

**AN INTERDISCIPLINARY APPROACH TO EVALUATING THE  
IMPACT OF AGRICULTURAL INTENSIFICATION ON BATS IN A  
DYNAMIC NEOTROPICAL LANDSCAPE**

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## Abstract

Almost 40% of the planet's ice-free surface has been converted to agriculture<sup>1</sup>. In this context, successful biodiversity conservation depends on maximizing the potential of dynamic agricultural landscapes to support native species, while allowing for sustainable agricultural production. This dissertation uses an interdisciplinary approach to evaluate impacts of agricultural intensification on the balance between conservation and production in the agricultural landscape of the San Juan – La Selva biological corridor in Costa Rica. In the first chapter, we describe and quantify how agricultural intensification in the form of pineapple plantations is driving changes in social, economic, and spatial characteristics of the landscape. Results indicate that pineapple expansion is simplifying and homogenizing both the local economy and the agricultural matrix between remnant forest cover. This creates a less diversified, more vulnerable economy and a landscape with lower potential for biodiversity conservation. The second chapter evaluates impacts of pineapple expansion on bat assemblages, and finds that this process is altering assemblage composition in remnant forest patches, resulting in higher proportions of frugivorous bats and lower proportions of insectivorous bats than in old-growth forests. We identify a potential threshold effect whereby patches surrounded by more than 50% forest can retain assemblage composition similar to that found in old-growth forest. In the third chapter, we use a landscape genetics approach to quantify the effect of pineapple expansion on functional connectivity for two widespread and abundant bat species, *Artibeus jamaicensis* and *Carollia castanea*. To support these analyses, in the fourth chapter we develop fourteen novel microsatellite markers for *C. castanea*. Results suggest that functional connectivity for *A. jamaicensis* remains high, but historical habitat loss and fragmentation and recent pineapple expansion have begun to disrupt gene flow for *C. castanea*. This work provides evidence for the importance of maintaining spatially and economically diverse land uses in complex agricultural landscapes, and fills important gaps in current understanding of how bat populations are responding to the trend of agricultural intensification in the tropics.

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<sup>1</sup> Ellis EC, Klein Goldewijk K, Siebert S, Lightman D, Ramankutty N (2010) Anthropogenic transformation of the biomes, 1700 to 2000. *Global Ecology and Biogeography* 19:589-606.

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I would like to first thank my family: my parents for supporting me through all of my adventures, and always letting me know they are proud of me, my Aunt Jeanne for teaching me to love and value nature, and especially my husband Dave, whose patience, support, and unconditional love made the last six years possible. You uprooted your life to be a part of this adventure, and it was a million times better with you by my side. I want to thank my teammates and friends Andre, Adina, Irene, and Ricardo for sharing all the wild ups and downs of this journey with me, and for always having the commitment to come back to the table and continue working toward our shared goals. You are impressive scientists and amazing friends, and I hope we can continue to work together in the future. I am grateful to my wonderful co-advisors Lisette and Bryan for guiding me through the complex challenges of an international, interdisciplinary PhD with patience and flexibility, and for teaching me to be a better thinker, writer, and scientist. Thank you to my field assistant Henry, who sat with me in the dark forests of Sarapiquí through rain and bugs and snakes and 2,180 bats, and never ran out of stories. Thank you to my friend Norma and her family for opening their home to me and sharing so many good laughs, long walks, and delicious, homemade Costa Rican meals. I am indebted to the landowners of Sarapiquí for generously giving me permission to work on their land and share their forests. Finally, thank you to all the great people in Idaho and Costa Rica for their support, advice, guidance, and friendship, especially my committee members Lee and Fabrice, the Waits Lab, the indomitable Nilsa, and my fellow IGERTers.

## **Dedication**

I dedicate this work to the wildlife of the San Juan-La Selva Biological Corridor. From the bossy kinkajous, bright tree frogs, and batty bats that I encountered almost every night in the forest, to the silent jaguars, ocelots, tapirs, and many other species that I knew shared the night with me but that I never had the luck to see: your lives deserve protection. I hope this work will be one tiny push toward a future where you have better odds of surviving, and thriving, and continuing to make nights in the forests of Sarapiquí mysterious and beautiful.

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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. Export crops such as pineapple are rapidly replacing multiuse landscape matrices comprised of pasture and smallholder crops with intensive 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 commodity crop expansion and 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 reveal that pineapple expansion produces social and environmental changes that affect local conservation. In particular, our data indicate pineapple production concentrates land, labor, and financial resources, which has a homogenizing effect on agricultural economy in the study region. This constrains alternative 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 the capacity for landscape level land use planning. 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 agricultural intensification.

Keywords: Agricultural Intensification, Biodiversity Conservation, Non-traditional Agricultural Exports, Rural Livelihoods, Pineapple, Costa Rica, Land Use Change, Commodity Crop Expansion

## 1. 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; Rudel et al., 2009b). Impacts of the expansion of agricultural intensification<sup>2</sup> 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<sup>3</sup>) 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

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<sup>2</sup> We define intensification as a multifaceted, nonlinear 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.

<sup>3</sup> 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.

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 the landscape, and increases the isolation of native plant and animal species in remnant forest patches (Rudel et al., 2009a). 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 social, economic and ecological consequences and tradeoffs 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 and tradeoffs of NTAE-driven agricultural intensification in specific regions throughout 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 within 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.

## **2. Theory**

### *2.1 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 among actors (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.

### **3. Materials and methods**

#### *3.1 Study region*

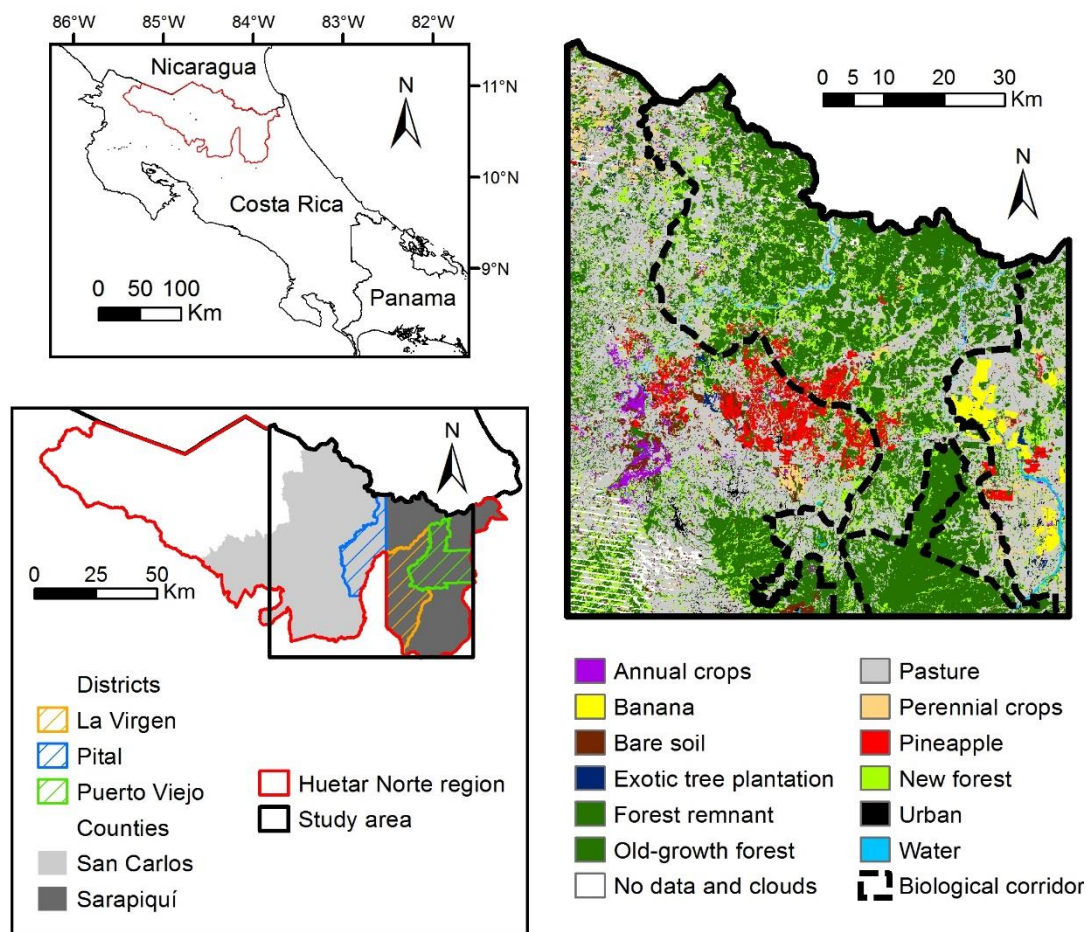
The study region (616,615 ha), was delimited by available remote sensing imagery and the Nicaraguan border (Fig. 1.1). It includes the landscapes within and surrounding the San Juan-La Selva (SJLS) biological corridor in northeastern Costa Rica (centered at 10.61°N, 84.13°W, Fig. 1). This region has 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).





**Fig. 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 1.1). 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.\*

| Districts                     | 1984         |           |       | 2011         |           |        |
|-------------------------------|--------------|-----------|-------|--------------|-----------|--------|
|                               | 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 where 43.8% forest cover is maintained with demonstrated 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.

### 3.2 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).

### 3.3 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 (Appendix B). 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). 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). More detailed information on the FRAGSTATS analysis is given in Appendix C.

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 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).

#### 4. 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 1.2). 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 1.2).

**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.

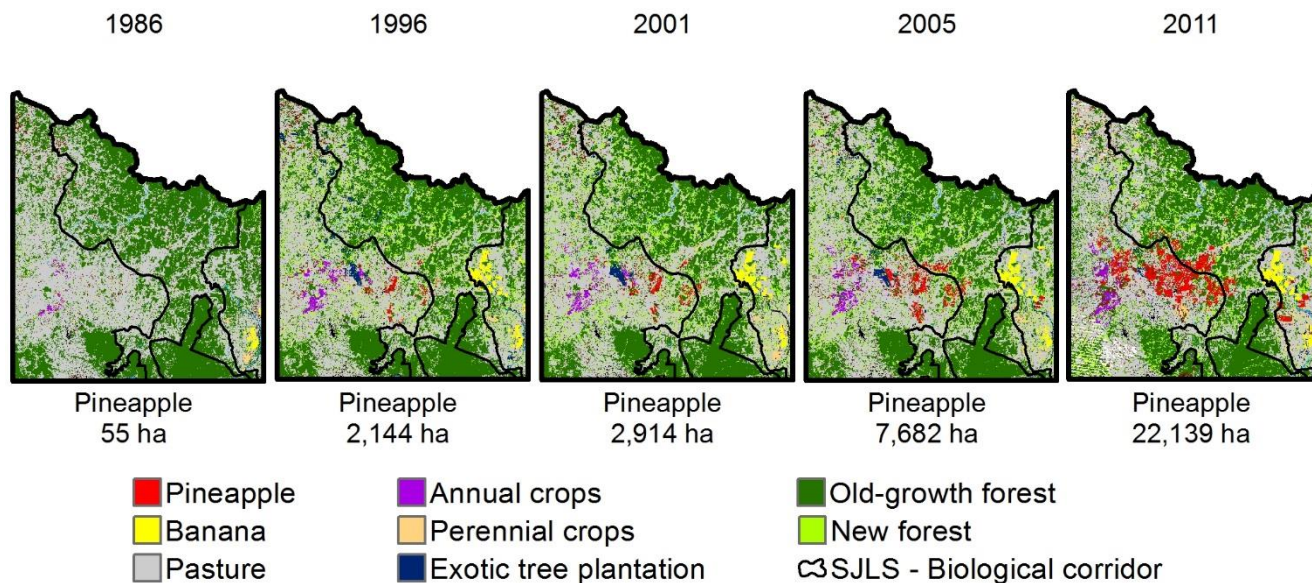
| Land cover type   | Area       |              |            |              | Subdivision |        | Isolation  |            |
|-------------------|------------|--------------|------------|--------------|-------------|--------|------------|------------|
|                   | CA<br>(ha) | PLAND<br>(%) | LPI<br>(%) | AREA<br>(ha) | SPLIT       | NP     | PROX       | ENN<br>(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.

#### *4.1 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 primary 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.



**Fig. 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 1.2). 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 1.2). Of total land dedicated to pineapple plantations in the study region, 78% occurs outside the SJLS biological corridor and 22% lies within (Table 1.3). 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 1.2 and 1.3) 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.

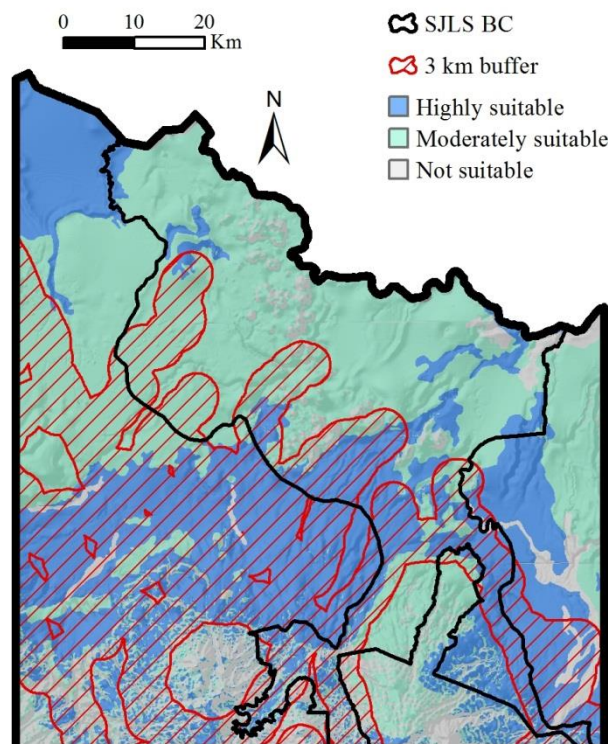
|         | Metric    | Old-growth forest | New forest* | Pasture | Pineapple |
|---------|-----------|-------------------|-------------|---------|-----------|
| Within  | PLAND (%) | 47.2              | 11.3        | 32.0    | 2.0       |
|         | LPI (%)   | 13.7              | 0.1         | 3.0     | 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.



**Fig. 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). Furthermore, in a survey of pineapple producers in the northern part of the corridor, Piñero and Díaz Ríos (2007) found it cost small and medium pineapple producers between .036-.013 cents to produce 1kilogram of fruit whereas it cost large producers .003 cents. 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.

**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:<br>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 and remnant trees | Pasture, sometimes with remnant trees and live fences<br><br>Density: 1 to 3 cow/ha. | Monoculture<br><br>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   |

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\*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.

The market structure of the pineapple sector also favors large-scale plantations over small pineapple farms. 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 infrastructural investments (Table 1.4), an expense most small farmers cannot afford (Piñero and Díaz Ríos, 2007). Large agribusinesses 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 smallholders 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 dual 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.

#### *4.2 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 1.2; 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 1.3); the largest old-growth forest patch covers almost 7% of the total study region (Table 1.2). 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 1.3).

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 1.3). 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 1.3).

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 1.5). 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 1.5), 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 1.5). 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. 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.” 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.

**Table 1.5.** Mean patch area and isolation metrics for forested land cover classes.

|                   | All patches |        |     | Share boundary with<br>pasture (n=3,559) |        |     | Share boundary with<br>pineapple (n = 402) |       |     |
|-------------------|-------------|--------|-----|--|--------|-----|--|-------|-----|
|                   | AREA        | PROX   | ENN | AREA                                     | PROX   | ENN | AREA                                       | PROX  | ENN |
|                   | (ha)        |        | (m) | (ha)                                     |        | (m) | (ha)                                       |       | (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; Guariguata 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. 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. 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 |



**Fig. 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.

### **4.3 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; an issue the leaders in the SJLS biological corridor initiative have identified, “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).

## **5. 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; Tschardt 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: Agricultural intensification alters bat assemblage composition and abundance in a dynamic Neotropical landscape

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**ABSTRACT:** The recent trend of agricultural intensification in tropical landscapes poses a new threat to biodiversity conservation. Conversion of previously heterogeneous agricultural landscapes to intensive plantation agriculture simplifies and homogenizes the landscape, reducing availability and connectivity of natural habitat for native species. To assess the impact of agricultural intensification on bats, we characterized the bat assemblage in the Sarapiquí region of Costa Rica, where heterogeneous land uses are being converted to intensive, large-scale pineapple plantations. In 2012 and 2013 we sampled bats in 20 remnant forest patches surrounded by varying proportions of pasture, mature forest, and pineapple and captured 1821 individual bats representing 39 species. We used ordination analyses to evaluate changes in species composition where pineapple is the main component of the agricultural matrix. We identified landscape metrics specifically correlated with pineapple and used multiple linear regression to test their effects on bat species richness, diversity, and guild-specific relative abundance. Results suggest pineapple expansion is driving changes in assemblage composition in remnant forest patches, resulting in new assemblages with higher proportions of frugivorous bats and lower proportions of insectivorous bats than in continuous mature forests. In addition, while pineapple does not diminish total bat species richness and diversity, the reduced forest cover and increased distance between forest patches in pineapple plantations has a significant negative impact on the relative abundance of insectivores. We also identify a potential threshold effect whereby patches surrounded by more than 50% forest can retain assemblage composition similar to that found in continuous mature forest.

**KEYWORDS:** agricultural intensification; bat conservation; habitat fragmentation; tropical agricultural landscapes

### INTRODUCTION

AN EMERGING LAND USE TREND THREATENS THE BALANCE BETWEEN PRODUCTION AND CONSERVATION IN TROPICAL AGRICULTURAL LANDSCAPES: agricultural intensification through the rapid spread of intensive plantation agriculture (Perfecto and Vandemeer 2008, Brannstrom 2009, Shaver *et al.* 2015). From oil palm plantations in Southeast Asia, to soybean cultivation in Brazil, to pineapple farms in Ghana and Costa Rica, intensive plantation crops are replacing heterogeneous landscapes of mixed crops and pasture (Wilcove and Pin Koh 2010, Arvor *et al.* 2012, Fagan *et al.* 2013), and this trend is predicted to continue (Wright *et al.* 2012). These crops are generally grown in

large-scale, monoculture plantations that homogenize the landscape, reduce total tree cover (Höbinger *et al.* 2012, Shaver *et al.* 2015), and increase agrochemical input (Tilman *et al.* 2002, Tscharntke *et al.* 2012). Resulting impacts on biodiversity are not well understood (Perfecto and Vandemeer 2008, Chazdon 2009), but preliminary studies show that intensive plantation agriculture can impede the movement of native species across the landscape (Vaughan *et al.* 2007, Blair and Melnick 2012), increase habitat fragmentation (Morton *et al.* 2006), and exacerbate biodiversity loss (Jackson *et al.* 2012, Karp *et al.* 2012).

Bats are an important component of tropical biodiversity that is likely to be affected by the spread of intensive plantation agriculture (Harvey and Villalobos 2007; Foster *et al.* 2011). Bats are abundant and widespread in the Neotropics and serve as the primary seed dispersers and pollinators for hundreds of native plant species (Fleming *et al.* 2009, Lobova *et al.* 2009). The importance of bats as key mutualists increases in fragmented landscapes, where maintaining viable populations in remnant forest patches depends on the exchange of seeds and pollen with other patches and with continuous mature forest (Hanson *et al.* 2007, Melo *et al.* 2009).

Previous studies of the response of bat species to conversion of forest to land uses such as agroforestry systems and pastures with scattered trees and live fences have shown that frugivorous bats have the capacity to be resilient to this type of landscape change, possibly because many of these species are generalists with large home ranges (Estrada *et al.* 1993, Garcia-Estrada *et al.* 2006, Harvey and Villalobos 2007). Nectarivorous bats are generally negatively affected by loss of forest cover (Quesada *et al.* 2003, Lobo *et al.* 2005), but when the agricultural matrix includes flowering crops that provide valuable food resources, species richness and abundance of nectarivores may be equal to or higher than in intact forest (Vaughan and Hill 1996, Garcia-Estrada *et al.* 2006, Harvey and Villalobos 2007). In contrast, insectivorous bats, especially those adapted to forage in dense vegetation, appear to be strongly negatively affected by loss of forest cover (Garcia-Morales *et al.* 2013).

However, intensive plantation agriculture is qualitatively and quantitatively different from land uses such as agroforestry systems and pastures with high tree cover, which are lower intensity because they retain a relatively complex vegetation structure and high tree cover, and have low agrochemical inputs per unit area (Benton *et al.* 2003, Tscharntke *et al.* 2012, Mendenhall *et al.* 2014). These types of land uses have high potential for biodiversity conservation because they can provide habitat and habitat connectivity for a variety of native species (Daily *et al.* 2003, Fischer and Lindenmayer 2007, Fahrig *et al.* 2011). In contrast, intensive plantation agriculture is characterized by extensive monocultures with low tree cover and high agrochemical inputs (Tscharntke *et al.* 2012, Shaver *et al.* 2015). Conversion of lower intensity land uses to intensive plantation agriculture



therefore poses a new challenge to bats, and few studies to date have examined how bat assemblages are responding.

In this study, we address the knowledge gap regarding the response of bats to rapidly spreading intensive plantation agriculture in the Sarapiquí region of northern Costa Rica, where the landscape is composed of forest embedded in an agricultural matrix dominated by pasture containing scattered trees, live fences, and small forest patches. This landscape is undergoing agricultural intensification as pastures with high tree cover are being converted to large-scale monoculture pineapple plantations that require more mechanization, more labor, and higher agrochemical use per unit area than pasture. Our objective was to evaluate how changes in landscape composition and configuration driven by this intensification process impact the composition, richness, diversity, and guild-specific relative abundance of the bat assemblage. We predicted that compared to continuous mature forest or patches within lower intensity agriculture, remnant forest patches where the matrix is dominated by pineapple plantations would exhibit 1) altered assemblage composition, 2) lower total species richness and diversity, and 3) guild-specific differences in relative abundance. Specifically, we predicted that that frugivorous species would show no difference in relative abundance across patches, while nectarivorous and insectivorous species would show lower relative abundance in patches where the matrix was dominated by pineapple as compared to continuous mature forest or to patches where the matrix was dominated by lower intensity agriculture.

## **METHODS**

**STUDY AREA** — The Sarapiquí region of Costa Rica (Fig. 1; 10°22'-10°43'n, 83°60'-84°20'w) lies in the wet tropical forest life zone (Holdridge *et al.* 1975) at elevations of 0-400 m. Mean temperature ranges from 30.2-31.9°C, and mean precipitation ranges from 28-61 cm per month (Organization for Tropical Studies 2012).

Land use history in the region reflects a pattern common in the tropics: initial human settlement and associated deforestation in the 1970s were followed by the establishment of pastures for cattle ranching in the subsequent decades. Over the past fifteen years, extensive and degraded pasturelands have been transitioning to intensively managed pineapple plantations (Morse *et al.* 2009; Fagan *et al.* 2013). The current landscape remains dominated by a mix of pasture and selectively logged mature forest (Boroumle and Finegan 2011), but pineapple is spreading rapidly. Between 2006 and 2010, Costa Rican land under pineapple cultivation increased from 22,400 ha to 45,000 ha (Barquero 2011), and more than half of this is in the Sarapiquí region (Blacio *et al.* 2010).

**BAT SAMPLING** — To select sampling sites, we used a 2011 land cover map created using high resolution (5 m<sup>2</sup>) Rapideye imagery (Shaver *et al.* 2015) to identify 20 remnant patches of mature forest between 20 and 60 has in size, separated by a minimum of 1.5 km, and representing a gradient of proportion of pineapple in the surrounding matrix (Fig. 1). Each sampling site was visited 3-4 times between January and May of 2012 and 2013, the driest portion of the year (Organization for Tropical Studies 2012). Sampling was restricted to this time period for logistical reasons, and although monthly variation in precipitation is minimal, patterns of richness, diversity, and guild-specific abundance may be different during the wetter portion of the year. We avoided sampling on rainy nights and during five nights around the full moon, as many bats are lunar phobic (Lang *et al.* 2006). On each visit we set ten 12 m x 3 m mist nets (Avinet, Dryden, NY) along forest trails and kept nets open from 6:00-10:30 pm. All captured bats were identified to species using Timm, Laval, and Rodriguez (1999), Laval and Rodriguez (2002), and Reid (2009). Prior to releasing each bat, we cut 2 cm of fur from the dorsal side, which allowed us to identify recaptured bats and avoid double-counting individuals. Our capture and handling procedures were consistent with standards for use of wild mammals in research established by the American Society of Mammalogists and met their animal care and use guidelines (IACUC protocol #2011-31)

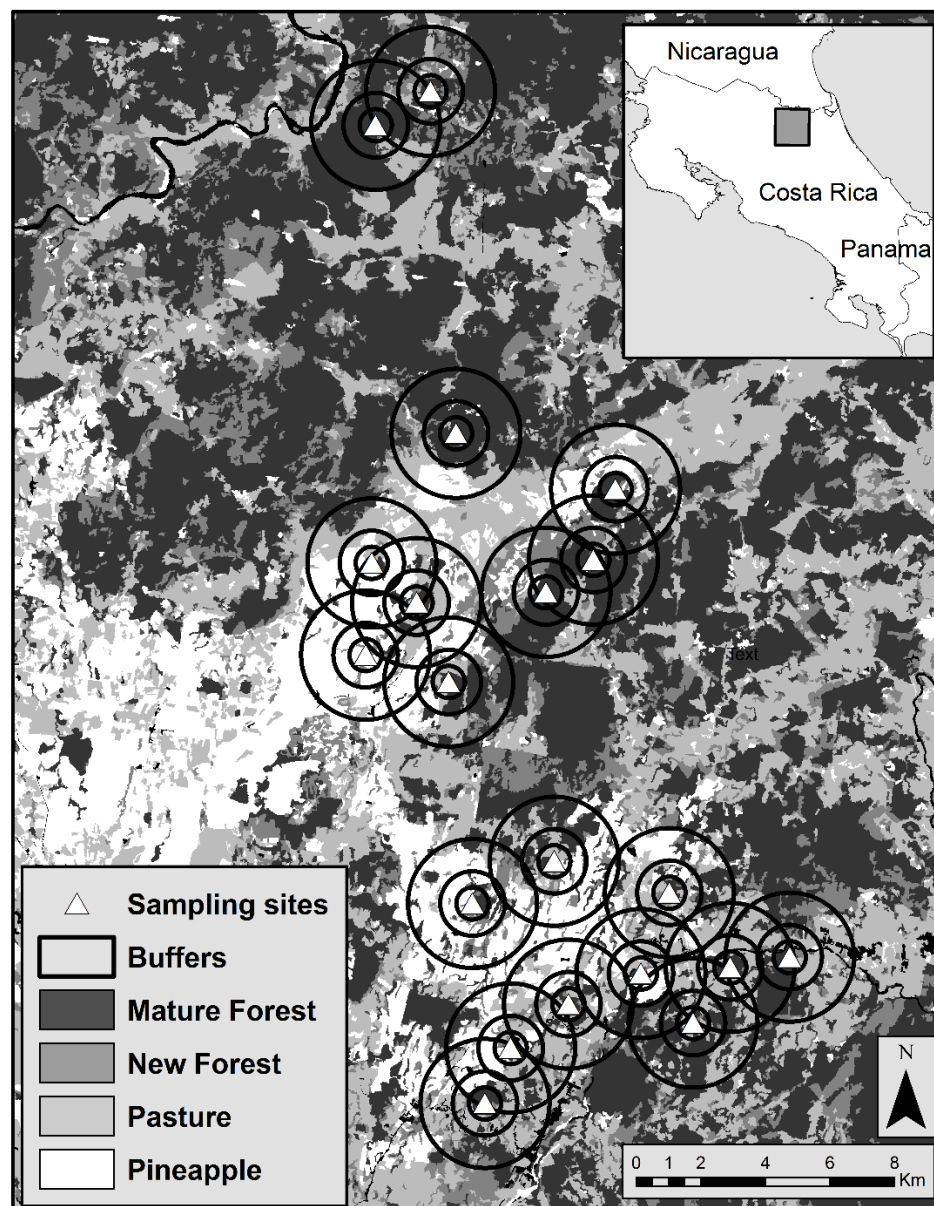


FIGURE 2.1 Sampling sites and 500 m, 1000 m, and 2000 m buffers. Species richness, diversity, and relative abundance was measured at sampling sites, and landscape metrics were measured inside each concentric buffer circle.

**DIVERSITY INDICES** — Four indices were calculated: species richness, species diversity, relative abundance of all species, and guild-specific relative abundance. We used EstimateS (v.9, Colwell 2013) to calculate the first-order jackknife estimator for species richness and the Shannon diversity index. We estimated relative abundance of all species by calculating captures per net-hour in each sampling site. To estimate guild-specific relative abundance, we separated species into guilds (sensu

Kalko *et al.* 1996) and then calculated captures per net-hour for 1) frugivores, 2) nectarivores, and 3) insectivores. We anticipated that separating species by guild, rather than foraging habitat (e.g. open space versus forest), would allow us to detect a more coherent response since food sources of many bats with similar foraging habitats would be affected differently by the changes in landscape composition and configuration driven by agricultural intensification. To test for spatial autocorrelation in our estimates, we used the Geary's C statistic in the *spdep* and *spatstat* packages in R (v.3.0.2; 2013). None of the indices showed significant ( $\alpha < 0.05$ ) spatial autocorrelation.

**Bat assemblage composition and agricultural intensification** — To assess differences in assemblage composition among sampling sites, we used a non-metric multidimensional scaling (NMDS) analysis, with the Bray-Curtis dissimilarity measure calculated on a matrix containing relative abundance data for all species at all sites. To evaluate whether differences in assemblage composition among sampling sites were related to the land cover type surrounding each site, we used a canonical correspondence analysis (CCA) constrained by percent land in forest, pasture, and pineapple. We used the 2011 land cover map in FRAGSTATs (v.4, McGarigal *et al.* 2012) to calculate these metrics within concentric circles centered on each sampling site, at three scales predicted to be relevant to habitat use and movement by bats: 500 m, 1000 m, and 2000 m (Fig. 1). The 500 m scale is large enough to contain the home range of several of the smallest species in the region (e.g., *Glossophaga commisarissi*, Lemke 1984; small frugivores, Bonaccorso *et al.* 2006), while the 2000 m scale is large enough to contain the home range of several of the largest species (e.g., *Artibeus jamaicensis*, Bernard and Fenton 2003). All ordination analyses were conducted with package *vegan* in R.

**SPECIES RICHNESS, DIVERSITY, AND RELATIVE ABUNDANCE IN RESPONSE TO AGRICULTURAL INTENSIFICATION** — Many studies investigating the effects of landscape change on bat assemblages are interested in how the composition and configuration of forest affect species richness, diversity, or relative abundance (Gorresen and Willig 2004, Klingbiel and Willig 2009, Avila-Cabadilla *et al.* 2012). Our goal was to take this approach one step further and identify changes in the composition and configuration of forest specifically caused by pineapple plantations. To do this, we conducted a preliminary analysis to test which changes in the composition and configuration of forest were more closely correlated with pineapple, versus those which were more closely correlated with pasture, which is still the dominant land use in the agricultural matrix in the majority of the study area. First, we used the 2011 land cover map in FRAGSTATs to calculate all available metrics (n=54) at the 500 m, 1000 m, and 2000 m scales for each sampling site, with mature forest as the focal class. Next, we ran each of the 54 metrics separately in two simple univariate regressions, first against percent land in pineapple and then against percent land in pasture. Of the fifty four available metrics, only three met

the criteria of being significantly correlated with percent land in pineapple but not significantly correlated with percent land in pasture: Euclidean distance between forest patches, edge density of mature forest, and edge contrast of mature forest (Appendix 3, Table S1). Edge density of mature forest refers to the amount of forest edge in the landscape per unit area; a landscape dominated by continuous mature forest would have lower edge density than a landscape with many long, narrow strips of forest such as live fences or riparian corridors. Edge contrast of mature forest is based on the dissimilarity in vegetation structure between two adjacent land cover types; a forest patch surrounded by a pasture with many scattered trees and live fences would have lower edge contrast than forest patch surrounded by pineapple. Since pineapple plantations have a significantly lower percent forest cover than pasture (Shaver *et al.* 2015), we also included the composition metric of percent land in mature forest. These four final metrics representing the changes in landscape composition and configuration caused by pineapple plantations are described in Table 2.1, and are hereafter referred to as agricultural intensification metrics.

**Table 2.1** Agricultural intensification metrics calculated with mature forest as the focal land use type, and their relationship to the percent of pineapple plantations at the same scale. Relationships were the same across all three scales.

| <b>Metric</b>   | <b>Abbreviation</b> | <b>Relationship</b>  |
|---|---------------------|--|
| Percent land in forest  | PFOR                | (-) As pineapple increases, forest cover decreases.  |
| Edge density of forest  | ED                  | (-) As pineapple increases, edge density of forest decreases.                                    |
| Mean Euclidean nearest neighbor distance between forest patches | ENN                 | (+) As pineapple increases, the mean nearest neighbor distance between forest patches increases. |
| Forest edge contrast index                                      | ECON                | (+) As pineapple increases, the proportion of forest with high contrast edges increases          |

Landscape configuration metrics are often correlated with the percent land in forest. To avoid multicollinearity problems, we used univariate linear regression to remove linear effects of associations between each of the three configuration metrics with the percent land in forest. The residuals of each regression were used with percent land in forest as predictor variables in all subsequent regression analyses (Gorresen and Willing 2004, Klingbeil and Willig 2009, Avila-Cabadilla *et al.* 2012). For simplicity, we continue to refer to each metric by its original name.

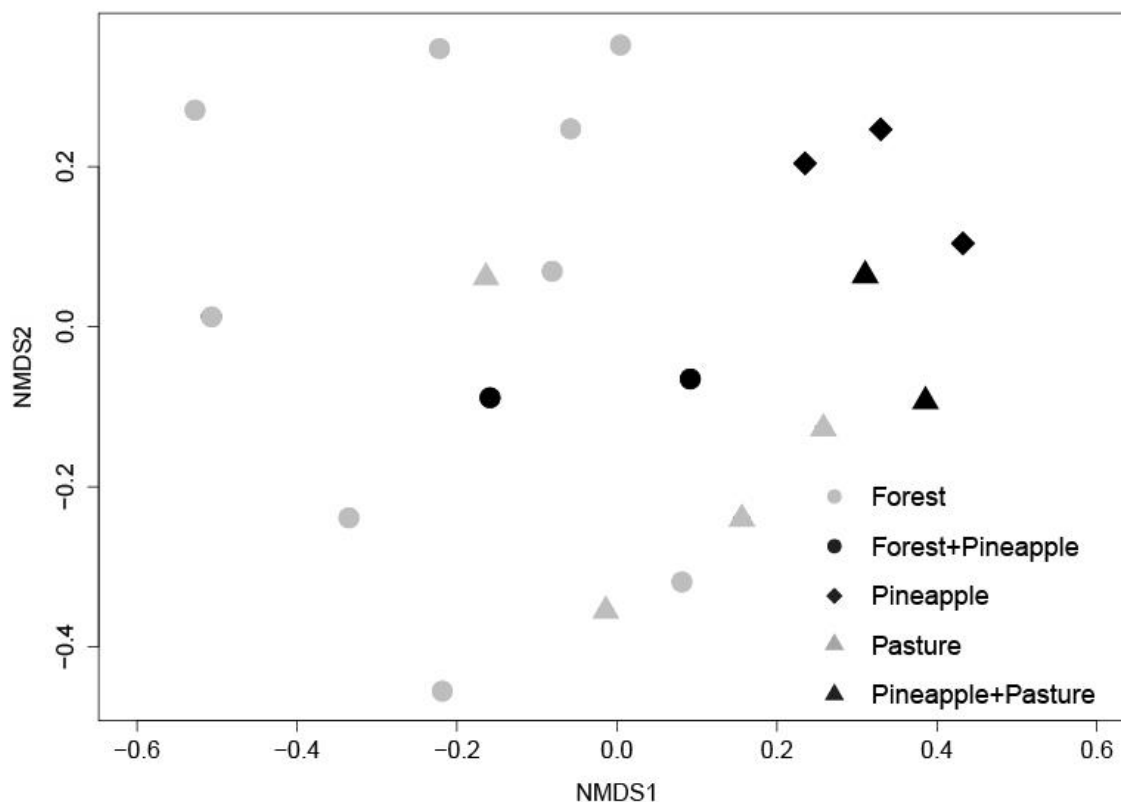
Relationships of the agricultural intensification metrics to 1) total species richness and diversity and 2) relative abundance of insectivores, nectarivores, and frugivores were quantified with

multiple linear regression in R. One sampling site emerged as an outlier with significant leverage in the majority of the regressions (Cook's distance > 0.5) and was removed from the final regression analyses. At the 2000 m scale, buffers for many of the sampling sites overlapped (Fig. 2.1). However, because there was no significant spatial autocorrelation in the diversity indices, we used all sites (minus the outlier) for analyses at this scale (Schank and Koehnle 2009, Zuckerberg *et al.* 2012).

## RESULTS

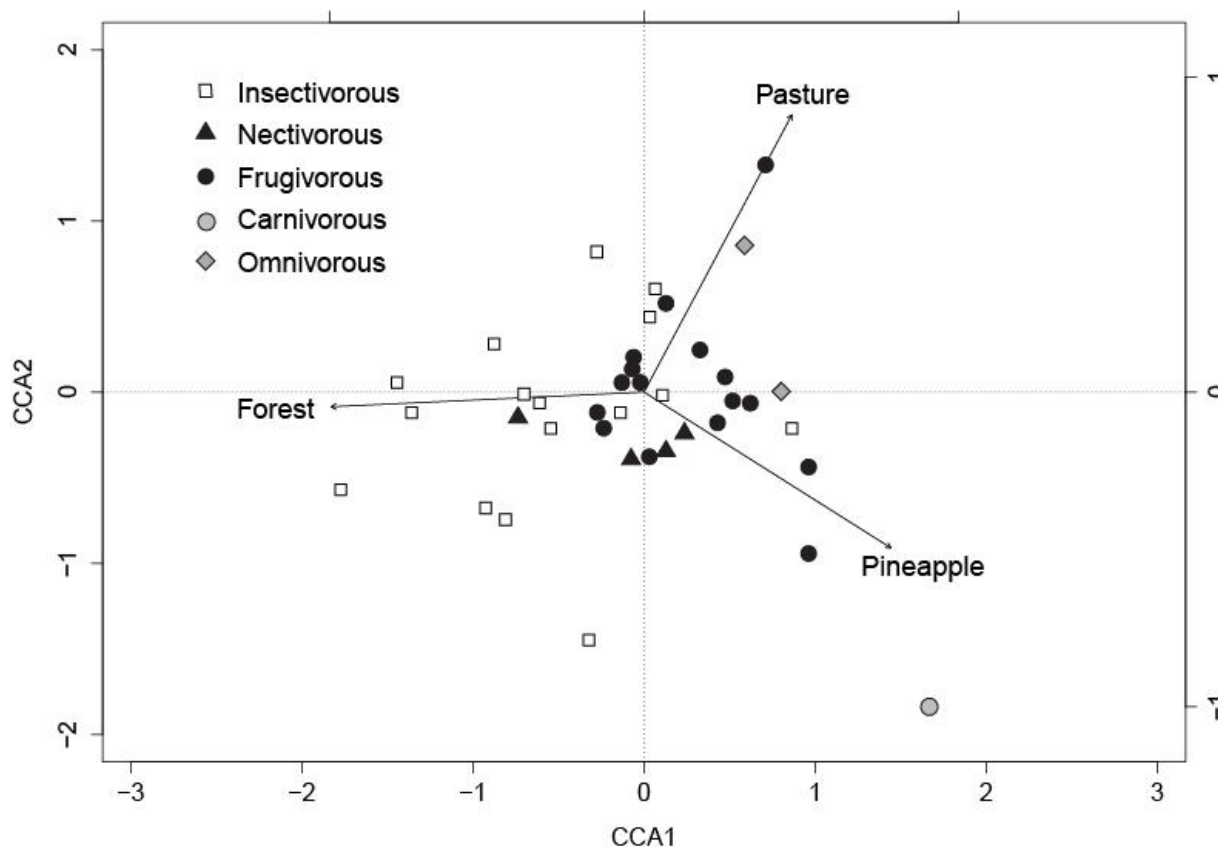
Sampling for a total of 2826 net-hours resulted in the capture of 1821 individual bats representing four families and 39 species (Appendix 3, Table S2). Bats in the family Phyllostomidae accounted for 95.7 percent of all captures, and the remainder were from the families Mormoopidae (2.7%), Vespertilionidae (1.0%), Emballonuridae (0.6%), and Thyropteridae (one individual). Twenty-two species were uniformly rare (<1% of captures).

**BAT ASSEMBLAGE COMPOSITION AND AGRICULTURAL INTENSIFICATION** — The NMDS ordination consisted of two axes that together explain 74.9 percent of the variation in the dissimilarity of the composition data with a reasonably low stress value of 0.201 (Fig. 2.2). The ordination grouped sampling sites where the matrix was dominated by pineapple, and sites surrounded by similar amounts of pineapple and pasture were also grouped near these sites. Sites surrounded primarily by forest or pasture did not exhibit any clustering pattern.



**FIGURE 2.2** Non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis dissimilarity index calculated on relative abundance data for the entire species set. Ordination stress was 0.201. Patches are labeled based on the dominant (>50%) land use surrounding each at the intermediate scale of 1000m. Where no single land use dominated, patches are labeled by the top two dominant land uses, with the more dominant one listed first.

The CCA ordination revealed clear differences in assemblage composition among patches surrounded primarily by forest, pineapple, or pasture. We focus on results from the analysis at 1000 m, as the results at the 500 m and 2000 m scales show similar patterns and explain similar amounts of variability in the species composition (21.4%, 21.6%, and 20.2%, respectively). The first canonical axis explained 59.4 percent of the constrained variability in the species composition data, and biplot scores indicate this axis was strongly negatively correlated with forest and positively correlated with pineapple (Fig. 2.3). The second canonical axis explained 28 percent of the constrained variability, and was strongly and positively correlated with pasture, and negatively and more weakly correlated with pineapple (Fig. 2.3).



**FIGURE 2.3** Canonical correspondence analysis ordination of all species at the 1000m scale. Each point is a separate species, labeled with a symbol that corresponds to that species' feeding guild. Land uses are represented by arrows, and the relative length of each arrow directly proportional to their importance in influencing bat assemblage composition

Examination of the species scores reveals guild-specific differences in the response to land cover type surrounding a sampling site (Fig. 2.3). The majority of insectivorous species had negative values on canonical axis one, indicating an association with greater forest cover (Fig. 3). The only insectivorous species with a strongly positive value on axis one was *Peropteryx kappleri*. In contrast, the majority of the frugivorous species were either clustered around the center of the ordination, indicating no strong responses to any axis, or distributed along the positive side of axis one, which was positively correlated with pasture and pineapple (Fig. 2.3). The two omnivorous species (*Phyllostomus discolor* and *Phyllostomus hastatus*) had positive values on canonical axis one, suggesting a higher tolerance of pasture and pineapple, while the single carnivorous species (*Vampyrum spectrum*) was closely positively aligned with pineapple (Fig. 2.3).



SPECIES RICHNESS, DIVERSITY, AND RELATIVE ABUNDANCE IN RESPONSE TO AGRICULTURAL INTENSIFICATION — None of the agricultural intensification metrics were significant predictors of total species richness or diversity at any scale. In contrast, analyses of guild-specific responses showed that several of the agricultural intensification metrics were significant predictors of relative abundance of insectivorous species at all scales, and the regression models explained a relatively high proportion of the variation in relative abundance (Table 2.2, adjusted  $r^2$  0.47-0.61). The percent land in mature forest maintained a significant positive relationship with relative abundance across all three scales, and the edge contrast of mature forest consistently showed a significant negative relationship. At the smallest scale of 500 m, relative abundance of insectivores was also negatively related to increasing distance between forest patches.

Similar to insectivores, the relative abundance of nectarivores was significantly positively associated with percent land in mature forest at the 500 m and 1000 m scales (Table 2). Compared to insectivorous species, less variation in relative abundance of nectarivores was explained by the agricultural intensification metrics (Table 2.2; adjusted  $r^2$  0.20-0.30). The relative abundance of frugivorous species was less strongly affected by the agricultural intensification metrics (Table 2.2; adjusted  $r^2$  0.19-0.46). The only significant predictor of relative abundance of frugivores was edge density of mature forest, which had a significant, negative relationship with relative abundance at all scales.

**Table 2.2** Multiple regression analyses of relative abundance of insectivorous, frugivorous, and nectarivorous bats as a function of the agricultural intensification metrics with mature forest as the focal land use type, at three scales. Metrics significant at  $\alpha=0.05$  are in bold.

|                     |                                     | 500 m         |              | 1000 m        |              | 2000 m        |              |
|---------------------|-------------------------------------|---------------|--------------|---------------|--------------|---------------|--------------|
| METRIC <sup>a</sup> |                                     | $\beta$       | <i>p val</i> | $\beta$       | <i>p val</i> | $\beta$       | <i>p val</i> |
| Insectivorous       | <b>PFOR</b>                         | <b>0.001</b>  | <b>0.007</b> | <b>0.001</b>  | <b>0.003</b> | <b>0.001</b>  | <b>0.001</b> |
|                     | ED                                  | 0.000         | 0.214        | 0.000         | 0.282        | 0.000         | 0.660        |
|                     | ENN                                 | <b>-0.001</b> | <b>0.048</b> | 0.000         | 0.965        | 0.000         | 0.685        |
|                     | <b>ECON</b>                         | <b>-0.001</b> | <b>0.037</b> | <b>-0.002</b> | <b>0.014</b> | <b>-0.001</b> | <b>0.035</b> |
|                     | <i>Adjusted Model r<sup>2</sup></i> | 0.471         |              | 0.525         |              | 0.613         |              |
| Nectarivorous       | <b>PFOR</b>                         | <b>0.001</b>  | <b>0.044</b> | <b>0.001</b>  | <b>0.028</b> | 0.001         | 0.054        |
|                     | ED                                  | -0.001        | 0.060        | -0.001        | 0.175        | -0.001        | 0.329        |
|                     | ENN                                 | 0.000         | 0.319        | 0.000         | 0.965        | 0.000         | 0.673        |
|                     | ECON                                | -0.001        | 0.106        | 0.000         | 0.884        | 0.000         | 0.833        |
|                     | <i>Adjusted Model r<sup>2</sup></i> | 0.308         |              | 0.223         |              | 0.207         |              |
| Frugivorous         | PFOR                                | 0.001         | 0.607        | 0.002         | 0.310        | 0.003         | 0.381        |
|                     | <b>ED</b>                           | <b>-0.007</b> | <b>0.010</b> | <b>-0.012</b> | <b>0.001</b> | <b>-0.013</b> | <b>0.037</b> |
|                     | ENN                                 | 0.000         | 0.778        | -0.004        | 0.051        | -0.002        | 0.319        |
|                     | ECON                                | -0.008        | 0.228        | 0.000         | 0.952        | 0.001         | 0.941        |
|                     | <i>Adjusted Model r<sup>2</sup></i> | 0.320         |              | 0.461         |              | 0.194         |              |

<sup>a</sup>PFOR = percent land in mature forest, ED = edge density of mature forest, ENN = mean Euclidean nearest neighbor distance between mature forest patches, and ECON = edge contrast index of mature forest.

## DISCUSSION

Our results suggest that the conversion of extensive pastures with many remnant trees, live fences, and small forest patches to pineapple monocultures is driving changes in assemblage composition in remnant forest patches, creating new assemblages that contain higher proportions of frugivorous bats and lower proportions of insectivorous bats than found in mature forests. While this process of agricultural intensification does not diminish the total species richness and diversity of the bat assemblage, it has a significant negative impact on the relative abundance of insectivores adapted to foraging in forests.

Findings from both ordination analyses support our hypothesis about the effects of agricultural intensification on assemblage composition. The NMDS ordination revealed that remnant forest patches surrounded by a minimum of 50 percent pineapple exhibited altered assemblage composition from that found in continuous mature forest or in other remnant forest patches where the matrix was

dominated by lower intensity agriculture such as pasture. The canonical axes identified by the CCA ordination confirmed this pattern, with the first axis strongly negatively correlated with percent land in forest and positively correlated with percent land in pineapple, and the second axis strongly positively correlated with percent land in pasture. If the differences in assemblage composition among patches were solely due to the loss of forest cover, the effects of pineapple and pasture would load onto a single axis opposite from forest. Instead, the first axis is dominated by the contrast between forest and pineapple, while the effect of pasture is partitioned out into the second axis (Fig. 2.3). This indicates that patches where the matrix is dominated by pineapple are characterized by an assemblage composition that is different not only from the composition in patches surrounded by forest, but also from the composition in patches where the matrix is dominated by pasture.

The CCA ordination further suggested that frugivorous species are tolerant of both habitat loss and fragmentation caused by lower intensity land uses such as pasture and the additional landscape alterations caused by agricultural intensification. Two frugivorous species, *Sturnira lilium* and *Uroderma bilobatum*, appear to be especially resilient. Like other frugivorous bats, these species' diets include a wide range of native plants found in both intact and disturbed forest (Fleming and Sosa 1994, Galindo-Gonzalez *et al.* 2000, Lopez and Vaughan 2007), and they are highly mobile even in fragmented landscapes (Loayza and Loiselle 2008, Meyer *et al.* 2009). Another species that the CCA indicates is resilient to agricultural intensification is the rare, threatened carnivore *Vampyrum spectrum*, which has previously been characterized as highly dependent on continuous mature forest (Peña-Cuellar *et al.* 2012, Vleut *et al.* 2012). However, several studies including ours have found *V. spectrum* in disturbed landscapes (Harvey and Villalobos 2007, Medina *et al.* 2007). The high mobility of this large bat may enable it to access isolated forest patches regardless of the dominant land use in the matrix. The only other threatened frugivorous species captured in this study, *Ectophylla alba*, did not show a strong response to agricultural intensification.

In contrast, the CCA revealed that the majority of insectivorous species are sensitive to habitat loss and fragmentation caused by both pasture and pineapple plantations. Only one insectivorous species, *P. kappeleri*, was strongly associated with the pasture and pineapple side of the first axis (Fig. 2.3). Unlike other insectivorous species captured, *P. kappeleri* is not adapted to foraging in dense vegetation, but rather is an aerial insectivore capable of foraging in open spaces, such as those found above pasture or pineapple (Kalko *et al.* 1996, Appendix 3, Table S2).

Overall, the ordination analyses suggest that the bat assemblage in remnant forest patches surrounded by intensive agriculture is likely to be characterized by a disproportionate abundance of frugivores, especially highly mobile species with a broad diet, a dearth of forest-dependent

insectivores, and potentially, an unexpectedly high number of large, highly mobile carnivores. The few existing studies that have sampled in intensive plantation crops such as banana have found bat assemblages characterized by a higher proportion of generalist species, especially large frugivores, and a lower proportion of nectarivorous and insectivorous species, especially insectivorous species adapted to forage in dense vegetation (Harvey and Villalobos 2007, Helbig-Bonitz *et al.* 2015). However, these studies sampled bats directly in intensive plantation crops, while our study sampled in remnant forest patches surrounded by intensive pineapple plantations. The alterations in assemblage composition we found in forest patches are nevertheless similar, suggesting that the effects of agricultural intensification on bat assemblage composition are pervasive across all land uses at the landscape scale, even within forest.

The NMDS ordination also revealed the existence of a potential threshold effect. The threshold effect concept proposes that there is a critical amount of habitat below which a species will become locally extinct (Lande 1987). In agricultural landscapes, this threshold depends on the species' reproductive rate and vagility, the configuration of habitat patches in the landscape, and the degree to which the species can use the intervening agricultural matrix as habitat (With *et al.* 1997, Fahrig *et al.* 2001). Few studies have examined threshold effects in bat assemblages, but Frey-Ehrenbold *et al.* (2013) measured bat activity in a European landscape undergoing agricultural intensification and identified guild-specific thresholds of habitat connectivity below which activity dropped significantly. In our study, the NMDS ordination plot shows that the three patches surrounded by more than 50 percent pineapple are clearly grouped together (Fig. 2.2). Patches surrounded by only slightly less pineapple would be expected to also group near these three patches. However, patches surrounded by only slightly less pineapple but at least 50 percent forest instead clustered with the patches surrounded by 60-100 percent forest. In addition, these patches contained several insectivorous species that were otherwise captured only in patches surrounded by 60-100 percent forest. This suggests that even in the presence of intensive plantation crops, if the surrounding landscape contains a high proportion of forest cover, patches have the potential to retain assemblage compositions similar to those found in intact forest. Further research is needed to determine whether this emerging pattern is indicative of a true threshold effect, and if so, to more precisely quantify that effect.

Contrary to our expectations, results from our regression analyses suggest that total species richness and diversity in remnant forest patches are resilient to agricultural intensification, and can remain similar to that found in continuous mature forest even as species composition undergoes dramatic shifts. Recent meta-analyses have shown that this is a common pattern in agricultural landscapes dominated by a mix of less intensive agriculture types (Garcia-Morales 2013, Mendenhall

*et al.* 2014), and here the pattern appears to hold even in remnant forest patches where intensive pineapple plantations are the main component of the matrix.

However, when the data were analyzed by feeding guild, we found a strong and varied response to the agricultural intensification metrics. Percent land in forest was consistently a significant predictor of higher relative abundance of insectivorous bats. This is not surprising, as the majority of insectivorous bats captured (12 of 17) were gleaning insectivores, which are specifically adapted to foraging in dense vegetation and depend on foliage and hollow trees for roosting sites (La Val and Rodriguez 2002). Although all agriculture results in some loss of forest cover, intensive plantation crops like pineapple cause measurably greater forest loss than lower intensity agriculture (Benton *et al.* 2003, Shaver *et al.* 2015), and will therefore have a stronger negative impact on the abundance of forest-dependent insectivorous species. The abundance of insectivores was also negatively affected by increased distance between remnant forest patches, and conversion of pasture to pineapple increases the minimum distance to the nearest forest patch (Table 2.1), as small-scale forest elements are usually eliminated to maximize production efficiency (Shaver *et al.* 2015). Insectivorous bats may be unable to cross these longer distances because their shorter and broader wings are adapted to slow, maneuverable flight in dense vegetation but make it difficult to quickly and efficiently cross large open spaces (Norberg and Rayner 1987).

The high agrochemical use in pineapple plantations may also contribute to making forest patches surrounded by pineapple less hospitable to insectivorous bats. Pineapple plantations apply fertilizers, fungicides, and pesticides year-round (Ingwersen 2012), and these agrochemicals can have an adverse effect on local insect populations (Echeverría-Sáenz *et al.* 2012). A study in coffee plantations in Mexico found that insectivorous bat abundance decreased in tandem with increased application of pesticides (García-Estrada *et al.* 2006). In our study, relative abundance of insectivores was negatively associated with high edge contrast of mature forest at all three scales, so the greater the proportion of mature forest edge shared with pineapple, the lower the relative abundance of insectivorous bats. This could potentially be a result of the application of agrochemicals in the pineapple, which would reduce the primary food source for insectivorous bats.

Nectarivorous species also showed a negative response to agricultural intensification. Although nectarivorous species can benefit from increased resource availability in agricultural land use types with high nectar and pollen availability (*e.g.*, shade cacao, banana plantations; Harvey and Villalobos 2007), the dominant agriculture types in the Sarapiquí region are pasture and pineapple, which do not provide food sources to nectarivorous bats. Therefore, nectarivorous species remain

dependent on the resources available within forest, and pineapple contains fewer small-scale forest elements than all other land use types (Shaver *et al.* 2015).

Frugivorous bats were the only feeding guild that showed no significant effect of loss of forest cover on relative abundance at any scale, which corroborates previous reports that frugivores are able to persist even in highly altered landscapes (Williams-Guillén 2010, Garcia-Morales 2013, Cisneros *et al.* 2014). Many frugivorous species are generalists (Lobova *et al.* 2009), which may contribute to their ability to find food resources even in landscapes impacted by agricultural intensification. In addition, many frugivorous bats have large home ranges and are capable of flying long distances (Morrison 1978, Loayza and Loiselle 2008, Meyer *et al.* 2009), which may allow them to move between remnant forest patches that are separated by intensive plantation agriculture. Although some studies suggest a slight increase in generalist frugivorous bats when forest is converted to agroforestry systems which provide additional food resources (Estrada and Coates-Estrada 1993, Pineda *et al.* 2005, Garcia-Morales *et al.* 2013), this is not to be expected in the case of conversion to pineapple because these plantations provide no known food resources.

Although they appear resilient to loss of forest cover, relative abundance of frugivorous bats was negatively impacted by increasing forest edge density at all three scales. The magnitude and direction of the effect of forest edge on bats likely depends in part on the degree of structural and functional contrast between the forest and the agriculture type in the matrix (Ewers and Didham 2006, Klingbiel and Willing 2009, Cortes-Delgado *et al.* 2011). Landscapes with low contrast edges, such as edge between mature forest and agroforestry systems providing food resources to bats, are more likely to show neutral or positive effects of edge density. In contrast, landscapes with high contrast edges, such as edge between mature forest and monoculture plantation agriculture, are more likely to show negative effects (Laurance *et al.* 2002, Ewers and Didham 2006). Because many forest remnants in this study were adjacent to pineapple, a large amount of high contrast edge was represented, which may explain why frugivorous bats exhibited negative relationships with edge density at multiple spatial scales.

This study provides the foundation for developing specific management recommendations to mitigate the impact of agricultural intensification on bat populations in agricultural landscapes throughout the Neotropics, especially in landscapes which retain significant forest cover. Our use of metrics specifically correlated with agricultural intensification allows us to go beyond simply documenting the effect of pineapple plantations on bat assemblages, and instead understand which aspects of this type of agriculture are driving declines in certain feeding guilds. For example, in our study area a federal forestry law mandates protection of all mature forest, but some pineapple

producers only conserve larger tracts of mature forest while eliminating small linear elements that interfere with the efficient operation of agricultural machinery (Shaver *et al* 2015). Our findings show that increased distance between forest patches at the 500 m scale is significantly correlated with lower abundance of insectivorous species; to protect these species the forestry law could be more comprehensively enforced so that pineapple producers retain small-scale forest elements between large forest patches that are more than 500 m apart. In tropical landscapes where intensive plantation crops such as pineapple are an important part of the local economy, these types of recommendations are more useful, and more likely to be implemented, than recommendations which simply state that the total area of intensive plantation agriculture must be reduced to facilitate biodiversity conservation.

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### Chapter 3:

## Comparative landscape genetics of two frugivorous bats in a biological corridor undergoing agricultural intensification

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### Abstract

Agricultural intensification in agricultural landscapes in the tropics poses a new threat to the ability of biological corridors to maintain functional connectivity for native species. In this study, we use a landscape genetics approach to evaluate impacts of pineapple plantations on two widespread and abundant frugivorous bats in a biological corridor in Costa Rica. We hypothesize that the larger, more mobile *Artibeus jamaicensis* will be less impacted by pineapple than the smaller *Carollia castanea*. In 2012 and 2013, we collected samples from 735 bats in 26 remnant forest patches surrounded by different proportions of pasture, crops, and pineapple. We used 10 microsatellite loci for *A. jamaicensis* and 16 microsatellite loci for *C. castanea* to estimate genetic diversity, relatedness, and gene flow. Regression analyses indicate that the proportion of pineapple surrounding patches has no impact on genetic diversity or relatedness of *A. jamaicensis*. However, for *C. castanea* the proportion of pineapple surrounding patches is significantly negatively correlated with genetic diversity, and positively correlated with relatedness. Least-cost transect approach analyses (LCTA) reveal that surprisingly, pineapple is the land cover type most permeable to gene flow for *A. jamaicensis*, while as expected, forest is the most permeable land cover type for gene flow of *C. castanea*. For both species, the LCTA indicated that development inhibits gene flow. This study answers the call for more landscape genetic research focused on tropical and agricultural landscapes, and highlights the value of comparative landscape genetics in biological corridor design and management.

**Keywords:** Biological corridor, bats, agricultural intensification, landscape genetics, tropical agricultural landscapes, least-cost transect approach

### Introduction

Over the past century, widespread conversion of tropical forests to agriculture has created complex landscapes composed of remnant forest embedded in a matrix of crops and pasture (DeClerck et al 2010). This conversion has caused widespread habitat loss and fragmentation, often leading to a loss of connectivity and interrupted gene flow between populations of native plants and animals (Fahrig et al. 2003, Chazdon 2009). Biological corridors are a widely used strategy to restore and protect habitat connectivity across these types of landscapes. In tropical regions, corridors are often designed based

on social and political considerations, rather than on an explicit analysis of the structural connectivity of remnant forest (Canet et al 2009). Although structural connectivity is important, the ultimate goal of a biological corridor is to protect functional connectivity, that is, the ability of native species to move between remnant forest patches regardless of the physical distance or land use type separating them, and to survive and successfully reproduce even in isolated patches (Belisle et al 2005, Kadoya 2009). Studies have found that many existing biological corridors in temperate regions have achieved this goal, and are successfully facilitating movement and resulting gene flow for a variety of species in diverse human-modified landscapes (Mech and Hallet 2001, Gilbert-Norton et al 2010, Sharma et al. 2013). Corridors in tropical regions may be similarly effective, but to date very few studies have tested functional connectivity for native species in tropical biological corridors.

In recent years the viability of biological corridors in tropical regions has been threatened by a new land use trend: the rapid spread of intensive plantation agriculture (Perfecto & Vandermeer 2008, Brannstrom 2009, Shaver et al. 2015). Across the global tropics, the agricultural matrix is transitioning from a diverse mixture of pasture and local market crops to intensive monoculture plantations of export crops such as oil palm, soybeans, and pineapple (DeClerck et al. 2010, Wilcove & Koh 2010, Arvor et al. 2012, Fagan et al. 2013). This trend is predicted to continue (Harvey et al 2005, Wright et al 2012). This transition threatens the ability of biological corridors to maintain functional connectivity for a range of species: it replaces land use types that are biodiversity friendly because they are structurally and/or compositionally similar to native vegetation (Brotons et al. 2003, Perfecto et al. 2003, Harvey et al. 2008, Prevedello & Vieira 2009) with large-scale, monoculture plantations that simplify and homogenize the agricultural matrix, reduce total tree cover (Höbinger et al. 2012, Shaver et al. 2015), and increase agrochemical inputs (Harvey et al 2005, Tschardt et al 2012). These characteristics suggest that intensive plantation agriculture has low potential to provide habitat connectivity, and the few existing studies of animal movement and gene flow through this type of agriculture support this hypothesis (Vaughan et al. 2007; Blair and Melnick 2012).

In the face of agricultural intensification, can tropical biological corridors continue to maintain functional connectivity for native species? Bats are an excellent focal group for testing the impact of intensive plantation agriculture on biological corridors, especially in the Neotropics; in this region bats are abundant and widespread, comprising 40-50% of total mammal species richness (Patterson et al. 2003), and serve as the primary seed dispersers and pollinators for hundreds of native plant species (Fleming et al. 2009; Lobo et al. 2009). Loss of functional connectivity for bats will also impact functional connectivity for mutualistic plants in remnant forest patches and impede the regeneration of new forest patches (Lobo & Mori 2004; Melo et al. 2009). Because bats are volant, it is often



assumed that they have high dispersal potential and will maintain functional connectivity regardless of habitat loss, fragmentation, and land use change, but it is uncertain whether this is true in landscapes experiencing agricultural intensification (Struebig et al 2011, Razgour et al 2014).

Population and landscape genetics approaches provide a useful framework for evaluating functional connectivity in these types of landscapes, but to date, very few studies have implemented these approaches to assess connectivity for Neotropical bats. Some of these have found no detectable impact of habitat loss and fragmentation. Populations of frugivorous bats in fragmented agricultural landscapes in Brazil and Costa Rica showed similar genetic diversity in forest fragments and continuous forest, and no significant genetic structure at scales of 20 to 120 km (McCulloch et al 2013, Ripperger et al 2014). In contrast, other studies have shown that isolated populations of frugivorous bats in human-modified landscapes can develop significant genetic structure even at fine scales of 20km or less (Meyer et al 2009, Ripperger et al 2013), and have provided evidence that this genetic structure is related to the amount of suitable habitat in the agricultural matrix (Ripperger et al 2013). Although no studies to date have specifically tested the impact of intensive plantation agriculture on genetic structure in Neotropical bats, evidence from non-genetic studies indicates that this type of agriculture may impede movement of some bat species (Phommaxy et al 2011, Cormier et al 2013, Freudmann et al 2015), and alter overall bat assemblage composition (Freudmann et al 2015; Cleary et al 2016).

In this study, we use a landscape genetics approach to evaluate the effects of land use change and agricultural intensification in a Costa Rican biological corridor on functional connectivity for two abundant and widespread frugivorous bat species. The land use history in the study area reflects a common pattern in the tropics: initial deforestation to establish pasture and local market crops created a complex landscape of remnant forest embedded in a diverse agricultural matrix, and in recent years, this diverse matrix is rapidly being replaced with large-scale monoculture pineapple plantations (Morse et al 2009, Fagan et al 2013). This study represents one of the few examples of tests of the efficacy of a biological corridor using a genetic approach (Christie and Knowles 2015), and also furthers biological corridor research by evaluating functional connectivity for multiple species simultaneously (Beier et al 2011). In addition, this study addresses several areas recognized as research priorities in landscape genetics. First, the majority of landscape genetics studies to date have predominantly focused on temperate species (Storfer et al 2010, Manel and Holderegger 2013; Cleary et al 2015) despite the fact that tropical regions are hotspots of biodiversity (Myers et al 2002). Second, comparative landscape genetics studies of multiple species in the same landscape remain scarce, but can provide valuable insight about shared responses to landscape features (Storfer et al

2010, Balkenhol et al 2015). Third, agricultural landscapes are underrepresented in landscape genetics studies, despite that fact that current assessments of global land use indicate that over 40% of the planet's ice-free surface has been converted to agriculture (Ellis et al 2010, Storfer et al 2010).

We focus on two frugivorous bat species with differential mobility (Meyer and Kalko 2008a): the Chestnut short-tailed bat, *Carollia castanea*, a small frugivore with low mobility, and the Jamaican fruit bat, *A. jamaicensis*, a larger, more mobile frugivore. We expect degree of mobility to be directly related to the impact of land use change on functional connectivity for these species; less mobile species, with small home ranges, short broad wings, and lower ratios of body size to wing area, are more vulnerable to fragmentation (Meyer et al 2008b, Burns and Broder 2014), and show higher genetic structure in fragmented landscapes (Meyer et al. 2009, Struebig et al 2011). We also expect diet to influence how these species are impacted by land use change. *C. castanea* is a specialist on plants in the family Piperaceae, and more than half of its diet is comprised of Piperaceae which occur in interior forest understory (Thies and Kalko 2004). In contrast, *A. jamaicensis* is a generalist which is known to feed on more than 100 species of plants, but its diet is dominated by figs (*Ficus*; Handley et al 1991, Ortega and Castro-Arellano 2001, Lopez and Vaughan 2007). Fig trees produce large crops of fruit but ripen asynchronously, creating a patchily distributed food resources that *A. jamaicensis* will travel long distances to locate (Morrison 1978).

We compare genetic diversity and mean relatedness for both species across the study area, and predict that *C. castanea* will exhibit lower genetic diversity and higher relatedness than the more mobile *A. jamaicensis*. We also estimate relatedness separately by sex for both species, and hypothesize that since most mammals exhibit male-biased sex dispersal (Greenwood 1980), relatedness will be higher for females in both species. To assess the impact of different land cover types and the expansion of pineapple on genetic diversity and relatedness, we test whether genetic diversity and relatedness are correlated with the percentage of each land cover type around each sampling site at relevant scales. We predict that higher percentages of forest surrounding a site will lead to higher genetic diversity and lower relatedness, and higher percentages of pineapple surrounding a site will be associated with lower genetic diversity and higher relatedness. We also predict that these effects will be stronger for *C. castanea* than for *A. jamaicensis*. To evaluate the effect of different land cover types on gene flow, we calculate overall genetic differentiation and pairwise genetic distance for each species, and hypothesize that *C. castanea* will show higher overall differentiation and more pairs of sampling deviating from panmixia than *A. jamaicensis*. Next, to identify the land cover type most permeable to gene flow of each species, we use a least-cost transect approach and maximum likelihood population

effects models introduced by Van Strien et al (2012). We predict that for both *C. castanea* and *A. jamaicensis*, intact forest cover will facilitate gene flow while pineapple and development inhibit it.

## Materials and Methods

### *Study site and field sampling*

This study was conducted in the San Juan-La Selva (SJLS) biological corridor and surrounding areas (Fig. 1; 10°22'-10°43'N, 83°60'-84°20'W). The SJLS region lies in the Holdridge Life Zone of wet tropical forests (Holdridge et al. 1975), at an elevation of 0-400m. Mean annual temperature is 25°C and mean annual precipitation is approximately 4000 mm (Sesnie et al 2009), with a dry season generally lasting from January to early April. The soils are acidic (pH ~4.5), primarily Inceptisols and Ultisols (Sollins et al. 1994), and support a forest dominated by *Pentaclethra maculosa* and palms (Sesnie et al. 2009).

Old-growth forest cover remained largely intact in this landscape until the 1970s, when human populations began to grow and government policies promoted the clearing of forests to establish subsistence crops and pasture for cattle ranching (Schelhas and Sanchez-Azofeifa 2006, Shaver et al 2015). As a result, total forest cover in the landscape decreased from 75.6% in 1960 to 48.5% in 1996 (Schelhas and Sanchez-Azofeifa 2006). In an effort to protect remaining forest cover, Costa Rica's 1996 Forest Law banned the clearing of old-growth forests, and effectively froze remaining old-growth forest patches in place on the landscape (Morse et al 2009). In 2001, forest conservation efforts were further strengthened by the establishment of the 246, 608 hectare SJLS biological corridor. The goal of the SJLS is to protect habitat connectivity between the Indio Maiz Biological Reserve in Nicaragua and Braulio Carrillo National Park in Costa Rica, while still allowing for agricultural productivity (Villate et al 2009). Indio Maiz and Braulio Carrillo represent an important link in the larger Mesoamerican Biological Corridor, a land use planning system that was initiated in 1997 to facilitate regional ecological connectivity and sustainable development from Mexico to Panama (DeClerck et al 2010).

During the same time period that forests were receiving increasing protection, agricultural intensification began to change the composition of the intervening agricultural matrix. Intensively managed pineapple plantations first arrived in the region in the early 1990s, and have been spreading rapidly since then, replacing extensive and degrading pasture in the agricultural matrix (Schelhas and Sanchez-Azofeifa 2006, Fagan 2013). Between 2006 and 2010, land in Costa Rica under pineapple cultivation increased from 22,400 to 45,000 hectares (Barquero 2011), and more than half of this is in the SJLS region (Blacio et al 2010). The spread of pineapple plantations has led to increased

availability of jobs in the SJLS region, which has driven high population growth and an associated expansion of urban areas and roads (Schelhas and Sanchez-Azofeifa 2006, Shaver et al 2015). The current landscape is characterized by a mix of old growth and secondary forest (43.8% of the landscape; hereafter referred to as “forest”), pasture (39.7%), pineapple and bare soil in preparation for planting pineapple (6.5%), smallholder crops and tree plantations (5.9%), and development, which includes urban areas and roads (~1.7%) (Shaver et al 2015).

To capture the range of possible responses of the frugivorous bat community in the SJLS region to this history of land use change, we selected two focal species at opposite ends of the spectrum of size and vagility. The Chestnut short-tailed bat (*Carollia castanea*) is a small frugivore weighing 11-16 g and having an average home range size of less than 7 hectares (Reid 2009, Bonaccorso et al. 2006). This bat occurs in lowland moist forests from Honduras to Bolivia, and is a feeding specialist with a strong reliance on plants in the Piperaceae family, especially those found in the understory of mature forest (Bonaccorso 1979, Thies & Kalko 2004). The Jamaican fruit bat (*Artibeus jamaicensis*) is a large frugivore weighing 29-51 g, also found in moist lowland forests from Mexico to Ecuador (Reid 2009). This large, vagile species is a feeding generalist that is known to disperse the seeds of at least 91 Neotropical plant species (Lopez & Vaughan 2007), and has been observed to fly up to 8 km between day roosts and feeding areas (Morrison 1978). Generation time of *A. jamaicensis* is approximately one year (Ortega and Castro-Arellano 2001). No direct information is available about the generation time of *C. castanea*, but the closely related *C. perspicillata* also has a generation time of approximately one year (Cloutier and Thomas 1992). Studies which report absolute abundance of both species in this region show that abundance is generally higher in *C. castanea* (Lopez and Vaughan 2007, Rex et al 2008, Cleary et al 2016), suggesting that *C. castanea* may have a larger effective population size than *A. jamaicensis* in the study area.

To select sampling sites, we first used a 2011 land cover map created using high resolution (5 m) RapidEye imagery (Shaver et al. 2015) to identify a set of patches of old-growth forest between 15-60 hectares in size, separated by at least 1.5km, and surrounded by different percentages of four land cover types relevant to agricultural intensification: forest, pasture, pineapple, and development. Next, we used older land cover maps from 1986, 1996, and 2001 to select patches from this set that have had approximately the same degree of isolation from other forest for the past 25 years. To provide an estimate of baseline gene flow in intact forest, we ensured that a subset of the patches were only separated by continuous forest. This process resulted in a final set of 26 sampling sites (Fig. 1). Sampling sites were visited 3-6 times between January and May of 2012 and 2013. On each visit we set ten 12 m x 3 m mist nets (Avinet, Dryden, NY) along forest trails and kept nets open from 6:00-

10:30 pm. We collected a tissue sample from the uropatagium of each *C. castanea* and *A. jamaicensis* captured; samples were stored in lysis buffer (100 mM NaCl, 50 mM EDTA, 50 mM Tris pH 8.0, 50 mM sucrose, 1% SDS). Our goal was to sample at least 20 individuals of each species in each sampling site (Hale et al 2012), but in some sites we were not able to reach this number due to small numbers of individuals caught in mist nets.

#### *Microsatellite genotyping and summary statistics*

Genomic DNA was extracted using a QIAGEN DNeasy Tissue kit, which was modified for use with lysis buffer by replacing the ATL buffer in the first step of the extraction with the same volume of lysis buffer from the sample tube. PCRs were conducted with multiplexed sets of 4-10 forward labelled primers (PCR conditions described in Appendix 4, Table S1). *A. jamaicensis* individuals were genotyped at 10 microsatellite loci, of which five were developed for *A. jamaicensis* (AjA151, AjA40, AjA47, AjA74, AjA80; Ortega et al. 2002) and five were cross-amplified from *A. literatus* (AL579, AL700, AL821, AL850, AL854; McCulloch et al 2011). *C. castanea* individuals were genotyped at 16 microsatellite loci, of which ten were developed for *C. castanea* (CC7, CC10, CC13, CC18, CC19, CC23, CC25, CC26, CC27, CC29; Cleary et al *in preparation*), five were cross-amplified from *Carollia brevicauda* (AAGG1, AAGG7, AAGG98, AAGG117, AAGG119; Bardeleben et al 2007), and one was cross-amplified from *A. literatus* (AL700; McCulloch et al 2011). Raw alleles were scored using Genemapper 3.7 (Applied Biosystems). To check for errors, 50% of samples from each species were run twice. Genotyping error rates were calculated using the R package ConGenR (Lonsinger and Waits 2015). All loci were tested for significant linkage disequilibrium and deviations from Hardy-Weinburg equilibrium using Genepop 4.2 (Raymond and Rousset 1995).

#### *Population genetic structure*

For all sampling sites, we identified private alleles and calculated unbiased expected heterozygosity for all individuals of each species using Genalex 6.5 (Peakall and Smouse 2012) and allelic richness using HP-rare (Kalinowski 2005). We estimated mean relatedness at all sampling sites for all individuals of each species using Lynch and Ritland's (1999) estimator in Genalex 6.5. To evaluate whether males and females within each species exhibited different population structure, we also identified private alleles and estimated mean relatedness separately by sex. To estimate genetic differentiation, we chose the metric  $G''_{st}$ . This metric is an analog of  $F_{st}$  that corrects for impacts of differences in heterozygosity and sample size among sampling sites, and is unbiased when a small number of populations are sampled (Meirmans and Hedrick 2011). First, we compared overall differentiation using only sampling sites sampled for both species using the global  $G''_{st}$  statistic calculated in Genalex 6.5. Next, we estimated pairwise genetic distance between all sites within

species using  $G''st$ , also in Genalex 6.5. Global  $G''st$  and pairwise  $G''st$  were also calculated for males and females separately for each species; only sampling sites with a minimum of five individuals of the respective sex were used in these calculations. Significance of pairwise  $G''st$  was tested by calculating the probabilities of all values with 999 permutations.

#### *Influence of landscape on genetic diversity and relatedness*

To test whether genetic diversity and relatedness in sampling sites have been influenced by surrounding land cover, we first used the 2011 land cover map in ArcGIS 10.1 to create circular buffers centered on each sampling site, at three scales predicted to be relevant to *C. castanea* and *A. jamaicensis*: 500m, 1000m, and 2000m. The smallest scale of 500m corresponds to the average long axis of the home range of *C. castanea* (Bonaccorso et al 2006), while the largest scale of 2000m has the potential to contain the average home range of *A. jamaicensis* (Morrison 1978, Bernard and Fenton 2003). Next, we used FRAGSTATs (v.4, McGarigal et al. 2012) to calculate the percentage of each land cover type within the circular buffers centered on each sampling site. We focused on the four land cover types identified above as relevant to agricultural intensification: forest, pasture, pineapple, and development. We used simple univariate regressions to test for significant relationships between the percentage of these four land cover types surrounding each site at each of the three scales and genetic diversity and relatedness of each species within that site. All regressions were performed using the R statistical package (R Core Development Team).

#### *Influence of landscape on genetic distance*

To evaluate how land use change in the SJLS has affected gene flow for the focal bat species, we used a least-cost transect analysis (LCTA) approach developed by Van Strien et al (2012). One of the most persistent challenges in landscape genetics is objectively estimating resistance distances between sampling sites (Spear et al 2010). A common approach is to first create a resistance surface where each land cover type or landscape feature is assigned a resistance value based on expert opinion, model optimization, or ecological field data (Spear et al 2010, Hall and Beissinger 2014), and then define resistance distance as the sum of the resistance values in a straight line or least cost path between sampling sites. These methods lack objectivity because they require the researcher to make *a priori* decisions about how different landscape features will affect gene flow for a given species. A potential solution is offered by transect-based approaches, which quantify the percentage of each land cover type within different buffer widths around straight lines connecting sampling sites and correlate these values with genetic distances (e.g. Pavlacky et al 2009, Goldberg and Waits 2010). Rather than assigning resistance values *a priori* to different land cover types, transect-based approaches allow researchers to directly test whether each land cover type facilitates or inhibits gene flow (Spear et al

2010). However, transect approaches based on straight line distances assume that dispersal occurs along a straight-line path, an unlikely hypothesis for many systems. The LCTA approach retains the objectivity of transect based approaches, and addresses the problem of the straight-line path by replacing straight line distances between sampling sites with least-cost paths, and then calculating the percentage of each land cover type inside transect widths around the least cost path. These percentages, combined with total transect length, can then be used as explanatory variables in models of the effect of the landscape on gene flow.

The first step in the LCTA approach is to create a set of binary resistance surfaces (RS) where each land cover type of interest has the opportunity to be considered optimal dispersal habitat. We continued to focus on the same four land cover types: pasture, forest, pineapple, and development. All analyses were conducted in ArcGIS 10.1 (ESRI) unless otherwise noted. For each land cover type, we created three RSs using the 2011 land cover map; in all RSs the land cover type being considered optimal dispersal habitat was given a value of 1, while all other land cover types were given a progressively higher resistance value ( $2^3$ ,  $2^6$ ,  $2^9$ ) in each of the three RSs. Separate least-cost path (LCP) analyses were conducted for each of the 12 resulting RSs, using the ArcGIS landscape genetics toolbox (Etherington 2010). To represent a scenario of isolation by Euclidean distance (IBD), we also created a set of LCPs that were simply straight line distances between all pairs of sampling sites. LCPs for the 12 RSs and the IBD scenario were buffered at three different transect widths (100 m, 400 m, and 800 m), since bats respond to land use change at multiple scales (Cleary et al 2016). The smallest scale of 100 m was chosen because given that home range size of *C. castanea* is approximately 7 ha, it is unlikely that land use inside a transect much narrower than 100 m would affect long-term gene flow of this species, much less of the larger *A. jamaicensis*. The largest scale of 800 m was the maximum scale at which heterogeneity in land cover proportions between LCPs was retained; at larger scales the land cover proportions in most LCPs approached average proportions across the entire study area. For each transect, we used Geospatial Modelling Environment (v 0.7.4.0, Beyer 2015) to calculate the total length of each LCP and the percentage of each of the four land cover types within the three transect widths. The final product of these analyses was 36 LCP datasets (4 land cover types x 3 resistance values x 3 transect widths), plus three LCP datasets for the IBD scenario (IBD least cost path x 3 transect widths). Each LCP dataset contained five explanatory variables for each pair of sampling sites: the total length of the least cost path, and the percentage of pasture, forest, pineapple, and development within the transect.

Next, we modeled the relationship between these explanatory variables and the response variable of genetic distance using the maximum-likelihood population effects (MLPE) method of Clarke et al.

(2002). The MLPE method was recently introduced to landscape genetics by Van Strien et al (2012), who proposed it as a solution to the problem of non-independence in pairwise genetic distance datasets. Unlike other linear mixed effect models, MLPE models incorporate a covariance structure specifically tailored for the dependency between pairwise values. For each species, we applied the MLPE approach to model the relationship between each explanatory variable and pairwise genetic distance ( $G^{\text{st}}$ ). Preliminary tests indicated the land cover variables showed high levels of collinearity, which would make interpretation of multi-variable models difficult. Therefore, we restricted our model sets to contain only univariate models. We used Akaike's information criterion (AIC), which has been shown to be effective for REML mixed model selection (Gurka 2006), to select the top model from each of the 39 LCP datasets, then reported the top two LCPs explaining most of the genetic differentiation for each species.

## Results

### *Microsatellite genotyping and summary statistics*

We collected sufficient genetic samples of *C. castanea* and *A. jamaicensis* from 24 of the 26 sampling sites (Table 3.1). After removing samples with incomplete or inconsistent genotypes, we assembled complete, reliable genotypes for 349 *C. castanea* individuals and 386 *A. jamaicensis* individuals. As a result of this screening, error rates in our final genotypes were very low: for *C. castanea* the false allele rate was 0.38% and the allelic dropout rate was 0.58%, and for *A. jamaicensis* the false allele and allelic dropout rates were 0.24% and 0.69%, respectively. Tests for deviations from Hardy-Weinberg equilibrium indicated that for *C. castanea* loci, 24 out of 384 (6.25%) Hardy-Weinberg tests were significant at  $\alpha = 0.05$ , with no patterns across loci or sites. No pair of loci was consistently in linkage disequilibrium across more than 3 sampling sites, out of 24 total sampling sites. For *A. jamaicensis* loci, 2 out of 240 (0.83%) Hardy-Weinberg tests were significant at  $\alpha = 0.05$ , with no patterns across loci or sites. No pair of loci was consistently in linkage disequilibrium across more than 4 sampling sites. Thus all loci were retained for the full analysis.

### *Population genetic structure*

Genetic diversity was high across the study area for both species. Average unbiased heterozygosity across all sampling sites was 0.721 for *C. castanea* and 0.740 for *A. jamaicensis*, and average allelic richness was 4.88 for *C. castanea* and 4.92 for *A. jamaicensis* (Table 3.1). The total number of private alleles across all sampling sites differed dramatically between species, with *C. castanea* having 19 while *A. jamaicensis* had only four. Estimates of mean relatedness at each sampling site found that for *C. castanea*, 9 sampling sites show relatedness significantly higher than the average and no sites show



relatedness significantly lower than average (Table 3.2). For *A. jamaicensis*, 6 sites show relatedness significantly higher than the average and one site shows relatedness significantly lower than average (Table 3.2). Estimates of relatedness separately by sex indicated that *C. castanea* males showed more sites ( $n = 7$ ) with higher than average relatedness than did *C. castanea* females ( $n = 3$ ) (Appendix 4, Table S3). *A. jamaicensis* males and females showed higher than average relatedness at approximately the same number of sites (males,  $n = 3$ ; females,  $n = 4$ ) (Appendix 4, Table S4).

Overall, levels of genetic differentiation across the study area were similar between *C. castanea* ( $G''st = 0.025$ ,  $p = 0.012$ ) and *A. jamaicensis* ( $G''st = 0.022$ ,  $p = 0.044$ ). At the level of the sampling site, significant pairwise  $G''st$  values were found for 47 pairs (17.1%) of *C. castanea* populations and 44 pairs (15.8%) of *A. jamaicensis* populations (Table 3.3). For *C. castanea*, more than half of the significant pairwise  $G''st$  values were from two patches: BT.9 and ER.36 (Appendix 4, Table S1a). These two patches were very isolated from continuous forest and surrounded by considerable amounts of pineapple (Fig. 3.1). In comparison, no patches separated only by continuous forest showed significant  $G''st$  (Appendix 4, Fig. S1a).

For *A. jamaicensis*, more than half of the significant pairwise  $G''st$  values were from two different patches: ER.19 and ER.23 (Appendix 4, Fig. S1b). These two patches are close together, but are not more isolated than average from continuous forest (Fig. 3.1). Estimates of global and pairwise  $G''st$  separately by sex indicated higher structure in *C. castanea* males than females: global  $G''st$  for females was not significant ( $G''st = 0.014$ ,  $p = 0.166$ ) and significant  $G''st$  values were only found for 6 of 171 (3.5%) pairs of populations, while global  $G''st$  for males was highly significant ( $G''st = 0.035$ ,  $p = 0.006$ ) and significant  $G''st$  values were found for 31 of 210 (14.3%) pairs of populations. In contrast, there was no significant difference in genetic structure between sexes in *A. jamaicensis*: global  $G''st$  was not significant for males ( $G''st = 0.016$ ,  $p = 0.197$ ) or females ( $G''st = 0.021$ ,  $p = 0.139$ ), and significant  $G''st$  values were found for males in 16 of 153 (10.4%) pairs of populations, and for females in 23 of 153 (15.0%) pairs of populations.

#### *Influence of landscape on genetic diversity and relatedness*

Regression analyses showed that genetic diversity and relatedness of *C. castanea* were correlated with the percentage of selected land cover types at all three scales evaluated using circular buffers (500 m, 1000 m, 2000 m; Fig. 2). Since the significance and direction of the relationships was the same across all three scales, for the sake of brevity we report only the results from the 1000 m radius buffer, but results from the 500 m and 2000 m are similar and available in supplementary materials (Appendix 4, Table S5). Allelic richness of *C. castanea* had a significant positive relationship with the percentage of forest cover ( $r^2 = 0.229$ ,  $p = 0.018$ ) and a significant negative relationship with pineapple ( $r^2 = 0.199$ ,

$p=0.029$ ). Relatedness of *C. castanea* had a significant negative relationship with the percentage of forest cover ( $r^2=0.185$ ,  $p=0.040$ ) and a highly significant positive relationship with pineapple ( $r^2=0.425$ ,  $p<0.001$ ). In contrast, genetic diversity and relatedness of *A. jamaicensis* showed no significant relationships with the percentage of these four land cover types at any scale.

#### *Influence of landscape on genetic distance*

The model representing the IBD scenario was never selected as top model for either species, which suggests that genetic differentiation in these species cannot be determined by distance alone, and that land cover does play a role in determining dispersal pathways for *C. castanea* and *A. jamaicensis*. The top two models for explaining genetic differentiation in *C. castanea* were not different from each other by more than 2 AIC, but were different from all other models by more than 2 AIC (Table 3.4). Both of these top models identified forest as the most likely dispersal habitat for this species, with a transect width of 100m and with the intermediate ( $2^6$ ) and highest ( $2^9$ ) resistance values, respectively. For *A. jamaicensis*, the first top model was different from the second top model by 8 AIC (Table 3.4). This top model was with pineapple as most likely dispersal habitat, with the highest resistance value ( $2^9$ ) and a transect width of 100 m. The AIC values of the second, third, and fourth models did not differ by more than 2 AIC, and all of these models also identified pineapple as the most likely dispersal habitat. For both species, the land cover variable in both top models was development, which had a negative impact on gene flow (positive correlation with  $G''st$ ) in all models.

## **Discussion**

Our findings suggest that despite agricultural intensification, the SJLS biological corridor is still effective at facilitating functional connectivity for *A. jamaicensis*, but historical habitat loss and fragmentation and the recent expansion of pineapple have begun to disrupt movement and resulting gene flow across the SJLS region for *C. castanea*. This study represents one of the few empirical tests of the efficacy of a biological corridor in the tropics, and one of the few tests of biological corridors in any ecosystem to implement a genetic approach (Paetkau et al 2009, Gilbert-Norton et al 2010, Christie and Knowles 2015, Waits et al 2016). The differences between these two frugivorous bats in the response of genetic diversity and gene flow to land use change show the importance of considering multiple species when designing and managing biological corridors. In addition, this study demonstrates that landscape genetics approaches can serve as valuable tools for understanding how to restore and protect functional connectivity in tropical agricultural landscapes, which are underrepresented in landscape genetics (Storfer et al 2010, Cleary et al 2015).

*Population genetic structure in an intensifying agricultural landscape*

Estimates of genetic diversity, relatedness, and genetic differentiation support our hypothesis that *C. castanea* has been more strongly impacted by recent land use change than *A. jamaicensis*. Although genetic diversity across the SJLS landscape is only marginally lower in *C. castanea* than in *A. jamaicensis*, other metrics suggest more pronounced differences between the species. More than 33% of the sampling sites showed higher than average relatedness for *C. castanea*, while only 25% of *A. jamaicensis* sites show higher than average relatedness. Sites with higher than average relatedness are at risk for inbreeding depression (Keller et al 2002), and this threat appears to be more immediate for *C. castanea*. In addition, *C. castanea* populations have almost five times as many private alleles as *A. jamaicensis* populations; the fact that so many alleles occur only in only a single *C. castanea* population may be due to low gene flow and genetic drift causing loss of shared alleles between populations (Slatkin et al 1985).

Previous studies of population genetic structure in *A. jamaicensis* have sampled fewer populations, but also found low relatedness within populations and low or no significant genetic differentiation across populations (Ortega et al 2003, Vasquez-Dominguez et al 2013). Both of these studies were conducted in fragmented agricultural landscapes at relatively small extents of less than 300 km<sup>2</sup>. However, McCulloch et al (2013) found that a closely related species of only slightly larger size, *Artibeus literatus*, showed no significant genetic structure across distances of more than 120 km in a region of Paraguay characterized by high forest fragmentation. This suggests that *A. jamaicensis* may be able to maintain genetic connectivity over a scale much larger than the SJLS biological corridor, as long as the landscape contains sufficient land use types that can provide roosting and foraging habitat. Genetic responses of *C. castanea* to land use change are not well documented, but Ripperger et al (2014) found no evidence of genetic structure in *C. castanea* in the same landscape as this study. However, Ripperger et al (2014) sampled on a finer spatial scale (~300 km<sup>2</sup>) and only in the southeastern part of the SJLS, which is characterized by lower forest fragmentation and less pineapple than the broader extent (~900 km<sup>2</sup>) over which our study sampled. The fact that *C. castanea* shows significant genetic structure in our study but not in Ripperger et al (2014) may be due to our sampling design capturing the response of *C. castanea* populations over a larger scale and to higher levels of forest fragmentation and agricultural intensification. In addition, Ripperger et al (2014) used mitochondrial DNA markers, which have a much slower mutation rate than nuclear DNA microsatellite markers, and are thus less capable of detecting responses to recent landscape change (Wang 2010, Cleary et al 2015). Since forest fragmentation and the expansion of pineapple happened very recently on an evolutionary

timescale, we would expect that only neutral nuclear DNA markers would already show a response to these processes.

Comparing genetic structure between these two types of markers in *C. castanea* in the SJLS region leads to an interesting insight about sex-biased dispersal in this species: higher genetic structure in microsatellites than in mitochondrial DNA suggests female-biased dispersal. This is contrary to our prediction that *C. castanea*, like the majority of mammals (Greenwood 1980), would show male-biased dispersal patterns. Although comparing data between two studies can be unreliable, additional support for female-biased dispersal is provided by data within each study. Separate analyses of genetic structure evaluating  $G''_{st}$  for male and female *C. castanea* in this study showed significant global genetic structure in males, but not in females. This is corroborated by relatedness estimates, where more sampling sites have higher than average relatedness in males than females. Ripperger et al (2014) also found significant genetic structure in males, but not in females. Female-biased dispersal is rare in mammals (Greenwood 1980) but evidence for it has been documented in several other Neotropical bat species (Nagy et al 2007, Nagy et al 2013), including one species in the same family as *C. castanea* (*Phyllostomus hastatus*; McCracken and Bradbury 1981).

#### *Influence of landscape on functional connectivity*

Landscape genetic analyses indicated that historical loss of forest cover and more recent expansion of pineapple plantations in the SJLS biological corridor are driving moderate loss of functional connectivity for *C. castanea*, but no effects were detected for *A. jamaicensis*. These results are in line with our hypotheses for *C. castanea*, but do not support our hypothesis that *A. jamaicensis* would also have a detectable response to recent land use change. Tests of pairwise population differentiation in *C. castanea* revealed that 30 of the 47 total significant pairwise  $G''_{st}$  values for *C. castanea* populations were accounted for by the two most isolated sampling sites surrounded by the highest percentage of pineapple (ER.36 and BT.9, Fig. 1, Fig. S1), indicating reduced gene flow between these sampling sites and the rest of the study area. The negative impact of pineapple plantations on *C. castanea* is also shown by the significantly lower allelic richness and higher relatedness in sampling sites surrounded by a high percentage of pineapple.

Although heterozygosity showed no response to the percentage of pineapple, allelic richness is known to respond more quickly to reduced gene flow than heterozygosity (Allendorf and Luikart 2009), and so the decline in allelic richness but not in heterozygosity may be attributed to the fact that pineapple has only been in the SJLS region for approximately 20 years, or 20 generations of *C. castanea* (Cloutier and Thomas 1992). Empirical and simulation landscape genetics studies have shown that there is a temporal lag between initial habitat loss and fragmentation and the appearance of detectable

responses in genetic diversity and differentiation (Spear and Storfer 2008, Anderson et al 2010, Landguth et al 2010). The significant negative relationship between allelic richness and the percentage of pineapple may be the first detectable response of *C. castanea* to agricultural intensification, and if functional connectivity is not restored then in the future a similar pattern is likely to appear in heterozygosity.

While pineapple plantations may impede gene flow for *C. castanea*, forest cover, as expected, seems to facilitate it. None of the sampling sites separated only by continuous forest showed significant pairwise  $G''st$  values, and regression analyses found a positive relationship between percentage of forest cover and allelic richness, and a negative one between percentage forest cover and relatedness. In addition, the LCTA analyses identified forest cover as the preferred dispersal habitat for this species. This dependence on forest cover may be due to the fact that although *C. castanea* consumes fruits from at least 21 different plant species, approximately half of its diet is comprised of fruits of Piperaceae species found in interior forest understory (Thies & Kalko 2004, Lopez and Vaughan 2007). Roosting habits of *C. castanea* are also dependent on forest; this species is not known to roost in man-made structures or agriculture, but rather appears to roost exclusively in earthen cavities among tree roots in riparian zones (Thies et al 2006, Bonaccorso et al 2006). Previous studies have found that *C. castanea* abundance is lower in modified agricultural areas than in interior forest (Medina et al 2007, Cortes-Delgado 2011), and is especially low in intensive agriculture like full-sun coffee plantations and oil palm plantations (Numa et al 2005, Freudmann et al 2015).

The negative impact of pineapple on functional connectivity for *C. castanea* is likely directly related to this species' dependence on forest cover. Like other intensive plantation crops, pineapple simplifies and homogenizes the landscape, creating an agricultural matrix that is structurally and compositionally extremely different from natural forest cover (Hobinger et al. 2012). A recent analysis of spatial characteristics of pineapple plantations in the SJLS region found that pineapple has lower total tree cover than all other agricultural land cover types except banana (Shaver et al. 2015). This analysis also showed that the conversion of pasture to pineapple increases the distance between remnant forest patches, because small-scale forest elements are usually eliminated in order to maximize production efficiency (Shaver et al 2015). Dispersing *C. castanea* individuals likely prefer to move through land cover types with more forest elements, spaced more closely together, such as pasture with scattered trees and live fences.

In contrast to *C. castanea*, the larger, more mobile *A. jamaicensis* showed a less marked response to landscape composition. Regression analyses showed no relationship between heterozygosity, allelic richness, or relatedness and land cover type surrounding sampling sites, and LCTA analyses indicated

that pineapple is actually the preferred dispersal habitat for this species. Although 44 of the 276 pairwise tests for population differentiation were significant, significant tests were not more evident between sites surrounded by a high percentage of pineapple than they were for sites surrounded by primarily forest (Fig. S2). The majority (65%) of significant tests were between sampling sites ER.19 and ER.23 (Fig. 1), but none of the tested landscape variables could explain this pattern. There may be undetected past or current processes that have reduced connectivity of these sites, such as a lack of concentrated food resources leading to low levels of migration into the site. The diet of *A. jamaicensis* likely contributes to its ability to maintain functional connectivity in the fragmented landscape of the SJLS biological corridor. This species is a generalist, consuming fruits of more than 100 different plant species (Ortega and Castro-Arellano 2001, Lopez and Vaughan 2007), including many early successional species (e.g. *Cecropia*) species used for live fences (e.g. *Spondias*) and local market crops commonly planted in the SJLS region, such as avocado, mango, and cashew (Ortega and Castro-Arellano 2001). This means that *A. jamaicensis* is not dependent on foraging in interior forest, but can also exploit food resources found in degraded forest, live fences, and small plots of local market crops. This flexibility in diet may be why previous studies have found *A. jamaicensis* at similar or higher abundances in modified agricultural areas as in forest (Estrada et al 1993, Faria et al 2007), even when the agricultural matrix is comprised of intensive plantation agriculture like full-sun coffee and oil palm (Numa et al 2005, Freudmann et al 2015).

Although *A. jamaicensis* feeds on a wide variety of plant species, two studies suggest almost 50% of its diet is figs (*Ficus*; Handley et al 1991, Lopez and Vaughan 2007), and we captured many individuals during this study at points close to fruiting *Ficus*. This strong reliance on figs may help explain why contrary to our hypotheses, results of the LCTA analysis suggest that *A. jamaicensis* is able to move through large-scale, monoculture pineapple plantations, despite the fact that it is not known to feed on pineapple (Ortega and Castro-Arellano 2001) and pineapple plantations contain few small-scale forest elements that could offer food resources or roosting habitat (Handley et al 1991, Shaver et al 2015). Unlike *Piper* plants, which produce few fruits over a prolonged period of time, fig trees reproduce with a “big bang” strategy, where all fruits on a tree ripen within a short amount of time (Thies and Kalko 2004). The high wing loading and high aspect ratio of *A. jamaicensis*' wings make it well-adapted for flying long distances to track these patchily distributed food resources (Norberg & Rayner 1987), so extensive, open pineapple plantations may serve as highly traversable flyways for *A. jamaicensis* to reach fruiting fig trees in nearby forest patches. In fact, radiotelemetry studies of *A. jamaicensis* have found that this species will travel 13 times farther to reach fruiting fig trees in a fragmented agricultural landscape than it does in an intact forested landscape (Morrison 1978). Although foraging movements are not the same as movement for dispersal, previous studies

have found that bat species which move long distances to track patchily distributed food resources also tend to have higher gene flow in fragmented landscapes (Meyer and Kalko 2009, McCulloch et al 2013).

One similarity in how the landscape affects functional connectivity for *C. castanea* and *A. jamaicensis* is that as predicted by our hypotheses, the LCTA analyses identified infrastructure development as a barrier to gene flow for both species. In this study, the majority of the land area categorized as development is represented by roads, since urban areas are restricted to the southern part of the SJLS biological corridor (Fig. 1). Since bats are volant, it is often assumed that roads will not represent an impediment to their movement. However, several studies have documented negative effects of roads on temperate zone bats, ranging from interruption of foraging movements (Zurcher et al 2010), avoidance of roads and surrounding areas due to noise and light pollution (Berthinussen and Altringham 2012), and direct mortalities due to collision with vehicles (Lesinski et al 2010), and these effects are likely similar for bats in the tropics. The expansion of pineapple plantations could exacerbate the impediment to gene flow posed by development; profitable pineapple production requires efficient market access, which is facilitated by the construction of new roads in and around plantations. Pitacuar (2010) found that in the SJLS region, pineapple production consistently occurs closer to road networks than other crops, pasture and forest.

#### *Implications for conservation*

The dual goals of the SJLS biological corridor are to protect functional connectivity for native species while allowing for sustainable agricultural production (Villate et al 2009). This study shows that the current landscape is succeeding in the first part of this goal for *A. jamaicensis*, but loss of forest cover and expansion of pineapple are straining the ability of *C. castanea* to maintain functional connectivity. Other bat species of the same or smaller size and similar life history traits as *C. castanea* may also be experiencing loss of functional connectivity. For example, the two other species of *Carollia* found in the SJLS, *C. perspicillata* and *C. brevicauda*, have similar home range sizes and diets as *C. castanea* (Bonaccorso et al 2006, Lopez and Vaughan 2007). Together these three *Carollia* species have been found to comprise more than 30% of bats captured in mist nets in other studies in the region (Rex et al 2008, Cleary et al 2016). Loss of functional connectivity for this considerable segment of local bat assemblages will mean a corresponding reduction in gene flow of the many plant species dependent on these species for seed dispersal, and some studies have already documented genetic structure in bat-dispersed canopy and understory tree species in fragmented agricultural landscapes (Aldrich et al 1998, Hanson et al 2008, Sanfiorenzo et al *in preparation*). The three *Carollia* species disperse primarily pioneer plant species (e.g. *Cecropia*, *Piperaceae*; Bonaccorso et al 2006, Lopez and

Vaughan 2007), so reduced movement of these bat species would disproportionately affect genetic diversity of plant communities in regenerating forests. However, other small frugivorous bats such as *Artibeus watsoni* have been documented dispersing seeds of many plant species associated with mature forest (e.g. *Calophyllum*, *Otoba*, *Quararibea*; Melo et al 2009), so disruptions to functional connectivity of these bat species would lead to reduced gene flow for interior forest plants as well. The importance of bat-mediated seed dispersal for both pioneer and mature forest plant species increases in fragmented agricultural landscapes like the SJLS, where many large and medium-sized mammalian seed dispersers such as tapir (*Tapirus bairdii*) and paca (*Agouti agouti*) may have been locally extirpated due to hunting pressure and habitat loss (Wright et al 2007, Melo et al 2009).

Our findings suggest a strategy of increasing forest cover inside of pineapple plantations would help to restore and protect functional connectivity and resulting gene flow for *C. castanea* and similar bat species in the SJLS biological corridor. Interior forest is probably the habitat most favorable to *C. castanea*, but forest edge can also potentially provide valuable food resources since they often provide high densities of early successional plants on which all three *Carollia* species feed (Lopez and Vaughan 2007). Therefore, even small, linear forest elements with a high edge-to-interior ratio could provide movement pathways for *C. castanea*, and retaining this type of forest cover within plantations requires minimal changes in cultivation methods. Reducing the total distance between remnant forest patches by retaining these small forest elements would also increase functional connectivity for other species in the SJLS biological corridor in which forest cover is positively related to gene flow, including the Chestnut-backed antbird (*Myrmeciza exsul*, Woltmann et al 2012), Thomas's fruit-eating bat (*Dermanura watsoni*, Ripperger et al 2012) and the litter frog (*Craugastor bransfordii*, Nowakowski et al 2015). Another recommendation stemming from our findings would be to minimize the growth of road networks, specifically paved roads which are likely to have a higher impact than the more common dirt roads due to increased traffic and higher levels of noise and light pollution. Limiting the expansion of paved roads would help maintain functional connectivity for bats and likely also for other native species for which roads represent movement barriers. If implemented, these management actions will also protect functional connectivity for the mutualistic plant species which depend on *C. castanea* and other small, frugivorous bat species for seed dispersal. Overall, our findings provide insight into the impact of agricultural intensification on the effectiveness of biological corridors in human-modified tropical landscapes, and emphasize the importance of an integrated view of connectivity for forest organisms when designing and managing biological corridors.



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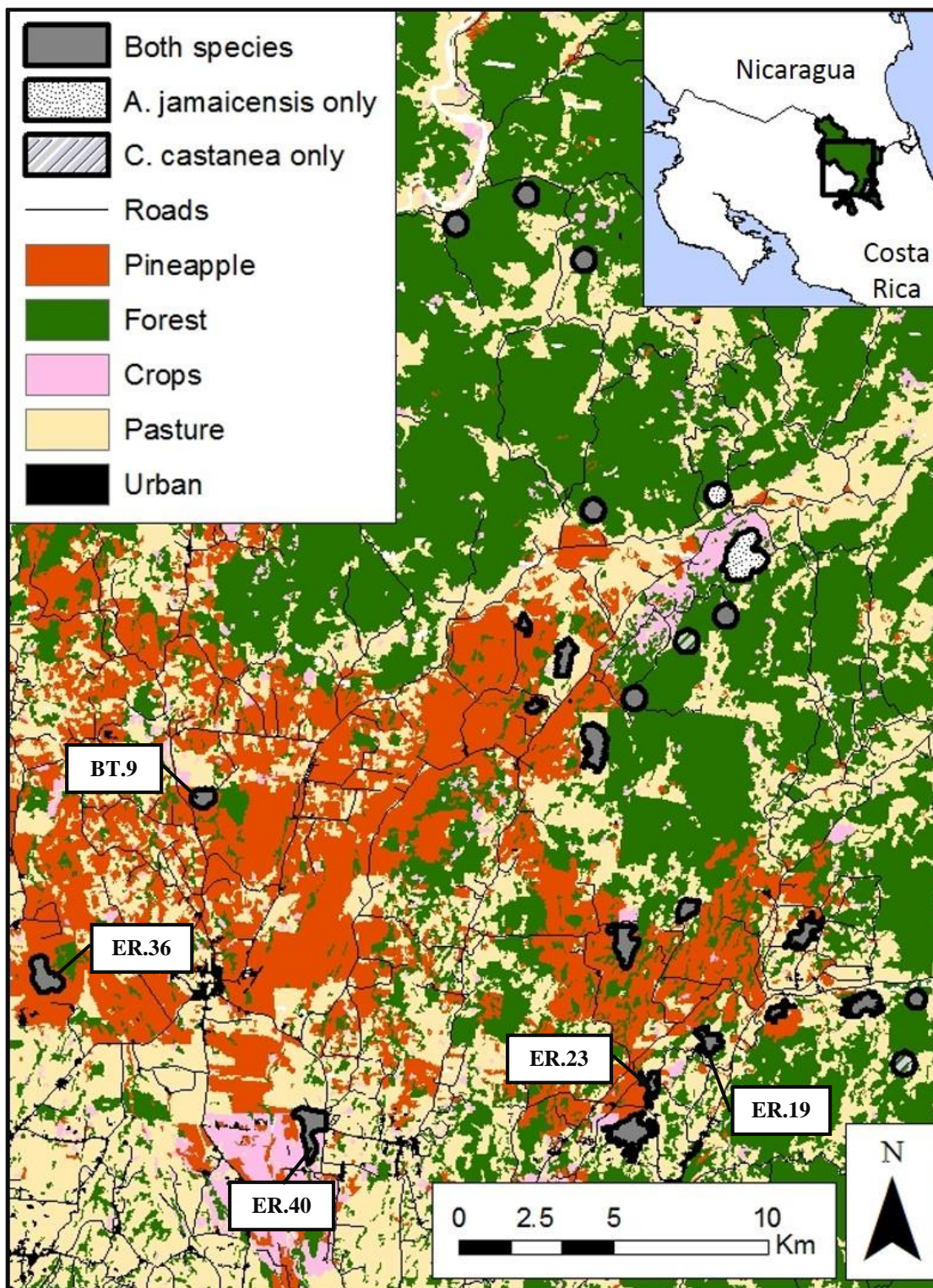
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## Tables and Figures

**Figure 3.1.** Map of the study area and sampling site. Selected sampling sites are labeled to facilitate interpretation of results.



**Table 3.1.** Sample size (N), year sampled (year), unbiased expected heterozygosity (uHe), allelic richness (AR), and number of private alleles (PA) of *Artibeus jamaicensis* and *Carollia castanea* in all sampling sites. Sites with fewer than five individuals sampled were not used in analyses for that species and are indicated by a “-”.

|                  | <i>Carollia castanea</i> |           |       |      |    | <i>Artibeus jamaicensis</i> |           |       |      |    |
|------------------|--------------------------|-----------|-------|------|----|-----------------------------|-----------|-------|------|----|
|                  | N                        | Year      | uHe   | AR   | PA | N                           | Year      | uHe   | AR   | PA |
| <b>BT4</b>       | 7                        | 2013      | 0.695 | 4.63 | 0  | 17                          | 2013      | 0.742 | 5.08 | 0  |
| <b>BT5</b>       | 8                        | 2013      | 0.766 | 5.19 | 0  | 22                          | 2013      | 0.763 | 5.06 | 0  |
| <b>BT9</b>       | 20                       | 2013      | 0.698 | 4.52 | 1  | 12                          | 2013      | 0.714 | 4.96 | 1  |
| <b>BTLL</b>      | 11                       | 2012      | 0.732 | 5.25 | 0  | 5                           | 2012      | 0.687 | 4.3  | 0  |
| <b>ER1</b>       | 22                       | 2012      | 0.740 | 4.99 | 2  | 27                          | 2012      | 0.751 | 5.07 | 1  |
| <b>ER14</b>      | 14                       | 2012/2013 | 0.714 | 4.92 | 1  | 17                          | 2012/2013 | 0.760 | 4.99 | 0  |
| <b>ER15</b>      | 12                       | 2012      | 0.732 | 4.78 | 1  | 13                          | 2012      | 0.747 | 4.99 | 0  |
| <b>ER19</b>      | 16                       | 2012/2013 | 0.701 | 4.63 | 1  | 10                          | 2012/2013 | 0.726 | 4.98 | 0  |
| <b>ER20</b>      | 13                       | 2012      | 0.728 | 5.06 | 0  | 12                          | 2012      | 0.732 | 4.93 | 0  |
| <b>ER23</b>      | 9                        | 2013      | 0.719 | 4.83 | 0  | 23                          | 2013      | 0.754 | 5.05 | 1  |
| <b>ER24</b>      | 20                       | 2013      | 0.742 | 4.95 | 1  | 18                          | 2013      | 0.739 | 4.99 | 0  |
| <b>ER27</b>      | 21                       | 2013      | 0.728 | 4.98 | 0  | 7                           | 2013      | 0.710 | 4.64 | 0  |
| <b>ER36</b>      | 11                       | 2013      | 0.692 | 4.77 | 3  | 24                          | 2013      | 0.747 | 4.97 | 0  |
| <b>ER40</b>      | 10                       | 2013      | 0.717 | 4.84 | 0  | 8                           | 2013      | 0.793 | 5.14 | 0  |
| <b>P1</b>        | 9                        | 2012      | 0.726 | 4.97 | 0  | 9                           | 2012      | 0.765 | 4.97 | 0  |
| <b>P10</b>       | 18                       | 2013      | 0.726 | 5.05 | 5  | 11                          | 2013      | 0.761 | 4.98 | 1  |
| <b>P11</b>       | 11                       | 2013      | 0.721 | 5.06 | 0  | 10                          | 2013      | 0.738 | 5.1  | 0  |
| <b>P12</b>       | 0                        | 2013      | -     | -    | -  | 27                          | 2013      | 0.752 | 4.97 | 0  |
| <b>P2</b>        | 8                        | 2013      | 0.729 | 5.05 | 0  | 12                          | 2013      | 0.716 | 4.79 | 0  |
| <b>P3</b>        | 11                       | 2012/2013 | 0.713 | 4.62 | 2  | 29                          | 2012/2013 | 0.750 | 4.92 | 0  |
| <b>P6</b>        | 2                        | 2013      | -     | -    | -  | 12                          | 2013      | 0.734 | 4.88 | 0  |
| <b>P9</b>        | 13                       | 2013      | 0.711 | 4.71 | 0  | 24                          | 2013      | 0.704 | 4.67 | 0  |
| <b>PD</b>        | 26                       | 2012      | 0.698 | 4.7  | 1  | 0                           | 2012      | -     | -    | -  |
| <b>PPA</b>       | 24                       | 2012/2013 | 0.722 | 4.92 | 1  | 16                          | 2012/2013 | 0.758 | 4.85 | 0  |
| <b>SV1</b>       | 15                       | 2013      | 0.730 | 4.9  | 0  | 21                          | 2013      | 0.726 | 4.87 | 0  |
| <b>SV2</b>       | 20                       | 2013      | 0.722 | 4.81 | 0  | 0                           | 2013      | -     | -    | -  |
| <b>Avg/Total</b> |                          |           | 0.721 | 4.88 | 19 |                             |           | 0.740 | 4.92 | 4  |

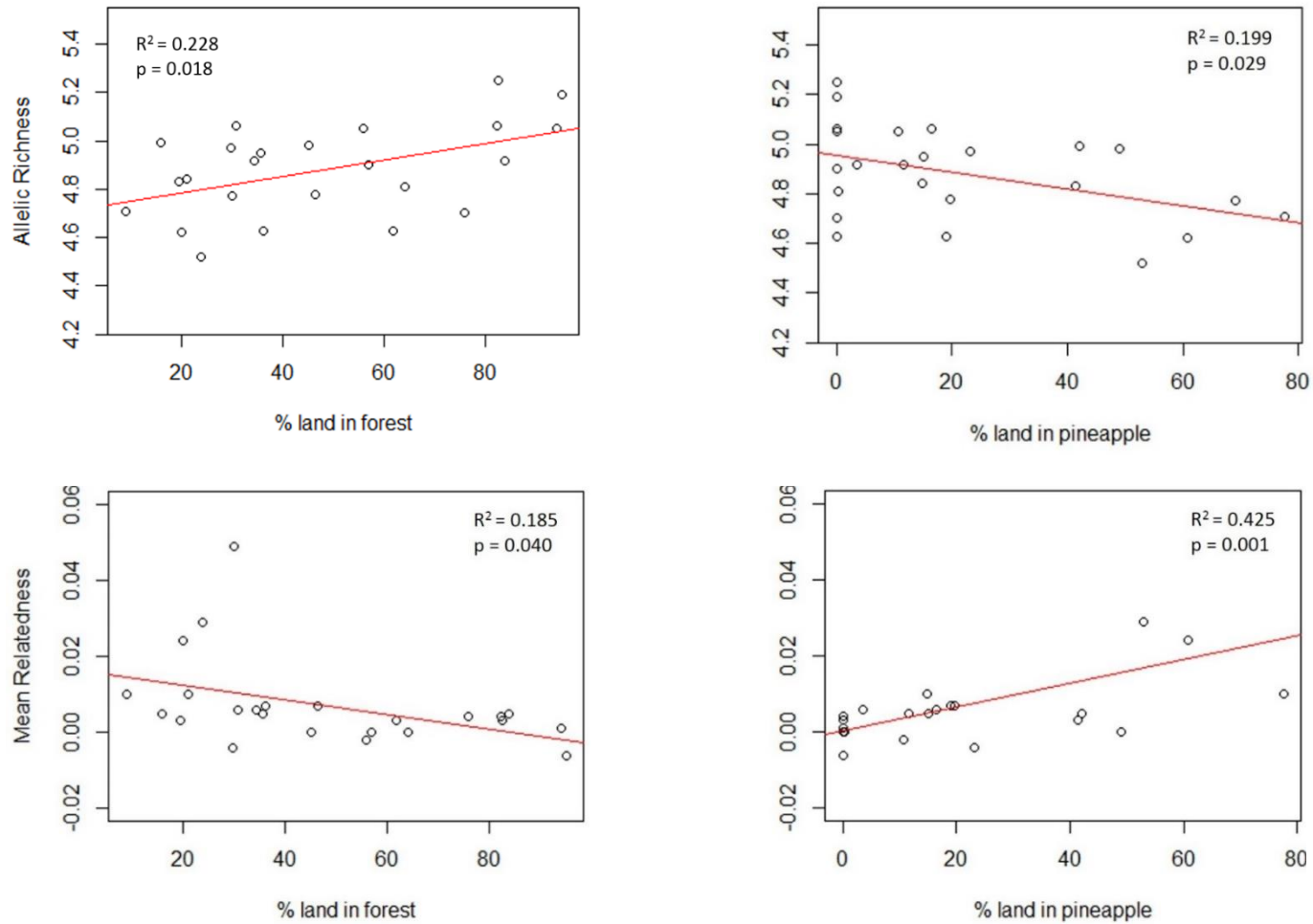
**Table 3.2.** Average (mean) relatedness for each sampling site for *Artibeus jamaicensis* and *Carollia castanea*. The left hand column of p-values corresponds to the test of whether a sampling site shows relatedness significantly higher than the average expected under permutation, while the right hand column corresponds to the test of significantly lower than the average. Significant values are in bold italics. Sites with fewer than five individuals sampled were not used in analyses and are indicated by a “-”.

|             | <i>Carollia castanea</i> |                           |                           | <i>Artibeus jamaicensis</i> |                           |                           |
|-------------|--------------------------|---------------------------|---------------------------|-----------------------------|---------------------------|---------------------------|
|             | Mean R                   | P(mean-rand >= mean-data) | P(mean-rand <= mean-data) | Mean R                      | P(mean-rand >= mean-data) | P(mean-rand <= mean-data) |
| <b>BT4</b>  | 0.003                    | 0.288                     | 0.713                     | <b>-0.009</b>               | 0.974                     | <b>0.027</b>              |
| <b>BT5</b>  | -0.006                   | 0.689                     | 0.312                     | 0.001                       | 0.240                     | 0.761                     |
| <b>BT9</b>  | <b>0.029</b>             | <b>0.001</b>              | 1.000                     | -0.001                      | 0.463                     | 0.538                     |
| <b>BTLL</b> | 0.003                    | 0.214                     | 0.787                     | 0.014                       | 0.169                     | 0.832                     |
| <b>ER1</b>  | <b>0.005</b>             | <b>0.020</b>              | 0.981                     | 0.002                       | 0.128                     | 0.873                     |
| <b>ER14</b> | 0.006                    | 0.070                     | 0.931                     | -0.005                      | 0.790                     | 0.211                     |
| <b>ER15</b> | 0.007                    | 0.066                     | 0.935                     | 0.002                       | 0.280                     | 0.721                     |
| <b>ER19</b> | <b>0.007</b>             | <b>0.021</b>              | 0.980                     | 0.006                       | 0.143                     | 0.858                     |
| <b>ER20</b> | 0.006                    | 0.088                     | 0.913                     | 0.000                       | 0.425                     | 0.576                     |
| <b>ER23</b> | 0.003                    | 0.274                     | 0.727                     | <b>0.008</b>                | <b>0.004</b>              | 0.997                     |
| <b>ER24</b> | <b>0.005</b>             | <b>0.029</b>              | 0.972                     | -0.002                      | 0.523                     | 0.478                     |
| <b>ER27</b> | 0.000                    | 0.306                     | 0.695                     | <b>0.021</b>                | <b>0.042</b>              | 0.959                     |
| <b>ER36</b> | <b>0.049</b>             | <b>0.001</b>              | 1.000                     | <b>0.004</b>                | <b>0.045</b>              | 0.956                     |
| <b>ER40</b> | 0.010                    | 0.066                     | 0.935                     | 0.000                       | 0.436                     | 0.565                     |
| <b>P1</b>   | -0.010                   | 0.896                     | 0.105                     | 0.004                       | 0.259                     | 0.742                     |
| <b>P10</b>  | 0.001                    | 0.254                     | 0.747                     | 0.007                       | 0.116                     | 0.885                     |
| <b>P11</b>  | 0.004                    | 0.185                     | 0.816                     | -0.001                      | 0.455                     | 0.546                     |
| <b>P12</b>  | -                        | -                         | -                         | -0.001                      | 0.376                     | 0.625                     |
| <b>P2</b>   | -0.002                   | 0.491                     | 0.510                     | -0.003                      | 0.580                     | 0.421                     |
| <b>P3</b>   | <b>0.024</b>             | <b>0.001</b>              | 1.000                     | <b>0.003</b>                | <b>0.039</b>              | 0.962                     |
| <b>P6</b>   | -                        | -                         | -                         | -0.002                      | 0.499                     | 0.502                     |
| <b>P9</b>   | <b>0.010</b>             | <b>0.016</b>              | 0.985                     | <b>0.008</b>                | <b>0.002</b>              | 0.999                     |
| <b>PD</b>   | <b>0.004</b>             | <b>0.014</b>              | 0.987                     | -                           | -                         | -                         |
| <b>PPA</b>  | <b>0.005</b>             | <b>0.020</b>              | 0.981                     | 0.002                       | 0.174                     | 0.827                     |
| <b>SV1</b>  | 0.000                    | 0.385                     | 0.616                     | <b>0.006</b>                | <b>0.020</b>              | 0.981                     |
| <b>SV2</b>  | 0.000                    | 0.279                     | 0.722                     | -                           | -                         | -                         |

**Table 3.3.** Population differentiation in *C. castanea* and *A. jamaicensis*. For each species, we report global  $G''st$  values and the percentage of pairs of populations with significant  $G''st$  values at  $\alpha=0.05$ , total and separately by sex. Significant global  $G''st$  values are in bold.

|   | <i>C. castanea</i>   |                      |               | <i>A. jamaicensis</i> |               |               |
|---|----------------------|----------------------|---------------|-----------------------|---------------|---------------|
|   | All                  | Males                | Females       | All                   | Males         | Females       |
| Global $G''st$ ( <i>p-value</i> )                       | <b>0.025 (0.012)</b> | <b>0.035 (0.006)</b> | 0.014 (0.166) | <b>0.022 (0.044)</b>  | 0.016 (0.197) | 0.021 (0.139) |
| Percentage of population pairs with significant $G''st$ | 17.1                 | 14.3                 | 3.5           | 15.8                  | 10.4          | 15.0          |

**Figure 3.2.** Genetic diversity (Ho, He, AR) and relatedness (Mean R) for *C. castanea* and *A. jamaicensis* were modeled as a function of the percentage of forest, pasture, pineapple, and urban in a circular buffer of radius 1000m. Only significant models are shown.



**Table 3.4.** Results from maximum likelihood population effects models with genetic distance ( $G''st$ ) as the response variable and results from the least-cost transect analyses as the explanatory variables. For the least-cost transect analyses, resistance surfaces were created with each land use type (forest, pasture, pineapple, and development) as the dispersal habitat, with three levels of resistance ( $2^3$ ,  $2^6$ ,  $2^9$ ) and three transect widths (100m, 400m, 800m). Shown are the top two models (based on AIC) for each species, and the variables in each model with direction of correlation.

| Species                     | Genetic distance | Dispersal habitat | Resistance level | Transect width | Variables       | AIC   | AIC weight |
|-----------------------------|------------------|-------------------|------------------|----------------|-----------------|-------|------------|
| <i>Carollia castanea</i>    | $G''st$          | Forest            | $2^6$            | 100m           | Development (-) | -1299 | 0.83       |
|                             |                  | Forest            | $2^9$            | 100m           | Development (-) | -1298 | 0.87       |
| <i>Artibeus jamaicensis</i> | $G''st$          | Pineapple         | $2^9$            | 100m           | Development (-) | -1166 | 0.54       |
|                             |                  | Pineapple         | $2^3$            | 100m           | Development (-) | -1158 | 0.53       |

**Chapter 4:**  
**Development and characterization of fourteen novel microsatellite markers for the chestnut short-tailed fruit bat (*Carollia castanea*), and cross-amplification to related species**

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## **ABSTRACT**

Rapid anthropogenic land use change threatens the primary habitat of the Chestnut short-tailed bat (*Carollia castanea*) throughout much of its range. Information on population genetic structure can inform management strategies for this widespread frugivorous bat, and effective protection of *C. castanea* will also benefit the more than 20 mutualistic plant species of which this bat is the primary seed disperser. To facilitate understanding of population genetic structure in this species, fourteen novel microsatellite markers were developed using restriction-site-associated DNA libraries and Illumina sequencing and tested on 28 individuals from 13 patches in Costa Rica. These are the first microsatellite markers developed for *C. castanea*. All loci were polymorphic, with number of alleles ranging from 2-11 and average observed heterozygosity of 0.631. Markers were also cross-amplified in three additional frugivorous bat species threatened by habitat loss and fragmentation: Sowell's short-tailed bat (*Carollia sowelli*), Seba's short-tailed bat (*Carollia perspicillata*), and the Jamaican fruit bat (*Artibeus jamaicensis*), and 11, 12, and 8 were polymorphic, respectively.

**Keywords** Microsatellite, *Carollia castanea*, Chestnut short-tailed bat, restriction-site-associated DNA

## **INTRODUCTION**

The Chestnut short-tailed bat (*Carollia castanea*) is a frugivorous bat which inhabits tropical forests from Honduras to Bolivia, and is the primary seed disperser of many pioneer plant species found in regenerating forests (Lopez and Vaughan 2007). Throughout this species' range, rapid conversion of native forest cover to agriculture is driving habitat loss and fragmentation, which

threatens the ability of *C. castanea* populations to maintain genetic connectivity. *C. castanea* has a small body size, limited home range of < 7 ha, and low wing loading, all of which are associated with lower vagility and increased vulnerability to fragmentation in bats (Bonaccorso et al. 2006, Meyer et al. 2008, Meyer et al. 2009). As a result, populations of *C. castanea* in fragmented agricultural landscapes are at risk of interrupted gene flow, genetic drift, and inbreeding, which reduce genetic diversity and adaptive capacity in the face of future perturbations (Willi et al. 2007, Mendez et al. 2011).

Although a previous study successfully cross-amplified 8 microsatellite loci developed for *C. brevicauda* to *C. castanea* (Bardeleben et al 2007), no loci have been developed specifically for this species. Here we develop and fully characterize fourteen polymorphic microsatellite markers specific to *C. castanea* using Illumina high-throughput sequencing. These novel markers can be used to quantify population genetic structure, identify populations that have become genetically isolated due to habitat loss and fragmentation, and evaluate correlations between genetic diversity, gene flow, and land use in fragmented agricultural landscapes. In addition, these markers can be used to increase understanding of mating and dispersal strategies in *C. castanea*; preliminary evidence of female-biased dispersal has been found in this species using mitochondrial DNA markers (Ripperger et al 2014), which is very rare in mammals (Greenwood 1980).

## **MATERIALS AND METHODS**

Samples of *C. castanea* were collected in 13 remnant forest patches in the San Juan-La Selva biological corridor in northern Costa Rica, where conversion of tropical lowland forest to agriculture has led to widespread habitat loss and fragmentation. Bats were captured using mist nets, and tissue samples of the uropatagium were collected using a 2mm diameter circular biopsy tool and stored in a 2mL tube with lysis buffer (Faure 2009).

Genomic DNA was extracted from tissue samples of three individuals using the Qiagen Blood and Tissue Kit. Libraries were prepared using a restriction-site-associated DNA approach (Etter et al 2011), and run on one lane of an Illumina® MiSeq250, which generated 3,179,284 sequences. Data was de-multiplexed and quality-cleaned using Stacks V.1.21 (Catchen et al 2013). A total of 2,300,295 cleaned sequences were run through the program QDD V.3.1 (Meglecz et al 2014) to identify microsatellites, filter out redundant sequences, and design primers. This process identified 10,558 sequences containing at least one microsatellite.

All sequences were screened using strict criteria to select only perfect microsatellites with di- or tetranucleotide motifs, at least five repeats, and low alignment scores with known transposable elements. From the candidate loci identified we selected 32 high-quality loci to test for amplification.



Unlabeled forward and reverse primers for these loci were synthesized through Applied Biosystems. Primers were diluted to a 10 $\mu$ M solution containing both forward and reverse primers, and tested on eight *C. castanea* individuals from eight different remnant forest patches. Individual amplifications were performed in a 7  $\mu$ L reaction containing 2  $\mu$ L template DNA, 2X Qiagen Multiplex PCR Master Mix, 0.5X Q solution, and 0.10  $\mu$ L of each 10  $\mu$ M primer solution. Cycling conditions consisted of a 15 minute initial denaturation at 95°C, followed by 15 cycles of a touchdown protocol of 94°C for 30s; 63°C for 90s; 72°C for 60s, and then 20 additional cycles of 94°C for 30s; 57°C for 90s; 72°C for 60s.

Amplification products were examined for polymorphism using standard gel electrophoresis with 3% agarose gels. Twenty loci were identified as potentially polymorphic. For these twenty loci, fluorescent labeled forward primers and unlabeled reverse primers were synthesized through Integrated DNA technologies and Applied Biosystems. Using the same amplification reactions and cycling conditions as for the previous step, these loci were amplified and amplification products were separated on an Applied Biosystems 3130xl Analyzer with LIZ500 internal size standard, and scored using GeneMapper 5 (Applied Biosystems). Fourteen of the twenty loci were identified as definitively polymorphic. These loci were multiplexed into two reactions (Table 4.1) and tested on 20 new *C. castanea* individuals from thirteen different remnant forest patches, in order to obtain estimates of population genetic parameters representative of the study area. Cycling conditions were the same as used above for both multiplexes. Amplification products were separated on an Applied Biosystems 3130xl Analyzer with LIZ500 internal size standard, and scored using GeneMapper 5 (Applied Biosystems). All loci were tested twice using DNA from the same *C. castanea* samples to ensure reliable results.

Number of alleles per locus ( $N_A$ ), observed heterozygosity ( $H_O$ ), expected heterozygosity ( $H_E$ ), and tests for departures from Hardy-Weinberg equilibrium ( $P_{HWE}$ ) were calculated in GenAlEx 6.5 (Peakall and Smouse 2012), and loci were screened for null alleles in CERVUS 3.0.7 (Kalinowski 2007). The program STRUCTURE v 2.3.4 (Pritchard et al 2000) was used to test for genetic structure in the data. We used an admixture model with correlated allele frequencies and ran the model for 10,000 generations, with a 10,000 generation burn-in, for one to 13 putative populations since the samples were collected from 13 different remnant forest patches, and it is possible that *C. castanea* populations in these patches represent 13 distinct genetic groups.

## RESULTS AND DISCUSSION

Primer sequences, size range of amplification product, and multiplex assignment for each of the fourteen microsatellite loci are presented in Table 4.1. All loci were in HWE with the exception of CC-30 ( $p = 0.003$ ), and all loci had null allele frequencies of  $<1\%$  except CC-24 (14%) and CC-30 (53%) (Table 4.1). The null allele rate in CC-24 is moderate and this locus was not out of HWE, so we consider it a reliable marker for use in *C. castanea*. The marker CC-30 was significantly out of HWE and showed a fairly high null allele rate in these analyses, so it is uncertain whether this marker will prove to be reliable for use in this species. Excluding these two potentially problematic loci, remaining loci had 2-11 alleles per locus, with an average observed heterozygosity of  $0.631 (\pm 0.227)$  (Table 4.1). Results from program STRUCTURE indicated that the most likely number of genetic groups was one ( $K=1$ ), with a posterior probability of  $\ln \Pr (X/K) = 0.47$ .

These fourteen novel microsatellite markers will facilitate future studies of population genetic structure in *C. castanea* and tests of whether levels of genetic diversity and gene flow in isolated populations are correlated with land use change and habitat loss and fragmentation. Understanding the impact of these processes on *C. castanea* is especially important since this bat is known to disperse at least 20 species of Neotropical plants (Lopez and Vaughan 2007). If *C. castanea* is able to maintain gene flow in fragmented landscapes, then these mutualistic plant species will also have a better chance of maintaining reproductive connectivity, genetic diversity, and recolonization capacity.

In addition, all loci were tested in three other related frugivorous bat species: Sowell's short-tailed bat (*Carollia sowelli*), Seba's short-tailed bat (*Carollia perspicillata*), and the Jamaican fruit bat (*Artibeus jamaicensis*). These species were chosen for testing because like *C. castanea* they face threats from habitat destruction throughout their range and together are key seed dispersers for hundreds of species of Neotropical plants (Ortega and Castro-Arellano 2001, Thies and Kalko 2004, Lopez and Vaughan 2007). Samples from these species were collected in the same remnant forest patches as *C. castanea* samples.

All loci were tested in two individuals of *C. sowelli*, three individuals of *C. perspicillata*, and three individuals of *Artibeus jamaicensis*. PCR conditions were the same as used above, but loci were tested separately to avoid the potential problem of overlapping alleles caused by shifting size ranges in the new species. Eleven loci amplified and were polymorphic for *C. sowelli*; these represent the first microsatellite markers available for this species. Twelve loci amplified and were polymorphic for *C. perspicillata*, and 8 loci amplified for *A. jamaicensis*, but only 6 were polymorphic (Table 4.2). Rates of polymorphism for these loci in these three species may be higher than reported here, since loci were tested in a small number of individuals of each species. Although microsatellite markers have

previously been cross-amplified to *C. perspicillata* and directly developed for *A. jamaicensis*, these additional markers will add resolution and power to future studies of genetic patterns in these species.

## TABLES

**Table 4.1** Microsatellite loci developed and characterized in 20 Chestnut short-tailed fruit bat (*C. castanea*) samples from Costa Rica. Fluorescent labels are in brackets. \*MP multiplex locus was assigned to,  $N_A$  number of alleles per locus,  $H_O$  observed heterozygosity,  $H_E$  expected heterozygosity.

| Locus | Repeat motif           | Primer 5'-3'   | Range (bp) | MP | $N_A$ | $H_O$ | $H_E$ | $P_{HWE}$ |
|-------|------------------------|--|------------|----|-------|-------|-------|-----------|
| CC-7  | (AC) <sub>13</sub>     | [PET]GAGTAACAAATAAGAGGGAAGTGGG<br>GCAACTGCTCACAACCTGTT | 292-300    | 1  | 5     | 0.800 | 0.715 | 0.385     |
| CC-10 | (AATG) <sub>7</sub>    | [FAM]TGCAGGGAAGATGAGAATGAACA<br>CAGGGCCTGGTGCATAGTAG   | 116-128    | 1  | 4     | 0.450 | 0.431 | 0.981     |
| CC-12 | (ACATAT) <sub>12</sub> | [VIC]ACAGACCAAGAACAGAGCTG<br>ATGATCTCTGAGCGCTCACA      | 236-420    | 1  | 11    | 0.929 | 0.870 | 0.389     |
| CC-13 | (AG) <sub>6</sub>      | [NED]CCGAGTCGTTTAGGCTGGTT<br>GCCCAACCCTGTCTTTGTC       | 181-185    | 1  | 2     | 0.500 | 0.455 | 0.658     |
| CC-18 | (AAGG) <sub>13</sub>   | [PET]AGCAGGACGTAAGACAGCAG<br>TTCCATTTTCATTGCTGTGGC     | 234-245    | 1  | 4     | 0.632 | 0.622 | 0.159     |
| CC-19 | (AC) <sub>18</sub>     | [PET]CCCTGCACCAAAATCAGCAAT<br>CTGCCAGCAATGCGTGAATG     | 120-142    | 1  | 6     | 0.650 | 0.703 | 0.558     |
| CC-20 | (AT) <sub>11</sub>     | [VIC]AGGAAGGGAGTCACCATGGT<br>CCAACCAGGTGTTAGTGCTA      | 178-226    | 2  | 8     | 0.550 | 0.700 | 0.257     |
| CC-23 | (AG) <sub>21</sub>     | [NED]CCTTCTATCTGTGACGCTGCT<br>TCACGCAACAAACAGTAAGTGA   | 226-256    | 1  | 10    | 0.750 | 0.781 | 0.898     |
| CC-24 | (ACAG) <sub>5</sub>    | [NED]GCAGGACAGGGAGCTTGA<br>ATCATAGAAAGTCGCTGTTGCT      | 136-140    | 2  | 2     | 0.368 | 0.494 | 0.267     |
| CC-25 | (AATG) <sub>8</sub>    | [NED]GTCTGTTTCTGCCTCTTTGGG<br>ATGGGTCACCGTGTCTTAGC     | 129-141    | 1  | 4     | 0.600 | 0.554 | 0.932     |

|       |                     |  |         |   |    |       |       |       |
|-------|---------------------|--|---------|---|----|-------|-------|-------|
| CC-26 | (AC) <sub>22</sub>  | [FAM]GAGGTACGCAGCCAGATGTG<br>ACTGCTTTCTGGTGCTTCTCA | 236-256 | 1 | 11 | 0.900 | 0.866 | 0.813 |
| CC-27 | (AC) <sub>21</sub>  | [FAM]GCAGGGAGTGGAGCATCATC<br>TGTTGCCAGGTTGTCACAGT  | 193-209 | 1 | 9  | 0.750 | 0.776 | 0.892 |
| CC-29 | (AC) <sub>12</sub>  | [VIC]ACCCTTGCTAGTCTGCCAAC<br>GAAGGCTCGGTCCTGCTC    | 220-230 | 1 | 6  | 0.850 | 0.785 | 0.660 |
| CC-30 | (AGGG) <sub>7</sub> | [VIC]AGGCAAACCCACAGACCAAA<br>CCAGTCTGTTCTCATTCCCGT | 119-131 | 2 | 3  | 0.100 | 0.329 | 0.003 |

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**Table 4.2** Cross-species amplification success, range in base pairs, and number of alleles ( $N_A$ ) for novel *C. castanea* loci in three related Phyllostomid species.

| <i>Carollia sowelli</i> |         |            |       | <i>Carollia perspicillata</i> |         |            |       | <i>Artibeus jamaicensis</i> |         |            |       |
|-------------------------|---------|------------|-------|-------------------------------|---------|------------|-------|-----------------------------|---------|------------|-------|
| Locus                   | Success | Range (bp) | $N_A$ | Locus                         | Success | Range (bp) | $N_A$ | Locus                       | Success | Range (bp) | $N_A$ |
| CC-7                    | 2/2     | 286-296    | 3     | CC-7                          | 3/3     | 286-296    | 4     | CC-7                        | 3/3     | 353-381    | 5     |
| CC-10                   | 2/2     | 124-146    | 3     | CC-10                         | 3/3     | 112-146    | 6     | CC-10                       | 3/3     | 112-138    | 4     |
| CC-12                   | 0/2     | --         | --    | CC-12                         | 0/3     | --         | --    | CC-12                       | 0/3     | --         | --    |
| CC-13                   | 2/2     | 153-163    | 2     | CC-13                         | 3/3     | 153-163    | 2     | CC-13                       | 0/3     | --         | --    |
| CC-18                   | 2/2     | 234-264    | 4     | CC-18                         | 3/3     | 210-292    | 5     | CC-18                       | 1/3     | 276-276    | 1     |
| CC-19                   | 2/2     | 124-132    | 2     | CC-19                         | 2/3     | 122-132    | 3     | CC-19                       | 2/3     | 116-122    | 3     |
| CC-20                   | 2/2     | 193-263    | 4     | CC-20                         | 3/3     | 165-221    | 6     | CC-20                       | 3/3     | 160-162    | 2     |
| CC-23                   | 0/2     | --         | --    | CC-23                         | 0/3     | --         | --    | CC-23                       | 0/3     | --         | --    |
| CC-24                   | 0/2     | --         | --    | CC-24                         | 2/3     | 125-133    | 2     | CC-24                       | 3/3     | 124-124    | 1     |
| CC-25                   | 2/2     | 135-145    | 3     | CC-25                         | 3/3     | 135-145    | 4     | CC-25                       | 0/3     | --         | --    |
| CC-26                   | 2/2     | 215-217    | 2     | CC-26                         | 3/3     | 215-219    | 3     | CC-26                       | 0/3     | --         | --    |
| CC-27                   | 2/2     | 185-197    | 2     | CC-27                         | 3/3     | 185-199    | 3     | CC-27                       | 3/3     | 188-192    | 3     |
| CC-29                   | 2/2     | 229-233    | 2     | CC-29                         | 3/3     | 213-239    | 4     | CC-29                       | 3/3     | 189-203    | 2     |
| CC-30                   | 0/2     | --         | --    | CC-30                         | 0/3     | --         | --    | CC-30                       | 0/3     | --         | --    |

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### **Competing Interests**

The authors declare no competing interests.

### **Author Contributions**

All authors conceived and designed the experiments. KAC collected the samples, performed the experiments, analyzed the data, wrote the paper, and prepared figures and/or tables. LW and PH contributed laboratory space and staff, and laboratory reagents/materials/analysis tools, and reviewed drafts of the paper.

### **Permissions**

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### **Data Deposition**

Gen Bank Accession Numbers: KX060618, KX060619, KX060620, KX060621, KX060622, KX060623, KX060624, KX060625, KX060626, KX060627, KX060628, KX060629, KX060630, KX060631

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## **Chapter 5:**

### **Conclusions, Recommendations, and Future Directions**

Katherine A. Cleary

This dissertation used an interdisciplinary approach to evaluate the impact of agricultural intensification on the linked social and ecological systems in the model landscape of the San Juan-La Selva (SJLS) biological corridor. Our interdisciplinary approach yielded insight about spatial patterns of pineapple expansion, the social and economic drivers of these patterns, and how these patterns are impacting bat assemblages and functional connectivity for frugivorous bats.

In this chapter, we synthesize our findings from across the four preceding chapters to draw conclusions, formulate management and policy recommendations, and suggest directions for future research. While some of the information presented here is specific to the SJLS region, our hope is that the majority of the recommendations and ideas for future research are also relevant in other tropical agricultural landscapes undergoing agricultural intensification.

#### **Conclusions**

In the first chapter, we found that intensive plantation agriculture simplifies and homogenizes both the local economy and the agricultural matrix between remnant forest patches. Fagan et al (2013) showed that pineapple expansion in this region occurs not by clearing old-growth forests to create new agricultural lands, but by converting smallholder crops, pasturelands, and young regenerating forests into pineapple plantations. Our analyses indicated that this conversion process measurably reduces total tree cover, increases the distance between remnant forest patches, and replaces heterogeneous mixes of pasture, trees, and crops with large, aggregated patches of monoculture pineapple plantations.

From a social and economic standpoint pineapple expansion has a similarly homogenizing effect. Pineapple production has high start-up costs, requires a large labor force, is most efficient on farms larger than 100 has. For these reasons, smallholders are excluded from the market and pineapple production is dominated by international agribusinesses. To acquire sufficient space for pineapple cultivation, these agribusinesses buy and lease large tracts of land from smallholders, who then lose their farm-based livelihoods and either seek off-farm employment or work for the pineapple producers. As a result, the percentage of non-pineapple crops cultivated in the region decreases, and the percentage of people who work in pineapple production increases. This transition creates a less

diversified economy that is more vulnerable to forces beyond the control of national policy, such as climate change and fluctuations in international market demand for pineapple.

In the second chapter, we assessed the impacts of pineapple expansion on bat assemblages in remnant forest cover. We found that conversion of smallholder crops and pastures with scattered trees, live fences, and small forest patches to pineapple monocultures is affecting several aspects of local bat assemblages. First, we assessed how bat assemblage composition is related to the proportion of different land uses in the agricultural matrix. We found that remnant forest patches where pineapple is the dominant land use in the matrix show bat assemblages characterized by higher proportions of frugivorous bats and lower proportions of insectivorous bats than assemblages in continuous old-growth forest. Interestingly, we identify a potential threshold effect whereby patches surrounded by a landscape containing at least 50% forest can retain assemblage composition similar to that found in continuous old-growth forest. Second, we tested for relationships between pineapple-driven changes in landscape composition and configuration and bat species richness, diversity, and guild-specific abundance. Findings indicate that total bat species richness and diversity do not appear to be affected by pineapple expansion, largely because disturbance-tolerant frugivorous species are occurring more often in remnant forest patches than in continuous old-growth forest. However, changes in landscape configuration have a significant negative impact on the relative abundance of insectivores adapted to foraging in forests. In particular, these insectivorous species are sensitive to the loss of forest cover, increased distance between forest patches, and high edge contrast of forest patches that our first chapter indicated are associated with conversion of croplands and pasture to pineapple.

In the third chapter, we moved from the assemblage level to the species level, and used a landscape genetics approach to evaluate the effects of pineapple expansion on functional connectivity for two widespread and abundant frugivorous bat species. We found that the larger, more mobile *Artibeus jamaicensis* seems to retain functional connectivity despite ongoing agricultural intensification. Genetic diversity and relatedness of this species were not significantly different in remnant forest patches surrounded by pineapple than in continuous old-growth forest. In addition, tests of how patterns of gene flow were correlated with land cover type showed that pineapple is actually the land cover type most permeable to gene flow for this species. In contrast, historical habitat loss and fragmentation and more recent pineapple expansion have impacted functional connectivity for the smaller, less mobile *Carollia castanea*. Populations of this bat in remnant forest patches surrounded by pineapple show lower genetic diversity and higher relatedness than populations in continuous old-growth forest, and tests for relationships between gene flow and land cover type identified forest as the

most likely dispersal habitat for this species. For both species, these analyses also identified development as an impediment to gene flow.

Our finding that functional connectivity has begun to be disrupted in *C. castanea* also has implications for other native plant and animal species in the SJLS region. There are at least 70 bat species found in this region (LaVal and Rodriguez 2002, Reid 2009), and many of these share with *C. castanea* the traits of small body size, limited home ranges, and restricted diets. These shared traits may imply a similar response to habitat loss and fragmentation and agricultural intensification. For example, in the same landscape Ripperger et al (2012) found significant genetic structure in the frugivorous bat *Artibeus watsoni*, which is slightly smaller than *C. castanea* and has a similarly limited home range of less than 10 hectares. Loss of functional connectivity for *C. castanea* and other small bat species will also disrupt functional connectivity for the hundreds of mutualistic plant species which depend on bats for critical pollination and seed dispersal services (Fleming et al. 2009; Lobo et al. 2009).

### **Management and Policy Recommendations**

Our findings provide a foundation for developing recommendations to mitigate impacts of agricultural intensification on social, economic, and ecological systems in agricultural landscapes throughout the Neotropics. Previous research on social-ecological systems has shown that maintaining diversity in all aspects of these systems, from the types of institutions participating in governance to the types of land cover in the agricultural matrix, enhances system resilience (Harvey et al 2005, Norberg et al 2008, Fahrig et al 2011). Our conclusions from the research in this dissertation also support the concept of diversity as the key to adaptability. Therefore, we suggest that the overall goal of management and policy in the SJLS region and similar tropical agricultural landscapes undergoing agricultural intensification should be to restore and maintain social, economic, and ecological diversity.

From a social and economic perspective, we suggest that Costa Rica consider changing agricultural policies to promote state-supported production of smallholder food crops. These types of policies were the norm in Costa Rica before the 1990s, when the country began participating in Structural Adjustment Programs (SAPs) as part of the conditions of debt restructuring through the World Bank. Under the SAPs, Costa Rica altered its agricultural policies toward a liberalized, globalized model promoting direct foreign investment and the production of large-scale export crops such as banana and pineapple (Edelman 1999). In this context, “state-supported” could mean tax incentives to cultivate crops destined for local or regional markets, or financial support for communities interested in forming agricultural cooperatives to give smallholders access to larger, more regulated markets outside of the region. These types of activities are already ongoing through some of the national universities, where

agronomy students assist rural cooperatives as part of a senior capstone project (personal communication, Guillermo Zamora, Pangola, Sarapiquí). Increased support for smallholders will help make crops destined for local or regional markets, such as cassava and plantain, more profitable, and will increase both economic diversity and food security.

Policies that encourage the cultivation of diverse smallholder crops will also benefit ecological systems, as diverse and heterogeneous landscapes are more likely to contain agriculture types that can be used for dispersal, foraging, and breeding habitat for native species (Daily et al 2003, Fahrig et al 2011). To further restore and maintain ecological diversity, our research suggests that policy should target two complementary goals: 1) increase the total amount of forest cover and 2) decrease the average distance between forest elements.

Costa Rica's Forestry Law already targets the first goal by prohibiting the clearing of old-growth forest and providing financial compensation for leaving land in forest through the system of payment for ecosystem services (Morse et al 2009). This law could be amended to include stronger incentives for protecting regenerating secondary forests. In our research secondary forests were grouped with old-growth forest in all analyses and therefore contributed to the positive correlations between forest cover and insectivorous and nectivorous species abundance and functional connectivity for *C. castanea*. Although smallholders engaged in agricultural production will not want to allow too much of their land to return to forest cover, an increase in the incentive provided by the payment for ecosystem services could make the rent of this land use competitive with some types of crop production. Increasing the total amount of forest cover in the landscape would also increase functional connectivity for other species in the SJLS biological corridor in which forest cover is positively related to gene flow, including the Chestnut-backed antbird (*Myrmeciza exsul*, Woltmann et al 2012), Thomas's fruit-eating bat (*Dermanura watsoni*, Ripperger et al 2012) and the litter frog (*Craugastor bransfordii*, Nowakowski et al 2015).

Other strategic amendments to the forestry law should be targeted not at smallholders, but at the large agribusinesses which dominate production of pineapple and other large-scale export crops. The forestry law to date focuses on regulating the overall composition of the landscape by arresting the proportion of forest on the landscape at the level it was in 1996 when the law was passed. We recommend adding a configuration requirement to the law to mandate the inclusion of forest cover within crop plots. Specifically, since our findings showed that pineapple plantations effectively increase the distance between remnant forest patches, we recommend the configuration requirement mandate that crop plots cannot extend continuously for more than 500m without any forest cover. Compliance with this requirement could be met by breaking up plantations with live fences or forest

buffer strips planted along roads within the farm, which would minimize the area lost to production. These strategically placed forest elements would increase connectivity for species like *C. castanea* which prefer to disperse through forest, and would mitigate the negative effects on insectivorous species of long distances between forest cover.

As mentioned above, this configuration requirement would by default be targeted at large agribusinesses, since smallholders are not likely to have enough land in cultivation to have crop plots that extend continuously for more than 500m. To formalize this, the law could include criteria to define what properties are mandated to comply with this new requirement. For example, since 76.5% of pineapple farms in the SJLS region are more than 100 hectares in size (MAG 2005), the law could specify that only farms larger than this minimum size are mandated to comply with the configuration requirement. This addition to the Forestry Law would also mitigate negative impacts of intensive plantation agriculture on biodiversity conservation in other large-scale export crops in Costa Rica, such as banana, sugar cane, and oil palm.

### **Future Directions**

Based on our interdisciplinary research findings and experience in the SJLS landscape, we have identified several research topics for future studies. A deeper understanding of these topics will contribute to finding a balance between production and conservation in tropical landscapes undergoing agricultural intensification.

The most recent study of what factors influenced smallholders' decisions about land use on their properties was completed by Morse et al (2009), using data collected several years earlier. This type of field-based social information is time-consuming to collect, but critical for understanding how policy changes will play out on real landscapes. We recommend that similar studies should be a priority in other tropical agricultural landscapes considering policy changes to mitigate the effects of agricultural intensification on social, economic, and ecological systems. In addition, we recommend that this study be repeated in the SJLS region before additional policy changes are made. Fagan et al (2013) and our study showed that the majority of pineapple expansion has happened after the data was collected for Morse et al (2009), and therefore the factors driving smallholders' decisions will likely have changed.

A clear research priority from the second chapter is to formally test the idea that remnant forest patches surrounded by an agricultural matrix which contains approximately 50% forest can retain bat assemblages similar to those found in continuous old-growth forest. In our research, we identified this potential threshold effect based on only two points. Future research could more rigorously test this hypothesis with a modified study design that included more patches surrounded by different threshold

amounts of forest in the surrounding matrix, and could potentially incorporate other taxa to determine whether this effect holds true beyond bat assemblages. Results from such a study would provide a valuable scientific foundation for landscape-level management recommendations about how much forest needs to be retained in a landscape to retain assemblage composition similar to that found in reserves.

Finally, our work on landscape genetics of two frugivorous bat species revealed several interesting patterns that merit additional study. We hypothesize that bat species of similar size and ecological traits as *C. castanea* are experiencing similar disruptions to gene flow. This hypothesis should be formally tested by future studies, particularly in insectivorous species, which our second chapter showed are generally more sensitive than frugivores to agricultural intensification. Many population genetics studies focus on large, charismatic species such as spider monkeys (Hagell et al 2013) or the almendro tree (Hanson et al 2008), but our work shows the importance of also evaluating small, forest dependent species which may be more vulnerable to habitat loss and fragmentation and agricultural intensification. For the larger, more mobile bat species, *A. jamaicensis*, our analyses identified pineapple as the preferred dispersal habitat. This is an unexpected finding that raises questions about how we hypothesize which land uses will more easier or harder for animals to traverse, especially volant animals. Future studies could focus on a mechanistic approach to determining the permeability of different land uses to bats, such as estimating energetic demands to travel over different land uses based on LIDAR-sourced data on air turbulence above each land use type. Finally, expanding our landscape genetics work into the realm of landscape genomics would provide interesting insights about whether agricultural intensification is driving adaptation in bat populations in isolated forest patches, and would allow identification of hotspots of adaptive potential that merit high priority for protection.

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## **Appendix 1. Letter of approval from University of Idaho Animal Care and Use Committee**

Subject: Protocol 2011-31 - Ecology and Conservation Genetics of Phyllostomid Bats in the Human-Dominated Landscape of San Juan-La Selva Biological Corridor  
Date: Wednesday, October 5, 2011 6:20 PM  
From: iacuc@uidaho.edu  
To: Lisette Waits <lwaits@uidaho.edu>  
Cc: <iacuc@uidaho.edu>  
Conversation: Protocol 2011-31 - Ecology and Conservation Genetics of Phyllostomid Bats in the Human-Dominated Landscape of San Juan-La Selva Biological Corridor

University of Idaho  
Animal Care and Use Committee

Date: Wednesday, October 05, 2011  
To: Lisette Waits  
From: University of Idaho  
Re: Protocol 2011-31  
Ecology and Conservation Genetics of Phyllostomid Bats in the Human-Dominated Landscape of San Juan-La Selva Biological Corridor

Your animal care and use protocol for the project shown above was reviewed and approved by the University of Idaho on Wednesday, October 05, 2011.

This protocol was originally submitted for review on: Tuesday, March 01, 2011  
The original approval date for this protocol is: Wednesday, October 05, 2011  
This approval will remain in affect until: Friday, October 05, 2012  
The protocol may be continued by annual updates until: Sunday, October 05, 2014

Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. For the first two years after initial approval of the protocol you will be asked to submit an annual update form describing any changes in procedures or personnel. The committee may, at its discretion, extend approval for the project in yearly increments until the third anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.

Brad Williams, DVM  
Campus Veterinarian  
University of Idaho 208-885-8958

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### Appendix 3: Supplementary Materials for Chapter 2

**Table S1.** Results of the preliminary analyses run to determine which landscape metrics best reflect the effects of agricultural intensification on the landscape. All metrics available in FRAGSTATs were calculated using mature forest as the focal habitat type. The value of each metric was then run in a simple univariate regression against 1) percent land in pineapple and 2) percent land in pasture, at the 500, 1000, and 2000 m scale. Only three metrics, Euclidean nearest neighbor distance between mature forest patches (ENN), edge density of mature forest (ED), and edge contrast of mature forest (ECON), were significantly related to percent land in pineapple and not significantly related to percent land in pasture. At the 500m scale, edge density was significantly related to both percent land in pineapple and percent land in pasture, but the sign of the relationship is opposite

|               | % land in pineapple |              | % land in pasture |              |
|---------------|---------------------|--------------|-------------------|--------------|
|               | Beta                | p val        | Beta              | p val        |
| <b>500 m</b>  |                     |              |                   |              |
| ENN           | <b>0.210</b>        | <b>0.000</b> | 0.017             | 0.751        |
| ED            | <b>-0.281</b>       | <b>0.032</b> | <b>0.436</b>      | <b>0.008</b> |
| ECON          | <b>0.809</b>        | <b>0.001</b> | 0.319             | 0.090        |
| <b>1000 m</b> |                     |              |                   |              |
| ENN           | <b>0.362</b>        | <b>0.000</b> | -0.003            | 0.968        |
| ED            | <b>-0.556</b>       | <b>0.007</b> | 0.162             | 0.386        |
| ECON          | <b>1.311</b>        | <b>0.000</b> | 0.229             | 0.446        |
| <b>2000 m</b> |                     |              |                   |              |
| ENN           | <b>0.268</b>        | <b>0.014</b> | -0.062            | 0.431        |
| ED            | <b>-0.521</b>       | <b>0.050</b> | 0.240             | 0.186        |
| ECON          | <b>1.272</b>        | <b>0.000</b> | 0.161             | 0.480        |

**Table S2.** Absolute abundance, guild assignment, and IUCN status of all species captured. Absolute abundance excludes recaptures, and guild assignment is sensu Kalko *et al.* (1996). *Dermanura watsoni* and *D. phaeotis* were not possible to differentiate reliably in the field and are grouped under *D. watsoni*

| <b>FAMILY</b>                    |                  |                       |                    |
|----------------------------------|------------------|-----------------------|--------------------|
| Subfamily                        | <b>Absolute</b>  |                       |                    |
| <i>Species</i>                   | <b>Abundance</b> | <b>Guild</b>          | <b>IUCN status</b> |
| <b>EMBALLONURIDAE</b>            |                  |                       |                    |
| <i>Cormura brevirostris</i>      | 2                | Insectivore: aerial   | Least concern      |
| <i>Peropteryx kappleri</i>       | 7                | Insectivore; aerial   | Least concern      |
| <b>MORMOOPIDAE</b>               |                  |                       |                    |
| <i>Pteronotus parnellii</i>      | 49               | Insectivore: aerial   | Least concern      |
| <b>PHYLLOSTOMIDAE</b>            |                  |                       |                    |
| Phyllostominae                   |                  |                       |                    |
| <i>Lonchorhina aurita</i>        | 2                | Insectivore: gleaning | Least concern      |
| <i>Macrophyllum macrophyllum</i> | 1                | Insectivore: gleaning | Least concern      |
| <i>Lamproncyteris brachyotis</i> | 2                | Insectivore: gleaning | Least concern      |
| <i>Micronycteris hirsuta</i>     | 14               | Insectivore: gleaning | Least concern      |
| <i>Micronycteris megalotis</i>   | 10               | Insectivore: gleaning | Least concern      |
| <i>Micronycteris minuta</i>      | 3                | Insectivore: gleaning | Least concern      |
| <i>Micronycteris schmidtorum</i> | 9                | Insectivore: gleaning | Least concern      |
| <i>Mimon crenulatum</i>          | 16               | Insectivore: gleaning | Least concern      |
| <i>Phyllostomus discolor</i>     | 8                | Omnivore: gleaning    | Least concern      |
| <i>Phyllostomus hastatus</i>     | 1                | Omnivore: gleaning    | Least concern      |
| <i>Tonatia saurophila</i>        | 14               | Insectivore: gleaning | Least concern      |
| <i>Lophostoma silvicolium</i>    | 15               | Insectivore: gleaning | Least concern      |
| <i>Lophostoma brasiliense</i>    | 20               | Insectivore: gleaning | Least concern      |
| <i>Trachops cirrhosus</i>        | 1                | Insectivore: gleaning | Least concern      |
| <i>Vampyrum spectrum</i>         | 3                | Carnivore: gleaning   | Near threatened    |
| Glossophaginae                   |                  |                       |                    |
| <i>Choeroniscus godmani</i>      | 17               | Nectarivore: gleaning | Least concern      |
| <i>Glossophaga commissarisi</i>  | 43               | Nectarivore: gleaning | Least concern      |
| <i>Glossophaga soricina</i>      | 6                | Nectarivore: gleaning | Least concern      |
| <i>Hylonycteris underwoodi</i>   | 42               | Nectarivore: gleaning | Least concern      |
| Caroliinae                       |                  |                       |                    |

|                               |     |                     |                 |
|-------------------------------|-----|---------------------|-----------------|
| <i>Carollia sowelli</i>       | 170 | Frugivore: gleaning | Least concern   |
| <i>Carollia castanea</i>      | 285 | Frugivore: gleaning | Least concern   |
| <i>Carollia perspicillata</i> | 264 | Frugivore: gleaning | Least concern   |
| <i>Artibeus jamaicensis</i>   | 277 | Frugivore: gleaning | Least concern   |
| <i>Artibeus literatus</i>     | 114 | Frugivore: gleaning | Least concern   |
| <i>Dermanura watsoni</i>      | 219 | Frugivore: gleaning | Least concern   |
| <i>Centurio senex</i>         | 1   | Frugivore           | Least concern   |
| <i>Chiroderma villosum</i>    | 4   | Frugivore: gleaning | Least concern   |
| <i>Ectophylla alba</i>        | 37  | Frugivore           | Near threatened |
| <i>Platyrrhinus helleri</i>   | 19  | Frugivore: gleaning | Least concern   |
| <i>Sturnira lilium</i>        | 9   | Frugivore: gleaning | Least concern   |
| <i>Uroderma bilobatum</i>     | 23  | Frugivore: gleaning | Least concern   |
| <i>Vampyressa nymphaea</i>    | 27  | Frugivore: gleaning | Least concern   |
| <i>Vampyressa pusilla</i>     | 12  | Frugivore: gleaning | Data deficient  |
| Desmodontinae                 |     |                     |                 |
| <i>Desmodus rotundus</i>      | 57  | Sanguivore          | Least concern   |
| <b>THYROPTERIDAE</b>          |     |                     |                 |
| <i>Thyroptera tricolor</i>    | 1   | Insectivore: aerial | Least concern   |
| <b>VESPERTILONIDAE</b>        |     |                     |                 |
| <i>Myotis nigricans</i>       | 17  | Insectivore: aerial | Least concern   |

## Appendix 4: Supplementary Materials for Chapter 3

**Table S1.** Multiplex setup and PCR conditions for a) *Carollia castanea* and b) *Artibeus jamaicensis*

a) Two multiplex PCRs for *Carollia castanea*

| Multiplex I Mix | uL for 1 |
|-----------------|----------|
| dH2O            | 0.260    |
| Master Mix (2x) | 3.500    |
| Q-solution (5x) | 0.700    |
| AAGG-1*(10uM)   | 0.100    |
| AAGG-7 (10uM)   | 0.020    |
| AAGG-98 (10uM)  | 0.070    |
| AAGG-119 (10uM) | 0.050    |
| AAGG-117 (10uM) | 0.200    |
| AL2_700 (10uM)  | 0.100    |
| Total           | 5.000    |

| PCR Cycling Conditions - Multiplex I |                  |                    |
|--------------------------------------|------------------|--------------------|
|                                      | Initial Denature | 94°C 15 min        |
| TD                                   | # of cycles :    | 15                 |
|                                      | Denature:        | 94°C 30 sec        |
|                                      | Annealing:       | 62C - 0.8°C 90 sec |
|                                      | Extension:       | 72°C 1 min         |
| Cycling                              | # of cycles :    | 20                 |
|                                      | Denature:        | 94°C 30 sec        |
|                                      | Annealing:       | 50°C 90 sec        |
|                                      | Extension:       | 72°C 1 min         |
|                                      | Cooldown         | 4°C 10 min         |

| Multiplex II Mix | uL for 1 |
|------------------|----------|
| dH2O             | 1.390    |
| Master Mix (2x)  | 3.500    |
| Q-solution (5x)  | 0.700    |
| CC-7 (10uM)      | 0.083    |
| CC-10 (10uM)     | 0.017    |
| CC-13 (10uM)     | 0.017    |
| CC-18 (10uM)     | 0.025    |
| CC-19 (10uM)     | 0.025    |
| CC-23 (10uM)     | 0.100    |
| CC-25 (10uM)     | 0.010    |
| CC-26 (10uM)     | 0.040    |
| CC-27 (10uM)     | 0.033    |
| CC-29 (10uM)     | 0.050    |
| CC-30 (10uM)     | 0.010    |
| Total            | 6.000    |

| PCR Cycling Conditions - Multiplex II |                  |                    |
|---------------------------------------|------------------|--------------------|
|                                       | Initial Denature | 94°C 15 min        |
| TD                                    | # of cycles :    | 15                 |
|                                       | Denature:        | 94°C 30 sec        |
|                                       | Annealing:       | 63C - 0.4°C 90 sec |
|                                       | Extension:       | 72°C 1 min         |
| Cycling                               | # of cycles :    | 20                 |
|                                       | Denature:        | 94°C 30 sec        |
|                                       | Annealing:       | 57°C 90 sec        |
|                                       | Extension:       | 72°C 1 min         |
|                                       | Cooldown         | 4°C 10 min         |

b) Multiplex PCR for *A. jamaicensis*

| Multiplex Mix     | uL for 1 |
|-------------------|----------|
| dH2O              | 0.525    |
| Master Mix (2x)   | 3.500    |
| Q-solution (5x)   | 0.700    |
| AjA40 F (10uM)    | 0.026    |
| AjA40 R (10uM)    | 0.026    |
| AjA47 F+R (10uM)  | 0.144    |
| AjA74 F (10uM)    | 0.025    |
| AjA74 R (10uM)    | 0.025    |
| AjA80 F+R (10uM)  | 0.072    |
| AjA84 F+R (10uM)  | 0.100    |
| AjA110 F+R (10uM) | 0.053    |
| AjA151 F (10uM)   | 0.133    |
| AjA151 R (10uM)   | 0.133    |
| AL2_579 (10uM)    | 0.100    |
| AL2_700 (10uM)    | 0.066    |
| A_713 (10uM)      | 0.066    |
| A_821 (10uM)      | 0.053    |
| AL2_850 (10uM)    | 0.053    |
| AL2_854 (10uM)    | 0.200    |
| Total             | 5.012    |

| PCR Cycling Conditions |                  |                     |
|------------------------|------------------|---------------------|
|                        | Initial Denature | 94°C 15 min         |
|                        | # of cycles :    | 15                  |
| TD                     | Denature:        | 94°C 30 sec         |
|                        | Annealing:       | 62°C - 0.8°C 90 sec |
|                        | Extension:       | 72°C 1 min          |
|                        | # of cycles :    | 25                  |
| Cycling                | Denature:        | 94°C 30 sec         |
|                        | Annealing:       | 50°C 90 sec         |
|                        | Extension:       | 72°C 1 min          |
|                        | Cooldown         | 4°C 10 min          |



**Table S2.** Sample size (N), year sampled (year), unbiased expected heterozygosity (uHe), allelic richness (AR), and number of private alleles (PA) of *Carollia castanea* and *Artibeus jamaicensis* males and females separately in all sampling sites. For each sex, only sites with a minimum of five individuals were used in analyses; sites with fewer than five individuals of that sex sampled were not used in analyses and are indicated with a “-”.

|              | <i>Carollia castanea</i><br>Females |           | <i>Carollia castanea</i><br>Males |           | <i>Artibeus jamaicensis</i><br>Females |           | <i>Artibeus jamaicensis</i><br>Males |           |
|--------------|-------------------------------------|-----------|-----------------------------------|-----------|--|-----------|--------------------------------------|-----------|
|              | N                                   | Year      | N                                 | Year      | N                                      | Year      | N                                    | Year      |
| <b>BT4</b>   | 3                                   | 2013      | 4                                 | 2013      | 9                                      | 2013      | 8                                    | 2013      |
| <b>BT5</b>   | 5                                   | 2013      | 2                                 | 2013      | 19                                     | 2013      | 3                                    | 2013      |
| <b>BT9</b>   | 9                                   | 2013      | 11                                | 2013      | 4                                      | 2013      | 8                                    | 2013      |
| <b>BTLL</b>  | 4                                   | 2012      | 7                                 | 2012      | 4                                      | 2012      | 1                                    | 2012      |
| <b>ER1</b>   | 8                                   | 2012      | 15                                | 2012      | 16                                     | 2012      | 11                                   | 2012      |
| <b>ER14</b>  | 7                                   | 2012/2013 | 9                                 | 2012/2013 | 9                                      | 2012/2013 | 11                                   | 2012/2013 |
| <b>ER15</b>  | 6                                   | 2012      | 6                                 | 2012      | 9                                      | 2012      | 5                                    | 2012      |
| <b>ER19</b>  | 6                                   | 2012/2013 | 10                                | 2012/2013 | 4                                      | 2012/2013 | 6                                    | 2012/2013 |
| <b>ER20</b>  | 6                                   | 2012      | 7                                 | 2012      | 6                                      | 2012      | 6                                    | 2012      |
| <b>ER23</b>  | 4                                   | 2013      | 5                                 | 2013      | 17                                     | 2013      | 6                                    | 2013      |
| <b>ER24</b>  | 6                                   | 2013      | 14                                | 2013      | 12                                     | 2013      | 6                                    | 2013      |
| <b>ER27</b>  | 10                                  | 2013      | 11                                | 2013      | 5                                      | 2013      | 2                                    | 2013      |
| <b>ER36</b>  | 6                                   | 2013      | 5                                 | 2013      | 18                                     | 2013      | 6                                    | 2013      |
| <b>ER40</b>  | 5                                   | 2013      | 5                                 | 2013      | 5                                      | 2013      | 3                                    | 2013      |
| <b>P1</b>    | 5                                   | 2012      | 4                                 | 2012      | 4                                      | 2012      | 5                                    | 2012      |
| <b>P10</b>   | 9                                   | 2013      | 10                                | 2013      | 3                                      | 2013      | 8                                    | 2013      |
| <b>P11</b>   | 4                                   | 2013      | 7                                 | 2013      | 7                                      | 2013      | 2                                    | 2013      |
| <b>P12</b>   | 0                                   | 2013      | 0                                 | 2013      | 18                                     | 2013      | 9                                    | 2013      |
| <b>P2</b>    | 3                                   | 2013      | 5                                 | 2013      | 8                                      | 2013      | 5                                    | 2013      |
| <b>P3</b>    | 5                                   | 2012/2013 | 6                                 | 2012/2013 | 13                                     | 2012/2013 | 16                                   | 2012/2013 |
| <b>P6</b>    | 0                                   | 2013      | 2                                 | 2013      | 4                                      | 2013      | 8                                    | 2013      |
| <b>P9</b>    | 8                                   | 2013      | 6                                 | 2013      | 9                                      | 2013      | 15                                   | 2013      |
| <b>PD</b>    | 12                                  | 2012      | 14                                | 2012      | 0                                      | 2012      | 0                                    | 2012      |
| <b>PPA</b>   | 7                                   | 2012/2013 | 17                                | 2012/2013 | 6                                      | 2012/2013 | 10                                   | 2012/2013 |
| <b>SV1</b>   | 5                                   | 2013      | 10                                | 2013      | 18                                     | 2013      | 4                                    | 2013      |
| <b>SV2</b>   | 9                                   | 2013      | 12                                | 2013      | 0                                      | 2013      | 0                                    | 2013      |
| <b>Total</b> | <b>134</b>                          |           | <b>192</b>                        |           | <b>204</b>                             |           | <b>149</b>                           |           |

**Table S3.** Average (mean) relatedness for each sampling site for *Carollia castanea* males and females separately. The left hand column of p-values corresponds to the test of whether a sampling site shows relatedness significantly higher than the average expected under permutation, while the right hand column corresponds to the test of significantly lower than the average. Significant values are in bold italics. Sites with fewer than five individuals sampled were not used in analyses and are indicated with a “-“.

|             | <i>Carollia castanea</i> Females |                           |                           | <i>Carollia castanea</i> Males |                           |                           |
|-------------|----------------------------------|---------------------------|---------------------------|--------------------------------|---------------------------|---------------------------|
|             | Mean relatedness                 | P(mean-rand >= mean-data) | P(mean-rand <= mean-data) | Mean relatedness               | P(mean-rand >= mean-data) | P(mean-rand <= mean-data) |
| <b>BT4</b>  | -                                | -                         | -                         | -                              | -                         | -                         |
| <b>BT5</b>  | -0.007                           | 0.575                     | 0.426                     | -                              | -                         | -                         |
| <b>BT9</b>  | 0.015                            | 0.014                     | 0.987                     | <b>0.047</b>                   | <b>0.001</b>              | 1.000                     |
| <b>BTLL</b> | -                                | -                         | -                         | -0.001                         | 0.372                     | 0.629                     |
| <b>ER1</b>  | -0.002                           | 0.395                     | 0.606                     | <b>0.011</b>                   | <b>0.003</b>              | 0.998                     |
| <b>ER14</b> | 0.012                            | 0.064                     | 0.937                     | 0.004                          | 0.181                     | 0.820                     |
| <b>ER15</b> | 0.011                            | 0.095                     | 0.906                     | 0.012                          | 0.107                     | 0.894                     |
| <b>ER19</b> | 0.006                            | 0.170                     | 0.831                     | <b>0.011</b>                   | <b>0.038</b>              | 0.963                     |
| <b>ER20</b> | -0.005                           | 0.532                     | 0.469                     | <b>0.018</b>                   | <b>0.027</b>              | 0.974                     |
| <b>ER23</b> | -                                | -                         | -                         | 0.008                          | 0.221                     | 0.780                     |
| <b>ER24</b> | -0.015                           | 0.848                     | 0.153                     | <b>0.011</b>                   | <b>0.008</b>              | 0.993                     |
| <b>ER27</b> | -0.006                           | 0.633                     | 0.368                     | 0.003                          | 0.161                     | 0.840                     |
| <b>ER36</b> | <b>0.069</b>                     | <b>0.001</b>              | 1.000                     | 0.015                          | 0.112                     | 0.889                     |
| <b>ER40</b> | -0.001                           | 0.408                     | 0.593                     | 0.009                          | 0.199                     | 0.802                     |
| <b>P1</b>   | -0.016                           | 0.820                     | 0.181                     | -                              | -                         | -                         |
| <b>P10</b>  | -0.008                           | 0.735                     | 0.266                     | 0.006                          | 0.122                     | 0.879                     |
| <b>P11</b>  | -                                | -                         | -                         | 0.009                          | 0.108                     | 0.893                     |
| <b>P12</b>  | -                                | -                         | -                         | -                              | -                         | -                         |
| <b>P2</b>   | -                                | -                         | -                         | 0.014                          | 0.129                     | 0.872                     |
| <b>P3</b>   | <b>0.048</b>                     | <b>0.001</b>              | 1.000                     | -0.006                         | 0.571                     | 0.430                     |
| <b>P6</b>   | -                                | -                         | -                         | -0.007                         | 0.642                     | 0.359                     |
| <b>P9</b>   | <b>0.012</b>                     | <b>0.019</b>              | 0.982                     | <b>0.007</b>                   | <b>0.030</b>              | 0.971                     |
| <b>PD</b>   | 0.004                            | 0.080                     | 0.921                     | -                              | -                         | -                         |
| <b>PPA</b>  | -0.012                           | 0.826                     | 0.175                     | <b>0.005</b>                   | <b>0.042</b>              | 0.959                     |
| <b>SV1</b>  | -0.021                           | 0.890                     | 0.111                     | -0.001                         | 0.374                     | 0.627                     |
| <b>SV2</b>  | 0.005                            | 0.130                     | 0.871                     | -0.006                         | 0.695                     | 0.306                     |

**Table S4.** Average (mean) relatedness for each sampling site for *Artibeus jamaicensis* males and females separately. The left hand column of p-values corresponds to the test of whether a sampling site shows relatedness significantly higher than the average expected under permutation, while the right hand column corresponds to the test of significantly lower than the average. Significant values are in bold italics. Sites with fewer than five individuals sampled were not used in analyses and are indicated with a “-“.

|             | <i>Artibeus jamaicensis</i> Females |                           |                           | <i>Artibeus jamaicensis</i> Males |                           |                           |
|-------------|-------------------------------------|---------------------------|---------------------------|-----------------------------------|---------------------------|---------------------------|
|             | Mean relatedness                    | P(mean-rand >= mean-data) | P(mean-rand <= mean-data) | Mean relatedness                  | P(mean-rand >= mean-data) | P(mean-rand <= mean-data) |
| <b>BT4</b>  | -0.015                              | 0.943                     | 0.058                     | -0.010                            | 0.748                     | 0.253                     |
| <b>BT5</b>  | -0.001                              | 0.369                     | 0.632                     | 0.001                             | 0.302                     | 0.699                     |
| <b>BT9</b>  | -                                   | -                         | -                         | -                                 | -                         | -                         |
| <b>BTLL</b> | -                                   | -                         | -                         | -                                 | -                         | -                         |
| <b>ER1</b>  | 0.005                               | 0.079                     | 0.922                     | -0.011                            | 0.873                     | 0.128                     |
| <b>ER14</b> | -0.010                              | 0.848                     | 0.153                     | -0.002                            | 0.342                     | 0.659                     |
| <b>ER15</b> | -0.005                              | 0.592                     | 0.409                     | 0.012                             | 0.163                     | 0.838                     |
| <b>ER19</b> | -                                   | -                         | -                         | -0.020                            | 0.939                     | 0.062                     |
| <b>ER20</b> | <b>0.022</b>                        | <b>0.035</b>              | 0.966                     | -0.026                            | 0.978                     | 0.023                     |
| <b>ER23</b> | <b>0.009</b>                        | <b>0.006</b>              | 0.995                     | -0.003                            | 0.430                     | 0.571                     |
| <b>ER24</b> | -0.009                              | 0.881                     | 0.120                     | <b>0.022</b>                      | <b>0.031</b>              | 0.970                     |
| <b>ER27</b> | 0.021                               | 0.082                     | 0.919                     | -                                 | -                         | -                         |
| <b>ER36</b> | -0.003                              | 0.560                     | 0.441                     | <b>0.020</b>                      | <b>0.047</b>              | 0.954                     |
| <b>ER40</b> | <b>0.028</b>                        | <b>0.038</b>              | 0.963                     | -                                 | -                         | -                         |
| <b>P1</b>   | -                                   | -                         | -                         | 0.019                             | 0.057                     | 0.944                     |
| <b>P10</b>  | -                                   | -                         | -                         | 0.005                             | 0.169                     | 0.832                     |
| <b>P11</b>  | -0.002                              | 0.454                     | 0.547                     | -                                 | -                         | -                         |
| <b>P12</b>  | -0.006                              | 0.802                     | 0.199                     | -0.005                            | 0.582                     | 0.419                     |
| <b>P2</b>   | -0.001                              | 0.370                     | 0.631                     | -0.002                            | 0.419                     | 0.582                     |
| <b>P3</b>   | <b>0.010</b>                        | <b>0.017</b>              | 0.984                     | -0.003                            | 0.463                     | 0.538                     |
| <b>P6</b>   | -                                   | -                         | -                         | -0.005                            | 0.571                     | 0.430                     |
| <b>P9</b>   | 0.002                               | 0.276                     | 0.725                     | <b>0.007</b>                      | <b>0.015</b>              | 0.986                     |
| <b>PD</b>   | -                                   | -                         | -                         | -                                 | -                         | -                         |
| <b>PPA</b>  | -0.021                              | 0.931                     | 0.070                     | 0.000                             | 0.328                     | 0.673                     |
| <b>SV1</b>  | 0.004                               | 0.067                     | 0.934                     | -                                 | -                         | -                         |
| <b>SV2</b>  | -                                   | -                         | -                         | -                                 | -                         | -                         |

**Table S5.** Results of simple univariate regressions of genetic diversity (Ho, He, AR) and relatedness (Mean R) as a function of the percentage of forest, pasture, pineapple, and urban in a circular buffer surrounding each sampling site of radius a) 500 m and b) 2000 m. Regressions where the percentage of land cover is a significant ( $\alpha=0.05$ ) predictor of the metric of diversity or relatedness are in bold.

a) 500 m circular buffer

|                             |               | FOREST         |               |               | PASTURE |        |             | PINEAPPLE      |               |               | DEVELOPMENT |        |               |
|-----------------------------|---------------|----------------|---------------|---------------|---------|--------|-------------|----------------|---------------|---------------|-------------|--------|---------------|
|                             |               | $\beta$        | $r^2$         | <i>pval</i>   | $\beta$ | $r^2$  | <i>pval</i> | $\beta$        | $r^2$         | <i>pval</i>   | $\beta$     | $r^2$  | <i>pval</i>   |
| <i>Carollia castanea</i>    | <b>Ho</b>     | 0.0003         | 0.0542        | <i>0.1421</i> | 0.0002  | 0.0078 | 0.6817      | -0.0004        | 0.1536        | 0.0582        | 0.0020      | 0.0012 | <i>0.8712</i> |
|                             | <b>He</b>     | 0.0002         | 0.0991        | <i>0.1341</i> | 0.0000  | 0.0000 | 0.9879      | -0.0002        | 0.1144        | 0.1060        | -0.0067     | 0.0291 | <i>0.4256</i> |
|                             | <b>AR</b>     | <b>0.0042</b>  | <b>0.3141</b> | <b>0.0044</b> | -0.0016 | 0.0148 | 0.5710      | <b>-0.0043</b> | <b>0.2809</b> | <b>0.0077</b> | -0.0903     | 0.0431 | <i>0.3306</i> |
|                             | <b>Mean R</b> | <b>-0.0002</b> | <b>0.1891</b> | <b>0.0337</b> | -0.0002 | 0.0665 | 0.2239      | <b>0.0003</b>  | <b>0.3963</b> | <b>0.0010</b> | -0.0002     | 0.0001 | <i>0.9674</i> |
| <i>Artibeus jamaicensis</i> | <b>Ho</b>     | 0.0000         | 0.0013        | <i>0.8677</i> | 0.0003  | 0.0199 | 0.5111      | -0.0002        | 0.0169        | 0.5451        | 0.0039      | 0.0025 | <i>0.8169</i> |
|                             | <b>He</b>     | 0.0001         | 0.0112        | <i>0.6232</i> | 0.0000  | 0.0006 | 0.9104      | -0.0003        | 0.0692        | 0.2141        | 0.0172      | 0.1010 | <i>0.1302</i> |
|                             | <b>AR</b>     | -0.0009        | 0.0168        | <i>0.5463</i> | 0.0029  | 0.0569 | 0.2617      | -0.0007        | 0.0087        | 0.6643        | 0.1233      | 0.0868 | <i>0.1622</i> |
|                             | <b>Mean R</b> | 0.0000         | 0.0070        | <i>0.6971</i> | -0.0001 | 0.0106 | 0.5791      | 0.0001         | 0.0408        | 0.3438        | 0.0018      | 0.0154 | <i>0.5639</i> |

b) 2000 m circular buffer

|                             |               | FOREST         |               |               | PASTURE |         |             | PINEAPPLE      |               |               | DEVELOPMENT |        |             |
|-----------------------------|---------------|----------------|---------------|---------------|---------|---------|-------------|----------------|---------------|---------------|-------------|--------|-------------|
|                             |               | $\beta$        | $r^2$         | <i>pval</i>   | $\beta$ | $r^2$   | <i>pval</i> | $\beta$        | $r^2$         | <i>pval</i>   | $\beta$     | $r^2$  | <i>pval</i> |
| <i>Carollia castanea</i>    | <b>Ho</b>     | 0.0004         | 0.1003        | 0.1317        | -0.0002 | 0.0115  | 0.6180      | -0.0003        | 0.0791        | 0.1831        | 0.0017      | 0.0046 | 0.7541      |
|                             | <b>He</b>     | 0.0003         | 0.1531        | 0.0586        | -0.0002 | 0.0170  | 0.5435      | -0.0002        | 0.0757        | 0.1931        | 0.0008      | 0.0024 | 0.8218      |
|                             | <b>AR</b>     | <b>0.0046</b>  | <b>0.2984</b> | <b>0.0058</b> | -0.0029 | 0.0391  | 0.3546      | <b>-0.0038</b> | <b>0.1794</b> | <b>0.0392</b> | -0.0079     | 0.0018 | 0.8456      |
|                             | <b>Mean R</b> | <b>-0.0003</b> | <b>0.2856</b> | <b>0.0071</b> | 0.0000  | -0.0455 | 0.9953      | <b>0.0003</b>  | <b>0.3092</b> | <b>0.0048</b> | 0.0034      | 0.0771 | 0.1889      |
| <i>Artibeus jamaicensis</i> | <b>Ho</b>     | -0.0001        | 0.0045        | 0.7550        | -0.0003 | 0.0127  | 0.5996      | 0.0002         | 0.0181        | 0.5306        | -0.0038     | 0.0130 | 0.5965      |
|                             | <b>He</b>     | -0.0001        | 0.0108        | 0.6295        | 0.0004  | 0.0401  | 0.3481      | -0.0001        | 0.0149        | 0.5702        | 0.0059      | 0.0648 | 0.2300      |
|                             | <b>AR</b>     | -0.0018        | 0.0485        | 0.3011        | 0.0051  | 0.1202  | 0.0969      | -0.0006        | 0.0046        | 0.7535        | 0.0528      | 0.0873 | 0.1609      |
|                             | <b>Mean R</b> | 0.0020         | 0.0026        | 0.8116        | -0.0002 | 0.1401  | 0.0715      | 0.0001         | 0.0634        | 0.2353        | -0.0010     | 0.0256 | 0.4551      |

**Figure S1.** Pairwise G'st values for all sampling sites for all individuals of a) *Carollia castanea* and b) *Artibeus jamaicensis*. Values significant at  $\alpha=0.05$  are in bold, and significant at  $\alpha=0.01$  are in bold italics.

a) *Carollia castanea* (Global G'st = 0.02, p = 0.012)

|              | BT.4  | BT.5  | BT.9               | BT.L               | ER.1               | ER.14              | ER.15              | ER.19              | ER.20              | ER.23 | ER.24              | ER.27              | ER.36              | ER.40              | P.1   | P.10  | P.11               | P.2  | P.3   |
|--------------|-------|-------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|-------|--------------------|--------------------|--------------------|--------------------|-------|-------|--------------------|------|-------|
| <b>BT.9</b>  | 0.02  | -0.02 |                    |                    |                    |                    |                    |                    |                    |       |                    |                    |                    |                    |       |       |                    |      |       |
| <b>BT.LL</b> | 0.05  | -0.04 | <i><b>0.09</b></i> |                    |                    |                    |                    |                    |                    |       |                    |                    |                    |                    |       |       |                    |      |       |
| <b>ER.1</b>  | 0.04  | -0.04 | <i><b>0.05</b></i> | -0.01              |                    |                    |                    |                    |                    |       |                    |                    |                    |                    |       |       |                    |      |       |
| <b>ER.14</b> | 0.04  | -0.04 | <i><b>0.05</b></i> | 0.01               | 0.02               |                    |                    |                    |                    |       |                    |                    |                    |                    |       |       |                    |      |       |
| <b>ER.15</b> | 0.05  | -0.01 | <i><b>0.11</b></i> | 0.04               | <i><b>0.05</b></i> | <i><b>0.06</b></i> |                    |                    |                    |       |                    |                    |                    |                    |       |       |                    |      |       |
| <b>ER.19</b> | 0.04  | 0.00  | <i><b>0.09</b></i> | 0.01               | 0.02               | 0.02               | 0.02               |                    |                    |       |                    |                    |                    |                    |       |       |                    |      |       |
| <b>ER.20</b> | -0.05 | -0.04 | <i><b>0.06</b></i> | -0.01              | 0.03               | 0.02               | 0.01               | 0.00               |                    |       |                    |                    |                    |                    |       |       |                    |      |       |
| <b>ER.23</b> | 0.04  | -0.01 | 0.05               | 0.02               | 0.00               | <i><b>0.06</b></i> | 0.03               | -0.02              | 0.02               |       |                    |                    |                    |                    |       |       |                    |      |       |
| <b>ER.24</b> | 0.03  | 0.01  | <i><b>0.09</b></i> | 0.01               | 0.02               | 0.04               | 0.02               | 0.03               | 0.03               | 0.02  |                    |                    |                    |                    |       |       |                    |      |       |
| <b>ER.27</b> | 0.01  | -0.06 | <i><b>0.03</b></i> | -0.01              | 0.01               | <i><b>0.04</b></i> | 0.03               | 0.02               | -0.01              | 0.01  | 0.02               |                    |                    |                    |       |       |                    |      |       |
| <b>ER.36</b> | 0.07  | 0.08  | <i><b>0.11</b></i> | <i><b>0.08</b></i> | <i><b>0.05</b></i> | <i><b>0.09</b></i> | <i><b>0.09</b></i> | <i><b>0.08</b></i> | <i><b>0.09</b></i> | 0.02  | <i><b>0.09</b></i> | <i><b>0.07</b></i> |                    |                    |       |       |                    |      |       |
| <b>ER.40</b> | 0.04  | 0.02  | <i><b>0.09</b></i> | 0.02               | 0.02               | 0.03               | 0.04               | <i><b>0.07</b></i> | 0.04               | 0.02  | 0.01               | 0.03               | 0.06               |                    |       |       |                    |      |       |
| <b>P.1</b>   | -0.03 | -0.04 | 0.02               | -0.03              | -0.03              | -0.02              | 0.00               | 0.01               | -0.03              | 0.01  | 0.00               | -0.03              | 0.02               | 0.01               |       |       |                    |      |       |
| <b>P.10</b>  | 0.00  | -0.03 | <i><b>0.05</b></i> | 0.01               | 0.03               | 0.02               | 0.00               | 0.01               | -0.01              | 0.02  | 0.03               | 0.00               | <i><b>0.06</b></i> | 0.02               | -0.03 |       |                    |      |       |
| <b>P.11</b>  | 0.02  | -0.03 | 0.04               | 0.01               | 0.03               | -0.02              | -0.01              | 0.01               | 0.00               | 0.01  | 0.04               | 0.04               | <i><b>0.10</b></i> | 0.04               | -0.05 | 0.01  |                    |      |       |
| <b>P.2</b>   | 0.07  | 0.00  | <i><b>0.09</b></i> | -0.04              | 0.02               | 0.03               | 0.05               | -0.01              | 0.00               | -0.02 | 0.00               | -0.03              | <i><b>0.07</b></i> | 0.04               | -0.03 | -0.02 | 0.03               |      |       |
| <b>P.3</b>   | 0.03  | 0.01  | 0.04               | 0.00               | 0.01               | 0.04               | 0.02               | <i><b>0.05</b></i> | 0.03               | -0.01 | 0.04               | 0.00               | 0.02               | 0.01               | 0.01  | 0.02  | 0.03               | 0.05 |       |
| <b>P.9</b>   | 0.04  | 0.01  | <i><b>0.13</b></i> | 0.01               | 0.02               | <i><b>0.06</b></i> | 0.03               | 0.04               | 0.03               | 0.02  | 0.01               | <i><b>0.04</b></i> | 0.06               | 0.00               | 0.02  | 0.02  | <i><b>0.06</b></i> | 0.03 | 0.02  |
| <b>P.D</b>   | -0.01 | 0.00  | <i><b>0.07</b></i> | 0.00               | <i><b>0.03</b></i> | <i><b>0.07</b></i> | 0.02               | 0.01               | 0.00               | 0.01  | <i><b>0.04</b></i> | 0.00               | <i><b>0.05</b></i> | <i><b>0.05</b></i> | -0.01 | 0.01  | 0.03               | 0.04 | 0.01  |
| <b>P.PA</b>  | -0.01 | 0.02  | <i><b>0.07</b></i> | 0.01               | <i><b>0.04</b></i> | <i><b>0.05</b></i> | 0.02               | <i><b>0.04</b></i> | -0.02              | 0.00  | 0.02               | 0.01               | <i><b>0.08</b></i> | 0.01               | -0.03 | -0.02 | 0.02               | 0.02 | 0.03  |
| <b>SV.1</b>  | 0.01  | -0.05 | 0.03               | -0.02              | -0.01              | -0.01              | 0.02               | 0.01               | -0.02              | -0.01 | 0.00               | 0.00               | <i><b>0.07</b></i> | 0.01               | -0.01 | 0.00  | 0.02               | 0.00 | 0.02  |
| <b>SV.2</b>  | 0.00  | -0.04 | <i><b>0.04</b></i> | -0.01              | 0.01               | 0.01               | 0.02               | 0.02               | 0.00               | 0.02  | 0.00               | -0.01              | 0.04               | 0.03               | -0.02 | 0.01  | 0.03               | 0.02 | -0.01 |

b) *Artibeus jamaicensis* (Global  $G^{*st} = 0.022$ ,  $p = 0.044$ )

|              | BT.4        | BT.5        | BT.9        | BT.L        | ER.1        | ER.14       | ER.15       | ER.19       | ER.20 | ER.23       | ER.24 | ER.27       | ER.36       | ER.40 | P.1   | P.10        | P.11  | P.12  | P.2   |  |
|--------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------|-------------|-------|-------------|-------------|-------|-------|-------------|-------|-------|-------|--|
| <b>BT.5</b>  | -0.03       |             |             |             |             |             |             |             |       |             |       |             |             |       |       |             |       |       |       |  |
| <b>BT.9</b>  | -0.01       | 0.01        |             |             |             |             |             |             |       |             |       |             |             |       |       |             |       |       |       |  |
| <b>BT.LL</b> | -0.02       | -0.01       | 0.06        |             |             |             |             |             |       |             |       |             |             |       |       |             |       |       |       |  |
| <b>ER.1</b>  | 0.00        | -0.01       | 0.00        | 0.02        |             |             |             |             |       |             |       |             |             |       |       |             |       |       |       |  |
| <b>ER.14</b> | 0.00        | 0.00        | 0.03        | 0.03        | 0.00        |             |             |             |       |             |       |             |             |       |       |             |       |       |       |  |
| <b>ER.15</b> | -0.02       | 0.00        | 0.02        | 0.07        | -0.01       | -0.02       |             |             |       |             |       |             |             |       |       |             |       |       |       |  |
| <b>ER.19</b> | <b>0.07</b> | <b>0.07</b> | <b>0.07</b> | <b>0.17</b> | <b>0.07</b> | <b>0.09</b> | <b>0.07</b> |             |       |             |       |             |             |       |       |             |       |       |       |  |
| <b>ER.20</b> | -0.05       | -0.01       | -0.04       | 0.00        | -0.03       | 0.01        | -0.02       | 0.03        |       |             |       |             |             |       |       |             |       |       |       |  |
| <b>ER.23</b> | <b>0.06</b> | <b>0.06</b> | 0.03        | <b>0.11</b> | <b>0.06</b> | 0.03        | 0.04        | <b>0.06</b> | 0.03  |             |       |             |             |       |       |             |       |       |       |  |
| <b>ER.24</b> | 0.01        | 0.01        | -0.01       | 0.04        | 0.00        | 0.00        | -0.01       | -0.02       | -0.03 | 0.02        |       |             |             |       |       |             |       |       |       |  |
| <b>ER.27</b> | 0.01        | 0.05        | -0.02       | 0.07        | 0.03        | 0.06        | 0.03        | 0.03        | 0.01  | 0.05        | -0.01 |             |             |       |       |             |       |       |       |  |
| <b>ER.36</b> | 0.01        | -0.01       | 0.04        | 0.04        | 0.01        | -0.01       | -0.03       | <b>0.06</b> | 0.01  | <b>0.07</b> | 0.00  | 0.07        |             |       |       |             |       |       |       |  |
| <b>ER.40</b> | 0.01        | 0.00        | <b>0.10</b> | 0.07        | 0.06        | 0.03        | 0.02        | 0.07        | 0.06  | <b>0.09</b> | 0.02  | 0.09        | 0.01        |       |       |             |       |       |       |  |
| <b>P.1</b>   | 0.00        | 0.02        | 0.02        | 0.13        | 0.03        | -0.03       | 0.00        | <b>0.09</b> | 0.00  | 0.06        | 0.05  | <b>0.10</b> | 0.03        | 0.06  |       |             |       |       |       |  |
| <b>P.10</b>  | -0.01       | 0.00        | <b>0.08</b> | -0.05       | <b>0.09</b> | 0.04        | 0.03        | <b>0.15</b> | -0.01 | <b>0.08</b> | 0.06  | <b>0.11</b> | 0.04        | 0.06  | 0.02  |             |       |       |       |  |
| <b>P.11</b>  | 0.00        | 0.03        | -0.01       | -0.01       | 0.03        | 0.00        | 0.03        | 0.07        | 0.00  | <b>0.06</b> | 0.03  | -0.02       | 0.02        | 0.08  | -0.01 | 0.04        |       |       |       |  |
| <b>P.12</b>  | -0.04       | -0.01       | 0.00        | 0.01        | 0.00        | -0.01       | -0.02       | <b>0.07</b> | -0.03 | 0.04        | 0.00  | 0.02        | 0.01        | 0.03  | -0.03 | -0.01       | -0.01 |       |       |  |
| <b>P.2</b>   | -0.05       | -0.02       | -0.03       | -0.03       | 0.00        | 0.02        | -0.02       | <b>0.08</b> | -0.02 | <b>0.08</b> | 0.02  | -0.01       | 0.00        | 0.03  | -0.01 | -0.01       | -0.01 | -0.01 |       |  |
| <b>P.3</b>   | 0.03        | 0.03        | <b>0.05</b> | 0.07        | 0.02        | -0.01       | 0.00        | 0.03        | 0.01  | <b>0.07</b> | 0.00  | <b>0.09</b> | 0.01        | 0.03  | -0.01 | <b>0.06</b> | 0.05  | 0.02  | 0.03  |  |
| <b>P.6</b>   | -0.04       | -0.03       | -0.03       | 0.01        | 0.01        | 0.00        | 0.00        | 0.03        | -0.03 | <b>0.05</b> | 0.00  | 0.05        | 0.03        | 0.02  | -0.01 | 0.02        | 0.00  | -0.01 | -0.04 |  |
| <b>P.9</b>   | -0.02       | 0.02        | 0.01        | 0.05        | 0.03        | 0.03        | 0.00        | <b>0.07</b> | 0.02  | <b>0.11</b> | 0.03  | 0.04        | <b>0.03</b> | 0.04  | 0.01  | <b>0.09</b> | 0.02  | 0.00  | -0.02 |  |
| <b>P.PA</b>  | -0.02       | -0.01       | 0.04        | -0.04       | 0.02        | -0.03       | 0.00        | <b>0.12</b> | 0.02  | <b>0.05</b> | 0.03  | 0.06        | 0.00        | 0.00  | 0.06  | 0.01        | 0.01  | -0.02 | -0.01 |  |
| <b>SV.1</b>  | 0.00        | 0.00        | -0.03       | 0.03        | -0.01       | 0.03        | -0.02       | 0.06        | -0.03 | 0.06        | -0.01 | 0.01        | 0.02        | 0.06  | 0.02  | <b>0.08</b> | 0.04  | 0.00  | -0.02 |  |