmental Technical Secretariat (Secretaría Técnica Nacional Ambiental).

Conclusions

Our results reveal how pineapple expansion produces social and environmental change with local conservation implications. In particular, our synthesis of data suggests that pineapple concentrates land, labor, and financial resources on the landscape, thereby increasing the homogeneity of the agricultural economy in the study region. When spatially heterogeneous pastures with tree cover or smallholder farms are converted to monoculture plantations dominated by agribusinesses, the loss of autonomy (i.e., land ownership or land use decision-making) constrains farm-based livelihoods, food security and agricultural diversity. Pineapple production also simplifies and homogenizes the agricultural matrix between forest patches. It further isolates old-growth forest patches, and reduces total tree cover, all of which are critical for maintaining connectivity of remnant forest patches. Since biodiversity in agricultural landscapes is positively associated with percent of tree cover and landscape heterogeneity, the continued spread of pineapple plantations is likely to have a negative effect on biodiversity conservation.

Despite pineapple's negative influence on some social and ecological components of the landscape, in some ways the SJLS region represents a best-case scenario. Strict and innovative regulatory and incentive schemes have successfully promoted retention of old-growth forest cover, and pineapple is just beginning to dominate agricultural land use. Spatially heterogeneous smallholder production systems and pasture with tree cover are still abundant within the corridor and contribute to forest connectivity.

To protect biodiversity and promote inclusive rural development in the face of pineapple expansion we propose several landscape-level policy and management approaches. First, sustainable production must be incentivized. In the SJLS region there has been unprecedented inter-institutional dialogue and coordination to develop an action plan for sustainable pineapple production, which is summarized in the PNP action plan. Second, policies that encourage landscape-level planning (Sayer et al., 2012) should be established to promote land use heterogeneity and economic diversity within the agricultural sector. Retaining smallholder agriculture as a viable livelihood should be a priority for both conservation and agricultural policy makers, as smallholders are critical contributors to rural poverty alleviation, food security, landscape heterogeneity and crop diversity (Dahlquist et al., 2007; Fisher et al., 2008; Perfecto and Vandemeer, 2008; Tscharntke et al., 2012). Third, landscape level planning should follow national level policies such as the Costa Rican 2021 carbon neutrality goal. This goal has already motivated several multinational agribusinesses to establish carbon neutral production strategies (Kilian et al., 2012). Agribusinesses could also commit to retaining more forest cover within plantations or to forest offset programs; this would contribute to their goals of offsetting carbon emissions while also increasing habitat connectivity. However, any investments toward carbon neutrality or sustainable production by agribusinesses need to be matched throughout the value chain by retailers in marketing and setting higher selling prices to offset these investments. Fourth, the Forestry Law of 1996 should be updated to more effectively target conservation and restoration of both riparian and secondary forest to promote increased habitat connectivity (Fremier et al., 2013) and move Costa Rica closer to its goal of carbon neutrality. Current conservation regulations in Costa Rica protect old-growth forest, while creating perverse incentives that block regrowth of secondary forest (Sierra and Russman, 2006; Morse et al., 2009; Fagan et al., 2013) despite evidence that secondary forests contribute to carbon sequestration (Pan et al., 2011).

Due to the global relevance of balancing local economic growth with biodiversity conservation, this Costa Rican case study can serve as a model against which to compare other regions currently undergoing rapid expansion of NTAE crop production. Indeed, understanding the social-ecological impacts of agricultural intensification in tropical regions is a critical piece of promoting the sustainability of rural agrarian development around the world. As shown in this study, landscapes operate as integrated social-ecological systems, and must be managed holistically to retain spatially and economically diverse land uses that support sustainable rural livelihoods and create a balance between agricultural production and biodiversity conservation.

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Chapter 2: Flower visitation and potential Pollinators for understory populations of a Neotropical canopy tree species, *Symphonia globulifera.*

Abstract

One difference between the forest canopy and the understory is that animals pollinate the majority of understory species in the tropical wet forest. Pollinators active in the understory are also different from those in the forest canopy and are adapted to the mesic conditions underneath the canopy. Recent studies have shown a relationship between visitation frequency and overall contribution to pollination, in which the most frequent pollinators in the majority of cases account for the most important pollinator for fruit set. In this study we used video cameras to observe flowers of understory *Symphonia globulifera* (Clusiacea) in the tropical west forest of Costa Rica. We quantified the timing, frequency and behavior of flower visitors to explore their potential contribution to pollination. A total of 82 flower visits were observed during 105 h of observations. Flowers were visited by ten insect species and one hummingbird species, the most active time period was 1200-1700 h followed by the time between 0500-1000 h. The time period with fewer visitors was 1700-2200 h, during this period we observed flowers visited in multiple instances by a cricket (Orthoptera). In this study the species of flower visitors with more interactions with flowers and which display a behavior of potential pollinators are the stingless bee *Tetragonisca angustula* and the hummingbird *Phaethornis longirostris*. Results from this study differ from those reported for canopy populations of *S. globulifera*, we do not observe perching birds and insects are predominant in contrast from observation in canopy populations of *S. globulifera*. These findings highlight the difference on flower visitors and potential pollinators between the forest canopy and the understory; flowers visitors in understory are different from those of canopy populations for the same tree species.

Introduction

Tropical wet forests (TWF) are unique ecosystems characterized by a distinct and immense diversity of taxa and complex vertical and horizontal structure. One characteristic that differentiates TWF from other forest biomes is that animals pollinate the great majority of tree species (Bawa et al. 1985; Dick et al. 2008). Differences in behavior and range of movement between pollinators influence the distance pollen is transported at a local scale, in addition the pollinator community can vary among forest vertical strata (Dick et al. 2008). Insects are the most important pollinator group, vertebrates like birds and bats also serve as pollinators for a lower fraction of TWF species (3-11%) (Dick et al. 2008; Fleming et al. 2009). Among insects, bees constitute the most important group in number and diversity of plant species pollinated (Bawa 1990).

Pollinator community surveys traditionally have been performed by direct observations and more recently by photographs and continuous video recording (e.g. Bawa 1990; Quesada et al. 2003; Tschapka 2003; Lortie et al. 2012; Bartos 2013). Identification of flower visitors and frequency of visits are integral components to evaluate animal pollination and provide an understanding of the plant-animal interactions that facilitate plant reproductive success (Stebbins 1970; Bawa 1990, Vazquez et al. 2005). There are two key components of pollinator actions that shape pollinator performance: frequency and effectiveness of flower visits (Ne'eman et al. 2010). Visit frequency can be simply defined as the number of visits to a flower per unit of time. Effectiveness, also called efficiency, is open to various interpretations and it relates to the pollinator´s behavior during flower visits (visit duration, contact with reproductive structures), and the amount of pollen carried away and deposited on receptive flowers (Sahli & Conner 2006; Ne'eman et al. 2009). Visitation frequency has been suggested, however, as an accurate surrogate of pollinators contribution to overall reproductive success (Vazquez et al 2005, Sahli & Conner 2006; Ne'eman et al. 2009). Metanalysis on plant-animal pollination datasets indicate the most frequent flower visitors account for >50% of the total pollination service (Vazquez et al 2005; Sahli & Conner 2006).

In this study we identified the organisms that visit *Symphonia globulifera* flowers. *S. globulifera* is a Clusiaceae distributed across the Neotropics, perching birds and hummingbirds have been suggested as the most important pollinators observed in canopy populations (Degen et al. 2004, da Silva Carneiro et al. 2007, Dick & Heuertz 2008). We surveyed understory *S. globulifera* flowers in mature lowland TWF forest sites of Costa Rica with video cameras to identify flower visitors. *S. globulifera* is an understory tree in our study area, while in other regions it is a canopy tree (Degen et al. 2004, da Silva Carneiro et al. 2007, Dick & Heuertz 2008). Thus, we hypothesized flower visitors of understory populations will be different from those of the canopy populations. We quantified the timing, frequency and behavior of flower visitors. Visitation frequency and foraging behavior are discussed to explore the potential contribution to pollination from the observed flower visitors. We also discuss differences between the results of our study with those of canopy populations of *S. globulifera*.

Materials and Methods

Study area

This study was conducted in three mature forest patches within the Sarapiquí County, part of the Heredia province located in the Caribbean lowlands of northern Costa Rica, centered at latitude 10.440588 North and longitude -84.115308 West. The study area is a 100 Km^2 polygon that contains all three research sites (Figure 2.1). This area is characterized by elevation that ranges from sea level to 300 m a.s.l.; terrain is a mixture of alluvial terraces, swamplands, and steep hills (Sesnie et al. 2008). Mean annual temperatures average 24˚C and mean annual precipitation, 4000 mm per year (Sesnie et al. 2008). Land use is dominated by pasture and recently pineapple has greatly expanded in the landscape. Other crops are also present intermixed mixed with mature and secondary forest patches (Shaver et al. 2015).

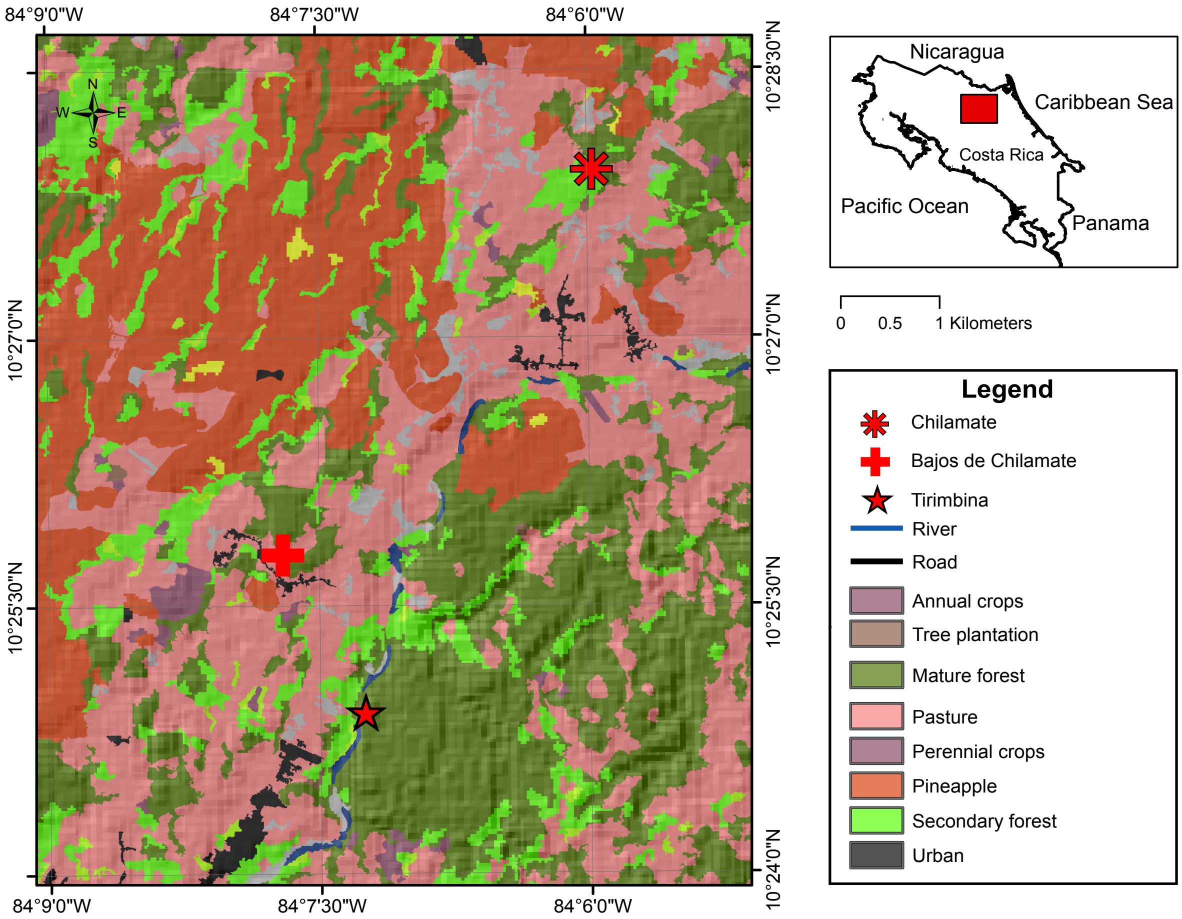

Figure 2.1 Study area, land cover and three sites were pollinator's observations took place.

Study species

Symphonia globulifera (Clusiaceae) is a shade tolerant tree species distributed in rain forests across the Neotropics and equatorial Africa (Dick & Heuertz 2008). It is the only recognized species in its genus found outside of Madagascar where 16 *Symphonia* species are present (Abdul-Salim 2002). Although *S. globulifera* are typically large canopy trees (Degen et al. 2004, Woodward 2005, Dick & Heuertz 2008), populations in the Sarapiquí region in Costa

Rica occur only as understory trees with an adult minimum reproductive size of 1 cm diameter at breast height (dbh, 1.3 m; personal observation). In French Guiana *S. globulifera* are large canopy trees that exist in two distinct forms, one with big leaves and flowers and the other with small leaves and flowers, they are treated as separate species by local forestry managers (Degen et al. 2004). None of this morphological variation has yet been considered sufficient to merit splitting of *S. globulifera* into more than one Neotropical species (Dick et al. 2003, Dick & Heuertz 2008).

Inflorescences of *S. globulifera* consist of 1-15 axillary flowers that are bisexual (Aldrich et al. 1998; Woodward 2005). Flowers are scarlet red, odorless, globose in shape and grow more or less vertically on branches. At anthesis, petals contort and form a chamber in which nectar accumulates. Access to the interior chamber for flower visitors is only possible at the apex between the incurved petals and the staminal tube. The staminal tube surrounds the pistil, anthers are adenate to the lobes of the staminal tube and open abaxially to display pollen immersed in a sticky, oily substance (Bittrich & Amaral 1996; Gill et al. 1998). A well-developed nectary surrounds the staminal tube at its base. The stigma is shaped like a five-lobed star, with small pores at the apices of each lobe (Bittrich & Amaral, 1996). Pollination of the small scarlet flowers has been described as facilitated by nectar-feeding birds, mainly hummingbirds (Bittrich & Amaral 1996; Gill et al. 1998; Woodward 2005) however, visits by bees from the Meliponini and Euglossini groups have been documented in our study area (Rincón et al. 1999). *S. globulifera* seeds are large 4–5 cm drupes consumed and dispersed by bats and monkeys (Aldrich et al. 1998). The species is usually > 90% outcrossed (Degen et al. 2004; da Silva Carneiro et al. 2007), although higher levels of self-fertilization (>10%) have been documented in disturbed habitats for the canopy populations in Costa Rica (Aldrich et al 1998).

Fieldwork

Trees were chosen based on the availability of flowers and accessibility from three mature forest sites that offered security for the video recording equipment. In total 25 flowers were observed, six flowers from one tree in Tirimbina, nine flowers from one tree in Chilamate and ten flowers from two trees (five each) in Bajos de Chilamate. Video recordings were performed during May and June 2013, using a Sony Digital Handycam HDR-SR10 with supplemented infrared light during nighttime. The cameras were placed inside waterproof cases, sufficiently close (less than 3 m) to the flower to allow clear vision of the anthers and stigma. Video recordings were made during flower anthesis in three time periods: 0500-1000 h, 1200-1700 h and 1700-2200 h. In total 105 hours of video recordings were analyzed to assess flower visits, 35 hours from each time period.

Data Analysis

Video observations and analyses were performed using Adobe Premier software, through visual identification of the arrival of flower visitors. Animals observed were only considered visitors if they touched the stigma, anther or consumed nectar from the flowers. Visits in which no contact was made with the stigma or anthers, or no nectar was consumed, were not considered. Most of these latter cases were of ants that roamed around the flower petals. For each pollinator visit the following data were recorded: still image; duration of visit; contact with the stigma or anther and if pollen or nectar feeding was observed. We considered pollen or nectar consumption if the buccal apparatus of the visitor touched the anther or accessed the nectar chamber and feeding behavior was displayed (Sakamoto et al. 2012). Still images from the video were selected and used for identification at la Selva Biological Station by R. Vargas. We calculated the visitation rate for each species, defining it as visits per flower per hour for each single recording period, then averaged across all observation periods.

Results

A total of 82 visits to *S. globulifera* flowers were observed during the 105 hours of evaluated video recordings. The flowers were visited by ten insect species and one hummingbird species (Table 2.1, Figure 2.2). We were unable to confidently identify two species, one small flying insect, probably a small Hymenopteran or Dipteran, and a nocturnal lepidopteran probably of the family Geometridae. These two visitors accounted for one observation each and were not considered in further analysis.

Table 2. 1 Species visiting flowers of *S. globulifera*, number of visits and frequency of visitation

Class, Order	Family	Species	N	Frequency
Insecta, Hymenoptera	Apidae	*Trigona sp. 1*	2	0.03
		Trigona sp. 2	1	0.01
		Trigona sp. 3	5	0.03
		Tetragonisca angustula	25	0.29
	Formicidae	*Pseudomyrmex sp. 1*	1	0.01
		Crematogaster sp. 1	7	0.10
		Solenopsis sp. 1	17	0.25
	Vespidae	*Polybia sp. 1*	3	0.04
Orthoptera	Tettigoniidae	*Tettigonia sp. 1*	9	0.09
Aves, Apodiformes	Trochilidae	*Phaethornis longirostris*	12	0.13

From the order Hymenoptera we observed four species of bees, from the Apidae family *Tetragonisca angustula* and three species from the genus *Trigona*. Various ants (Formicidae) were observed: one species of *Pseudomyrmex, Crematogaster* and *Solenopsis*. One wasp (Vespidae) from the genus *Polybia* was also present. Additionally we recorded one species of hummingbird (Trochilidae) *Phaethornis longirostris* and one cricket (Tettigoniidae), *Tettigonia* sp.1

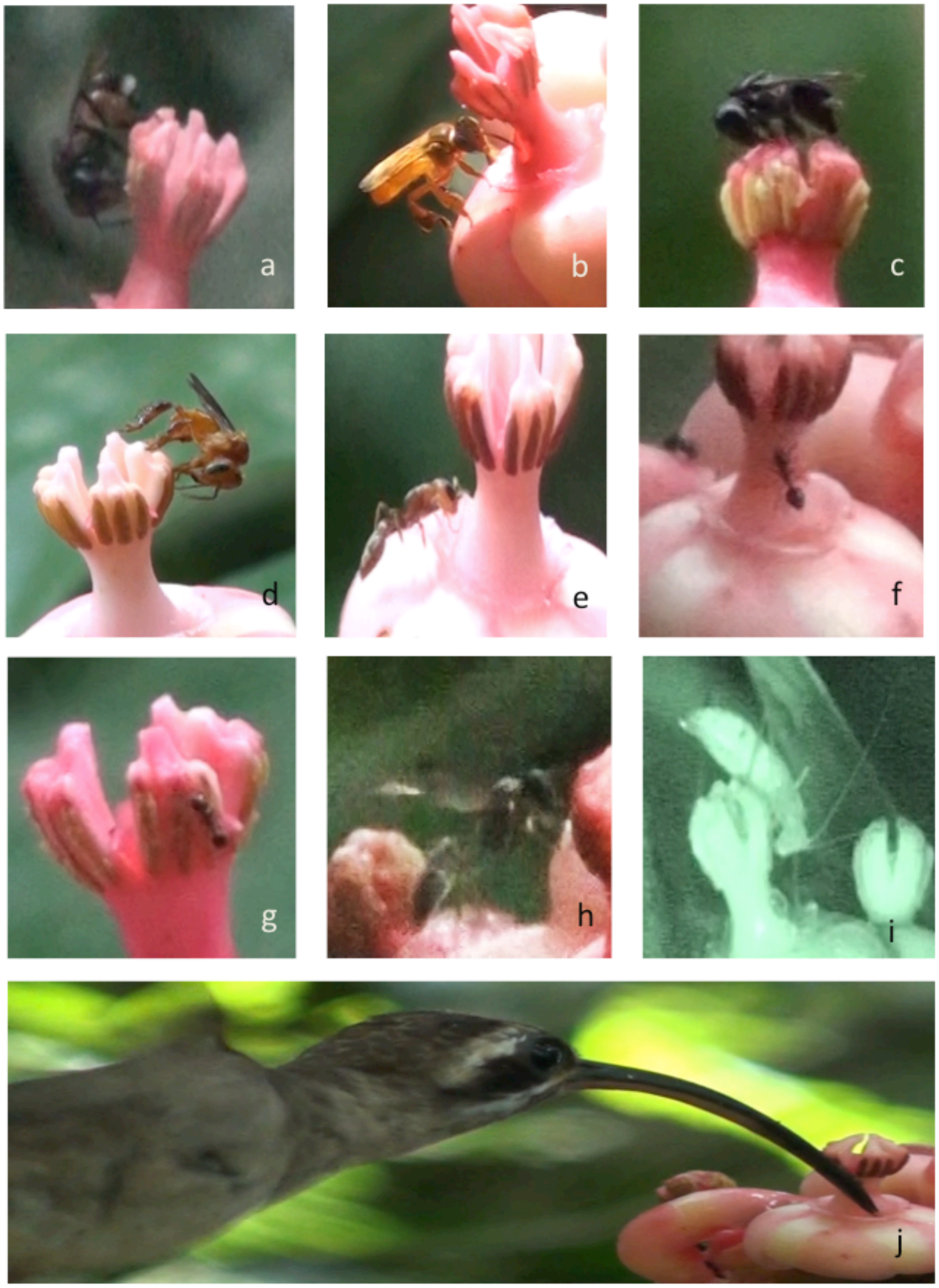

Figure 2.2 Symphonia globulifera flower visitors (a) Trigona sp. 1, (b) Trigona sp. 2, (c) Trigona sp. 3, (d) Tetragonisca angustula, (e) Pseudomyrmex sp. 1, (f) Crematogaster sp. 1, (g) Solenopsis sp. 1, (h) Polybia sp. 1, (i) Tettigonia sp. 1, (j) Phaethornis longirostris.

Considering all observations together the bee *Tetragonisca angustula* was the most frequent flower visitor. It was followed by the ant *Solenopsis* sp. 1, which was present on many occasions during diurnal observation periods. The hummingbird *Phaethornis longirostris* ranked third in visitation frequency with 12 observed visits. Other flower visitors were observed which account for a lower visitation frequency (Table 1). Visitation activity varied between time periods (Table 2.2). Visitors were more abundant during the 1200-1700 h time period with seven species recorded during this period. We only observed *Pseudomyrmex sp. 1*, *Crematogaster sp. 1, Polybia sp. 1* during this period. In contrast, *Trigona sp. 3*, *Tetragonisca angustula*, *Solenopsis* sp. *1* and *Phaethornis longirostris*, were observed during both (0500-1000 h; 1200-1700 h) time periods. The least active time period was between 1700-2200 h; the only visitor observed more than once during 1700-2200 h was a cricket (*Tettigonia* sp. 1), which was only observed during this period.

Table 2. 2 Number of *S globulifera* flower visits and frequency of visits by time period.

	0500-1000 h		1200-1700 h		1700-2200 h	
Species	N	Frequency	N	Frequency	N	Frequency
Trigona sp. 1	2	0.07	0	0.00	0	0
Trigona sp. 2	1	0.03	0	0.00	0	0
Trigona sp. 3	1	0.03	4	0.09	0	0
Tetragonisca angustula	11	0.37	14	0.33	0	0
Pseudomyrmex sp. 1	0	0.00	1	0.02	0	0
Crematogaster sp. 1	0	0.00	7	0.16	0	0
Solenopsis sp. 1	10	0.33	7	0.16	0	0
Polybia sp. 1	0	0.00	3	0.07	0	0
Tettigonia sp. 1	0	0.00	0	0.00	9	1
Phaethornis longirostris	5	0.17	7	0.16	0	0
TOTAL	30		43		9	

Table 2. 3 Foraging behavior metrics for visitors of *S. globulifera* flowers.

Species	Visitation rate (Number of visits/hour)	% of visits that contact stigma	contact anther	fed nectar	fed pollen	Visit duration Mean	SD	Min-Max
Trigona sp. 1	0.02	100	100	0	50	11	1.4	10-12
Trigona sp. 2	0.01	0	100	0	0	5	0	5-5
Trigona sp. 3	0.06	80	80	20	80	11.5	13.4	2-21
Tetragonisca angustula	0.28	80	84	16	76	118.2	142.6	4-562
Pseudomyrmex sp. 1	0.01	0	0	100	0	181	0	181-181
Crematogaster sp. 1	0.06	0	0	100	0	74.57	95.5	16-289
Solenopsis sp. 1	0.21	71	88	29	76	143.65	105.1	18-453
Polybia sp. 1	0.03	100	100	0	67	18	11.5	7-30
Tettigonia sp. 1	0.09	78	100	33	100	515	545.3	115-1445
Phaethornis superciliosus	0.13	83	90	100	0	8.44	11	1-36

Visitation rate calculated as the average number of visits per hour reveals the number of interactions per unit of time. The highest visitation rate was for the bee *Tetragonisca angustula* with 0.28 visits flower^{-1} h^{-1}, followed by the ant *Solenopsis sp. 1* with 0.21 visits flower^{-1} h^{-1} and the hummingbird *Phaethornis longirostris* with 0.13 visits flower^{-1} h^{-1} (Table 3). Other diurnal visitors showed lower visitation rates, some of which represent a single visit (Table 2.1 and 2.3). During the 1700-2200 period *Tettigonia* accounted for 0.9 visits flower^{-1} h^{-1}.

Foraging behavior during flower visits varied between species (Table 2.3). Eight species came in contact with the anthers during flower visits; only two ant species (Pseudomyrmex sp. 1 and Crematogaster sp. 1) did not touch the anthers while visiting flowers. Considering the species that touched the anther, six were observed consuming pollen, that is, their buccal apparatus touched the anther area. We observed that seven species came in contact with the stigma while consuming pollen or nectar during flower visits. Observations showed that seven species consumed nectar from flowers; the ants *Pseudomyrmex* and *Crematogaster* only visited flowers to consume nectar and did not touch the anther or stigma. The hummingbird *P. longirostris* was the only species capable of accessing the internal chambers formed by the flower petals where nectar is accumulated employing the long beak and tongue to consume the available nectar. During the short visits of *P. Longirostris,* we witnessed direct contact between the upper beak and flower anthers and stigmas. Immediately after these visits we observed ants that consume nectar residues in the locations where *P. longirostris* inserted its beak. In general visits by the ant *Solenopsis* sp.1 consisted of constant roaming around the flower, and we observed pollen and nectar consumption during flower visits. They moved over the anther multiple times during a visit and in some cases individuals were present near or on the petals of the flower for the entire filming period. The other ant species, *Pseudomyrmex sp.1,* and *Crematogaster* sp.1, were less frequent visitors and showed a similar behavior of roaming around the flower and consuming nectar.

The stingless bee *T. angustula* was the most frequent flower visitor with a mean visit duration of 110 seconds. This bee spent most of the time eating and collecting pollen, most of its body touched the anther and on many occasions the abdomen and legs contacted the stigma. Three species of *Trigona* bees also visited *S. globulifera* flowers, these visits were less frequent and their duration was shorter. One species of wasp, *Polybia* sp., was also observed three times, during visits it consumed pollen and roamed around the flower coming in contact with the stigma. *Tettigonia* sp. 1 was the only visitor during the 1700-2200 hours observation period. It was observed after sunset touching the anther and stigma, this species accounts for the longest duration of visits with a mean value of 515 seconds. During visits

Tettigonia sp. 1 spent most of the time consuming pollen and many parts of the upper body came in contact with anthers and on some occasions touched the stigma.

Discussion

This research provides evidence that a different community of pollinators facilitates pollination of forest understory plant species. Our results show that frequent flower visitors observed in understory populations of *S. globulifera* are distinctive from those reported for canopy populations of the same species. Nine insect species and one hummingbird are the most common and abundant flower visitors for *S. globulifera* in the understory populations of the Sarapiquí region in Heredia, Costa Rica. Suggesting that insects and understory birds facilitate *S. globulifera* pollination in contrast with canopy populations of *S. globulifera* in South America were perching birds are suggested as the main pollinators (Bittrich & Amaral 1996, Gill et al. 1998).

Flower visitors documented by this study are known to play an important role in the pollination of many plants in the TWF understory. Bees (Apidae) are often the most frequent visitors of flowers and the predominant pollinators for most plants and ecosystems (Winfree et al. 2011, Neff & Simpson 1993). Hummingbirds (Trochilidae) are found only in the Americas, and include 328 flower-visiting species (Winfree et al. 2011). In TWF hummingbirds are responsible for the pollination of herbaceous monocots in the genus *Heliconia* and also regularly visit flowers from a wide range of species. In some cases hummingbirds have been reported as nectar thieves and not true pollinators (Muchhala et al. 2008; Hadley et al. 2014). Ants visiting flowers are usually considered non-pollinating insects (Chacoff & Aschero 2014; Dutton and Frederickson, 2012; Hull and Beattie, 1988). However there is evidence that ants can be pollinators since they are common flower visitors and are able to carry pollen that results in seed set (de Vega et al. 2009; Ashman & King 2005, Kawakita and Kato, 2002). Orthopterans are well known for herbivory, and this insect order is not typically considered as pollinators (Schuster 1974; Proctor et al., 1996). However, Micheneau et al. (2010) reported that in wet lowlands forests the orchid *A. cadetii* seems to rely on the pollination of an orthopteran to achieve fruit set.

During flower visits we observed that most insects came in contact with the anther and displayed pollen consumption behavior during flower visits. For these flower visitors it appears that the reward for visits was the pollen and oil solution present at the anther (Bittrich & Amaral 1996). Many flower visitors made contact with the stigma and may therefore be potential pollinators. It is during this stigmatic contact that transfer of pollen that results in ovule fertilization could occur. Visits from *Pseudomyrmex* and *Crematogaster* ants did not involve contact with the anther or stigma and may represent simply a search for nectar without any potential contribution to flower pollination. *Solenopsis* sp. 1 ants, one of the most frequent flower visitors, displayed similar behavior, though they moved all over the flower, and we observed pollen consumption and brief contact with the stigma. However, we saw that individuals from this species tend to stay in a single group of flowers for many hours exhibiting an opportunistic behavior after hummingbird visits at which ants seemed to be consuming nectar residues left on flower petals after the feeding. For these reasons we conclude that the potential of ants as pollinators for *S. globulifera* is minimal. The presence of ants did not seem to discourage other flower visitors since in many occasions flower visits occurred with the presence of ants roaming on the flower petals.

Our results contrast markedly with studies of canopy populations of *S. globulifera.* In undisturbed lowland TWF of French Guyana the most frequent and persistent flower visitors were five perching bird species of the family Thraupidae (Gill et al. 1998). Hummingbirds were also reported as persistent flower visitors, while no insects were observed and all flower visits were diurnal (Gill et al. 1998), while in Sarapiquí, the cricket *Tettigonia* sp. visited flowers during the 1700-2200 hr period. In disturbed lowland TWF of Brazil, also for canopy populations, two species of Trochiline hummingbirds were the most frequent flower visitors (Bittrich & Amaral 1996). Insect visitors were also observed including *Trigona* bees. *Trigona* bees displayed destructive behavior by chewing petals to access nectar damaging or completely destroying the flowers; therefore, they act as nectar thieves, not pollinators for these populations (Bittrich & Amaral 1996).

The most frequent flower visitors are likely the most important pollinators since visitation rate is a strong predictor of pollinators' performance (Vazquez et al. 2005). We quantified interactions with the visitation rate per hour, this rate allows us to identify species with the most frequent interactions and therefore with greater potential for the pollination of *S. globulifera*. Our results suggest that considering foraging behavior and visitation rate the bee *T. angustula* and the hummingbird *P. longirostris* have the greatest potential contribution to the pollination of *S. globulifera*. Most flower visitors exhibit foraging behavior that involved contact with anther and stigma, suggesting a possible contribution to pollination. The frequent visitors usually contribute disproportionately to the plant's reproductive success, even if their effectiveness is relatively low (Vazquez et al. 2005).

Tetragonisca angustula was the most frequent flower visitor, behavior during flower visits involved the consumption of pollen and in many instances their body parts came in contact with the stigma of the flowers. Visitation rate for this species is the highest (0.28 visits flower^{-1} h^{-1}) more than twice that of the hummingbird (0.13 visits flower^{-1} h^{-1}). In this sense this is the flower visitor with the greatest interaction with *S. globulifera* flowers in this landscape. Not only it is a more frequent flower visitor than the hummingbird, the duration of visits is also longer allowing for lengthier flower interaction time and contact with the flower stigma. This stingless bee is distributed from Mexico to Argentina, one of the most widespread bee species in the Neotropics (Silveira et al. 2002; Camargo & Pedro 2013). They are generalists in their habits and have been identified as pollinators of many Neotropical plant species (Braga et al. 2012).

Hummingbirds were observed as frequent flower visitors for this understory tree population, which concurs with observations in other populations of canopy *S. globulifera* across the Neotropics (Bittrich & Amaral 1996; Gill et al. 1998). *P. longirostris* behavior during flower visits suggests they are potential pollinators since they come in contact with the anther and stigma while consuming nectar from flowers. Visitation rate for this species was 0.13 visits flower^{-1} h^{-1}; visits were short (mean 8 seconds); however, we observed contact between the upper beak and the anther and stigma on more than 80% of visits. *P. longirostris* is a known *Heliconia* specialist (Snow and Texeira 2005); evidence suggest this hummingbird species is tolerant of some degree of forest fragmentation, associated to the amount of forest habitat remnant in the landscape (Hadley & Betts 2009; Volpe et al. 2014). *P. longirostris* is associated with understory habitats of mature and old secondary forests (Johnsgard 1997, Skutch and Dunning 1979).

Conclusion

Previous study on canopy populations of S. *globulifera* described perching birds and hummingbirds as the most frequent flower visitors with no insects reported as potential pollinators. In this understory population our results describe a different community of visitors to *S. globulifera* flowers. 12 species belonging to 5 families were observed visiting flowers, 11 species of insects and one hummingbird. This suggests a shift in flower visitors between canopy and understory populations, emphasizing the difference between canopy and understory dynamics even for the same species. Our results show different pollinators from those of canopy populations pointing to possible hurdles for gene flow between canopy and understory population given that the most frequent pollinators observed by this study are associated with the forest understory rather than the forest canopy. Species observed visiting flowers in both canopy and understory populations are *Trigona* bees, suggested as nectar thieves. Nonetheless, these bees could be responsible for pollen exchange between canopy and understory population. Further research in this area is needed to better understand gene flow between canopy and understory population of *S. globulifera*.

Species with more interactions with flowers and which display a behavior of potential pollinators are *T. angustula* and *P. longirostris*, during visits they come in contact with anther and stigma which can allow pollen transfer between conspecific adults resulting in effective pollination and seed production. Hummingbirds and bees, even if tolerant to forest fragmentation, require forest habitat to persist in the landscape (Brosi et al. 2008; Volpe et al. 2014). Thus, it is likely that forest fragmentation and subsequent land uses in the matrix can influence patterns of movement for these species and consequently the exchange of pollen for *S. globulifera* and other understory species throughout the landscape.

The present study increases understanding of flower visitors and pollination in the tropical forest understory specifically for *S. globulifera*. Further research on the deposition of pollen by each species can contribute to a more in depth evaluation of individual pollinator's contribution to overall reproductive success. Effects of forest fragmentation should also be assessed for potential pollinators and *S globulifera* populations in order to better understand the implications of fragmentation and habitat loss on the resilience of understory plant-animal mutualistic relationships throughout the TWF.

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Chapter 3: Forest fragmentation and agricultural intensification reduce genetic diversity and increase genetic structure within understory populations of Symphonia globulifera (Clusiaceae).

Abstract

Tropical wet forests are unique ecosystems characterized by a distinct and immense diversity of species and complex vertical and horizontal structure, composed of plant species almost exclusively outcrossed. Forest fragmentation and consequent land cover change are known to have an overall strong negative impact on plant reproduction with different effects on individual plant species. Most studies of the impacts of fragmentation on tropical tree species have focused on canopy tree species, especially those that are valuable for timber, which represent a low fraction of plant species present in tropical forests. Using nine nuclear DNA microsatellite loci, we examined the effects of forest fragmentation and landscape composition on the genetic diversity and structure of understory *Symphonia globulif*era in the wet lowland tropical forests of northeastern Costa Rica. Our study site encompasses the San Juan-La Selva biological corridor and surrounding areas, and within this fragmented landscape we compare genetic diversity and genetic structure across three life stages (adults, saplings, seedlings) using 324 samples per cohort and 9 nuclear DNA microsatellite loci. We predicted lower genetic diversity and increased genetic structure between the younger life stages since these individuals were likely established under current fragmentation conditions and limited movement of seed and pollen will promote greater differentiation between populations. Results followed these expectations with significantly lower numbers of alleles, observed heterozygosity, Shannon's information index, and allelic richness in seedlings compared to adults. Inbreeding, measured by F_{IS}, exhibited an increasing trend from adults

(0.009) to seedlings (0.029). We predicted genetic diversity of seedlings and saplings would be correlated with the percent of forest in the contemporary surrounding landscape while there would be no correlation for adults. Land cover maps for the years 1986, 1996, 2001 and 2011 were used to relate landscape composition with genetic parameters. The amount of forest and pasture surrounding remnant mature forest patches positively correlated with the observed patterns of genetic diversity. Genetic differentiation among adult trees was low (mean pairwise G’st = 0.15), probably reflecting larger population sizes and broad gene flow in the past. Genetic differentiation of seedlings was significantly higher (mean pairwise G’st = 0.22) probably reflecting smaller effective population sizes and more restricted gene flow in the current landscape. Results from this study provide evidence that forest fragmentation and land use change have significantly reduced genetic diversity, increased inbreeding and reduced gene flow for *S. globulifera* understory populations. However, gene flow and diversity were significantly higher inside the biological corridor than outside, indicating that conservation measures are providing a positive impact for this species.

Introduction

In recent decades, tropical forests have experienced high rates of deforestation, which have reduced many large continuous forests to isolated patches in fragmented landscapes (Gardner *et al.* 2009). The resulting landscape is a composite of many different land uses that aggregate to form an agricultural and human infrastructure matrix in which forest fragments are imbedded and subject to many different perturbation regimes. The conservation of natural areas and the promotion of connectivity among these areas have emerged as an important approach to achieve biodiversity conservation in many regions (Laurance 2008; Harvey *et al.* 2008). Biological corridors have been implemented with the goal to improve functional connectivity of ecosystem processes and maintain landscape resilience (DeClerck *et al.* 2010; Cushman *et al.* 2013). Biological corridors have been shown to provide an overall increase in functional connectivity with greater benefits for the movement of invertebrates, nonflying vertebrates, and plants than for birds (Gilbert-Norton *et al.* 2010).

Tropical wet forests are unique ecosystems characterized by a distinct and immense diversity of species and complex vertical and horizontal structure. In the tropical wet forest, one characteristic that differentiates this system from others is that animals pollinate the majority of tree species (Dick et al. 2008, Bawa 1985). Low population density and high dependence on animal-vectored pollination and seed dispersal make many tropical tree species particularly vulnerable to fragmentation effects (Bawa *et al.* 1985; Nason & Hamrick 1997; Hanson *et al.* 2007; Aguilar *et al.* 2008). Pollination and seed dispersal processes impact genetic diversity and genetic structure in plant species (Schaberg *et al.* 2008; Ashley 2010). Animal-mediated seed dispersal exerts a strong influence on the distribution and dynamics of tropical forest tree communities (Nathan & Muller-Landau 2000; Wright 2002). Genetic diversity is critical to the long-term persistence of tree populations, since trees have limited mobility, are slow to reach reproductive maturity, and are likely to encounter significant environmental change during their lifetime (Hamrick 2004; Lowe *et al.* 2005; Schaberg *et al.* 2008). Forest fragmentation and consequent land cover change are known to have an overall strong negative effect on plant reproduction such as increased inbreeding and genetic structure, reduced pollination neighborhood and decreases in seed dispersal distances; with different effects on individual plant species (Schaberg *et al.* 2008; Eckert *et al.* 2010).

Genetic monitoring has been employed as an effective tool to evaluate historical and current levels of genetic diversity and the effectiveness of conservation strategies

(Aravanopoulos 2011; Schwartz *et al.* 2006). Genetic monitoring is defined as the quantification of temporal changes in population genetic metrics or other population data generated using molecular markers (Schwartz *et al.* 2006). The goal of genetic monitoring is to assess the current status of genetic diversity and structure and to quantify relevant changes with the goal of preserving the long-term adaptive evolutionary potential of a species (Hansen *et al.* 2012). Focusing on observed temporal genetic changes, potential drivers of genetic change can be deduced and their relative importance can be evaluated (Lowe *et al.* 2005; Hoffmann & Willi 2008; Stetz *et al.* 2011). Such an early detection mechanism can maximize the chances of implementing management decisions that seek to promote conservation and functional connectivity.

The goal of this research was to conduct genetic monitoring of an understory tree species *Symphonia globulifera,* in the San Juan La Selva biological corridor (SJLS), part of the Mesoamerican corridor, a regional biodiversity conservation initiative. The main goal of the SJLS corridor is to improve connectivity between the Braulio Carillo national park in Costa Rica and the Indio Maiz reserve in Nicaragua while promoting better livelihoods for its communities (Villate *et al.* 2009). Within this region in Costa Rica, two studies have evaluated temporal genetic change across a fragmented landscape for canopy and canopy emergent trees. Hanson et al. (2008) found a small increase in genetic structure between adults and progeny for *Dipteryx panamensis* a canopy emergent tree. In the same landscape, Davies et al. (2013) evaluated genetic diversity and gene flow within adults and progeny of secondary forests for the canopy tree *Vochysia ferruginea.* No significant decrease in genetic diversity was found, and high gene flow across the landscape was detected. One key remaining question is how understory tree species, which are a large proportion of the tree species of tropical forests, respond to the impacts of habitat fragmentation in this biological corridor.

In this study, we use 9 microsatellite markers and survey three cohorts (adults, saplings, and seedlings) of understory tree *Symphonia globulifera* in 18 mature forest patches within the SJLS fragmented landscape. The overall goal of this research is to understand how forest fragmentation and the composition of the surrounding agricultural matrix influence genetic diversity and genetic structure of an understory tree species. We specifically address the following research questions: What are the estimates of genetic diversity within sampled forest sites for adults, saplings and seedlings and are there differences between cohorts? What are the estimates of genetic structure between forest site for adults, saplings and seedlings and are there differences between cohorts? Are genetic diversity and genetic structure related to the land use composition of the surrounding landscape? Are populations in the SJLS biological corridor retaining higher genetic diversity and gene flow than areas outside the corridor? We expect to see lower genetic diversity and increased genetic structure between the younger life stages since these individuals were probably established under current fragmentation conditions and limited movement of seed and pollen will promote greater differentiation between populations. Genetic diversity of seedlings and samplings will be related with the contemporary surrounding landscape while there will be no relation for the adult cohort established during the earlier fragmentation stage. We expect areas inside the SJLS corridor to have higher genetic diversity and less genetic structure than areas outside the corridor, since the corridor retains more forest and is less fragmented than surrounding areas.

Study Site

The study region includes landscapes within and surrounding the San Juan-La Selva (SJLS) biological corridor in northeastern Costa Rica (centered at 10.618 N, 84.138 W, Figure 1). Most of the region is composed of lowland areas that are characterized by tertiary volcanic rock and alluvial terraces and flood plains that range from 0 to 400 m in elevation (Sesnie *et al.* 2009). This region has a mean annual temperature of 26.5 ^{o}C, annual precipitation ranging from 3000 to 4500 mm (Grieve et al., 1990; McDade et al., 1994), and lies within a wet tropical forest life zone (sensu Holdridge et al., 1975). Mature and second-growth forests currently cover 50% of the land area (Morse *et al.* 2009; Fagan *et al.* 2013; Shaver *et al.* 2015), retaining high tree species diversity and showing quick regeneration rates (Guariguata et al., 1997; Schedlbauer et al., 2007; Chazdon et al., 2009b; Norden et al., 2009; Sesnie et al., 2009; Bouroncle and Finegan, 2011).

The land use and land cover change history in the SJLS region reflects a recent pattern in the tropics where intensive agriculture followed initial human colonization and associated deforestation (Lambin, Geist & Lepers 2003). The opening of the SJLS region in the 1970s and 1980s drove massive deforestation; redistributive land reform led to the eventual dominance of smallholder farms and pasturelands (Schelhas & Sánchez-Azofeifa 2006). In the late 1980s, the policies driving this land rush officially ended, replaced by policies simultaneously encouraging forest conservation. The 1996 Forestry Law of Costa Rica, instituted a national ban on mature forest clearing; this theoretically ‘‘froze’’ remaining forest patches on the landscape (Watson et al. 1998; Morse *et al.* 2009).

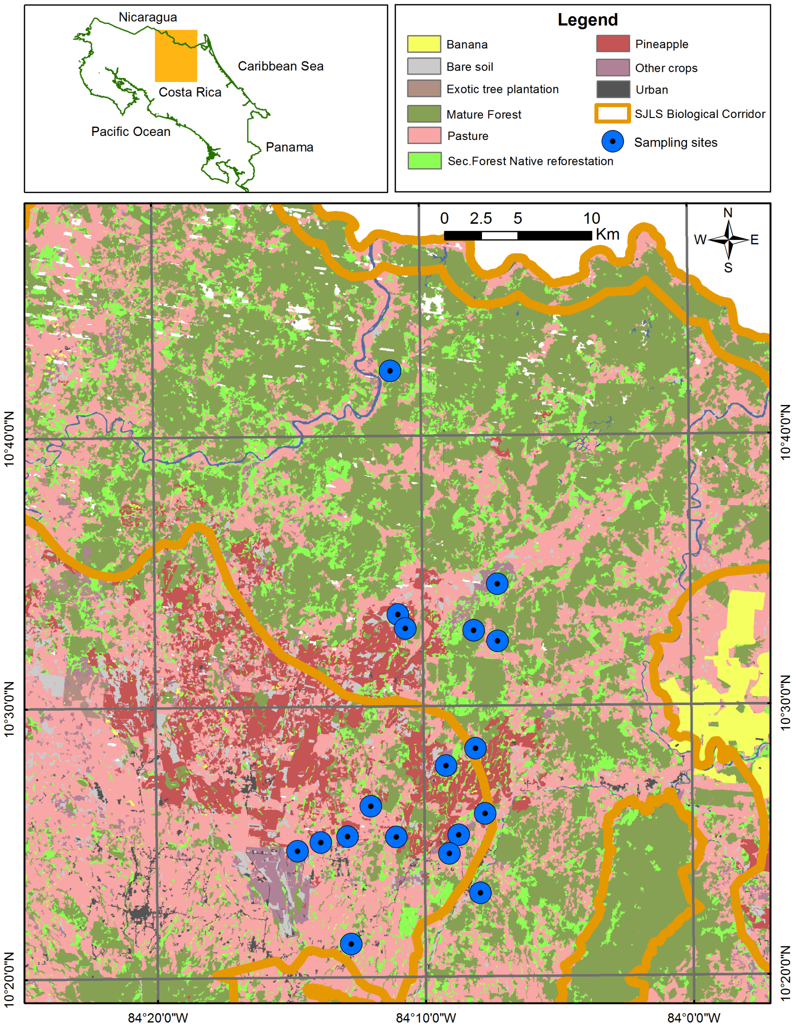

Figure 3. 1 Study landscape depicting land cover, location of surveyed mature forest patches and the SJLS Biological corridor.

Study species

Symphonia globulifera (Clusiaceae) is a shade tolerant tree species distributed in rain forests across the Neotropics and equatorial Africa (Dick & Heuertz 2008). Although *S. globulifera* are typically large canopy trees (Degen et al. 2004, Woodward 2005, Dick & Heuertz 2008), populations in the northern Caribbean lowlands of Costa Rica occur only as understory trees with an adult minimum reproductive size of 1 cm diameter at breast height (dbh, 1.3 m; personal observation). In French Guiana, *S. globulifera* are large canopy trees that exist in two distinct forms, one with big leaves and flowers and the other with small leaves and flowers, they are treated as separate species by local forestry managers (Degen et al. 2004). None of this morphological variation has yet been considered sufficient to merit splitting of *S. globulifera* into more than one Neotropical species (Dick et al. 2003; Dick & Heuertz 2008).

Observations of the small scarlet red flowers suggest bees and hummingbirds as the most important pollinators in this region (Chapter 2). *S. globulifera* seeds are large 4–5 cm drupes consumed and dispersed by bats and monkeys (Aldrich *et al.* 1998). The species is usually > 90% outcrossed (Degen *et al.* 2004; da Silva Carneiro *et al.* 2007), although higher levels of self-fertilization (>10%) have been documented in disturbed habitats for the canopy populations in Costa Rica (Aldrich et al 1998).

Methods

Field sampling

S. globulifera individuals were sampled in 18 mature forest patches (Figure 3.1). We employed historical vegetation cover maps to ensure forest patches were isolated for the past 20 years and with a surrounding landscape that includes the major land covers present in the region (pasture, pineapple, forest and other - seems weird to include other). In each forest patch we identified, georeferenced and recorded height and diameter at breast height (dbh) for 54 *S. globulifera* individuals. Adults were considered as individuals with diameter at breast height (dbh, 1.3 m) $\geq$ 1 cm, , saplings as individuals < 1 cm dbh and height > 50cm. Seedlings were considered as individuals < 50cm height. Leaves samples from a total of 972 *S. globulifera* trees (54 individuals per forest patch) were collected in all forest patches (Figure. 3.1). Leaves were cleaned with 90% Ethanol and distilled water then placed in silica gel and stored at room temperature till DNA extraction.

DNA extraction, microsatellite amplification and genotyping

Genomic DNA was extracted from about 0.02 g of dried leaves using the Biosprint Plant extraction Kit (QIAgen, UK, Ltd) following the protocol provided by the manufacturer and eluted in150 ul of molecular grade water. PCRs were conducted with multiplexed sets of 9 forward labeled primers (Table 3.1) of which four were previously developed for canopy populations (Aldrich *et al.* 1998; Degen *et al.* 2004; Vinson *et al.* 2005) and 5 microsatellite markers developed for these understory populations (supplemental material in prep). The conditions for the 7 uL PCR were 0.02 uM SG06, 0.2 uM SG10, 0.03 uM SG18, 0.01 uM SGC4, 0.01 uM SG11, 0.01 uM SG12, 0.01 uM SG13, 0.02 uM SG14 and 0.01 uM SG15 for each primer pair, along with 1x Qiagen Master Mix, 0.5x Qiagen Q solution, and 1 uL DNA extract. The PCR profile was an initial denaturation step at 94˚C for 15 minutes followed by a touchdown of 10 cycles at 94˚C for 30 seconds, 58˚C for 90 seconds, and 72˚C for 60 seconds with a 0.5˚C decrease in the annealing temperature at each cycle followed by 30 cycles at 94˚C for 30 seconds, 53˚C for 90 seconds, and 72˚C for 60 seconds. Primers SG11 and SG14 were labeled with 6-FAM dye, SGC4 and SG12 were labeled with NED dye, Sg06, SG13 and SG15 were labeled with VIC dye, and SG10 and SG18 were labeled with PET dye. PCR products were run on a 3130xl Genetic Analyzer using Genescan 500 LIZ Size Standard (Applied Biosystems Inc., Foster City, California).

Table 3. 1 Loci, primer sequences, range size and number of alleles used for analysis.

Locus	**Primer sequences**	**Size range**	**# alleles**	**Source**
Sg06	F 5′ACGACATTGGGAGGTTTCAG-3′ R 5′CTGAAACTTAAGGTGCCAAGAA-3′	179-311	65	Vinson et al. (2005)
Sg10	F 5′ACCACAAGTTCACCCTCACC-3′ R 5′GCTACGAAACCAAAGAACTAACAA-3′	111-225	56	Vinson et al. (2005)
Sg11	F 5’ GATCTTCCTGCATGCCTCAC-3’ R 5’GACTTTGGCATGAACTTCCG-3’	218-264	19	This study
Sg12	F 5’ CAAAGCTAGAGTGACTTTGAAGC -3’ R 5’ TTTCACTCGCTCTTTGACACA -3’	96-150	18	This study
Sg13	F 5’ TTTAGTATCAGTACCACCCTCTTCAA -3’ R 5’ GAATGTGATAATGACAAGAAGATTGC -3’	142-181	13	This study
Sg14	F 5’ GGAGCTAGTTTGGAGTTGTCA -3’ R 5’ GGTAACAAGGAGAAGCTGCC -3’	65-177	56	This study
Sg15	F 5’ TGCAAGAGAACTCACATCCG -3’ R 5’ CCCTCTTGACTCTTCCAACAA-3’	96-146	25	This study
Sg18	F 5’TCTTTTGCCTTTTTAGTTGA-3’ R 5’TGAGGATTGTTTGCCCAGAA-3’	284-322	14	Degen et al (2004)
SgC4	F 5’CGGTGATTAGCGTGTCCTTT-3’ R 5’AACTGGCAAGCGTATAGGACC-3’	136-201	25	Aldrich et al (1998)

Alleles were scored using Genemapper 4.1 (Applied Biosystems). To check for genotyping errors, 30% of samples were run twice. Genotyping error rates were calculated using the R package ConGenR (Lonsinger & Waits 2015). All loci were tested for significant deviations from Hardy-Weinburg equilibrium using Genepop 4.2 (Raymond & Rousset 1995). Critical significance levels were adjusted for multiple comparisons using Bonferroni corrections. We also tested for linkage disequilibrium between all pairs of loci across all populations using Fisher’s global test in GENEPOP (web ver. 4.1.10; Raymond & Rousset 1995).

Genetic diversity

We estimated standard measures of genetic diversity separately for all life stages, including the average number of alleles per locus (A), effective alleles (N_e), observed heterozygosity (H_o), Shannon information index (I), and the inbreeding coefficient (F_{IS}) using

Genalex 6.5 (Peakall and Smouse 2012). Allelic richness was obtained using the R package diveRsity and calculated using 1000 re-samples (n = smallest sample per pop for each locus), with replacement per population and locus (Keenan *et al.* 2013). Significance of differences between means for each cohort was evaluated using t tests with the InfoStat statistical package (Di Rienzo et al. 2014).

Genetic structure

To estimate genetic differentiation, we used GenAlex 6.5 (Peakall & Smouse 2012) to perform an AMOVA to obtain global Fst values. We estimated pairwise genetic Fst and G'st between all sites and within each cohort (adults, seedling, saplings) with GenAlex 6.5 (Peakall & Smouse 2012), statistical significance was tested by calculating the probabilities of values with 9999 permutations. In addition, we tested for spatial autocorrelation of populations using relatedness (r) between site pairs (within adults, saplings and seedlings) with 2 Km distance classes. The statistical significance of r was determined using 9999 permutation and bootstrap replicates. To test for isolation by distance on pairwise G'st we performed Mantel tests between the geographic distance and G'st within each cohort. Statistical significance was determined using 9999 permutation and bootstrap replicates.

Relationships between genetic diversity, genetic structure and the composition of the surrounding landscape.

To assess whether genetic diversity and genetic structure in sampling sites have been influenced by surrounding land cover, we used four land cover maps representing the landscape in 1986, 1996, 2001 (Sesnie *et al.* 2008) and 2011 (Shaver *et al.* 2015). Using

ArcMap 10.1 (ESRI 2012), we extracted land cover for circular buffers centered on each sampled forest site, at four diameters: 500m, 1000m, 1500m, and 2000m. Then we used FRAGSTATs (v.4, McGarigal et al. 2012) to calculate the percentage of each land cover type within the circular buffers. The land cover types were: forest, pasture, pineapple and others (urban-infrastructure, annual and perennial crops and water) which are not always present in the landscape and when present represent less than 10%. We used simple univariate regressions to test for significant relationships between the percentage of land cover types surrounding each site at each of the scales and genetic diversity and relatedness within that site. All regressions were performed using the InfoStat statistical package (Di Rienzo et al. 2014).

Comparison between inside and outside the SJLS biological corridor.

To compare sites within and outside the SJLS biological corridor, we compared the means of genetic diversity parameters and mean pairwise G'st between the two areas, for each cohort, using t-tests. Nine of the 18 sampled sites are located outside the corridor and the remaining 9 sites are inside the SJLS biological corridor (Figure 3.1). We standardized the increase in genetic distance dividing by the geographic distance between each population in order to compare the mean increase between areas inside and outside the SJLS.

Results

DNA extraction, microsatellite amplification and genotyping

We genotyped 972 *S. globulifera* individuals*,* 324 from each cohort, adults (mean height 3.96m ±1.81m, mean dbh 2.77 ±1.63cm), saplings (mean height 1.23m ±0.52m, mean dbh 0.63 ±0.20cm) and seedlings (mean height 0.36m ±0.14m). All nine microsatellite loci showed reliable PCR amplification and were polymorphic (Table S1). All samples with a minimum of 8 complete genotyped loci were retained and missing data accounted for only 2% of genotypes. Error rates in our final genotypes were low: the false allele rate was 0.43% and the allelic dropout rate was 1.34%. We found 19 deviations from HWE out of 486 comparisons (9 loci x 18 pop x 3 cohorts), deviations varied across cohorts (4 adults, 8 saplings, 7 seedlings) after Bonferroni correction. No patterns across loci were observed; therefore, we kept all loci for further analysis. No significant linkage disequilibrium (LD) was found between all pairs of these 9 loci (Table S2).

Genetic diversity

Genetic diversity (reflected by alleles per locus (N_a), effective alleles (N_e), Shannon information index (I), observed (H_o) and allelic richness (Ar)) was higher in adults than in seedlings (Table 3.2). The inbreeding coefficient values for all cohorts were low (F_{IS} =0.009–0.029) and not significantly different from zero, although there was a rising trend in the seedling cohort.

Table 3.2 Genetic diversity parameters. N_a = No. of Different Alleles, N_e = No. of Effective Alleles), I = Shannon's Information Index , H_o = Observed Heterozygosity, Ar= Allelic richness, F_{IS} =Fixation index. Bold values represent statistical different results from adult cohort ($p < 0.05$).

	N_a	**N_e**	**I**	**H_o**	**Ar**	**F_{IS}**
Adults N=324	11.278 ±0.437	7.004 ±0.357	1.977 ±0.047	0.781 ±0.011	8.908 ±0.654	0.009 ±0.014
Saplings N=324	10.574 ±0.391	6.452 ±0.329	1.906 ±0.045	0.754 ±0.012	8.921 ±1.024	0.026 ±0.012
Seedlings N=324	**9.883 ±0.346**	**5.968 ±0.263**	**1.858 ±0.042**	**0.748 ±0.013**	**8.732 ±0.569**	0.029 ±0.019

Genetic structure

A comparison of Fst values using AMOVA revealed an increase in population genetic structure among cohorts. Adult Fst, at 0.031, was lower than sapling and seedling Fst (0.039 and 0.048, respectively) suggesting an increase in population genetic structure across time, adults represent reproductive events in the past and seedlings represent resent reproductive events. With all Fst values significantly different from cero. Adults and saplings did not show any significant relation between geographical distance and relatedness (r) (Figure 3.2). In contrast to adults and saplings, seedlings displayed a positive relationship with relatedness; spatial genetic autocorrelograms deviated significantly from the null hypothesis of no relation at 2, 4 and 6 Km (p= 0.001, 0.0001, 0.005).

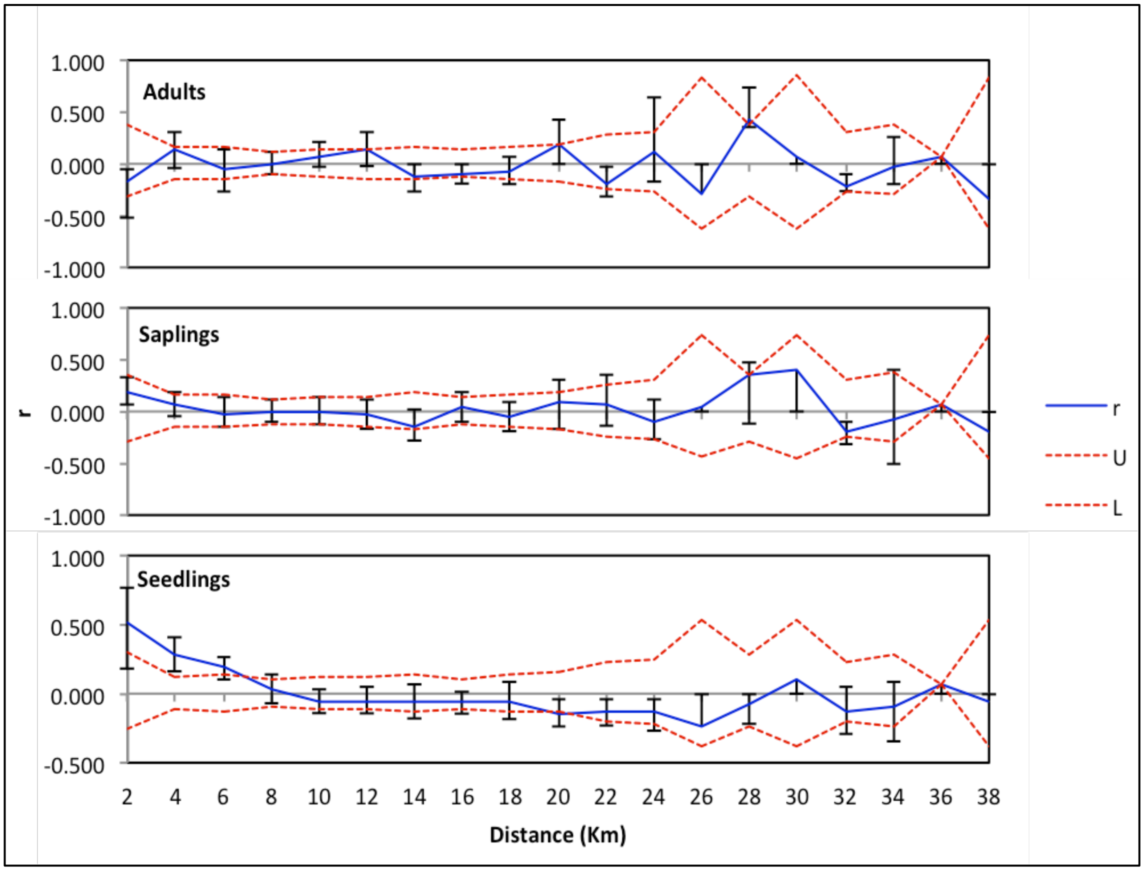

Figure 3.2 Spatial genetic structure autocorrelograms for adults, saplings, and seedlings, respectively. 'r' is the relatedness coefficient of Queller & Goodnight as implemented in GenAlex. Error bars are 95% confidence intervals for the value of r. Upper and lower bounds (U and L) represent the 95% confidence intervals for the null hypothesis (no spatial structure)

Mean pairwise G’st values were significantly higher in seedlings (G’st = 0.22) than in adults (G’st = 0.15) when compared across cohorts. All comparisons were significant with p value = 0.001 and t values of -4.20 for adults-saplings, -12.89 for adults-seedlings and -7.87 for saplings-seedlings indicating less genetic structure between the adults and higher genetic structure within the seedlings. Pairwise Fst also presented a similar trend, increasing from 0.03 in adults to 0.04 in seedlings with saplings presenting an intermediate value of 0.035. Mantel tests showed no significant relationships between G´st and geographic distance for adults and saplings. However, seedlings showed a significant relationship with distance ($R^2 = 0.18$, $p<0.0001$) (Figure S11).

Table 3.3 G’st and Fst genetic distance t test comparisons between cohorts (± standard deviation). Bold values represent statistical difference from adult cohort, * represent statistical difference from sapling cohort ($p < 0.05$).

Cohort	G'st	Fst
Adults	0.15±0.068	0.030 ±0.007
Saplings	**0.17±0.075**	**0.035 ±0.010**
Seedlings	**0.22±0.065***	**0.040 ±0.008***

The null hypothesis of panmixia was rejected for pairwise G’st comparisons among populations; all comparisons were significantly higher than random for the overall data set (Table S4- S9). Comparisons across cohorts revealed an increase in population genetic distance for seedlings within most forest sites, only two sites IDA and PAG did not present an increase in G’st from adults to seedlings. Saplings presented a similar pattern of higher G’st values than adults; in this case five sites (IDA, NOR, PAG, PAZ, RIO and TAB) did not present an increase in G’st from adults to saplings (Figure 3.3).

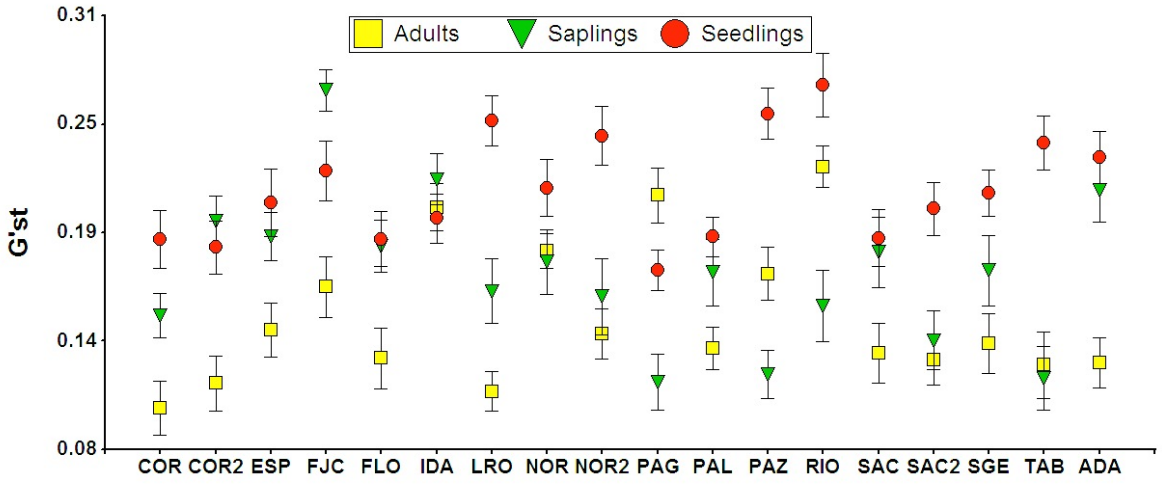

Figure 3. 3 Mean G'st of all pairwise comparisons for each site within adult, sapling and seedling populations of *Symphonia globulifera* (error bars represent the standard error of the mean).

Considering the increase in mean pairwise G'st across sampling sites, the amount of increase varies among sites (Figure 3.3). Spatial locations of surveyed sites suggest that populations with the higher increase are located close to the most fragmented and agricultural dominated areas, but not in all cases as for the site ADA which is within the less fragmented area of the corridor (Figure 3.4).

Relationships between genetic diversity, genetic distance and the composition of the surrounding landscape.

Regression analyses indicate that the number of alleles N, Shannon's information index I, observed heterozygosity Ho and allelic richness Ar for saplings are significantly positively related to the amount on forest within a 500m diameter buffer around sites in the year 2001 (Table 3.4). The amount of pasture within a 500m buffer surrounding the sites was also significantly positively related to allelic richness for the 2001 land cover data. Shannon's information index, observed heterozygosity and allelic richness of seedlings were significantly positively related with the percentage forest area in the year 2011 within a 1500m and 2000m buffer diameter surrounding the site. For adults we found that the area of pineapple at 2000m in 2001 was positively associated with genetic distance G'st. No other significant relationships with the percentage of land cover types at any scale were found in the dataset.

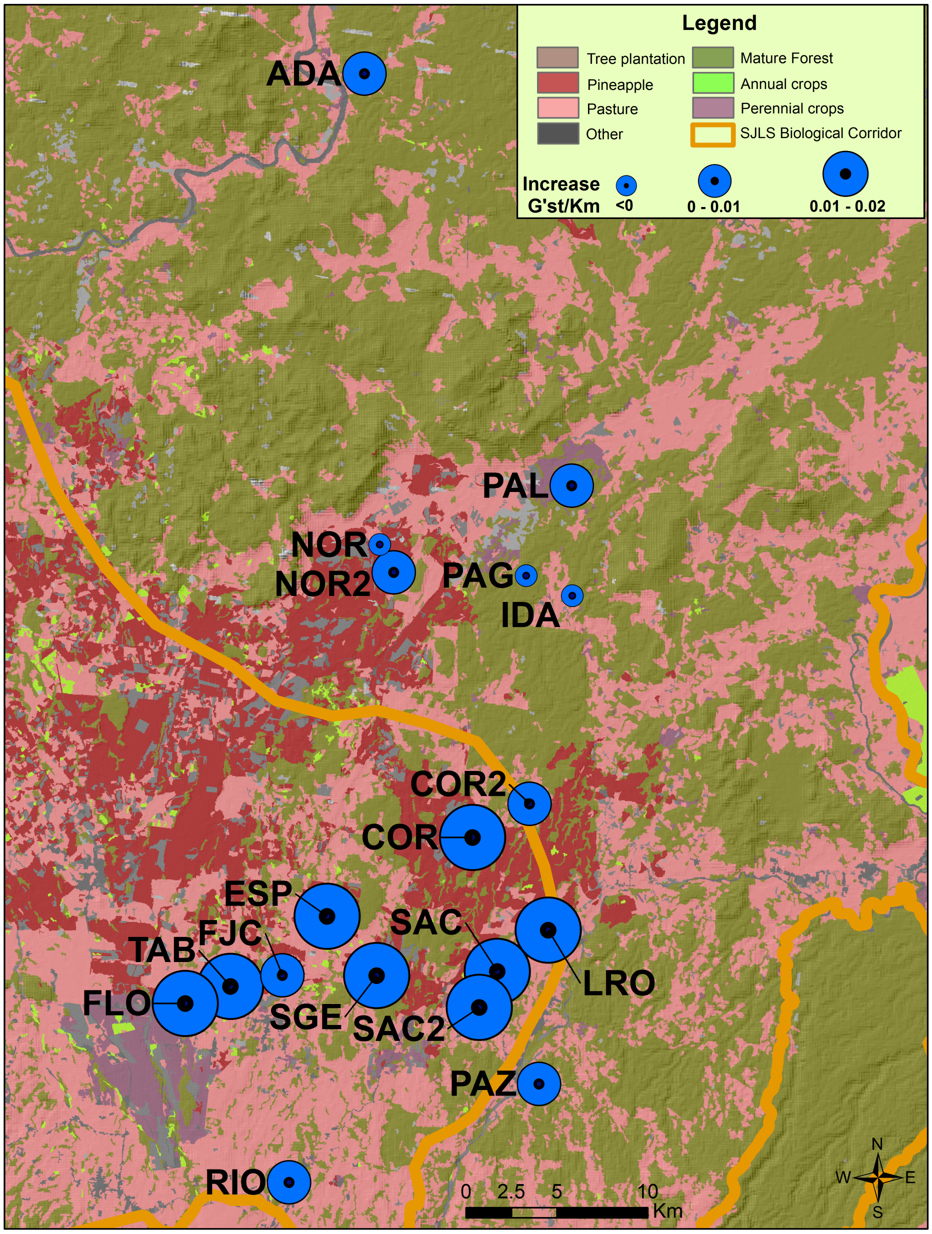

Figure 3. 4 Labeled sample locations and G'st/Km increase values between adults and seedlings, 2011 land cover data.

Table 3.4 Adjusted R^2 and p values for regressions of genetic metrics on percentage of forest and pasture at different scales around forest patches. N_a = No. of Different Alleles, I = Shannon's Information Index, H_o = Observed Heterozygosity, Ar= Allelic richness and G'st=mean pairwise distance between all pairs. Bold represents statistical significance. Considers the landscape surrounding the samples sites at 500m, 1500 m and 2000m buffer diameters.

Cohort		Adults	Saplings	Saplings	Seedlings	Seedlings
Land cover date		2001	2001	2001	2011	2011
Buffer size m		2000	500	500	1500	2000
% Land Cover		Pineapple	Forest	Pasture	Forest	Forest
N_a	Adj R^2	0.02	**0.22**	**0.21**	0.11	0.15
	p-value	0.267	**0.030**	**0.030**	0.100	0.060
I	Adj R^2	0	**0.19**	**0.16**	0.13	**0.17**
	p-value	0.600	**0.040**	**0.055**	0.080	**0.050**
H_o	Adj R^2	0	0.00	0.00	**0.19**	**0.22**
	p-value	0.320	0.620	0.790	**0.040**	**0.030**
Ar	Adj R^2	0	**0.18**	**0.16**	**0.17**	**0.22**
	p-value	0.360	**0.046**	**0.054**	**0.050**	**0.030**
G'st	Adj R^2	**17**	0	0	0	0
	p-value	**0.05**	0.593	0.675	0.34	0.455

Comparison between inside and outside the SJLS biological corridor.

Comparisons between nine sites inside and nine sites outside the SJLS biological corridor revealed statistically significant differences between the observed heterozygosity in the adult cohort with higher levels inside the corridor. The number of effective alleles is significantly lower in seedlings outside the corridor when compared to seedlings inside the corridor (t test p value = 0.004 and t value = -3.35). Other diversity metrics did not present statistical significance, however all present a trend of reduction of genetic diversity in sites outside the limits of the SJLS biological corridor when compared to sites within the SJLS biological corridor.

G'st values in contrast are lower between sites outside the corridor than inside the corridor in all cohorts. This comparison was not considered accurate since distances between sites are greater within the corridor than outside the corridor and distance is related to G'st patterns in seedlings. To address this we calculated the increase in G’st values from adults to seedlings, and then accounted for distance (G’st increase/Km). Comparison for the increase of G’st after accounting for geographic distance revealed less mean increase of G’st inside the corridor than areas outside. G’st increase inside the corridor presented a mean value of 0.004, in contrast G’st values outside the presented a mean G’st increase of 0.011. These values are significantly different (p=0.03, t=2.412) from each other, and present a lower increase of G’st values from adults to seedlings within the SJLS corridor. Some of the description above should go in methods

Table 3. 5 Comparison of means between 9 sites outside and 9 sites inside the SJLS biological corridor. N_a = No. of Different Alleles, I = Shannon's Information Index, H_o = Observed Heterozygosity, Ar= Allelic richness. Bold represents statistical significance from adults, * represent statistical significance form saplings and ** represent statistical significance between the same group inside and outside the corridor. Variance from the mean within parenthesis.

	Inside SJLS corridor			Outside SJLS corridor		
Genetic parameter	Adults N=162	Saplings N=162	Seedlings N=162	Adults N=162	Saplings N=162	Seedlings N=162
N_a	11.296 (1.046)	10.654 (2.171)	**10.173** **(0.636)**	11.259 (1.17)	10.494 (1.266)	**9.593*** **(0.435)**
N_e	7.093 (0.662)	6.366 (2.007)	**6.366**** **(0.367)**	6.915 (0.874)	6.247 (0.918)	**5.571**** **(0.138)**
I	1.99 (0.013)	1.905 (0.039)	1.896 (0.008)	1.963 (0.01)	1.906 (0.018)	**1.82** **(0.005)**
H_o	0.795** (0.001)	0.752 (0.005)	0.761 (0.002)	0.766** (0.001)	0.756 (0.001)	0.735 (0.002)
Ar	9.201 (0.467)	8.752 (1.176)	**8.481** **(0.301)**	9.109 (0.492)	8.632 (0.681)	**8.032** **(0.163)**
F_{IS}	-0.001 (0.001)	0.025 (0.003)	0.026 (0.002)	0.024 (0.002)	0.03 (0.002)	0.036 (0.002)
G'st	0.155 (0.0041)	0.171 (0.0056)	**0.218*** **(0.0033)**	0.141 (0.005)	0.173 (0.0058)	**0.216*** **(0.0045)**
G’st increase/Km		0.004** (0.00005)			0.011** (0.00005)	

Discussion

Genetic diversity

Populations of *S. globulifera* in mature remnant patches across the study sites exhibit a pattern of higher genetic diversity in adults relative to seedlings. We found a significantly lower number of alleles, observed heterozygosity and allelic richness in the seedlings than in adults. Higher levels of genetic diversity in adults likely reflect high levels of historic gene flow and higher effective population size during the early fragmentation period of this landscape. We believe that adults represent reproductive events during the early fragmentation period, and the saplings and seedlings represent recent post fragmentation reproductive events. Results point to a reduction in genetic diversity and increase in genetic structure evidenced by stepwise changes across cohorts (time), saplings always represent intermediate values of genetic diversity between the adults and seedlings within the same sites. This suggests gene flow might have changed due to the reduced abundance and changed spatial distribution of adult trees, and perturbation of gene flow mechanisms (pollination and seed dispersal) following human disturbance and forest fragmentation (Sodre, Cavalcanti & Ferreira 2005; Aguilar *et al.* 2008; Yineger, Schmidt & Hughes 2014; Christie & Knowles 2015). Therefore, reduced gene flow among remnant patches might have contributed to the production of seedlings with lower genetic diversity.

Genetic Structure

Analysis of population genetic structure among patches for the adult cohort revealed lower levels of population differentiation that can reflect the early fragmentation state of formerly continuous forests across the study region. In contrast saplings and seedlings exhibit an increase in population differentiation across all sites. Limited gene flow after forest fragmentation promotes an increase in genetic structure among post fragmentation generations (Aldrich *et al.* 1998; Sodre *et al.* 2005; Farwig, Braun & Böhning-Gaese 2007). Geographic distance did not show any significant relationships relatedness and genetic distance among the adults and saplings. However, significant positive relationships were detected between relatedness and geographical distance at two four and six kilometers in the seedlings, which represent generations born under current forest fragmentation conditions. This suggests that the current landscape structure and composition stimulates genetic structuring at short distances in contrast with previous generations where no correlation of relatedness with distance was detected. The same case is evident from Mantel correlations between G'st and geographic distance - no relationship for distance exists in the adults and saplings. In contrast, seedlings showed for a significant relationship between genetic distance and geographic distance. During this period, geographic distance has not changed, the most drastic change that has occurred is the reduction of forest and the introduction of agricultural production in between sites (Fagan *et al.* 2013; Shaver *et al.* 2015). These modifications have resulted in an increased role played by the distance and land covers between remnant forests in determining genetic structure across the landscape.

Significant increases in pairwise G'st from adults to seedlings among all sampled sites suggest *Symphonia globulifera* populations in the region are experiencing a change in gene flow dynamics after forest fragmentation and agricultural intensification. These patterns coincide with theoretical predictions of the effects on genetic structure after landscape fragmentation; increase differentiation between populations over successive generations (Nason & Hamrick 1997). Reduction of local gene dispersal by pollen and seeds can reduce the effective population size; this can be dangerous for populations of tree species as it increases the risk of loss of advantageous alleles, fixation index of deleterious alleles, and of local extinction (Oddou-Muratorio, Klein & Austerlitz 2005; Eduardo *et al.* 2008; Oddou-Muratorio & Klein 2008).

Relationships between genetic diversity, genetic distance and the composition of the surrounding landscape.

Results from this study illustrate how gene flow dynamics for an understory tree species are influenced by landscape fragmentation. The adult cohort established during the early fragmentation period shows no relation to landscape composition for the years 1986 and 1996. Interestingly, the amount of pineapple in the year 2001 was positively related with the patterns of G'st for the adult cohort. We interpret this result as a relationship between forest fragmentation and G'st rather than a direct influence of pineapple production. Adults were established before the significant amounts of pineapple were present in the landscape. However, pineapple was mainly established in extensive pasture areas that lay within the most fragmented region of the landscape (Shaver *et al.* 2015), in this sense areas under pineapple cultivation in 2001 were the areas subject to greater fragmentation and land cover change between the 1996 and 2001 period. This intensification and increased change within the agricultural matrix influenced the genetic differentiation between the adult cohort across sites. In saplings we find significant positive relationships between allelic richness and the amount of pasture and forest (positive) within a 500m surrounding the site. Sites surrounded by higher amounts of forest and pasturelands accounted for higher diversity within the saplings. Finally in seedlings the increase in the amount of surrounding forest at the regional scale (1.5- 2 Km) increases genetic diversity present at each site, which coincides with the distance at which we start to find a relationship between kinship relationships and geographic distance. This highlights the importance of forest cover between the existent mature forest patches to promote functional connectivity and reduce inbreeding among populations (Christie & Knowles 2015).

Results for *S. globulifera* regarding reduction in genetic diversity and inferred gene flow after forest fragmentation are more pronounced than results for canopy tree species within the SJLS landscape. Hanson et al. (2008) found a small increase in genetic structure between adults and progeny within the San Juan-La Selva biological corridor for *Dipteryx panamensis,* a canopy emergent tree. There was no difference of G'st values between cohorts and progeny in three habitats (continuous forest, forest fragments or trees in pasture), differences were observed only for isolated trees within pastures located > 1.5 km from continuous forest or forest fragments (adults G'st=0.046, progeny G'st=0.065). These results suggest the persistence of gene flow across the landscape after forest fragmentation for this canopy emergent species (Hanson *et al.* 2008). Davies et al. (2013) evaluated the consequences of secondary forest colonisation on the mating patterns and genetic diversity of the canopy tree *Vochysia ferruginea*. Mature forest populations had higher allelic richness and expected heterozygosity than secondary forest populations. There were no significant differences in genetic metrics between progeny and adults in mature forests. Genetic diversity was higher in progeny compared with adults in the secondary forest populations suggesting high genetic connectivity for this canopy emergent tree across the landscape (Davies *et al.* 2013).

Higher erosion in genetic diversity and the increase in population differentiation observed in *S. globulifera* can be associated with differences between pollinators across forest strata. In our study, understory trees of this species received more visits by specialized pollinators such as hummingbirds, large beetles, and sphingid moths than did canopy trees in another study (Bawa 1990, Chapter 2). Patterns of increase in genetic structure after landscape fragmentation have also been documented for canopy populations of *S. globulifera* in other regions (Aldrich et al. 1998; Degen et al. 2004; Vinson et al. 2005; da Silva Carneiro et al. 2007) and other tree species such as *Caesalpinia echinata* in tropical dry seasonal forests of Brazil (Sodre *et al.* 2005) and *Prunus africana* in montane tropical rainforest of Madagascar (Yineger et al. 2014).

Comparison between inside and outside the SJLS biological corridor.

Sites within the SJLS biological corridor had significantly higher genetic diversity than sites outside the biological corridor. This was predicted as the amount of forest inside the corridor is higher and landscape is less fragmented (Shaver *et al.* 2015) and shows the value of the corridor to maintaining biodiversity and functional connectivity. However the trend in reduction of genetic diversity and increase in genetic differentiation across cohorts is present both inside and outside the SJLS corridor. We believe that current landscape structure and composition within the corridor has promoted higher gene flow dynamics when compared to areas outside the SJLS biological corridor, evidenced by lower increase of G'st values within the corridor. Within this region the majority of remnant mature forest areas are located inside the SJLS corridor, in order to ensure the corridor is providing its intended function it is necessary the increase of forest habitat to provide functional connectivity across the agricultural matrix. Initiatives that seek to increase the amount of forest within the matrix and in-between remnant mature forests can aid in maintaining genetic diversity and reduce genetic differentiation for *S. globulifera* and other species part of the forest understory. Conservation and expansion of riparian areas have been suggested as a viable alternative that can promote connectivity across the landscape and at the same time maximizing the provision of ecosystems services (Fremier *et al.* 2013). The approach applied in this study can serve as a tool for genetic monitoring of functional connectivity across the SJLS biological corridor and other fragmented landscape (Stetz *et al.* 2011; Hansen *et al.* 2012).

Conclusion

Results we present here denote a significant reduction of gene flow and genetic diversity in seedlings of *S. globulifera* across all sampled mature forest patches. Seedlings represent reproductive events that occurred across an agricultural matrix that increased in pineapple production from 7,682 ha in 2005 to 22,139 in 2011 representing a 3 fold increase of area dedicated to pineapple production in a 6 year period (Fagan *et al.* 2013; Shaver *et al.* 2015). Higher genetic diversity and gene flow between forest patches for the adults will be expected as it coincides with a less and recently fragmented forest and pasturelands as the primary land use in the matrix. Pasturelands have been shown to retain forest remnant trees that can provide for the movement of pollinators and seed dispersers across the landscape (Manning, Fischer & Lindenmayer 2006; Medina *et al.* 2007; Harvey & González Villalobos 2007; Hanson *et al.* 2008; Milder *et al.* 2010). Saplings display a decrease in genetic diversity however not significantly different from the adults. This reduction can be interpreted as a result of the consolidation on the agricultural matrix and increase in land use-land cover change dynamics within the agricultural matrix.

Further research that evaluates the contribution of land cover types to gene flow will be useful for policy and management. Understanding which land covers facilitate or restrict gene flow will be crucial to design strategies that promote functional connectivity. Conservation efforts should also consider potential pollinators and seed dispersers as they play a critical role for *S. globulifera* reproductive and dispersal dynamics. The results presented here demonstrate that the amount of forest surrounding a patch is an important driver for retaining genetic diversity, thus conservation efforts must focus not only on the particular forest patch but also on protecting forest elements in the surrounding region to maintain genetic diversity and promote gene flow across fragmented landscapes.

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Supplemental Material

Table 3. 6 Hardy Weinberg test for 9 loci within each cohort p-values. Bold are significant after Bonferoni correction (0.05/9 = 0.0056).

	POP	Sg06	Sg10	Sg18	Sg11	Sg12	Sg13	Sg14	Sg15	SgC4
Adults	ADA	0.1345	*0.0457*	0.4097	0.172	0.7145	0.7334	0.2421	0.2258	0.2637
	COR	0.1405	0.1493	0.356	0.0631	*0.0468*	0.6762	0.0692	0.9879	*0.0122*
	COR2	0.0799	0.2809	0.208	0.9591	0.8362	0.7361	0.1682	0.5269	0.6765
	ESP	0.0465	0.4127	0.4591	0.5353	**0.0028**	0.5903	0.8166	0.696	0.2289
	FJC	1	0.3221	0.9067	0.5805	0.0171	*0.0763*	0.4862	**0.0001**	0.3921
	FLO	0.085	0.1602	0.837	0.866	0.3361	0.2751	0.9689	0.8557	0.8499
	IDA	0.1536	0.2044	0.033	0.5809	0.643	0.9838	0.3627	0.9985	0.775
	LRO	0.2102	0.1619	0.9412	0.1051	0.8344	0.6529	0.6293	0.4669	0.5423
	NOR	0.8329	1	0.833	0.1766	0.6436	0.4309	0.2794	0.4351	0.5437
	NOR2	1	1	1	0.7491	0.1228	0.7087	0.8092	**0.0024**	0.8165
	PAG	0.4128	*0.0192*	0.4084	0.2617	0.6878	0.4193	*0.0111*	0.6783	0.8031
	PAL	*0.0082*	0.938	0.5373	*0.035*	0.7028	0.679	0.5027	0.3961	0.4405
	PAZ	0.2831	0.3809	0.6655	0.6521	0.7851	0.0735	0.1027	0.2088	0.9262
	RIO	0.0549	0.7872	0.19	0.3031	0.1973	0.2532	0.8189	0.4836	0.8341
	SAC	**0.0029**	0.4299	0.2712	0.2835	0.6981	0.8671	0.5029	0.5923	0.5626
	SAC2	0.5379	0.2679	0.8363	0.1643	0.1151	0.4567	0.4533	0.9605	0.7811
	SGE	1	0.8089	0.1108	*0.025*	0.815	0.4875	0.6976	0.16	0.316
	TAB	*0.0401*	1	0.6352	0.3491	0.1636	0.7925	0.215	0.3554	0.1105
Saplings	ADA	0.7547	0.2746	0.1424	*0.0366*	0.1454	1	0.0929	0.8105	0.7649
	COR	*0.0316*	0.5543	0.4181	0.8279	0.2319	0.2206	0.2273	0.5381	0.3075
	COR2	0.1106	0.6862	0.0362	0.4984	0.2243	0.3178	**0.0001**	0.048	0.9828
	ESP	*0.0393*	0.1748	0.1773	*0.0283*	0.1117	0.1917	0.4053	0.0515	0.6198
	FJC	0.91	0.1833	0.5324	0.7213	0.6588	0.4063	0.9954	0.6638	0.3068
	FLO	0.8492	0.5796	0.771	0.1413	0.3429	1	0.69	0.0433	*0.0271*
	IDA	0.9534	0.6473	1	0.0626	*0.018*	0.5711	0.6871	0.4746	0.7771
	LRO	0.4596	0.27	0.7313	0.9919	0.8515	0.3414	**0.0001**	0.509	0.3158
	NOR	0.5244	0.7255	0.2821	**0.0017**	0.9927	0.1671	**0.0001**	*0.071*	0.7854
	NOR2	*0.0386*	0.5344	0.1752	0.8166	0.7715	0.6007	0.0516	0.8523	0.4338
	PAG	0.1442	0.0764	0.5887	*0.0227*	0.2792	0.3352	0.3765	0.8474	*0.0283*
	PAL	1	0.3875	0.9314	0.7088	0.7873	0.8322	0.5246	0.4601	0.3434
	PAZ	0.3197	0.665	0.3717	0.3774	0.0566	0.2542	0.374	0.6056	0.6696
	RIO	0.1924	0.8571	0.7536	0.3917	0.5458	0.4476	0.3989	**0.0002**	0.2292
	SAC	0.174	0.5023	0.4171	0.161	0.2663	0.7801	0.7003	0.5803	0.5792
	SAC2	**0.0001**	0.7323	0.8728	0.083	0.1257	0.3133	**0.0001**	0.4937	*0.0337*
	SGE	0.4581	0.2016	0.0571	*0.0384*	0.7483	0.7579	0.2168	*0.0418*	*0.0112*
	TAB	**0.0001**	0.1841	0.1016	0.2232	0.291	0.2302	0.1903	0.153	0.5019
Seedlings	ADA	0.0918	0.1515	0.9122	0.8443	0.5649	0.7848	0.3579	*0.0751*	0.8248
	COR	0.1139	0.1601	0.7795	0.616	0.5348	0.574	0.234	**0.0001**	0.1544
	COR2	0.9172	0.2435	0.1937	0.0951	0.8627	0.8848	0.1488	**0.0038**	*0.0065*
	ESP	*0.0272*	0.6089	0.9767	0.6028	0.5737	0.1198	0.2287	0.9253	0.1112
	FJC	0.4015	0.1665	1	0.2744	*0.0161*	0.9307	0.4117	0.2947	0.3489
	FLO	**0.0034**	0.0598	0.3369	0.282	0.1887	0.6848	**0.002**	0.3836	0.174
	IDA	0.7323	0.3217	0.3263	0.267	0.2849	0.1715	0.9976	0.7936	0.3095
	LRO	0.5914	0.2308	0.0521	0.4342	0.6266	0.7731	0.2943	0.1306	0.088
	NOR	1	0.7053	0.2064	*0.0141*	0.9976	0.613	0.0779	0.0536	0.8086
	NOR2	*0.0018*	0.5061	*0.039*	0.4348	0.5424	0.1408	0.3464	*0.0296*	0.702
	PAG	0.0896	1	0.6963	0.2572	0.0862	0.7915	0.9193	0.6159	0.1878
	PAL	0.9617	0.7681	0.1695	0.8909	0.8652	0.3715	0.4637	**0.0011**	*0.0098*
	PAZ	0.1104	0.1108	0.4869	0.9514	0.9185	0.2278	0.3675	0.3327	0.1921
	RIO	0.1669	0.7199	0.915	0.2014	0.0435	0.282	0.202	0.1342	0.4699
	SAC	0.4072	0.0466	0.7176	*0.0274*	0.487	0.1646	0.2171	0.2757	0.2341
	SAC2	0.0842	0.6044	0.4517	0.0629	0.7817	0.8155	0.9073	**0.0032**	0.1007
	SGE	0.1383	0.9574	0.3625	0.4729	**0.0047**	0.439	*0.049*	0.4588	0.9203
	TAB	0.3151	0.8807	0.3905	0.0917	0.0662	0.7573	0.2165	0.6374	0.8084

Table 3.7 Genotypic linkage disequilibrium p-value for each locus pair across all populations (Fisher's method).

Loci pair		Adults			Saplings			Seedlings		
Locus 1	Locus 2	Chi2	df	P-Value	Chi2	df	P-Value	Chi2	df	P-Value
Sg06	Sg10	7.241	12	0.8413	0.000	14	1.0000	7.573	22	0.9981
Sg06	Sg18	1.934	14	0.9999	9.558	22	0.9899	25.498	26	0.4910
Sg10	Sg18	3.603	18	0.9999	10.671	24	0.9912	16.419	30	0.9789
Sg06	Sg11	6.468	14	0.9533	6.326	22	0.9995	21.519	26	0.7147
Sg10	Sg11	9.894	18	0.9353	3.900	24	1.0000	13.574	32	0.9982
Sg18	Sg11	21.971	36	0.9681	21.714	36	0.9711	36.760	36	0.4335
Sg06	Sg12	6.415	14	0.9549	14.165	22	0.8955	19.024	26	0.8354
Sg10	Sg12	6.092	18	0.9958	8.245	24	0.9988	18.950	32	0.9672
Sg18	Sg12	30.407	34	0.6444	22.151	36	0.9660	32.637	36	0.6294
Sg11	Sg12	21.967	36	0.9682	27.347	36	0.8496	28.183	36	0.8206
Sg06	Sg13	12.596	14	0.5585	6.601	22	0.9994	9.216	26	0.9990
Sg10	Sg13	15.648	18	0.6171	11.324	24	0.9865	30.522	32	0.5414
Sg18	Sg13	36.688	36	0.4368	33.524	36	0.5869	50.008	36	0.0604
Sg11	Sg13	32.573	36	0.6324	24.218	36	0.9328	36.499	36	0.4455
Sg12	Sg13	15.019	34	0.9980	17.200	36	0.9966	27.449	36	0.8463
Sg06	Sg14	10.484	14	0.7260	12.393	22	0.9487	7.293	26	0.9999
Sg10	Sg14	0.000	18	1.0000	0.000	24	1.0000	33.772	32	0.3818
Sg18	Sg14	6.298	32	1.0000	22.618	36	0.9598	27.755	36	0.8358
Sg11	Sg14	20.314	32	0.9456	14.098	36	0.9996	28.779	36	0.7983
Sg12	Sg14	3.469	32	1.0000	17.912	36	0.9949	20.207	36	0.9844
Sg13	Sg14	16.271	32	0.9905	21.578	36	0.9725	14.032	36	0.9996
Sg06	Sg15	7.606	14	0.9088	2.122	22	1.0000	13.590	26	0.9780
Sg10	Sg15	0.000	20	1.0000	0.000	24	1.0000	8.629	32	1.0000
Sg18	Sg15	18.205	34	0.9877	15.639	36	0.9988	24.301	36	0.9311
Sg11	Sg15	10.405	34	1.0000	10.684	36	1.0000	24.092	36	0.9353
Sg12	Sg15	11.752	34	0.9999	28.923	36	0.7927	21.322	36	0.9751
Sg13	Sg15	29.961	34	0.6660	37.144	36	0.4161	17.916	36	0.9949
Sg14	Sg15	4.123	32	1.0000	10.079	36	1.0000	11.707	36	1.0000
Sg06	SgC4	5.327	14	0.9807	8.544	22	0.9954	7.205	26	0.9999
Sg10	SgC4	6.941	20	0.9969	6.022	24	0.9999	18.938	32	0.9673
Sg18	SgC4	10.626	36	1.0000	20.456	36	0.9826	22.339	36	0.9636
Sg11	SgC4	20.600	36	0.9815	11.244	36	1.0000	24.909	36	0.9179
Sg12	SgC4	16.287	36	0.9981	21.412	36	0.9742	28.777	36	0.7984
Sg13	SgC4	15.881	36	0.9985	18.235	36	0.9940	35.311	36	0.5012
Sg14	SgC4	5.676	32	1.0000	25.648	36	0.8999	33.614	36	0.5826
Sg15	SgC4	7.256	34	1.0000	14.519	36	0.9995	14.654	36	0.9994

Table 3.1 Population genetic parameters for each cohort in each population standard, deviation within parenthesis.

Population genetic parameter	Cohort	**ADA**	**COR**	**COR2**	**ESP**	**FJC**	**FLO**	**IDA**	**LRO**	**NOR**
Na	Adults	11.667 (±1.915)	11 (±1.979)	9.222 (±1.451)	10.111 (±1.852)	9.889 (±1.467)	10.333 (±1.633	11.333 (±1.537	10.889 (±1.550	9.667 (±1.691)
	Saplings	11.333 (±1.434)	10.556 (±1.425)	10.889 (±1.736)	8.667 (±1.202)	8.333 (±1.000)	10.111 (±1.495)	12.111 (±1.783)	9.222 (±1.526)	9.000 (±1.886)
	Seedlings	11.111 (±1.628)	10.222 (±1.451)	10.000 (±1.748)	9.444 (±1.132)	9.556 (±1.733)	8.556 (±1.281)	11.222 (±1.722)	9.444 (±1.733)	10.333 (±2.048)
Ne	Adults	7.851 (±1.671)	6.123 (±1.486)	5.831 (±1.078)	5.706 (±1.259)	5.992 (±1.172)	5.974 (±1.072)	6.861 (±1.195)	6.684 (±1.453)	5.476 (±0.775)
	Saplings	6.978 (±1.260)	6.263 (±1.329)	7.108 (±1.333)	4.662 (±0.910)	4.459 (±0.879)	5.335 (±0.920)	7.253 (±1.512)	5.756 (±1.021)	6.052 (±1.611)
	Seedlings	6.2323 (±1.137)	6.114 (±0.948)	6.928 (±1.483)	5.307 (±0.844)	5.842 (±1.339)	5.398 (±0.784)	7.091 (±1.337)	5.710 (±1.285)	7.033 (±1.572)
I	Adults	2.043 (±0.213)	1.924 (±1.178)	1.818 (±0.184)	1.809 (±0.209)	1.851 (±0.193)	1.908 (±1.178)	2.026 (±0.167)	1.956 (±0.185)	1.842 (±0.170)
	Saplings	2.033(±0.166)	1.958 (±0.153)	1.988 (±0.204)	1.622 (±0.203)	1.614 (±0.174)	1.848 (±0.169)	2.072 (±0.186)	1.812 (±0.184)	1.755 (±0.212)
	Seedlings	1.949 (±0172)	1.964 (±0.142)	1.941 (±0.193)	1.793 (±0.169)	1.738 (±0.252)	1.797 (±0.151)	2.014 (±0.194)	1.766 (±0.212)	1.929 (±0.213)
Ho	Adults	0.771 (±0.062)	0.716 (±0.043)	0.772 (±0.042)	0.753 (±0.052)	0.759 (±0.048)	0.808 (±0.037)	0.796 (±0.034)	0.747 (±0.064)	0.807 (±0.043)
	Saplings	0.735 (±0.055)	0.802 (±0.045)	0.827 (±0.068)	0.636 (±0.071)	0.710 (±0.061)	0.778 (±0.037)	0.741 (±0.040)	0.778 (±0.043)	0.790 (±0.058)
	Seedlings	0.764 (±0.041)	0.778 (±0.035)	0.710 (±0.052)	0.733 (±0.049)	0.685 (±0.077)	0.673 (±0.038)	0.821 (±0.058)	0.722 (±0.059)	0.75 (±0.069)
He	Adults	0.802 (±0.056)	0.786 (±0.029)	0.773 (±0.042)	0.756 (±0.045)	0.764 (±0.050)	0.784 (±0.039)	0.814 (±0.034)	0.791 (±0.044)	0.781 (±0.033)
	Saplings	0.809 (±0.038)	0.800 (±0.027)	0.803 (±0.048)	0.694 (±0.067)	0.701 (±0.054)	0.767 (±0.038)	0.807 (±0.038)	0.775 (±0.042)	0.754 (±0.046)
	Seedlings	0.786 (±0.045)	0.807 (±0.024)	0.801 (±0.038)	0.756 (±0.050)	0.713 (±0.080)	0.782 (±0.032)	0.808 (±0.038)	0.743 (±0.059)	0.794 (±0.043)
uHe	Adults	0.825 (±0.058)	0.808 (±0.030)	0.795 (±0.043)	0.778 (±0.046)	0.786 (±0.051)	0.807 (±0.040)	0.837 (±0.035)	0.814 (±0.045)	0.803 (±0.036)
	Saplings	0.832 (±0.039)	0.823 (±0.027)	0.826 (±0.049)	0.714 (±0.069)	0.721 (±0.055)	0.789 (±0.039)	0.830 (±0.040)	0.797 (±0.043)	0.776 (±0.047)
	Seedlings	0.809 (±0.047)	0.830 (±0.025)	0.823 (±0.039)	0.778 (±0.051)	0.733 (±0.083)	0.804 (±0.033)	0.831 (±0.040)	0.764 (±0.061)	0.817 (±0.044)
F	Adults	0.047 (±0.023)	0.085 (±0.050)	-0.012 (±0.053)	0.006 (±0.026)	-0.004 (±0.048)	-0.037 (±0.030	0.018 (±0.036)	0.061 (±0.048)	-0.047 (±0.068)
	Saplings	0.092 (±0.051)	-0.008 (±0.055)	-0.021 (±0.045)	0.084 (±0.047)	-0.008 (±0.028)	-0.021 (±0.034)	0.081 (±0.025)	-0.004 (±0.024)	-0.057 (±0.063)
	Seedlings	0.022 (±0.028)	0.030 (±0.048)	0.113 (±0.048)	0.021 (±0.042)	0.044 (±0.045)	0.134 (±0.045)	-0.007 (±0.035)	0.025 (±0.028)	0.064 (±0.058)
Ar	Adults	10.417 (±4.043)	10.46 (±4.121)	9.588 (±3.689)	9.133 (±3.903)	8.89 (±3.972)	9.566 (±4.145)	8.568 (±3.687)	9.359 (±4.265)	9.724 (±4.268)
	Saplings	7.847 (±3.538)	8.29 (±3.371)	7.591 (±3.107)	8.291 (±3.037)	7.724 (±3.256)	7.666 (±3.157)	7.064 (±2.450)	9.672 (±3.638)	10.089 (±4.532)
	Seedlings	8.934 (±3.299)	8.647 (±2.944)	8.541 (±3.877)	7.927 (±2.702)	7.933 (±3.810)	7.476 (±2.784)	9.26 (±3.739)	7.847 (±3.803)	8.496 (±4.348)

Table 3.2 Continued. Population genetic parameters for each cohort in each population, standard deviation within parenthesis.

Population genetic parameter	Cohort	**NOR2**	**PAG**	**PAL**	**PAZ**	**RIO**	**SAC**	**SAC2**	**SGE**	**TAB**
Na	Adults	11.111 (±1.775)	12.667 (±2.248	10.111 (±1.703	9.445 (±1.396)	11.222 (±1.786)	9.333 (±1.633)	10.222 (±1.690	10.333 (±1.443)	11.444 (±2.129)
	Saplings	10.222 (±1.839	13 (±2.211)	9.000 (±0.972)	9.444 (±1.482)	10.000 (±1.130)	8.667 (±1.616)	9.111 (±1.274)	9.222 (±1.222)	10.222 (±1.588)
	Seedlings	10.000 (±1.414	11.000 (±1.633	9.222 (±1.289)	9.222 (±1.526)	9.444 (±1.365)	9.000 (±1.364)	10.556 (±1.780)	9.222 (±0.983)	10.333 (±1.323)
Ne	Adults	6.791 (±1.488)	8.277 (±2.119)	6.248 (±1.186)	5.875 (±1.094)	6.589 (±1.355)	5.365 (±1.227)	6.207 (±1.268)	6.057 (±1.025)	7.235 (±1.531)
	Saplings	6.864 (±1.744)	8.835 (±2.212)	5.078 (±0.820)	5.074 (±0.885)	5.986 (±1.091)	4.724 (±0.979)	5.359 (±1.060)	5.238 (±1.086)	6.212 (±1.141)
	Seedlings	5.943 (±1.169)	6.861 (±1.423)	5.673 (±0868)	5.823 (±1.266)	5.788 (±1.328)	5.306 (±0.975)	5.539 (±0.958)	4.933 (±0.829)	5.908 (±0.863)
I	Adults	1.966 (±0.193)	2.081 (±0.227)	1.889 (±0.202)	1.825 (±0.192)	1.962 (±0.204)	1.706 (±0.219)	1.876 (±0.206)	1.917 (±0.165)	2.013 (±0.218)
	Saplings	1.920 (±0.200)	2.157 (±0.209)	1.771 (±0.137)	1.736 (±0.194)	1.892 (±0.161)	1.605 (±0.228)	1.721 (±0.215)	1.781 (±0.162)	1.898 (±0.199)
	Seedlings	1.858 (±0.187)	1.978 (±0.194)	1.867 (±0.149)	1.765 (±0.210)	1.791 (±0.188)	1.751 (±0.189)	1.832 (±0.213)	1.797 (±0.122)	1.913 (±0.165)
Ho	Adults	0.784 (±0.056)	0.784 (±0.042)	0.762 (±0.046)	0.802 (±0.048)	0.790 (±0.044)	0.704 (±0.060)	0.759 (±0.067)	0.778 (±0.037)	0.827 (±0.046)
	Saplings	0.809 (±0.052)	0.809 (±0.035)	0.747 (±0.039)	0.735 (±0.055)	0.772 (±0.049)	0.685 (±0.084)	0.704 (±0.072)	0.722 (±0.061)	0.741 (±0.032)
	Seedlings	0.712 (±0.054)	0.827 (±0.036)	0.796 (±0.044)	0.741 (±0.060)	0.759 (±0.058)	0.679 (±0.052)	0.741 (±0.061)	0.765 (±0.055)	0.802 (±0.052)
He	Adults	0.791 (±0.040)	0.801 (±0.047)	0.773 (±0.052)	0.767 (±0.047)	0.788 (±0.043)	0.721 (±0.060)	0.772 (±0.051)	0.792 (±0.035)	0.809 (±0.037)
	Saplings	0.788 (±0.041)	0.824 (±0.038)	0.757 (±0.038)	0.737 (±0.053)	0.787 (±0.035)	0.685 (±0.072)	0.724 (±0.067)	0.753 (±0.041)	0.786 (±0.043)
	Seedlings	0.768 (±0.050)	0.793 (±0.041)	0.790 (±0.030)	0.747 (±0.054)	0.757 (±0.043)	0.746 (±0.054)	0.749 (±0.060)	0.765 (±0.029)	0.791 (±0.039)
uHe	Adults	0.813 (±0.042)	0.824 (±0.048)	0.797 (±0.053)	0.789 (±0.048)	0.810 (±0.045)	0.741 (±0.061)	0.794 (±0.052)	0.815 (±0.036)	0.832 (±0.038)
	Saplings	0.810 (±0.042)	0.848 (±0.039)	0.778 (±0.039)	0.758 (±0.055)	0.809 (±0.036)	0.704 (±0.074)	0.744 (±0.069)	0.774 (±0.043)	0.808 (±0.045)
	Seedlings	0.790 (±0.051)	0.816 (±0.043)	0.813 (±0.031)	0.768 (±0.055)	0.779 (±0.045)	0.767 (±0.056)	0.771 (±0.062)	0.787 (±0.029)	0.813 (±0.041)
F	Adults	0.015 (±0.035)	0.009 (±0.045)	-0.002 (±0.046)	-0.055 (±0.049)	-0.004 (±0.024)	0.016 (±0.044)	0.030 (±0.038)	0.014 (±0.032)	-0.025 (±0.039)
	Saplings	-0.030 (±0.046	0.016 (±0.017)	0.013 (±0.020)	0.003 (±0.018)	0.022 (±0.036)	0.011 (±0.059)	0.021 (±0.043)	0.052 (±0.043)	0.043 (±0.046)
	Seedlings	0.067 (±0.046)	-0.050 (±0.032	-0.019 (±0.06)	0.014 (±0.023)	0.002 (±0.034)	0.084 (±0.034)	0.005 (±0.040)	-0.001 (±0.059)	-0.012 (±0.031)
Ar	Adults	9.209 (±4.903)	9.197 (±4.111)	8.259 (±3.281)	8.48 (±3.699)	8.033 (±3.182)	9.639 (±4.775)	8.644 (±3.774)	8.783 (±4.014)	8.841 (±4.314)
	Saplings	9.825 (±4.414)	9.213 (±4.791)	8.303 (±4.152)	9.172 (±3.618)	8.814 (±3.211)	8.951 (±3.072)	9.519 (±4.086)	8.257 (±3.673)	10.177(±3.857)
	Seedlings	8.286 (±3.169)	9.157 (±3.645)	8.014 (±2.916)	7.789 (±3.5408)	7.93 (±3.336)	7.617 (±3.084)	8.389 (±3.678)	7.833 (±2.233)	8.534 (±3.035)

Table 3.3 Adult's pairwise G'st value between populations below the diagonal, bold indicates significance at α 0.05. Probability, P(rand >= data) based on 9999 permutations is shown above diagonal.

ADA	COR	COR2	ESP	FJC	FLO	IDA	LRO	NOR	NOR2	PAG	PAL	PAZ	RIO	SAC	SAC2	SGE	TAB	
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	ADA
0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	COR
0.056	**-0.013**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	COR2
0.110	**0.112**	**0.103**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	ESP
0.186	**0.075**	**0.117**	**0.151**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	FJC
0.075	**0.076**	**0.098**	**0.064**	**0.091**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	FLO
0.126	**0.152**	**0.179**	**0.246**	**0.263**	**0.244**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	IDA
0.092	**0.034**	**0.044**	**0.100**	**0.113**	**0.086**	**0.092**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	LRO
0.117	**0.137**	**0.160**	**0.189**	**0.253**	**0.191**	**0.160**	**0.119**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	NOR
0.129	**0.019**	**0.052**	**0.164**	**0.085**	**0.123**	**0.200**	**0.095**	**0.181**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	NOR2
0.106	**0.103**	**0.213**	**0.244**	**0.182**	**0.267**	**0.193**	**0.204**	**0.177**	**0.191**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	PAG
0.143	**0.092**	**0.126**	**0.165**	**0.179**	**0.150**	**0.209**	**0.112**	**0.200**	**0.109**	**0.177**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	PAL
0.191	**0.169**	**0.137**	**0.147**	**0.217**	**0.137**	**0.289**	**0.131**	**0.171**	**0.190**	**0.298**	**0.105**	0.000	0.000	0.000	0.000	0.000	0.000	PAZ
0.273	**0.205**	**0.221**	**0.213**	**0.316**	**0.195**	**0.263**	**0.206**	**0.252**	**0.214**	**0.332**	**0.172**	**0.227**	0.000	0.000	0.000	0.000	0.000	RIO
0.109	**0.137**	**0.107**	**0.062**	**0.173**	**0.102**	**0.244**	**0.098**	**0.202**	**0.176**	**0.233**	**0.052**	**0.124**	**0.199**	0.000	0.000	0.000	0.000	SAC
0.110	**0.075**	**0.067**	**0.168**	**0.127**	**0.153**	**0.218**	**0.124**	**0.201**	**0.109**	**0.186**	**0.052**	**0.147**	**0.185**	**0.061**	0.000	0.000	0.000	SAC2
0.132	**0.154**	**0.126**	**0.111**	**0.145**	**0.079**	**0.213**	**0.095**	**0.213**	**0.144**	**0.256**	**0.097**	**0.137**	**0.229**	**0.090**	**0.032**	0.000	0.000	SGE
0.123	**0.138**	**0.123**	**0.053**	**0.125**	**0.011**	**0.226**	**0.091**	**0.204**	**0.183**	**0.265**	**0.095**	**0.097**	**0.191**	**0.019**	**0.115**	**0.021**	0.000	TAB

Table 3.4 Saplings pairwise G'st values between population below the diagonal, bold indicates significance at α 0.05. Probability, P(rand >= data) based on 9999 permutations is shown above diagonal.

ADA	COR	COR2	ESP	FJC	FLO	IDA	LRO	NOR	NOR2	PAG	PAL	PAZ	RIO	SAC	SAC2	SGE	TAB	
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	ADA
0.102	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	COR
0.146	**0.110**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	COR2
0.133	**0.194**	**0.181**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	ESP
0.327	**0.232**	**0.253**	**0.356**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	FJC
0.127	**0.053**	**0.111**	**0.205**	**0.230**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	FLO
0.142	**0.158**	**0.148**	**0.187**	**0.267**	**0.178**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	IDA
0.292	**0.143**	**0.206**	**0.229**	**0.252**	**0.231**	**0.214**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	LRO
0.302	**0.200**	**0.257**	**0.227**	**0.280**	**0.251**	**0.222**	**0.076**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	NOR
0.266	**0.165**	**0.260**	**0.205**	**0.313**	**0.222**	**0.271**	**0.092**	**0.121**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	NOR2
0.197	**0.124**	**0.180**	**0.139**	**0.246**	**0.153**	**0.163**	**0.086**	**0.092**	**0.013**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	PAG
0.249	**0.213**	**0.227**	**0.176**	**0.312**	**0.259**	**0.227**	**0.174**	**0.168**	**0.144**	**0.038**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	PAL
0.172	**0.092**	**0.208**	**0.103**	**0.193**	**0.143**	**0.191**	**0.121**	**0.134**	**0.139**	**0.051**	**0.038**	0.000	0.000	0.000	0.000	0.000	0.000	PAZ
0.233	**0.155**	**0.223**	**0.180**	**0.313**	**0.186**	**0.291**	**0.150**	**0.147**	**0.108**	**0.073**	**0.165**	**0.036**	0.000	0.000	0.000	0.000	0.000	RIO
0.238	**0.189**	**0.271**	**0.186**	**0.319**	**0.260**	**0.322**	**0.193**	**0.193**	**0.121**	**0.117**	**0.175**	**0.113**	**0.072**	0.000	0.000	0.000	0.000	SAC
0.212	**0.123**	**0.154**	**0.166**	**0.218**	**0.142**	**0.288**	**0.122**	**0.165**	**0.100**	**0.112**	**0.143**	**0.087**	**0.066**	**0.077**	0.000	0.000	0.000	SAC2
0.322	**0.174**	**0.280**	**0.219**	**0.267**	**0.232**	**0.284**	**0.157**	**0.113**	**0.113**	**0.094**	**0.123**	**0.114**	**0.135**	**0.125**	**0.121**	0.000	0.000	SGE
0.221	**0.108**	**0.181**	**0.166**	**0.210**	**0.184**	**0.226**	**0.020**	**0.078**	**0.053**	**0.046**	**0.097**	**0.060**	**0.089**	**0.141**	**0.010**	**0.071**	0.000	TAB

Table 3.5 Seedlings pairwise G'st values between population below the diagonal, bold indicates significance at α 0.05. Probability, P(rand >= data) based on 9999 permutations is shown above diagonal.

ADA	COR	COR2	ESP	FJC	FLO	IDA	LRO	NOR	NOR2	PAG	PAL	PAZ	RIO	SAC	SAC2	SGE	TAB	
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	ADA
0.226	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	COR
0.249	**0.032**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	COR2
0.204	**0.138**	**0.169**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	ESP
0.209	**0.254**	**0.232**	**0.131**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	FJC
0.277	**0.142**	**0.121**	**0.142**	**0.192**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	FLO
0.174	**0.159**	**0.157**	**0.239**	**0.303**	**0.198**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	IDA
0.316	**0.234**	**0.151**	**0.342**	**0.269**	**0.266**	**0.262**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	LRO
0.150	**0.216**	**0.256**	**0.240**	**0.203**	**0.255**	**0.157**	**0.251**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	NOR
0.222	**0.284**	**0.230**	**0.313**	**0.239**	**0.270**	**0.215**	**0.174**	**0.069**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	NOR2
0.140	**0.139**	**0.161**	**0.159**	**0.186**	**0.152**	**0.107**	**0.236**	**0.134**	**0.175**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	PAG
0.221	**0.155**	**0.136**	**0.174**	**0.237**	**0.176**	**0.141**	**0.178**	**0.238**	**0.262**	**0.182**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	PAL
0.326	**0.245**	**0.245**	**0.210**	**0.203**	**0.191**	**0.286**	**0.338**	**0.261**	**0.354**	**0.268**	**0.206**	0.000	0.000	0.000	0.000	0.000	0.000	PAZ
0.338	**0.243**	**0.203**	**0.314**	**0.374**	**0.184**	**0.235**	**0.260**	**0.341**	**0.299**	**0.254**	**0.255**	**0.339**	0.000	0.000	0.000	0.000	0.000	RIO
0.277	**0.123**	**0.158**	**0.107**	**0.203**	**0.080**	**0.191**	**0.271**	**0.262**	**0.286**	**0.164**	**0.149**	**0.183**	**0.200**	0.000	0.000	0.000	0.000	SAC
0.203	**0.215**	**0.210**	**0.135**	**0.080**	**0.146**	**0.250**	**0.302**	**0.213**	**0.292**	**0.167**	**0.179**	**0.204**	**0.292**	**0.157**	0.000	0.000	0.000	SAC2
0.243	**0.196**	**0.251**	**0.237**	**0.285**	**0.154**	**0.196**	**0.196**	**0.264**	**0.269**	**0.193**	**0.125**	**0.234**	**0.130**	**0.162**	**0.229**	0.000	0.000	SGE
0.200	**0.228**	**0.194**	**0.312**	**0.255**	**0.284**	**0.151**	**0.264**	**0.186**	**0.221**	**0.129**	**0.238**	**0.281**	**0.376**	**0.267**	**0.236**	**0.286**	0.000	TAB

Table 3.6 Adults pairwise Fst values between populations below the diagonal, bold indicates significance at α 0.05. Probability, P(rand >= data) based on 9999 permutations is shown above diagonal.

	ADA	COR	COR2	ESP	FJC	FLO	IDA	LRO	NOR	NOR2	PAG	PAL	PAZ	RIO	SAC	SAC2	SGE	TAB
ADA	0.000	0.047	0.016	0.002	0.001	0.003	0.001	0.001	0.002	0.002	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.001
COR	**0.008**	0.000	0.456	0.003	0.009	0.010	0.001	0.082	0.002	0.188	0.003	0.001	0.001	0.001	0.001	0.011	0.001	0.001
COR2	**0.014**	**0.000**	0.000	0.007	0.001	0.003	0.001	0.056	0.001	0.030	0.001	0.001	0.001	0.001	0.002	0.010	0.001	0.001
ESP	**0.024**	0.021	**0.020**	0.000	0.001	0.014	0.001	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.022	0.001	0.002	0.041
FJC	**0.037**	**0.014**	**0.023**	**0.032**	0.000	0.002	0.001	0.001	0.001	0.003	0.001	0.001	0.001	0.001	0.001	0.003	0.001	0.001
FLO	**0.018**	**0.014**	**0.018**	**0.013**	**0.019**	0.000	0.001	0.009	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.007	0.337
IDA	**0.024**	**0.028**	**0.033**	**0.048**	**0.054**	**0.047**	0.000	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
LRO	**0.021**	0.007	0.009	**0.021**	**0.024**	**0.017**	**0.019**	0.000	0.001	0.002	0.001	0.001	0.001	0.001	0.003	0.001	0.003	0.003
NOR	**0.022**	**0.022**	**0.027**	**0.034**	**0.047**	**0.033**	**0.028**	**0.022**	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
NOR2	**0.030**	0.004	**0.011**	**0.036**	**0.019**	**0.026**	**0.043**	**0.021**	**0.036**	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
PAG	**0.022**	**0.019**	**0.041**	**0.049**	**0.038**	**0.052**	**0.038**	**0.042**	**0.032**	**0.042**	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001
PAL	**0.033**	**0.019**	**0.026**	**0.036**	**0.040**	**0.032**	**0.044**	**0.025**	**0.039**	**0.026**	**0.039**	0.000	0.003	0.001	0.039	0.036	0.001	0.001
PAZ	**0.040**	**0.033**	**0.028**	**0.031**	**0.048**	**0.028**	**0.060**	**0.028**	**0.033**	**0.043**	**0.063**	**0.024**	0.000	0.001	0.001	0.001	0.001	0.002
RIO	**0.055**	**0.041**	**0.045**	**0.046**	**0.070**	**0.041**	**0.055**	**0.045**	**0.049**	**0.050**	**0.071**	**0.040**	**0.051**	0.000	0.001	0.001	0.001	0.001
SAC	**0.023**	**0.025**	**0.020**	**0.013**	**0.036**	**0.020**	**0.047**	**0.020**	**0.036**	**0.038**	**0.047**	**0.011**	**0.026**	**0.043**	0.000	0.027	0.004	0.224
SAC2	**0.024**	**0.014**	**0.013**	**0.034**	**0.027**	**0.030**	**0.043**	**0.025**	**0.037**	**0.024**	**0.038**	**0.011**	**0.032**	**0.040**	**0.012**	0.000	0.110	0.001
SGE	**0.030**	**0.031**	**0.026**	**0.025**	**0.033**	**0.017**	**0.046**	**0.021**	**0.042**	**0.034**	**0.056**	**0.023**	**0.032**	**0.053**	**0.020**	0.007	0.000	0.164
TAB	**0.026**	**0.026**	**0.024**	**0.011**	**0.027**	0.002	**0.045**	**0.019**	**0.037**	**0.040**	**0.054**	**0.021**	**0.021**	**0.041**	0.004	**0.024**	0.005	0.000

Table 3.7 Saplings pairwise Fst values between populations below the diagonal, bold indicate significance at α 0.05. Probability, P(rand >= data) based on 9999 permutations is shown above diagonal.

	ADA	COR	COR2	ESP	FJC	FLO	IDA	LRO	NOR	NOR2	PAG	PAL	PAZ	RIO	SAC	SAC2	SGE	TAB
ADA	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
COR	**0.028**	0.000	0.001	0.001	0.001	0.024	0.001	0.001	0.001	0.001	0.003	0.001	0.004	0.001	0.001	0.001	0.001	0.002
COR2	**0.045**	**0.029**	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
ESP	**0.037**	**0.044**	**0.047**	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
FJC	**0.095**	**0.057**	**0.070**	**0.087**	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
FLO	**0.039**	**0.014**	**0.032**	**0.052**	**0.063**	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
IDA	**0.046**	**0.043**	**0.045**	**0.051**	**0.077**	**0.053**	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
LRO	**0.069**	**0.027**	**0.046**	**0.045**	**0.053**	**0.051**	**0.050**	0.000	0.018	0.008	0.011	0.001	0.001	0.001	0.001	0.001	0.001	0.243
NOR	**0.071**	**0.038**	**0.057**	**0.044**	**0.059**	**0.054**	**0.052**	**0.012**	0.000	0.001	0.005	0.001	0.001	0.001	0.001	0.001	0.005	0.014
NOR2	**0.066**	**0.033**	**0.060**	**0.042**	**0.069**	**0.051**	**0.066**	**0.016**	**0.020**	0.000	0.296	0.001	0.001	0.001	0.003	0.003	0.002	0.059
PAG	**0.049**	**0.025**	**0.042**	**0.029**	**0.054**	**0.035**	**0.040**	**0.015**	**0.016**	0.002	0.000	0.080	0.051	0.022	0.002	0.001	0.009	0.061
PAL	**0.068**	**0.048**	**0.059**	**0.041**	**0.077**	**0.066**	**0.062**	**0.034**	**0.033**	**0.030**	0.008	0.000	0.090	0.001	0.001	0.001	0.003	0.003
PAZ	**0.044**	**0.019**	**0.050**	**0.022**	**0.044**	**0.034**	**0.048**	**0.021**	**0.023**	**0.026**	0.010	0.008	0.000	0.124	0.003	0.005	0.001	0.036
RIO	**0.057**	**0.031**	**0.052**	**0.037**	**0.068**	**0.043**	**0.070**	**0.025**	**0.024**	**0.019**	**0.013**	**0.034**	0.007	0.000	0.015	0.021	0.002	0.007
SAC	**0.059**	**0.038**	**0.063**	**0.038**	**0.071**	**0.060**	**0.078**	**0.033**	**0.033**	**0.022**	**0.021**	**0.036**	**0.021**	**0.013**	0.000	0.008	0.003	0.001
SAC2	**0.056**	**0.027**	**0.039**	**0.036**	**0.052**	**0.035**	**0.074**	**0.023**	**0.030**	**0.020**	**0.022**	**0.032**	**0.018**	**0.013**	**0.015**	0.000	0.001	0.334
SGE	**0.083**	**0.037**	**0.068**	**0.047**	**0.062**	**0.056**	**0.072**	**0.029**	**0.020**	**0.022**	**0.018**	**0.027**	**0.023**	**0.026**	**0.024**	**0.025**	0.000	0.031
TAB	**0.052**	**0.021**	**0.040**	**0.032**	**0.044**	**0.040**	**0.052**	0.003	**0.012**	0.009	0.008	**0.019**	**0.011**	**0.015**	**0.024**	0.002	**0.013**	0.000

Table 3.8 Seedlings pairwise Fst values between populations below the diagonal, bold indicates significance at α 0.05. Probability, P(rand >= data) based on 9999 permutations is shown above diagonal.

	ADA	COR	COR2	ESP	FJC	FLO	IDA	LRO	NOR	NOR2	PAG	PAL	PAZ	RIO	SAC	SAC2	SGE	TAB
ADA	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
COR	**0.043**	0.000	0.138	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001
COR2	**0.049**	0.006	0.000	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.001
ESP	**0.045**	**0.029**	**0.036**	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
FJC	**0.053**	**0.060**	**0.056**	**0.036**	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
FLO	**0.056**	**0.028**	**0.024**	**0.032**	**0.048**	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.004	0.001	0.001	0.001
IDA	**0.034**	**0.029**	**0.029**	**0.051**	**0.071**	**0.039**	0.000	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.002
LRO	**0.073**	**0.051**	**0.034**	**0.084**	**0.074**	**0.062**	**0.057**	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
NOR	**0.031**	**0.041**	**0.049**	**0.052**	**0.050**	**0.051**	**0.030**	**0.057**	0.000	0.012	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
NOR2	**0.046**	**0.055**	**0.046**	**0.070**	**0.062**	**0.056**	**0.042**	**0.042**	**0.014**	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
PAG	**0.029**	**0.026**	**0.031**	**0.035**	**0.046**	**0.031**	**0.020**	**0.054**	**0.027**	**0.036**	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001
PAL	**0.045**	**0.030**	**0.027**	**0.039**	**0.059**	**0.036**	**0.027**	**0.041**	**0.047**	**0.054**	**0.037**	0.000	0.001	0.001	0.001	0.001	0.001	0.001
PAZ	**0.074**	**0.053**	**0.054**	**0.052**	**0.056**	**0.044**	**0.061**	**0.085**	**0.058**	**0.082**	**0.060**	**0.047**	0.000	0.001	0.001	0.001	0.001	0.001
RIO	**0.074**	**0.051**	**0.043**	**0.075**	**0.098**	**0.041**	**0.049**	**0.065**	**0.073**	**0.067**	**0.056**	**0.056**	**0.083**	0.000	0.001	0.001	0.002	0.001
SAC	**0.063**	**0.027**	**0.035**	**0.027**	**0.056**	**0.019**	**0.041**	**0.069**	**0.058**	**0.066**	**0.037**	**0.034**	**0.047**	**0.050**	0.000	0.001	0.001	0.001
SAC2	**0.046**	**0.046**	**0.046**	**0.033**	**0.022**	**0.034**	**0.054**	**0.076**	**0.047**	**0.068**	**0.038**	**0.041**	**0.051**	**0.071**	**0.040**	0.000	0.001	0.001
SGE	**0.052**	**0.041**	**0.053**	**0.055**	**0.075**	**0.034**	**0.041**	**0.049**	**0.056**	**0.058**	**0.042**	**0.028**	**0.057**	**0.031**	**0.040**	**0.055**	0.000	0.001
TAB	**0.041**	**0.043**	**0.038**	**0.069**	**0.063**	**0.058**	**0.029**	**0.060**	**0.037**	**0.046**	**0.026**	**0.048**	**0.063**	**0.082**	**0.060**	**0.053**	**0.062**	0.000

Table 3.9 Linear regression results between the genetic metrics as response variable and the land use composition as the predictors.

Cohort	Land cover	Buffer	% Land Cover	Na		Ne		I		Ho		AR		Fis		G'st	
				Adj R^2	p-value	Adj R^2	p-value	Adj R^2	p-value	Adj R^2	p-value	Adj R^2	p-value	Adj R^2	p-value	Adj R^2	p-value
Adults	2001	500	Forest	0.00	0.74	0.00	0.99	0.00	0.52	0.00	0.39	0.00	0.71	0.00	0.73	0	0.7292
Adults	2001	500	Pasture	0.00	0.79	0.00	0.95	0.00	0.54	0.00	0.41	0.00	0.74	0.00	0.74	0	0.6863
Adults	2001	500	Pineapple	0.00	0.42	0.00	0.36	0.00	0.70	0.00	0.62	0.00	0.48	0.00	0.53	0	0.6237
Adults	2001	1000	Forest	0.00	0.86	0.00	0.75	0.00	0.47	0.00	0.63	0.00	0.73	0.00	0.92	0	0.8483
Adults	2001	1000	Pasture	0.00	0.97	0.00	0.84	0.00	0.61	0.00	0.77	0.00	0.89	0.00	0.88	0	0.9338
Adults	2001	1000	Pineapple	0.00	0.70	0.00	0.79	0.00	0.89	0.00	0.43	0.00	0.84	0.00	0.59	0.004	0.3165
Adults	2001	1500	Forest	0.00	0.81	0.00	0.60	0.00	0.38	0.00	0.55	0.00	0.65	0.00	0.93	0	0.6991
Adults	2001	1500	Pasture	0.00	0.95	0.00	0.72	0.00	0.53	0.00	0.81	0.00	0.80	0.00	0.82	0	0.9695
Adults	2001	1500	Pineapple	0.00	0.34	0.00	0.53	0.00	0.71	0.00	0.34	0.00	0.45	0.00	0.32	0.1	0.1038
Adults	2001	2000	Forest	0.00	0.80	0.00	0.56	0.00	0.38	0.00	0.47	0.00	0.63	0.00	0.98	0	0.6381
Adults	2001	2000	Pasture	0.00	0.87	0.00	0.67	0.00	0.51	0.00	0.78	0.00	0.72	0.00	0.85	0	0.8387
Adults	2001	2000	Pineapple	0.02	0.27	0.00	0.48	0.00	0.61	0.00	0.36	0.00	0.37	0.00	0.31	**0.17**	**0.0521**
Saplings	2001	500	Forest	**0.22**	**0.03**	0.13	0.08	**0.19**	**0.04**	0.00	0.62	**0.18**	**0.05**	0.08	0.14	0	0.5933
Saplings	2001	500	Pasture	**0.21**	**0.03**	0.14	0.07	0.16	0.06	0.00	0.79	0.16	0.05	0.11	0.10	0	0.6753
Saplings	2001	500	Pineapple	0.00	0.74	0.00	0.71	0.00	0.69	0.09	0.13	0.00	0.79	0.06	0.18	0	0.7926
Saplings	2001	1000	Forest	0.11	0.10	0.04	0.21	0.10	0.11	0.00	0.75	0.08	0.13	0.07	0.15	0	0.6294
Saplings	2001	1000	Pasture	0.11	0.09	0.05	0.18	0.08	0.13	0.00	0.89	0.08	0.14	0.09	0.13	0	0.4637
Saplings	2001	1000	Pineapple	0.00	0.50	0.00	0.96	0.00	0.47	0.06	0.18	0.00	0.51	0.00	0.43	0	0.8246
Saplings	2001	1500	Forest	0.08	0.13	0.02	0.28	0.10	0.11	0.00	0.56	0.07	0.15	0.02	0.25	0	0.7312
Saplings	2001	1500	Pasture	0.11	0.09	0.04	0.22	0.09	0.12	0.00	0.79	0.08	0.13	0.07	0.15	0	0.5244
Saplings	2001	1500	Pineapple	0.00	0.97	0.00	0.68	0.00	0.89	0.00	0.49	0.00	0.95	0.00	0.59	0	0.757
Saplings	2001	2000	Forest	0.09	0.13	0.02	0.26	0.11	0.10	0.00	0.48	0.08	0.14	0.00	0.33	0	0.8626
Saplings	2001	2000	Pasture	0.12	0.08	0.04	0.20	0.11	0.10	0.00	0.75	0.10	0.11	0.07	0.16	0	0.661
Saplings	2001	2000	Pineapple	0.00	1.00	0.00	0.71	0.00	0.90	0.00	0.55	0.00	0.96	0.00	0.66	0	0.6924
Seedling	2011	500	Forest	0.00	0.52	0.00	0.47	0.00	0.36	0.00	0.47	0.00	0.39	0.00	0.79	0.01	0.3121
Seedling	2011	500	Pasture	0.00	0.98	0.00	0.75	0.00	0.87	0.00	0.75	0.00	0.92	0.00	0.57	0	0.3767
Seedling	2011	500	Pineapple	0.00	0.44	0.00	0.58	0.01	0.31	0.10	0.11	0.00	0.33	0.03	0.22	0	0.8315
Seedling	2011	1000	Forest	0.01	0.31	0.00	0.71	0.01	0.30	0.08	0.13	0.03	0.24	0.04	0.22	0.01	0.2974
Seedling	2011	1000	Pasture	0.00	0.70	0.00	0.91	0.00	0.98	0.00	0.76	0.00	0.78	0.00	0.85	0	0.5135
Seedling	2011	1000	Pineapple	0.00	0.84	0.00	0.98	0.00	0.57	0.08	0.14	0.00	0.66	0.03	0.22	0	0.7512
Seedling	2011	1500	Forest	0.11	0.10	0.01	0.30	0.13	0.08	**0.19**	**0.04**	0.17	0.05	0.08	0.13	0	0.3405
Seedling	2011	1500	Pasture	0.00	0.43	0.00	0.39	0.04	0.21	0.00	0.57	0.00	0.32	0.00	0.94	0.06	0.1604
Seedling	2011	1500	Pineapple	0.00	0.79	0.00	0.97	0.00	0.61	0.10	0.11	0.00	0.63	0.08	0.14	0	0.942
Seedling	2011	2000	Forest	0.15	0.06	0.06	0.18	**0.17**	**0.05**	**0.22**	**0.03**	**0.22**	**0.03**	0.10	0.11	0	0.455
Seedling	2011	2000	Pasture	0.00	0.39	0.03	0.23	0.09	0.12	0.00	0.48	0.03	0.24	0.00	0.90	0.06	0.1658
Seedling	2011	2000	Pineapple	0.00	0.97	0.00	0.84	0.00	0.90	0.06	0.17	0.00	0.84	0.08	0.14	0	0.7238

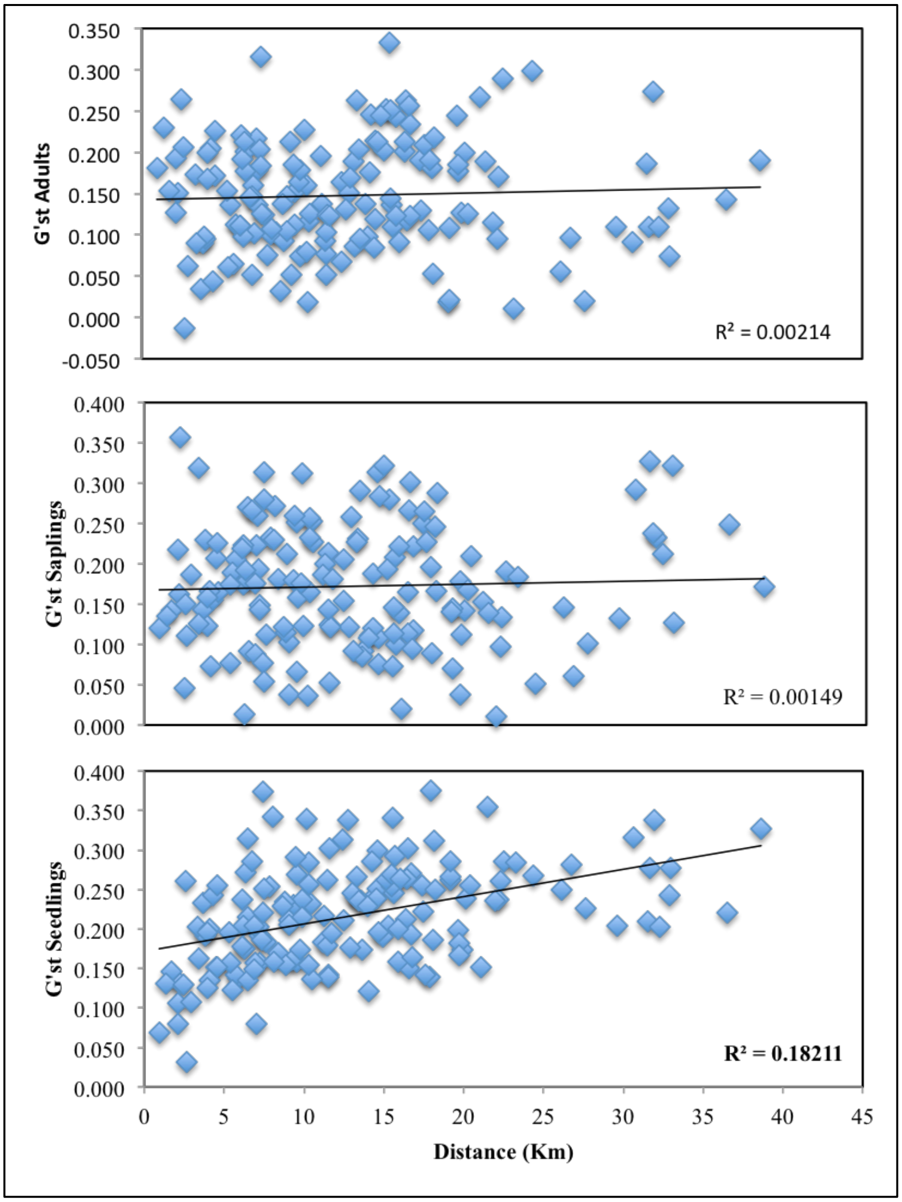

Figure 3. 5 Mantel correlation results between G'st and geographic distance for adults, saplings and seedlings, bold R^2 indicate statistical significance based on 9999 permutations.

Table 3.17 Comparison of means between inside and outside the SJLS for each cohort.

Cohort	Variable	Group 1	Group 2	n(1)	n(2)	Mean(1)	Mean(2)	LL(95)	UL(95)	pVarHom	T	p-value
Adults	Na	outside SJLS	inside SJLS	9	9	11.26	11.3	-1.09	1.01	0.8787	-0.07	0.9413
Adults	I	outside SJLS	inside SJLS	9	9	1.96	1.99	-0.13	0.08	0.7516	-0.55	0.5929
Adults	Ho	outside SJLS	inside SJLS	9	9	0.77	0.8	-0.06	-5.90E-04	0.6866	-2.16	**0.0459**
Adults	AR	outside SJLS	inside SJLS	9	9	9.11	9.2	-0.78	0.6	0.9437	-0.28	0.7811
Adults	Fis	outside SJLS	inside SJLS	9	9	0.03	0.01	-0.01	0.05	0.1824	1.58	0.1328
Adults	Gst	outside SJLS	inside SJLS	9	9	0.14	0.15	-0.05	0.03	0.8233	-0.73	0.4747
Adults	Ne	outside SJLS	inside SJLS	9	9	6.92	7.09	-1.05	0.7	0.7044	-0.43	0.6725
Saplings	Na	outside SJLS	inside SJLS	9	9	10.49	10.65	-1.47	1.15	0.4623	-0.26	0.7985
Saplings	I	outside SJLS	inside SJLS	9	9	1.91	1.91	-0.17	0.17	0.2915	0.01	0.989
Saplings	Ho	outside SJLS	inside SJLS	9	9	0.76	0.75	-0.05	0.06	0.1032	0.15	0.8845
Saplings	AR	outside SJLS	inside SJLS	9	9	8.63	8.75	-1.08	0.84	0.4562	-0.26	0.795
Saplings	Fis	outside SJLS	inside SJLS	9	9	0.03	0.03	-0.04	0.04	0.4443	0.1	0.9203
Saplings	Gst	outside SJLS	inside SJLS	9	9	0.17	0.17	-0.04	0.04	0.7179	0.1	0.9218
Saplings	Ne	outside SJLS	inside SJLS	9	9	6.25	6.66	-1.62	0.8	0.2894	-0.72	0.4838
Seedlings	Na	outside SJLS	inside SJLS	9	9	9.59	10.17	-1.31	0.15	0.6036	-1.68	0.1121
Seedlings	I	outside SJLS	inside SJLS	9	9	1.82	1.9	-0.16	4.80E-03	0.617	-2	0.0633
Seedlings	Ho	outside SJLS	inside SJLS	9	9	0.74	0.76	-0.07	0.02	0.9245	-1.19	0.2515
Seedlings	AR	outside SJLS	inside SJLS	9	9	8.03	8.48	-0.93	0.03	0.4045	-1.98	0.0655
Seedlings	Fis	outside SJLS	inside SJLS	9	9	0.04	0.03	-0.04	0.05	0.6019	0.23	0.8218
Seedlings	Gst	outside SJLS	inside SJLS	9	9	0.22	0.22	-0.03	0.03	0.7375	-0.13	0.8971
Seedlings	Ne	outside SJLS	inside SJLS	9	9	5.57	6.37	-1.3	-0.29	0.1896	-3.36	**0.004**

Table 3.18 Comparison of means between each cohort separate analysis for areas inside and outside the SJLS.

Position SJLS	Variable	Group 1	Group 2	n(1)	n(2)	Mean(1)	Mean(2)	LL(95)	UL(95)	pVarHom	T	p-value
outside SJLS	Na	{adult}	{sapling}	9	9	11.26	10.49	-0.34	1.87	0.9135	1.47	0.1607
	Na	{adult}	{seedling}	9	9	11.26	9.59	0.77	2.56	0.1835	3.95	**0.0012**
	Na	{sapling}	{seedling}	9	9	10.49	9.59	-0.02	1.82	0.152	2.07	**0.0547**
	I	{adult}	{sapling}	9	9	1.96	1.91	-0.06	0.17	0.4296	1.02	0.3235
	I	{adult}	{seedling}	9	9	1.96	1.82	0.06	0.23	0.4099	3.46	**0.0032**
	I	{sapling}	{seedling}	9	9	1.91	1.82	-0.02	0.19	0.1135	1.71	0.1075
	Ho	{adult}	{sapling}	9	9	0.77	0.76	-0.02	0.04	0.3557	0.7	0.4957
	Ho	{adult}	{seedling}	9	9	0.77	0.74	-0.01	0.07	0.1212	1.77	0.0961
	Ho	{sapling}	{seedling}	9	9	0.76	0.74	-0.02	0.06	0.5125	1.06	0.3032
	AR	{adult}	{sapling}	9	9	9.11	8.63	-0.29	1.24	0.6559	1.32	0.2052
	AR	{adult}	{seedling}	9	9	9.11	8.03	0.5	1.65	0.1394	3.99	**0.001**
	AR	{sapling}	{seedling}	9	9	8.63	8.03	-0.05	1.25	0.0592	1.96	0.0677
	Fis	{adult}	{sapling}	9	9	0.03	0.03	-0.04	0.03	0.9597	-0.3	0.759
	Fis	{adult}	{seedling}	9	9	0.03	0.04	-0.05	0.03	0.3005	-0.4	0.6697
	Fis	{sapling}	{seedling}	9	9	0.03	0.04	-0.04	0.04	0.3239	-0.2	0.8577
	Gst	{adult}	{sapling}	9	9	0.14	0.17	-0.07	0.01	0.6349	-1.7	0.1161
	Gst	{adult}	{seedling}	9	9	0.14	0.22	-0.11	-0.04	0.4244	-4.8	**0.0002**
	Gst	{sapling}	{seedling}	9	9	0.17	0.22	-0.08	-0.01	0.2081	-2.5	**0.0258**
	Ne	{adult}	{sapling}	9	9	6.92	6.25	-0.28	1.61	0.9462	1.5	0.1538
	Ne	{adult}	{seedling}	9	9	6.92	5.57	0.61	2.08	0.0173	4.01	**0.0021**
	Ne	{sapling}	{seedling}	9	9	6.25	5.57	-0.09	1.44	0.0148	1.98	0.0765
inside SJLS	Na	{adult}	{sapling}	9	9	11.3	10.65	-0.63	1.91	0.3221	1.07	0.2989
	Na	{adult}	{seedling}	9	9	11.3	10.17	0.21	2.04	0.4973	2.6	**0.0194**
	Na	{sapling}	{seedling}	9	9	10.65	10.17	-0.7	1.67	0.1019	0.86	0.4012
	I	{adult}	{sapling}	9	9	1.99	1.91	-0.08	0.25	0.1314	1.13	0.2771
	I	{adult}	{seedling}	9	9	1.99	1.9	-0.01	0.2	0.5204	1.97	0.0662
	I	{sapling}	{seedling}	9	9	1.91	1.9	-0.15	0.17	0.0364	0.12	0.9041
	Ho	{adult}	{sapling}	9	9	0.8	0.75	-0.01	0.1	0.0354	1.74	0.1089
	Ho	{adult}	{seedling}	9	9	0.8	0.76	-3.70E-03	0.07	0.2812	1.91	0.0737
	Ho	{sapling}	{seedling}	9	9	0.75	0.76	-0.07	0.05	0.2718	-0.3	0.7588
	AR	{adult}	{sapling}	9	9	9.2	8.75	-0.46	1.35	0.2128	1.05	0.3091
	AR	{adult}	{seedling}	9	9	9.2	8.48	0.1	1.34	0.5485	2.46	**0.0254**
	AR	{sapling}	{seedling}	9	9	8.75	8.48	-0.59	1.13	0.071	0.67	0.5129
	Fis	{adult}	{sapling}	9	9	0.01	0.03	-0.06	0.01	0.0374	-1.4	0.1783
	Fis	{adult}	{seedling}	9	9	0.01	0.03	-0.05	0.01	0.0691	-1.6	0.1257
	Fis	{sapling}	{seedling}	9	9	0.03	0.03	-0.04	0.04	0.7673	-0	0.9732
	Gst	{adult}	{sapling}	9	9	0.15	0.17	-0.06	0.02	0.9131	-0.9	0.385
	Gst	{adult}	{seedling}	9	9	0.15	0.22	-0.1	-0.03	0.491	-3.7	**0.0019**
	Gst	{sapling}	{seedling}	9	9	0.17	0.22	-0.08	-0.01	0.5613	-2.8	**0.0128**
	Ne	{adult}	{sapling}	9	9	7.09	6.66	-0.72	1.59	0.1377	0.8	0.4337
	Ne	{adult}	{seedling}	9	9	7.09	6.37	0.01	1.44	0.4208	2.15	**0.0471**
	Ne	{sapling}	{seedling}	9	9	6.66	6.37	-0.84	1.42	0.0269	0.56	0.5838

Appendix

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