Title
Behavior, Ecology and Genetics of Geoffroy's Tamarin (Saguinus geoffroyi)

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Publication Date
2010

Peer reviewed|Thesis/dissertation

Behavior, Ecology and Genetics of Geoffroy's Tamarin
(*Saguinus geoffroyi***)**

by

Samuel Luis Díaz-Muñoz

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor in Philosophy

in

Integrative Biology

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Eileen A. Lacey, Chair
Professor Craig Moritz
Professor Justin S. Brashares

Spring 2010

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Samuel Luis Díaz-Muñoz

Abstract

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Cooperative behavior in reproductive contexts is rare among animals, especially males. Tamarins exhibit a rare breeding system called cooperative polyandry, in which a single breeding female mates with two or more males to produce fraternal twins and the males cooperate in caring for the infants by carrying young for the first 10 weeks of their lives. This peculiar breeding system raises questions about the adaptive consequences of male behavior. The nature of the breeding system also prompts questions about the ecological context and genetic consequences of this social organization. My research attempted address fundamental questions in behavior, ecology and evolutionary biology through the lens of individual behavior. Tamarins oftentimes inhabit disturbed habitats, however detailed space use within fragmented habitats is not well characterized. I used fine scale spatial and behavioral data in order to quantify habitat preference of *S. geoffroyi* in a heterogenous urban-forest landscape in central Panama. Using home range-based analyses and a novel method, first passage time analysis, I showed that tamarins spend significantly more time in secondary forest habitat and are more likely to forage and engage in social behavior in forest as compared to human-modified habitats. I examined the role of two aquatic barriers of varying age in creating population genetic structure in *S. geoffroyi*. I found that there was significant population differentiation across the Chagres River, an older, established riverine barrier and smaller, but detectable population structure across the Panama Canal, a recent anthropogenic riverine barrier. Finally, I examined the possible adaptive benefits of cooperative male parental care using genetic analyses of paternity and relatedness. I found that males in a group are often related and that they share paternity over multi-year associations. My results suggest that indirect and direct fitness benefits may play a role in maintaining male-male cooperation in tamarins.

I dedicate this dissertation to my mother, Carmen Rita Muñoz Berríos,
for giving me life and never doubting me.

Dedico este trabajo a mi madre, Carmen Rita Muñoz Berríos,
por darme vida y nunca dudar que este día llegaría.

CONTENTS

Acknowledgements

This work would have not been possible without the aid of a large group of people throughout the years when I conducted my doctoral studies. Following is an attempt to thank most people who contributed in one way or another to this endeavor:

First and foremost, I owe a huge debt of gratitude to my advisor and mentor, Dr. Eileen A. Lacey. She is an exceptional teacher, mentor and advisor for whom I have the highest regard. She encouraged me from the initial stages of my research to be independent and think big. Even though she was initially hesitant to have me study primates, once I convinced her of the opportunities in the study of Callitrichines, she was supportive and helpful in all stages. Eileen is an excellent editor and it is –in no small part – due to her assistance in crafting numerous grant applications that I was able to fund this project from the ground up. Eileen was active in providing suggestions throughout the project and was a source of comfort, encouragement and advice when things did not go the expected way. In the final stages she was instrumental in editing the dissertation and the manuscripts for publication. She provided the big picture and the significance of my findings (always emphasizing the *biology* of the organisms) at a time when I was somewhat overwhelmed by the minutiae of finalizing a dissertation. Eileen embodies many characteristics that seem to be lost in academia: a hard worker, a passionate teacher, an accessible mentor and a brilliant but understated scientist. Going forward Eileen will be for me a model of what an academic scientist should be. The members of the Lacey Lab were instrumental in providing feedback, support, editing, sharing successful applications and mentoring: Alan Krakauer, Emily DuVal, Maria Soares, Lauryn Benedict, Mark Hauber, Dustin Rubenstein, Wly dos Santos, Matt MacManes, Julie Woodruff and Rachel Walsh.

I would like to thank all the members of my academic committee: Justin S. Brashares, Craig Mortiz, Per Palsbøll and Walt Koenig. Dr. Moritz, in particular, provided detailed and extremely insightful comments which have greatly improved this dissertation. They were not only there to provide the requisite meetings and paperwork but were important guides and mentors throughout the development of my project. They are all admirable scientists, veritable giants in their fields and I derive important lessons and inspiration from each of their careers.

The Department of Integrative Biology provided significant institutional and logistic support without which this research project would have not been possible. The staff provided considerable logistical support and were always looking to help facilitate research. Rebecca Pauling and Mei Griebenow were instrumental in keeping me on track with academic requirements. Fellow grad students provided a nurturing and supportive community where cooperation and sharing was at the core of interactions.

The Museum of Vertebrate Zoology was my home at UC Berkeley. I am honored to have been a part of this vibrant community with a rich academic history. The support staff was essential in facilitating many logistical challenges. I would especially like to thank Anne Caulfield, Molly Mitchell, Anna Ippolito, John Stenske, Stephen Long and Mary Dunn. Monica Albe provided multiple opportunities to engage in outreach activities and show me the inner workings of the collection. Lydia Smith provided much assistance in the Evolutionary Genetics Lab, which she manages so ably. My office mates provided innumerable comments, revisions, distractions and words of support: William B. Monahan, Ricardo Pereira, Angela Ribeiro, Andrew Rush, Jérôme Fuchs, Hanneline Smit, Rob Bingham, Kristen Ruegg and Alan Krakauer. My go-to people for my incessant lab questions were Matt K. Fujita, William B. Monahan and Matt MacManes. William B.

Monahan deserves further special mention as a mentor, teacher, supporter, colleague and dear friend. Many colleagues and life-long friends will be the legacy of my years at the museum.

My research was generously supported by several institutions and foundations. The Museum of Vertebrate Zoology provided funds from the Carl Koford Memorial grant, the Hendrickson Research grant and the Louise Kellog Fund. The Department of Integrative Biology provided Summer Research Funds. Sigma Xi provided several Grants in Aid of Research. The American Society of Mammalogists provided a Grant in Aid of Research. The National Science Foundation supported this research project with a Dissertation Improvement Grant (#0608467).

This research project was authorized by the National Authority of the Environment of the Republic of Panama and import of samples under CITES was authorized by the US Fish and Wildlife Service. The animal handling and use procedures in this project were approved by the UC Berkeley Institutional Animal Care and Use Committee (MAP #R224-030) and followed the published guidelines for animal use of the American Society of Mammalogists.

I received invaluable assistance in the field from a large number of people. First, I would like to thank Maribel Tejada Gonzalez and Angel Joel Sosa Bartuano. Both were hard working enthusiastic students which helped me over multiple years. They generously shared their wealth of knowledge and passion for Panamanian natural history and taught me much about the culture of Panamá, helping me to understand and feel the historical ties between Panamá and Puerto Rico. The "darting crew" generously donated their time and expertise in the capture and handling of primates: Robert Lessnau, C. Rose Laughter, Mary Elizabeth Martin, Hully Hummel Border, Tiffany Marie Burgess and Jennifer Leean Eddington. Diorene J. Smith, DVM also provided ongoing onsite veterinary assistance. Sonia Sánchez Navarro and Evangelina López Vdovenko also provided field assistance at a crucial stage in my research.

The Smithsonian Tropical Research Institute brought together a unique community of researchers, providing stories that will enrich my life for years to come. I thank Biff Bermingham for being my sponsor at STRI and providing very useful advice and encouragement in the initial stages of my work. Marcela Paz and Orelis Arosemena not only provided logistical support, but were easily among the most helpful and warm people at the institution. STRI provided facilities that greatly improved the quality of life in the field and allowed me to sustain engagement with the scientific community from the field.

The pioneering work of many researchers (esp. PA Garber, AW Goldizen) inspired this research project and provided the necessary ground work and context. I derive particular inspiration from AW Goldizen's work which emphasized long-term field work and a behavioral ecological approach to understanding mating systems regardless of the taxon under study.

My financial needs were covered throughout my career allowing me to log the extensive field time necessary for this dissertation. The University of California, Berkeley offered a Chancellor's Opportunity Fellowship; the National Academies and the Ford Foundation provided a Ford Predoctoral Fellowship and the University of California Office of the President awarded a UC Dissertation Year Fellowship.

I would like to thank my mother, Carmen Rita Muñoz Berríos, who believed in me so matter-of-factly, that she took this doctorate as a given. She always provided perspective, support and celebration throughout the years. She was the connection to my homeland and my family in Puerto Rico when I was away for long periods of time. The family of my dearest friend Carlos Bustamante Migone, served as my adoptive family in the Bay Area, along with the Alarcón family. They have made of Berkeley a true second home for me. My brother David Díaz Muñoz came to the Bay Area mid-way through my graduate career. He brought with him a piece of home and his youth, vibrancy and passion which have brightened my days. Berkeley was the stage of the fortunate encounter with my life-long partner and love: my wife Rebecca Calisi provided tough love, support, encouragement and reminded me that I could be writing papers as I write these acknowledgements.

INTRODUCTION

The evolution of cooperative behavior has been a paradox since the birth of Darwinian evolutionary theory (Darwin 1859). Natural selection should act against individuals which forgo reproduction in order to assist others' reproductive efforts. Species that live in cooperative groups have thus been the focus of substantial theoretical and empirical research (reviewed by: Cockburn 1998, Koenig et al. 1992, Jennions & MacDonald 1994). This body of research has not only elucidated the mechanisms that favor the evolution of cooperation in social species (Clutton-Brock 2002, Griffin & West 2003), but has also revealed that these species differ markedly from their non-social heterospecifics in a suite of biological characteristics, including ecology, morphology and patterns of genetic variation.

Because the fitness benefits of cooperation are not always clear, much research has focused on the fitness benefits that promote the maintenance of cooperation among individuals. Seminal work by Hamilton (1964) demonstrated that natural selection could explain apparent altruism if individuals helped conspecifics that shared some proportion of their genes. Kin selection theory (Maynard-Smith 1964) predicted that cooperating individuals would indirectly increase their fitness, thereby assuring the permanence of their genes "for" cooperation in the population. This theory led to an explosion of interest in the role of kin selection in cooperative societies and much empirical work focused on testing predictions from kin selection theory. Later work was more critical of kin selection ideas and empirical studies have shown that some cooperative behaviors may be explained by direct fitness benefits. Recent work emphasizes the effects of both indirect and direct fitness benefits and attempts to assess their relative roles.

A particularly interesting subset of cooperative breeding is male cooperation in reproductive contexts. Males of a variety of species cooperate in reproductive contexts, for example in performing courtship displays (McDonald & Potts 1994, Krakauer 2005, DuVal 2007) or forming coalitions to compete for female access (Packer et al. 1991, Connor et al. 1992). These observations are at odds with typical predictions for males (Bateman 1948, Trivers 1974), which are expected to be competitors for reproductive opportunities. The high cost of male cooperation in breeding suggests that adaptive benefits are accrued from these behaviors, which may solve this evolutionary puzzle.

The aggregation of individuals into cooperative groups can have consequences in other aspects of a species' biology. Much research has highlighted the consequences of a social lifestyle to a species' ecology, physiology, neurobiology, morphology and genetics and in some cases has created important advances in these fields. For example, comparative neurobiological work has shown that species that vary their social organization also vary in their receptor distribution of neurohormones (Beery et al. 2008), contributing to the understanding of the role of these hormones in social and non-social species alike. Social species also differ in selection on genes mediating immunocompetrence (Hambuch & Lacey 2002) and have created insights into hypotheses concerning the evolution of MHC genes (Piertney & Oliver 2006). Social species also differ markedly in characteristics such as effective population size and sex biased dispersal patterns causing characteristic signatures in the genetic landscape (Amos & Harwood 1998, Sugg et al. 1996) and sometimes impacting speciation (Pamilo et al. 1997).

My dissertation research was motivated by the unusual social system of Geoffroy's tamarin monkeys. This project studied tamarin cooperative behaviors and their repercussions for some aspects of tamarin biology. I used *Saguinus geoffroyi* as a system to test hypotheses about the evolution of cooperation, specifically the potential adaptive benefits of male cooperation in breeding. Unlike other systems where male cooperation is present, *S. geoffroyi* is distinct due to the intensive parental care involved in male-male cooperation in reproduction, further adding to the complexity of the evolutionary paradox of cooperation. The remainder of my dissertation used knowledge of behavior of individuals

to investigate the ecological context and the population consequences of this breeding system, while attempting to answer fundamental questions in conservation biology and population genetics.

Chapter one focuses on the movement ecology of tamarins in a diverse urban-forest matrix. I used fine scale spatial and behavioral data in order to quantify habitat use by *S. geoffroyi* in a heterogenous urban-forest landscape in central Panama. Using home range-based analyses and first passage time analyses designed to examine individual movement, I tested whether tamarins showed a preference for forested habitat as compared to other, more disturbed habitat types. I also tested whether tamarins modified their movement patterns when engaged in foraging or social behavior. The study illustrates the use of fine scale spatial data in studying animal movements in modified habitats to aid biodiversity conservation efforts.

Chapter two focuses on the population genetics of *S. geoffroyi* in the Panama Canal Zone, with particular reference to the role of aquatic barriers in creating genetic differentiation. In this study, I use the Panama Canal watershed as an ideal test case of the effects of riverine barriers of varying ages in creating population structure. I use data from mitochondrial and microsatellite loci to investigate whether there was population genetic structuring in sampling localities on either side of an old (Chagres River) and novel (the Panama Canal) riverine barrier to examine whether the they are important barriers to gene flow that have produced detectable population structure. The results contribute to understanding of the effect of the timing and appearance of physical barriers on genetic structure in populations.

The final chapter examines the adaptive benefits of cooperative male parental care in *S. geoffroyi*. Tamarins exhibit a rare breeding system called cooperative polyandry in which a single breeding female mates with two or more males to produce fraternal twins and the males cooperate in caring for the infants, particularly by carrying young for the first 10 weeks of their lives. Given that males are expected to compete for mating opportunities and provide little parental care, tamarins represent a doubly puzzling case of male cooperation and raises the question of the adaptive benefits involved. I test two critical predictions of the indirect and direct fitness benefits hypotheses: whether male adult group mates are related and how they distribute paternity among themselves. This chapter provides a distinctive example of male-male cooperation, which adds to the comparative picture of the benefits associated with this form of cooperation.

My dissertation provides novel insights into the evolution of cooperative breeding in vertebrates, which sheds light on the evolution of cooperation in our own species. I have used the lens of individual behavior in order to understand the context and consequences of the cooperative lifestyle of *S. geoffroyi*, while taking the opportunity to address fundamental questions in ecology and evolutionary biology. This work provides conservation-relevant data and much needed life-history information that will improve our knowledge of this important biomedical model species.

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CHAPTER 1

Fine Scale Movement Analysis Reveals Preference for Forest in a Disturbance-tolerant Primate (*Saguinus geoffroyi*) in an Urban-forest Matrix

ABSTRACT

Forest destruction and disturbance are the major causes of species declines worldwide, especially in the tropics. The realization that the matrix consists of heterogeneous habitat types which animals often use, has led to an interest in the ecology of animals living in human-modified landscapes. Given the increasing prevalence, and hence conservation value of disturbed landscapes, the study of animal movements in modified habitats has become a priority for biodiversity conservation. Here I use fine scale spatial and behavioral data in order to quantify habitat preference in a reportedly disturbance-tolerant primate (*Saguinus geoffroyi*) inhabiting a heterogeneous urban-forest matrix in central Panama. Using home range-based analyses and First Passage Time analysis, which allows dynamic evaluation of individual movement, I show that tamarins spend significantly more time in forest habitat and are more likely to forage and engage in social behavior in forest habitats as compared to other modified types present. My results suggest that even purported disturbance-tolerant species may have important habitat requirements that should be incorporated into conservation planning to preserve ecosystems that may provide crucial ecosystem services. Spatial and behavioral data must be collected and analyzed at appropriate scales in order to best understand the biological context and potential causes of movements. My fine-scale approach can be generalized to other species to aid local conservation efforts and provide mechanistic movement models to complement landscape level models of habitat conservation.

INTRODUCTION

Forest destruction and disturbance are the major causes of species declines worldwide, especially in tropical forests, which harbor a large portion of the terrestrial biota (Meffe and Carroll, 1997; Pimm *et al.*, 1995; Laurance, 1999). Species exhibit a variety of reactions to forest disturbance, with some disappearing quickly after fragmentation and others thriving in the face of significant disturbance (Laurance, 2008). Understanding how animals move through an increasingly disturbed landscape is critical to preserve the remaining species in these landscapes.

Recently, it has been recognized that the habitat matrix surrounding forests is not homogenous and that different types of matrix habitats can influence habitat connectivity and local population dynamics (Laurance, 2008). Some species are assumed to be disturbance-tolerant based on presence in a disturbed area, however, presence may not accurately reflect species habitat requirements. Animals may vary space use and behavior in different habitat types (Sekercioglu *et al.*, 2007; Presley *et al.*, 2009). Putative disturbance-tolerant species may actually rely on remnant forest fragments, spending considerable time or conducting critical life history activities (*e.g.* nesting) in these habitats (Cohen and Lindell, 2005; Sekercioglu *et al.*, 2007). Therefore, a fine-scale perspective of the behavior and habitat use are essential to preserving species in remnant fragments.

Primates are not only affected by disturbance and destruction of their habitat, but also by human exploitation such as capture for the pet trade (Duarte-Quiroga and Estrada, 2003), bush meat (Bowen-Jones and Pendry, 1999; Milner-Gulland *et al.*, 2003; Brashares *et al.*, 2004), vehicle

impacts and fires (Peres *et al.*, 2003). However, some primate species persist in the face of habitat disturbance (Peres *et al.*, 2003). These primates can preserve important ecosystem services such as seed dispersal (Catenacci *et al.*, 2009) and serve as flagship species to rally conservation efforts in biodiversity hotspots (Kleiman and Mallinson, 1998). Given that primates are most often studied within relatively pristine habitats, recent reviews suggest that more studies of primates in disturbed areas will aid conservation (Chapman and Peres, 2001). Studies of primates in fragmented habitats have been conducted (*e.g.* Anderson *et al.*, 2007; Estrada and Coates-Estrada, 1996; Onderdonk and Chapman, 2001), however very few have examined habitat use in detail, especially at the forest-urban interface.

Geoffroy's tamarin (*Saguinus geoffroyi*) is considered a disturbance-tolerant primate (Moynihan, 1970), studies of which may provide insights into species interactions with a human dominated landscape. *S. geoffroyi* occurs in the Panama Canal watershed where it inhabits a unique area in the tropics (Rompré *et al.*, 2008). While many studies have focused on the agricultural-forest interface, the proximity of two major cities between large forested areas provides an opportunity to study the impact of urban development in tropical forests, particularly in Latin America where urbanization is projected to be a major source of habitat disturbance (Mcdonald *et al.*, 2008).

In this paper, I use data from a field study of Geoffroy's Tamarin living in an urban-forest matrix to examine how habitat and specific behaviors influence movement patterns. I use home range-based analyses and a method, First Passage Time analysis (FPT), that allows dynamic evaluation of movement at the scale at which Geoffroy's tamarins make movement decisions. In particular, I address the following questions:

(1) Do tamarins show a preference for forest habitat over disturbed habitats, either when establishing a home range or using areas within their home range?

(2) Are tamarins more likely to exhibit specific behaviors (*e.g.* foraging and social interactions) in different habitat types?

(3) Do tamarins change their movement patterns in certain habitat types or when engaged in specific activities (*e.g.* foraging and social interactions)?

METHODS

Study species — Geoffroy's Tamarins (*Saguinus geoffroyi*) are Callitrichine primates that inhabit tropical forests mainly in Panama and Colombia (Hershkovitz 1977). They are cooperative breeders that live, travel and forage in social groups usually composed of 3-9 individuals, including multiple males (Dawson, 1976). They have an omnivorous diet composed of fruits and insects (Dawson, 1976; Garber, 1986) with a small but important component of plant exudates (Garber, 1984; Sussman and Kinzey, 1984). Tamarins are reported to occupy a variety of habitat types and to prefer secondary forest and edge habitats (Dawson, 1979; Rylands, 1993) and have been regarded as disturbance-tolerant (Moynihan, 1970).

Study site — The rural town of Gamboa, Colón Province (9.118º, -79.698º) is located at the confluence of the Chagres River and the Panama Canal in the Republic of Panama, approximately 25 kilometers northwest of Panama City. Gamboa is surrounded by Soberanía National Park, which consists primarily of semi-deciduous, lowland tropical moist forest (Condit *et al.*, 2001). Rainfall is highly seasonal, with over 80 percent of annual rain falling in the wet season from Mid-April to Mid-December (Panama Canal Authority; Smithsonian Tropical Research Institute).

Gamboa contains a 22.04 ha section of disturbed secondary forest in the center of the town (Fig. 1A). Private residences and roads border this forest patch, with trees and shrubs in backyards providing some level of vegetative connectivity between subsections of the patch as well as gallery forest corridors within the study area (Fig. 1A). Anecdotal evidence suggests that tamarins have inhabited the town of Gamboa for at least 26 years (R. Condit, G. S. Gilbert, A. E. Herre, E. G. Leigh and S. J. Wright, pers. comm). Human population density is low (population 341, Contraloría General de la República de Panamá, 2000) and consists primarily of native residents, and a transient population of researchers (Smithsonian Tropical Research Institute) and ecotourists. Thus, direct human exploitation of tamarins is not significant, although tamarins are subject to mortality from vehicle collisions (Díaz-Muñoz, pers. obs.). While Gamboa is far from the urban centers of the Canal Zone, human modifications of the landscape are typical of urban and suburban environments. Thus, this study site provides an unusual opportunity to examine how habitat affects the behavior and movements of tamarins in advance of the projected growth of urban areas close to protected areas in Panama (Rompré *et al.*, 2008).

Captures and marking — I captured tamarins using hand-activated live traps baited with bananas as described by Garber *et al.* (1993) or by blow-darting (BioJect, Blowguns Northwest, Richland WA) with tranquilizer darts (Pnueu-Dart, Williamsport, PA). In both cases, I anesthetized individuals using a cocktail of Ketamine (7.5mg/kg) and Zoletil (3.75 mg/kg Vibrac S. A., Carros Cedex, France). I released individuals back to their social group after they were fully recovered from anesthesia. Capture methods resulted in no significant injuries. All animals recovered from captures and integrated into their groups. Individuals remained integrated into their social groups and injury free 2 months after the end of this current study (Díaz-Muñoz, pers. obs.). All procedures were approved by the UC Berkeley Institutional Animal Care and Use Committee and followed the guidelines of the American Society of Mammalogists (Gannon and Sikes, 2007).

I marked individuals for visual recognition using either (1) ball-link chains with colored beads or (2) shaving portions of the tail (for individuals which had not attained full adult body size). I attached radio collars (RI-2DM, Holohil Systems Ltd, Ontario Canada) to a subset of individuals using a ball-link chain. The mass of radiotransmitters was 20g, which represented <4.5 percent of adult body mass. I detected radio signals using a receiver (TRS-200S, Wildlife Materials, Carbondale IL.) with a custom-built three-element antenna, which provided detectability throughout the entire 88 ha study area. I used radiotelemetry for occasional initial location of groups, but not for following. All groups were highly habituated to human observers and could be readily followed for hours; their activities were not visibly disturbed by human presence.

Data collection: behavior, habitat and localities — Together with a trained observer, I conducted focal follows on three groups representing the entire Gamboa population of tamarins. I quantified behavior of individuals at 10 minute intervals during scan samples (Altmann, 1974) of groups; each visible individual was scored as engaged in Foraging, Resting, Social Behavior or Traveling. Resting included lying on branches (with no weight on limbs) and other stationary behaviors, such as sitting still while visually scanning the surroundings. Social behavior included affiliative and agonistic behaviors in both inter- and intragroup interactions.

I identified the locations of foraging and social behavior because they were (among the broad behavioral categories) expected to most closely relate to survival and reproduction. I

defined major foraging bouts as sample periods when ≥ 50 percent of individuals in the group were foraging. Major social bouts were similarly defined as sample periods when ≥ 50 percent of individuals in the group were engaged in social behavior.

During focal observations, I recorded the habitat type where behaviors were performed. Because I was interested in testing the influence of anthropogenic disturbance on the movement patterns of tamarin groups, I classified habitat into five broad types based on the degree of human disturbance and amount of forest cover:

a) Forest: the 22 hectare forest fragment in the town. Secondary growth with medium canopy height that contained ample gaps. Lowest level of human disturbance (e.g. humans occasionally walking through) and most forest cover.
b) **Gallery:** continuous strip of vegetation strongly influenced by edge effects. Composed of fruiting trees, lianas and shrubs which often offer structural connectivity to other habitat types. Often adjacent to urban structures such as roads and houses, presenting a higher level of disturbance and less cover than Forest.
c) **Backyard:** Areas immediately adjacent to houses, which included grass and one or more trees, often fruiting trees such as mango (*Magnifera indica*) and Malay apple (*Syzygium malaccense*). High levels of human activity and varying levels of forest cover, but some vegetation often present.
d) **Pasture:** Continuous patch of grass (*i.e.* grass field at a park) with no woody vegetation. Despite low levels of current human activity, this habitat had no forest cover and provides no vertical structure for arboreal animals.
e) **Urban:** Human-built landscape features such as houses and roads. No forest cover or vegetation and highest degree of human disturbance and activity.

To build a locality data set for spatial analyses, I recorded group locations at the center of the group every 10 minutes during focal follows using a Garmin GPSmap 60CSx (Garmin Ltd. Olathe KS). The sample interval was chosen to balance the tradeoff between the time necessary to record habitat and behavioral data (Martin and Bateson, 1993) and accurately discretizing the movement trajectory (Pace in Millspaugh and Marzluff, 2001).

Home range estimation — I used three methods of home range estimation: Minimum Convex Polygon (MCP), Kernel Density Estimator (KDE) and Nearest Neighbor Local Convex Hull measurements (LoCoH). MCP estimates draw a convex polygon around the outermost localities (Mohr, 1947); in contrast, the KDE method calculates the probability density of locations to create a utilization distribution, which is used to estimate home range area (Worton, 1989). The LoCoH method was developed by Getz and Wilmers (2004) to produce a home range estimation method that would perform well in landscapes with abrupt edges between features. It constructs minimum convex polygons (hulls) between a set of k nearest-neighbor points and, by taking the union of these hulls, constructs isopleths representing a specified percentage of the points (*i.e.* the x% isopleth, analogous to the isopleths of a fixed kernel estimation). The optimal number of k is determined by examining the effect of k on home range size estimates until a stable value is achieved (Ryan *et al.*, 2006).

Multiple studies suggest that reducing sampling effort can obscure biologically important phenomena (De Solla *et al.*, 1999; Borger *et al.*, 2006) by underestimating home range sizes and

eliminating areas that may be important to animals. To examine the effect of sampling frequency on the robustness of home range size estimates, I re-sampled the entire data set using a 40 min sampling interval to create an effective 4 fold decrease in the sampling frequency and compared the two size estimates using a t-test.

I conducted all home range analyses using the package ADEHABITAT (Calenge, 2006), within the statistical program R (R Development Core Team, 2008). I used ARCGIS 9.3 (ESRI, Redlands CA) and GOOGLE EARTH 5.0 (Google, Mountain View CA) for the spatial visualization of data.

Habitat selection — Compositional analysis is a statistical tool used to compare proportional data, such as that generated from activity budgets or diet. Aebischer *et al.* (1993) developed tools to apply compositional analysis to studies of habitat use. This procedure tests for deviations from random use and employs analysis of proportions to assess preference for each habitat, while taking into account the prevalence of other habitat types. Given that habitat selection is a hierarchical process that involves several "decisions" on the animal's part, these analyses use Johnson's (1980) definitions of selection order where establishment of the home range is considered second order selection and usage of areas within the home range is deemed third order selection. Compositional analysis of habitat use requires definition of available habitat versus used habitat. In the case of home range establishment (second order selection), the proportion of habitat types in the study area is compared to the proportion of habitat types in the (individual) home ranges. For use within home ranges (third order habitat selection), the proportion of habitat types within the home range is compared to the proportion of localities in each habitat type within the home range.

In order to quantify habitat availability, I utilized a true color satellite image (DigitalGlobe, Longmont CO) with a spatial resolution of 60 cm to classify the study area into the five habitat types described above. I defined the study area by drawing a square that encompassed the largest home range estimates outlined above (Kazmaier *et al.* 2001). Alternate study site definitions (10 percent fixed-width buffer strip around MCP home ranges and a town perimeter, based on limits imposed by water and large cleared areas, see Fig 1) yielded qualitatively similar results. I quantified the proportion of each habitat type in the study area and within each home range by drawing polygons in ARCMAP (ArcGIS 9.3 ESRI, Redlands CA), calculating their area, and dividing by the total study site area or home range, respectively.

I used the tools in the package adehabitat (Calenge, 2006) within the statistical program R (R Development Core Team 2008), to conduct compositional analysis of habitat use. I evaluated whether there was evidence of non-random habitat use and used a ranking matrix to determine which habitats were preferred relative to others.

First passage time analysis — Home range estimates tend to be constructed using spatial data aggregated over relatively large time scales, thereby capturing patterns of space use resulting from movements. First passage time (FPT) analysis, a method with roots in physics, allows a dynamic evaluation of the process of movement. When complemented with fine scale behavioral data, FPT allows elucidation of two questions important to conservation: 1) at which spatial scale are animals making movement decisions? and 2) what factors (behavioral state, habitat type) influence movement decisions at scales relevant to the animals?

FPT analysis examines the time that it takes a random walker to move outside of a circle of a given radius (r) and thus gives a measure (FPT) of the time spent in a circle of that radius.

High FPT values can be caused by an increase in turning rate and/or reduction in speed. Conversely, low FTP values signal an increase in speed or more directional travel. High FPT values have been taken by some authors as evidence of possible area restricted search, which may indicate important foraging areas (Fauchald and Tveraa, 2003). However, apparent evidence of area restricted search may also reflect other activities such as sleep or rest (Pinaud and Weimerskirch, 2007). Therefore, I use the term area restricted movement (ARM) to describe this change in movement pattern.

Since FPT vales are dependent on the scale of movement (*i.e.* radius of theoretical circle, *r*), I identified the scale of restricted movement by locating the maximum variance of FPT (log transformed) for each trajectory (Fauchald and Tveraa, 2003) using a wide range of possible values of *r* (1-500m). I then plotted the FPT values along the movement trajectory to identify high FPT areas where tamarins were changing their movement patterns (*i.e.* potential areas of restricted movement). Areas of restricted movement were defined by standardizing FPT values for each point to a standard normal distribution (mean=0, SD =1) and selecting values which were greater than 1 standard deviation from the mean FPT value for the movement trajectory. This provides a quantitative method for objectively determining areas of high FPT values (Barraquand and Benhamou, 2008). These values were projected geographically onto the study area using a 10m grid, based on the mean value of *r* across all analyzed trajectories. An example of these steps is illustrated in Fig. 2.

I examined whether ARM's were more likely to occur in certain habitat types to test if tamarin groups were altering their movement in response to different habitat types. In order to determine if changes in movement patterns corresponded to changes in behavioral state, I tested whether ARM's were associated with major foraging or social events by comparing the proportion of these events that occurred inside ARM's to the proportion of all sample points where these foraging bouts had been observed. The hypothesis that major foraging and social events were more likely to occur inside ARM's was tested using a binomial test with Yate's continuity correction.

ArcGIS 9.3 with Spatial Analyst and HawthTools extensions (Beyer, 1994) was used for GIS analyses. FPT analyses were conducted using the package adehabitat (Calenge, 2006), within the statistical program R (R Development Core Team, 2008.).

Results

Captures and marking — I captured the entire population of *S. geoffroyi* from Gamboa, which was composed of 17 tamarins. These individuals comprised three social groups (Table 1), as determined by the consistent spatial and temporal association of the same individuals during all observations and their aggressive interactions with other groups. I attached radiocollars to two individuals from two groups (the third group was not outfitted with radio collars because individuals had not yet grown to their full adult body mass). While the time to find the remaining group (group A) may have differed slightly, this did not result in obviously biased sampling in terms of the total duration or timing of observations. Group A was observed the longest total time and ranked second in number of days observed. This group also did not significantly differ from others in the distribution of observations throughout the day (see below).

Behavior, habitat and localities — A total of 100 hrs of observation were conducted on the three study groups over 36 days, from 19 March to 14 May 2008. While space use and behavior may vary beyond this period, it represents the transition from dry to rainy season that is likely to

reflect a range of resource availability on the site. More importantly, it represents peak birth season, a time of extensive cooperative infant care which is the most energetically demanding time for tamarin groups (Tardif *et al.*, 1993). Each group was followed for 36 ± 9.17 hrs over 12 ± 3 days. The mean observation time per day and group was 3.01 ± 0.31 hours. A total of 694 localities (231.3 ± 57.50 localities per group) were recorded. Each locality was associated with behavioral and habitat data. The distribution of observations throughout the day (on an hourly basis) did not differ among the three groups (Kolmogorov-Smirnov tests: $D = 0.25$ $p= 0.8475$, $D = 0.0833$ $p=1$, $D = 0.25$ $p= 0.8475$).

Analysis of behavioral data identified 68 major foraging events involving the three groups in Gamboa. Over 80 percent of these events were conducted in forest habitat and 93.9 percent of these were conducted in forest and gallery forest habitat combined. A total of 52 major social events were identified, 75 percent of which occurred in forest habitat and 86.5 percent occurred in forest and gallery habitat combined. Both feeding and social events were significantly more likely to be found in forest habitat as compared to the proportion of forest habitat (36.09 %) in the study site (binomial test, foraging: $p < 0.001$; social: $p < 0.001$).

Home range estimation — Estimates of home range size calculated using the 100% MCP's, 95% KDE's and the LoCoH are presented in Table 2. Estimates were not significantly different from each other when sampling frequency was decreased to 40 min intervals, despite a decrease from 694 points to 181 total localities (Welch two sample t-test MCP: $t = -0.573$, $df = 3.894$, $p = 0.598$, KDE: $t = 0.563$, $df = 3.829$, $p = 0.605$, LoCoH: $t = 0.140$, $df = 3.92$, $p = 0.896$).

There were consistent differences in home range size among the estimation methods, with LoCoH producing the smallest estimates and KDE producing the largest estimates. The relative differences in home range size between groups were largely preserved across methods (Table 2). Differences occur because of the way these methods estimate home range. Specifically, the sharp boundaries between suitable and unsuitable habitat yield a homerange with "holes" or un-utilized areas. The LoCoH method has generally smaller estimates than MCP and KDE because this method tends to exclude these unused areas. The MCP does not exclude these areas because the method includes all areas within a polygon bounded by the outer points, whereas KDE includes them because it assumes a normal distribution based on a fixed probability of occurrence around each point. These methodological idiosyncrasies have consequences for methods of habitat selection, as discussed below.

Habitat selection — The 80 ha study area was composed of 36.1% forest, 8.18% gallery, 19.21% backyard, 7.21% pasture and 29.3% urban. For simplicity, I present the mean proportion of habitat types of all three home range estimation methods (Fig. 3); estimates derived from each method provide comparable results.

Compositional analysis of habitat use (third order selection) revealed non-random use of habitat types within MCP home ranges (Lambda= 0.003, $df = 4$, $p = 0.001$); KDE and LoCoH home range estimates did not reveal statistically significant deviations from random habitat associations (KDE: Lambda= 0.297, df=4, p=0.456; LoCoH: Lambda= 0.001, df=4, p=0.526) within home ranges. For LoCoH nonrandom associations may have been lacking because the home range was well defined and did not include unused areas, possibly indicating that the selection occurred at the level of the establishment of the home range (second order selection). Consistent with this idea there was statistically significant evidence for nonrandom use of habitat

types in the establishment of the LoCoH home range within the study site (Lambda=0.031, df=4, p=0.034).

Habitat selection within MCP home ranges and establishment of LoCoH home ranges within the study site revealed clear and consistent patterns of preference. The ranking matrix (Table 3) shows that forest habitat was significantly preferred over all other habitat types, followed by gallery forest, while pasture and urban habitats were not preferred (MCP results shown, LoCoH results consistent).

First passage time analysis — To determine whether tamarin groups were changing their movement patterns according to habitat features or behavioral events, I conducted FPT analysis on 24 independent trajectories from the 3 groups (mean ± SD: 8 ± 1 trajectories per group, separated by on average 10.57 days). The average radius for analysis (*i.e.* scale of search *sensu* Fauchald and Tveraa (2003)) was 8.7 m (SD= 8.5), indicating that tamarins were changing their movement patterns at that spatial scale. The projection onto geographical space of areas 1 SD above the mean FPT yielded a total of 47 areas of restricted movement (ARM's). Using the satellite image, I determined that 70.21 percent of these grid cells were in forest habitat, which was significantly different from the overall availability of forest (36.09%) in the study site (exact binomial test: $p < 0.001$).

To determine whether area restricted movement was associated with major foraging or social events, I plotted these events onto the map of ARMs. Major foraging events were significantly more likely to be observed in ARMs (binomial test: X-squared = 14.366, df = 1, $p < 0.001$). Likewise, major social events were also significantly more likely to occur inside ARM's (binomial test: X-squared = 93.301, df = 1, $p < 0.001$). Over 65 percent (65.95) of the ARM gird cells had at least one social and/or foraging event.

Discussion

The results of this study suggest that tamarins, a reportedly disturbance-tolerant primate, show a clear preference for forest, both in their usage and establishment of home ranges and in changes in their movement trajectories. Moreover, tamarins seem to preferentially engage in foraging and social behaviors in forest habitats. While tamarins were not expected to prefer human modified landscapes, the strong behavioral preference for conducting vital life history activities in forest habitat suggests that these may be critical to their long term persistence.

This study employs data at a scale seldom collected for tropical mammals and illustrates how combining the analysis of movement trajectories with fine scale observations of habitat and behavior can yield a better understanding of the causes of animal movements in a fragmented landscape. Results highlight the importance of conservation approaches that quantify behavioral patterns in heterogeneous landscapes (Wiens, 1976; Presley et al., 2009), given the profound relevance of behavior to dispersal (Bowler and Benton, 2005) and landscape connectivity (Baguette and Van Dyck, 2007).

Home range — Few published papers have reported home range sizes of free-ranging tamarin groups and even fewer have used established estimators (*e.g.* MCP, KDE, etc.). Instead, researchers typically report estimates based on quadrat maps of a field site, which roughly correspond with the MCP method. Dawson (1976) reported home ranges of 26 and 32 ha for this species in a relatively undisturbed habitat in central Panama. Other tamarin species of similar

diet and body size have been reported to have home range sizes of 32-55 ha (*S. mystax*: Lottker *et al.*, 2004) and 56.2-41.7 ha (*S. nigricollis*: de la Torre *et al.*, 1995). Thus, it is an open question whether the considerably smaller MCP home range estimates presented here (7.9-16.5 ha) are due to reduced habitat availability at the Gamboa site or alternatively, due to the limited time frame of study; tamarins may increase their range in response to seasonal changes in resources. Home range sizes of free-ranging callitrichines generated using standardized methods are critical for establishing criteria for habitat management both inside and outside of reserves.

Home range estimation methods differed in their performance as has been previously reviewed. Notably, the LoCoH method performed well in this study and may be of particular use in the study of animal movement in fragmented habitats in a variety of species (badgers: Huck *et al.*, 2008; dolphins: Elwen *et al.*, 2009; buffalo: Ryan *et al.*, 2006; Korte, 2008; primates: Campos and Fedigan, 2009).

Habitat selection — Results suggest that Geoffroy's tamarins have a strong preference for forest and gallery forest habitat, but avoid urban and pasture habitat relative to their availability. While tamarins will cross roads or make use of anthropogenic structures such as fences and roofs (Díaz-Muñoz, pers. obs.), these movements are sporadic and are always used to reach another habitat type. Support for this comes from the observation that these movements did not noticeably affect habitat analyses even at this fine spatial scale. Thus, even though the species is often labeled "disturbance-tolerant" and is found in urban areas, the least preferred habitats are only used sporadically.

My results also highlight the importance of using appropriate home range estimation methods. I found differences in compositional analysis of habitat use according to which method was used to define the home ranges, in particular the LoCoH method. Using this method, I found no statistical evidence for preference for areas within the home range; my analyses suggest that this may have occurred because the home range was well defined and did not include unused areas, possibly indicating that the selection occurred at the level of the establishment of the home range (second order selection). Alternatively, still finer scale data are needed to determine selection within this well defined home range estimate. This underscores the assertions of Johnson (1980) that (a) habitat selection is a hierarchical process and (b) the use of the method of home range estimation can greatly affect what the researcher deems "available" to the animal and contradicts Aebischer *et al.* (1993) who suggest that their method may be used with any home range estimation method. This should be kept in mind when conducting similar studies and could be an interesting avenue for future methodological research on compositional analysis of habitat use (Millspaugh *et al.*, 2006).

Behavior and first passage time analysis — FPT analysis provided a means to use fine-scale locality data to determine the scale at which tamarins changed their movement patterns. The collection of on-the-ground data on habitat and behavior enabled an understanding of the context of tamarins' movement decisions. The results suggest that tamarin groups change their movement patterns in forest habitat in order to spend more time in this habitat type. Additionally, f oraging and social behavior were significantly more likely to occur within areas of restricted movement, suggesting that tamarins are altering their movement patterns in response to suitable foraging sites and suitable areas for grooming, intergroup interactions and other social behaviors. The FPT approach revealed movement patterns and behavioral activities that would have been obscured at

coarser resolutions or using other methodological approaches.

Conservation implications — With regard to tamarin conservation, these results suggest that in disturbed settings, management at local scales should focus on preservation of forested areas and connectivity between these areas with habitats that contain some vegetative cover and limited human disturbance. The absence of primates can have marked effects on forest structure and composition (Beckman and Muller-Landau, 2007; Nunez-Iturri and Howe, 2007) and small-bodied primates such as tamarins are often the only remaining primates in disturbed areas to disperse seeds (Peres and Palacios, 2007; Catenacci *et al.*, 2009). Thus, the persistence of tamarins in disturbed areas is likely to have positive effects on forest function, particularly in the increasingly valuable urban areas (Dearborn and Kark, 2009).

More broadly, this study suggests that caution should be exercised when assuming a disturbance-tolerant species will persist over the long term in fragmented habitats (Cohen and Lindell, 2005) and emphasizes the importance of understanding the ecology of human modified landscapes, especially in tropical forests (Gascon *et al.*, 1999; Lindenmayer *et al.*, 2008; Chazdon *et al.*, 2009). Even in a case where these animals face few threats from direct exploitation, they may still be adjusting their movement and conducting important behaviors in forest habitat. In other localities, the combination of a reduction of preferred habitat and direct exploitation may lead to more rapid local extinction and concomitant loss of ecosystem services.

Finally, this study highlights the importance of integrating of data on organismal and environmental causes of movement to better understand how movement processes scale up, as proposed by the emerging movement ecology paradigm (Nathan *et al.*, 2008). Fine-scale descriptions of movement are important for informing conservation and management strategies at the local scale (Schwartz, 1999; Rouget, 2003) and can often be used to predict and explain patterns at larger scales (Revilla *et al.*, 2004; Patterson *et al.*, 2008).

This study contributes to the understanding of the processes underlying individual movement and provides a framework that can be applied both in local and large scale conservation planning. Hopefully, it will stimulate further research on animals that persist in the face of disturbance, including integration of information at the scale at which these species perceive the environment.

Acknowledgments

I would like to thank: the Natural Authority of the Environment of the Republic of Panama (ANAM) for authorization to conduct this project. R. Lessnau, C. R. Laughter, M. E. Martin and D. J. Smith who generously provided their expertise in trapping and handling animals. E. A. Lacey, C. Moritz, J. S. Brashares, A. C. O. Burton, W. B. Monahan, L. Benedict, D. R. Rubenstein, J. W. Alves-dos Santos, J. Woodruff, M. MacManes provided critical feedback on the manuscript. The Smithsonian Tropical Research Institute for providing a unique community of researchers and facilities to support research. C. A. Christen and the Smithsonian Latino Initiatives Pool for providing GIS training. A. J. Sosa-Bartuano for invaluable assistance in the field and a wealth knowledge of Panamanian natural history. This work was funded by the Museum of Vertebrate Zoology and Department of Integrative Biology, the American Society of Mammalogists, Sigma Xi and NSF DDIG grant 0608467. SLDM was supported by the Ford Foundation Predoctoral Fellowship and the Chancellor's Opportunity Fellowship from UC Berkeley.

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Table 1. Composition of study groups in Gamboa. This represented the entire population of tamarins in Gamboa during the study period.

Group	Males/Females	Infants	Total
A	2/0	0	2
B	3/2	1	6
C	4/3	2	9

Table 2. Home range area estimates for each group of S. geoffroyi in Gamboa using minimum convex polygon (MCP), kernel density estimates (KDE), and nearest-neighbor convex hull methods (LoCoH) on the entire locality data set.

Group	MCP (ha)	95% KDE (ha)	LoCoH k-50 (ha)
A	7.89	12.91	4.35
B	16.51	29.42	11.9
C	9.92	12.73	7.64

Table 3. Ranking matrix of selection of habitats within MCP home ranges, based on compositional analysis of habitat use. Results for establishment of LoCoH home ranges in the study site were qualitatively similar. When the habitat in the row is used more than the habitat in the column there is a "+", if it is used less there is a "-" Significant differences are shown by triple signs and **bold** typeface. Ranks indicate that the most preferred habitat (4) to the least preferred habitat (0).

	Forest	Gallery	Backyard	Pasture	Urban	Rank
Forest		+	**+++**	**+++**	**+++**	4
Gallery	-		+	**+++**	**+++**	3
Backyard	**---**	-		**+++**	**+++**	2
Pasture	**---**	**---**	**---**		**+++**	1
Urban	**---**	**---**	**---**	**---**		0

Figure 1. Study area and home ranges plotted on satellite image. (A) Study area (B) 100% MCP (C) 95% KDE (D) LoCoH k-30. Home ranges from left to right correspond to groups A, B and C. Home ranges plotted using all localities.

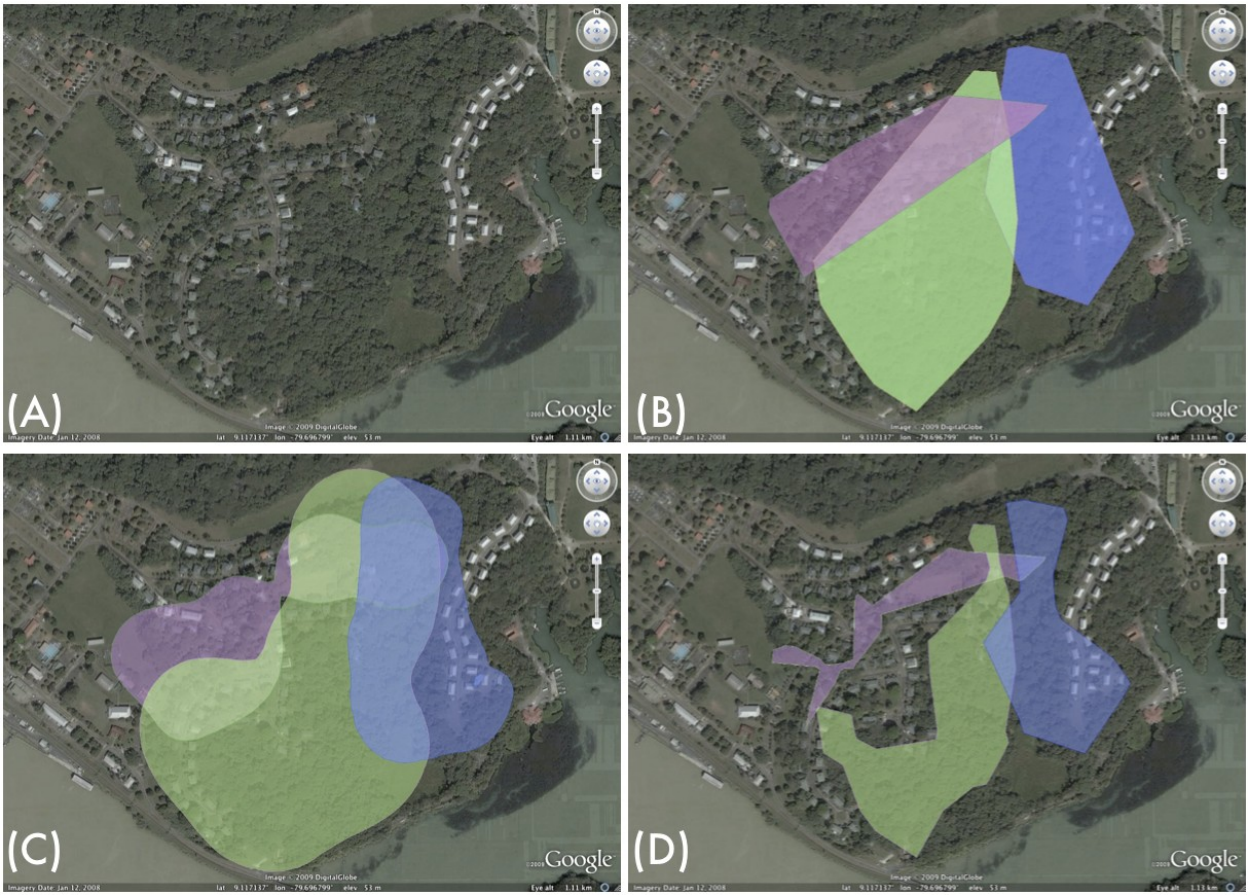


Figure 2. Example of the steps in First Passage Time analysis. (A) Plot of the variance of log(FPT) against the radius (in meters) of circles in order to identify the scale of search. The maximum variance in this example is at 16 meters and circled in red. (B) Plot of the first passage time across a circle with a radius of 16 meters against the time of day of the movement path. Areas of high FPT (ARM's) are circled. These are areas which are 1 SD over the mean FPT value (noted by the red line). Two (C) The geographic location of two major foraging events identified by behavioral observations (yellow globes) are plotted on a satellite image containing the analyzed trajectory in red. Note how movement is more tortuous around the location of the feeding events, which took place in gallery forest. Note how the movements between these areas are relatively straight.

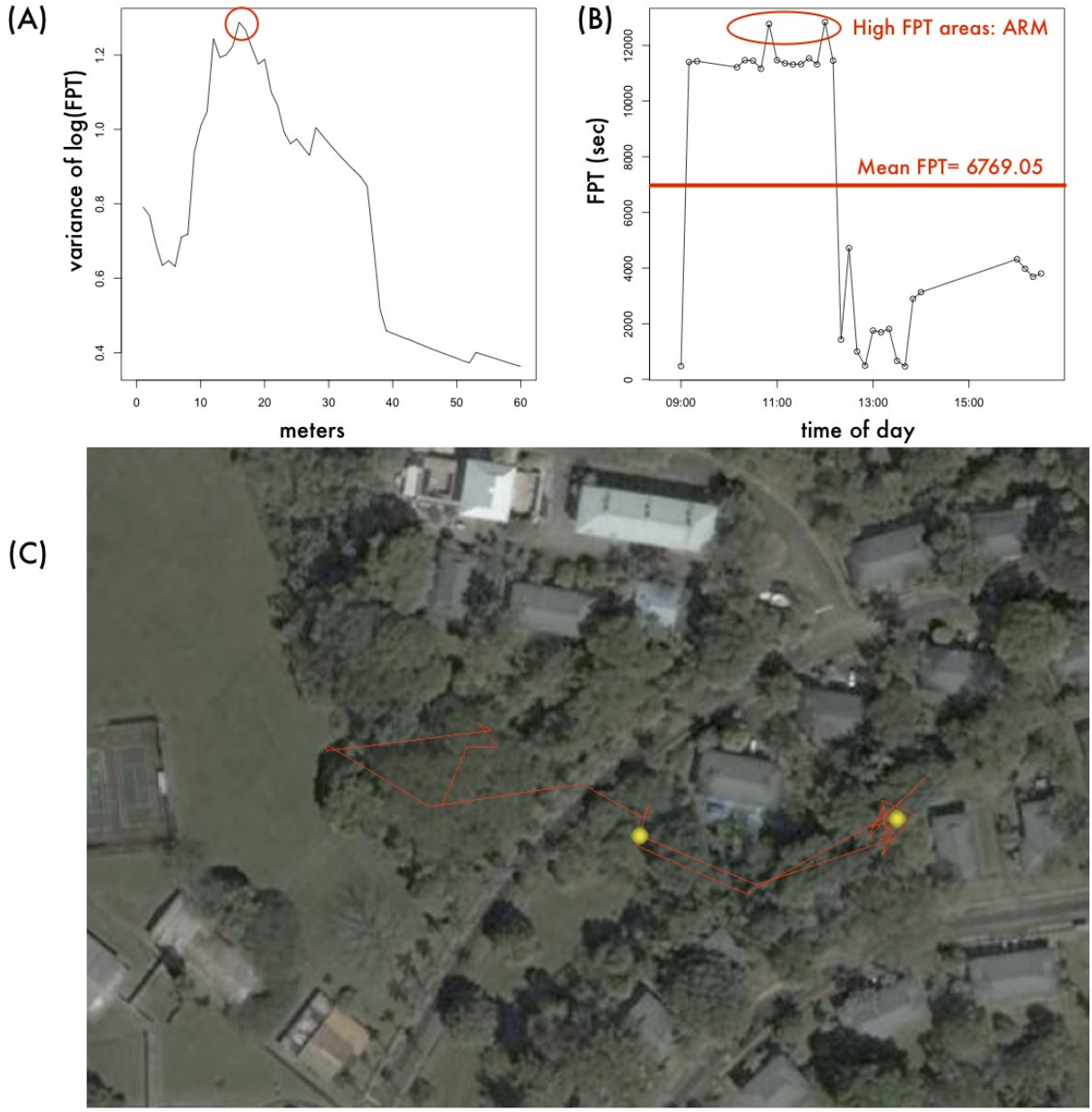
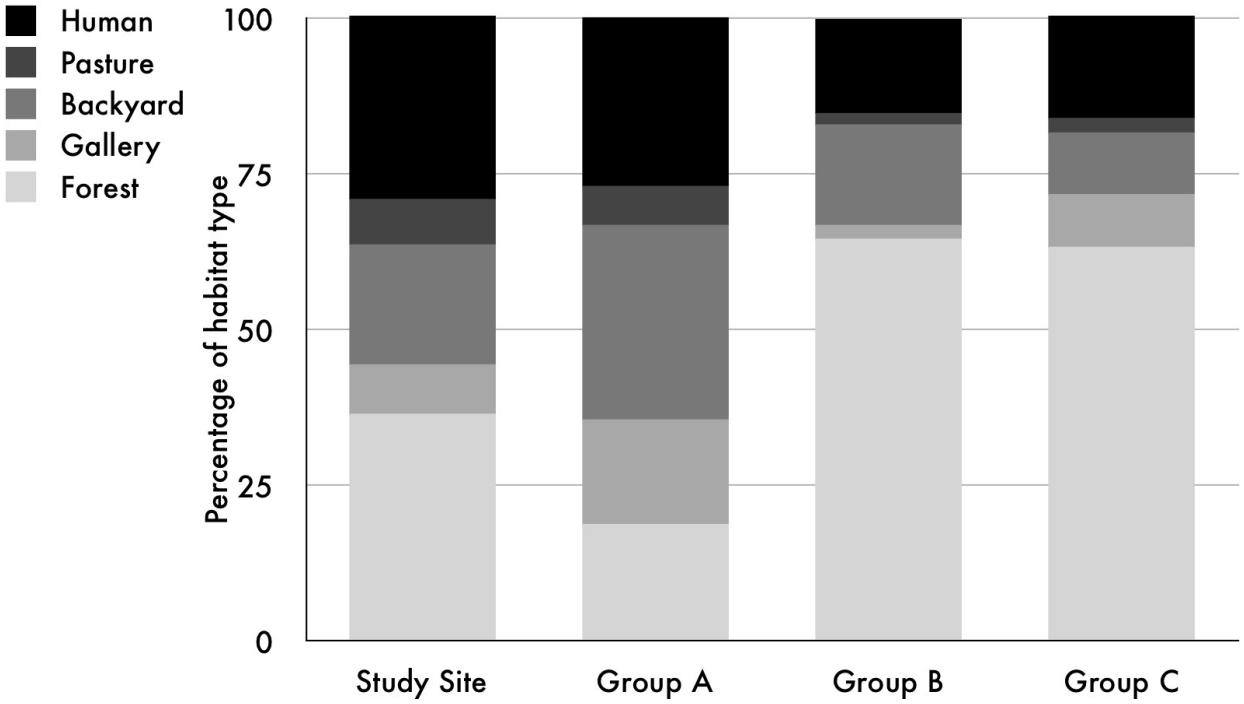


Figure 3. Proportion of each habitat type within the study area and within the home ranges of three groups. The proportion of habitat types presented is the mean value of all three home range estimation methods.



CHAPTER 2

The role of riverine barriers in fine scale population genetic structure of Geoffroy's Tamarin (*Saguinus geoffroyi*) in the Panama Canal Watershed

ABSTRACT

The role of physical barriers in promoting population divergence and genetic structuring is well known. While it is well established that animals can show genetic structuring at small spatial scales, less well resolved is how the timing of the appearance of barriers affects population structure. This study uses the Panama Canal watershed as a test of the effects of old and novel riverine barriers in creating population structure in *Saguinus geoffroyi*, a small cooperatively breeding neotropical primate. Mitochondrial sequences and microsatellite genotypes from three sampling localities revealed genetic structure across the Chagres River and the Panama Canal, suggesting that both waterways may act as barriers to gene flow. F-statistics and exact tests of population differentiation suggest population structure on either side of both riverine barriers. Genetic differentiation across the Canal, however, was less than observed across the Chagres. Accordingly, Bayesian clustering algorithms detected between two and three populations, with localities across the older Chagres river always assigned as distinct populations. While conclusions must be regarded as preliminary, this study adds to the evidence indicating that riverine barriers create genetic structure across a wide variety of taxa in the Panama Canal watershed and highlights the potential of this study area for discerning modern from historical influences on observed patterns of population genetic structure.

INTRODUCTION

The distribution of genetic variability across geography is affected by multiple biotic and abiotic factors (Avise 2004, Loveless & Hamrick 1984), including mode of reproduction, vagility, philopatry and geography. The role of physical barriers in promoting population divergence and structure is well known (Avise & Felley 1979, Preziosi & Fairbairn 1992). While it is well established that animals can show genetic structuring at very small spatial scales (Selander 1970), the timing of the origin of those patterns has been more difficult to discern because they may be the combined result of contemporary processes (Zellmer & Knowles 2009) as well as longer term historical events (Bowen & Avise 1990). Thus, the timing and appearance of physical barriers and how quickly these affect genetic structure in populations remains a topic of interest (Matocq et al. 2000, Vandergast et al. 2007, Zellmer & Knowles 2009).

Landscape features that constitute barriers vary among species. For instance, differences in elevation contribute to population differentiation in two amphibians, the blotched tiger salamander (Spear et al. 2005) and the Columbia spotted frogs (Funk et al., 2005). Conversely, pacific jumping mice (*Zapus trinotatus*) readily bound large topographic barriers, with gene flow explained more appropriately by habitat connectivity. Even relatively new and small barriers can affect the population structure of animals. Epps et al. (2005) showed that recent (~ 40 yrs) anthropogenic barriers have caused a marked decline in genetic diversity, in a large vagile mammal, the desert bighorn sheep (*Ovis canadensis nelsoni*). Similarly, anthropogenic barriers which red grouse could theoretically cross in one flight acted as barriers to gene flow (Piertney et al. 1998).

Bodies of water promote genetic differentiation in a variety of terrestrial species. Sea lochs explain most of the genetic differentiation among populations of red deer in the Scottish

highlands (Pérez-Espona et al. 2008). Quéméré et al. (2010) found that the Manankolana River was the major barrier to gene flow for the golden crown sifaka (*Propithecus tattersalli*) in the Daraina region of Madagascar. Bodies of water may also be barriers for volant animals, as reported by (Meyer at al. 2009) for some bat species. Rivers can serve as barriers for amphibians, as reported for the alpine stream frog (*Scutiger spp.*) in the Hengduan Mountains of China (Li et al. 2009).

Although riverine barriers have been implicated as barriers to gene flow in a variety of species, less is known about how the timing of the appearance of these barriers affect gene flow. The Panama Canal is one of the largest modifications of the hydrographic landscape undertaken by humans. Because the addition of this major riverine barrier is well documented historically and the Panama Canal is embedded in the center of a dynamic watershed with older riverine barriers, it presents an ideal opportunity to test the influence of the timing of physical, riverine barriers in population genetic differentiation. Before the construction of the Canal, the Chagres River flowed along the Atlantic slope of the Isthmus, while only small costal streams drained the Pacific slope (Meek & Hildebrand 1916). Geologic studies indicate that the Panamanian Istmus was formed by sometime in the Pliocene (Coates et al. 2004, Kirby et al. 2008) with little tectonic activity after the late Pliocene (Coates et al. 2004) indicating that by then the topographic composition of the Panama Canal watershed was probably similar to that observed today. This suggests that the Chagres was in place as a major riverine barrier well before the estimated divergence time (0.7 Mya, Evans et al., 1998) of Geoffroy's tamarin from its sister species (*S. oedipus*). Thus, while the Chagres River had been a major part of the central isthmian basin in paleontological time, the completion of the Panama Canal in 1914 created a novel riverine barrier, which is expected to have affected the movements and, hence, gene flow in a multitude of species.

This study uses the Panama Canal watershed to test the effects of an old and a novel riverine barrier in creating population structure in Geoffroy's tamarin *Saguinus geoffroyi*, a small neotropical primate. Tamarins represent an appropriate study species for this study for several reasons. First, previous work has shown that riverine barriers are important for structuring primate populations in general (Wallace 1852) and for the diversification of tamarins in particular. Peres et al. (1996) showed that cytochrome-b haplotypes corresponded to phenotypically distinct morphs of subspecies of Saguinus fuscicollis on opposite sides of the Rio Juruá in Amazonian Brazil. Furthermore, the authors showed that gene flow (and intergradation of color morphs) increased towards the narrow headwater streams of the river, as predicted by Wallace (1852). Second, among the primates inhabiting areas close to the Panama Canal (howler monkeys: *Alouatta palliata* and capuchin monkeys: *Cebus capuchinus*), tamarins may be most likely to exhibit rapid population differentiation in response to landscape changes. Tamarins exhibit high intragroup relatedness (Huck et al. 2005), suggesting some degree of population viscosity and a greater likelihood of showing genetic structure. In contrast, howler monkeys inhabiting an isolated island in the Panama Canal watershed for > 90 years showed no genetic evidence of a population bottleneck (Milton et al. 2009), suggesting little population viscosity and a near panmictic mating pattern.

To examine the role of rivers in creating population genetic structure at a small spatial scale, I sampled three populations distributed across two prominent riverine barriers – the Panama Canal and the Chagres River – to test whether the age of a physical barrier to gene flow has an effect on the level population genetic structure. I predict that there will be significant differentiation between populations separated by the Chagres, whereas differentiation across the

Panama Canal will be more modest, owing to its novelty as a barrier. To test these predictions, I: 1) examine differences in genetic variability among sampling localities at mtDNA and microsatellite loci 2) use F-statistics and AMOVA at mtDNA and microsatellite loci to investigate population genetic structure between sampling localities and 3) use Bayesian clustering algorithms to determine the number of likely populations.

Methods

Study Sites — Three areas within the Panama Canal watershed (provinces of Panama and Colón, Republic of Panama) were targeted for sampling (Figure 1): (a) The Soberanía field site is located within the boundaries of Soberanía National Park, close to Camino de Plantación (9.076º, -79.659º). (b) The Gamboa field site, just outside the park boundaries, is located in and around the rural town of Gamboa, Colón Province (9.118º, -79.698º). (c) The Panama West locality (8.957º, -79.668) is west of Panama City across the Panama Canal. The Soberania and Gamboa field sites were selected to provide a comparison across the Chagres River; these samples were collected as part of a separate study on cooperative breeding in *S. geoffroyi* (Chapter 3). The Panama West locality was selected to capture the potential effect of the Panama Canal as a barrier. Samples from Soberanía National Park and Gamboa were field collected, whereas the samples from western Panama Province were obtained from museum skins collected by Dawson (1976) and housed at the Michigan State University Museum (Table 6). The use of museum samples imposed limitations on the study, specifically: they represented a sample from a wider geographic area as compared to the other localities and represented a time period ~ 30 years earlier than field-collected samples (field samples: 2005-2009; museum samples: 1973-1974). However, I opted to use museum samples due to the fact that areas directly across the Panama Canal from Soberanía have limited accessibility (due to deposits of unexploded ordnance from military activities) and tamarin populations at some localities in Western Panama have been extirpated due to growing urbanization. Utilizing museum resources allowed a broader sampling than would have been possible with field efforts and allowed looking at both of the riverine barriers of interest, albeit in a preliminary way.

Captures and Sample Collection — Tamarins from Gamboa and Soberanía were captured using hand-activated live traps baited with bananas as described by Garber et al. (1993) or by blow-darting (BioJect, Blowguns Northwest, Richland WA) with tranquilizer darts (Pnueu-Dart, Williamsport, PA). Individuals from the Soberanía population were only captured in traps and were not anesthetized. To prevent excessive stress, handling time was limited to < 15 min and manipulations were constrained to marking and sampling hair. Soberanía individuals were released immediately at the capture site. Gamboa adults were captured using blow-darting; infants and juveniles were trapped because they were too small to safely dart. In both trapping and darting, I anesthetized Gamboa individuals using Ketamine (7.5mg/kg) and Zoletil (3.75 mg/kg Vibrac SA, Carros Cedex, France). Gamboa animals were anesthetized to enable collection of morphological data for a separate study (Chapter 3). Gamboa individuals were handled for 48 ± 14 min and were placed in a pet kennel for 3.67 ± 2.13 hrs until fully recovered. Respiration, heart rate and internal body temperature of anesthetized individuals were measured throughout handling procedures to monitor animal condition. To minimize potential injuries, individuals were darted at feeding stations that were eye-level above the ground. After darting, individuals were followed by two field assistants with a mesh net to catch anesthetized individuals which

strayed from the feeding station. All capture and handling procedures were approved by the UC Berkeley Institutional Animal Care and Use Committee and followed the guidelines of the American Society of Mammalogists (Gannon & Sikes 2007).

Field collected samples included: (a) Hair samples plucked from the base of the tail and saved in coin envelopes and stored dry and (b) Ear tissue collected from the pinnae using surgical scissors and stored in RNA *later* and frozen at -20ºC until extraction. Soberania animals were represented by hair samples and Gamboa individuals were represented by hair and tissue samples. To verify the reliability of microsatellite genotypes from hair samples, all Gamboa individuals were genotyped using tissue samples to corroborate the results obtained from hair samples.

Museum skins were preserved as dry flat skins. Surgical scissors were used to extract a ca. 1 mm^2 piece of tissue from the edge of the flat skin. The tissue samples were stored in an empty microcentrifuge tube until extraction within the week. A list of sampled individuals is included in Table 6.

DNA Extraction — Genomic DNA was extracted using Qiagen DNA Micro kits (Qiagen, Valencia, CA), according to manufacturer instructions for each sample type. Museum tissue samples were soaked in 70% ethanol for 24 hrs prior to extraction. DNA yield was quantified using a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Hair and museum skin samples were extracted in a "clean" room dedicated to low-copy sample extractions. Both sampling and extraction negative controls were used to monitor for possible contamination at every step of the genetic workflow.

Mitochondrial Sequencing — Mitochondrial sequences are extensively used in population genetic studies (Avise 2004) owing to their uniparental inheritance and their relatively rapid sequence evolution, especially in the mitochondrial control region (Hoelzel et al. 1991). A 520 bp fragment from the mitochondrial control region was amplified using primers designed to be genus-specific for *Saguinus (*Table 1, Cropp et al. 1999). To design primers which would amplify a short fragment suitable for skin and hair samples, control region sequences derived from tissue-extracted DNA from the Gamboa population were used. A 700bp section of the control region was amplified – using "universal" primers MVZ 121/70 derived from (Kocher et al. 1989, Palumbi 1996) – to design primers SGDL1-F and SGDL1-R using Primer3 (Rozen & Skaletsky 2000), as implemented in Geneious 4.8.5 (Biomatters, Auckland, NZ) . PCR reactions were performed on ABI 2720 (Applied Biosystems, Foster City, CA) or BioRad iCycler (Bio-Rad, Hercules, CA) thermocyclers using fluorescently labelled primers. Cycling conditions were 94ºC for 4 min; 94ºC for 1 min, 52ºC for 1 min, 72ºC for 75 s, repeated 30 times; 72ºC for 10 min. Polymerase Chain Reaction volume was 10 µL with: 40 ng of genomic DNA, 1µL of 10X PCR Buffer (Applied Biosystems, Foster City, CA), 2.5mM MgCl2, 0.8µL 10 mg/mL BSA, 0.4 mM of each DNTP, 3pm of each primer and 0.5 U of Taq polymerase (Invitrogen, Carlsbad, CA). Amplification was confirmed via TBE-agarose gel electrophoresis and cleaned products of amplification using Exo-SAP IT (Affymetrix, Cleveland, OH) . PCR products were fluorescently labelled utilizing ABI Big Dye 3.1 (Applied Biosystems, Foster Ciy, CA) and sequenced amplicons in an ABI 3730 automated sequencer (Applied Biosystems, Foster Ciy, CA). Alignment of sequences was accomplished using CodonCode Aligner v3.5.6 (CodonCode, Dedham, MA) and Geneious 4.8.5 (Biomatters, Auckland, NZ). To ensure sequence accuracy, sequences from both strands (derived from each forward and reverse primer) belonging to the same individual were obtained, aligned

and manually edited. The consensus sequence of each individual was used for further analyses.

Microsatellite Genotyping — In order to maximize the chances of detecting population structure across the Panama Canal, microsatellite loci were genotyped. Due to their higher mutation rates (Jarne & Lagoda 1996), microsatellites provide information about genetic structure over shorter time scales than mtDNA, and provide a multilocus biparental perspective on population genetic structure. Seven polymorphic microsatellite loci from previously published studies (Bohle & Zischler 2002, Escobar-Páramo 2000) were amplified on ABI 2720 (Applied Biosystems, Foster City, CA) or BioRad iCycler (Bio-Rad, Hercules, CA) thermocyclers using fluorescently labelled primers. Cycling conditions followed the mitochondrial protocol with the following modifications: 35 cycles of amplification and locus-specific annealing temperature (see Ta Table 3). To genotype samples in an ABI 3730 automated sequencer (ABI, Foster City, CA), 1μL of PCR product was added to 8.8 μL of formamide with 0.2 μL of GeneScan 500-LIZ size standard (ABI, Foster City, CA). Genotypes were scored manually using Genemapper 4.0 (ABI, Foster City, CA). To ensure robustness of genotyping, homozygote genotypes from the museum skin samples and field collected hair samples were genotyped from at least 2 independent PCR reactions. A subset of samples from Gamboa were genotyped from both hair and tissue samples. Additionally, a subset of samples from each population was genotyped de-novo from independent extractions.

Loci were checked for evidence of null alleles and genotyping errors using the program MICROCHECKER 2.2 (Oosterhout et al. 2004). Microsatellite loci were tested for deviations from Hardy-Weinberg and linkage disequilibrium using FSTAT 2.93 (Goudet, 1995).

Genetic Diversity — The program DnaSP 5.1 (Librado & Rozas 2009) was used to calculate nucleotide (π) and haplotypic (*h*) diversity for mitochondrial sequence data. Number of alleles, expected and observed heterozygosities for microsatellite loci were calculated using ARLEQUIN 3.1 and GENALEX 6.1 (Peakall & Smouse 2006). Allelic richness and private allelic richness using the rarefaction method (to control for sample size differences) were calculated in using HP-RARE 1.1 (Kalinowski 2005). Differences between populations in microsatellite genetic diversity statistics were tested using ANOVA. Statistical tests are two-tailed and means are reported with their standard deviations (mean ± SD), unless otherwise noted.

Population Genetic Structure — Mitochondrial haplotype relationships were examined using a parsimony network as calculated by TCS 1.22 (Clement et al. 2000), with a 95% connection limit. An exact test of population differentiation based on mitochondrial haplotype frequencies (Raymond & Rousset 1995) with 105 randomization steps was conducted in ARLEQUIN 3.1 (Excoffier et al. 2005). Pairwise F-statistics were calculated in ARLEQUIN to investigate population genetic structure via mtDNA and microsatellite data. Differences in FST values between populations were tested using 10,100 permutations. Analysis of molecular variance, AMOVA, (Excoffier et al. 1992) was used to examine the amount of genetic variance explained by within and among population (i.e., sampling locality) variation.

Population Assignment — To determine the probable number of populations represented by the data set three different Bayesian clustering methods were used, due to previously reported variability in performance of these methods (Waples & Gaggiotti 2006, Rowe & Beebee 2007)

and the somewhat different algorithms implemented and information used in each program. STRUCTURE (Pritchard et al. 2000) was run with no previous population information (USEPOPFLAG=0), using an admixture model and assuming allele frequencies were correlated. Four replicate runs at k=1-5 were run using a burin of 10^6 and a run length of 10^9. The optimal k value was selected using the highest Pr(X | k) values (Pritchard et al. 2000). An assignment test in GENECLASS2 (Piry et al. 2004) was conducted to determine the probability that each individual was assigned to its own "population", in this case representing the sampling locality. An alternate population definition collapsing the Soberanía and Panama West individuals into one population, was also run. Individuals were assigned to a population using the criterion of Rannala & Mountain (1997) and the probability of these assignments was calculated using a Monte-Carlo resampling technique (Piry et al. 2004) based on 10,000 simulated individuals. The Type I error was set at 0.01. GENELAND was used as a third method of population clustering because it incorporates spatial data in order to identify genetic discontinuities in a spatially explicit fashion, which is relevant to the question at hand. First, the most probable number of k populations was determined using the MCMC over 5 x 10^5 iterations (Coulon et al. 2004) using the uncorrelated model. Five replicates of this process were conducted to ensure consistency of results (Rowe & Beebee 2007). Spatial maps of the posterior probability of population membership were generated by using the posterior probability of population membership obtained from the MCMC simulation. The course of the Chagres River and the Panama Canal (extracted from a satellite photo) was overlaid on the population map, in order to investigate the coincidence of geographic barriers with the population limits calculated by GENELAND.

RESULTS

Genetic samples were collected from a total of 59 *S. geoffroyi* from across the Panama Canal watershed. The number of individuals from which microsatellite (and mitochondrial) data were collected in each population were: Gamboa: 19 (17), Panama West: 22 (22) and Soberanía: 18 (14).

Microsatellite Genotyping — Microsatellite loci showed no evidence of null alleles or genotyping errors. Loci did not show evidence of departure from Hardy-Weinberg equilibrium or linkage disequilibrium in any of the populations after correction for multiple tests at the 0.05 nominal level. Genotyping of hair samples and ear tissue samples from Gamboa yielded identical genotypes at all loci, suggesting that genotypes from hair samples were reliable.

Genetic Diversity — Analysis of mitochondrial sequences revealed 8 variable sites and 8 haplotypes. Haplotype and nucleotide diversity in each population is presented in Table 2. As expected for a larger geographic sample, the Panama West population had the largest number of haplotypes, but it did not have the greatest nucleotide diversity, as did Gamboa (Table 2). Microsatellite locus-specific measures of genetic diversity along with population means are presented in Table 3. Measures of allelic diversity at microsatellite loci were not significantly different between sampling localities (ANOVA's: Observed heterozygosity p=0.8008, unbiased heterozygosity p=0.5118, allelic richness p=0.3991, rarefaction-calculated private allelic richness p=0.3882). Gamboa appeared to have the lowest allelic diversity across measures, but these differences were not statistically significant. The lack of difference in microsatellite diversity included the Panama West locality, despite the differences in the nature of the sampling regime (larger geographic extent,

different time frame).

Population Structure — The haplotype network (Figure 1) revealed sharing of haplotypes between the Panama West and Soberanía sites. The number of haplotypes observed in the Panama West locality was larger than at the other localities. The Gamboa locality was composed of only two haplotypes, including one unique to the locality (47 % of individuals); the other haplotype was shared with the Panama West locality. An exact test based on haplotype frequencies suggested strong evidence for differentiation of three populations ($P < 0.0001$).

Permutation analyses of FST values yielded significantly different ($P < 0.001$) values between all pairwise comparisons of sampling localities (Table 4). All FST values were statistically significantly different from zero. FST values calculated from mitochondrial sequence data were in general over two times larger than those calculated from microsatellite data. FST values calculated from localities across the Panama Canal were ca. half of those across the Chagres River, suggesting greater differentiation across the older riverine barrier.

AMOVA for mitochondrial data attributed 34.28% of variance to among group (sampling locality) variation, compared to 11.11% for microsatellite data (Table 5). The fixation index and among population variance components calculated by both AMOVA's differed significantly from random expectation ($P < 0.0001$).

Population Assignment — The three Bayesian methods yielded different estimates for most probable number of populations. STRUCTURE detected $k = 2$ as the most likely number of populations, with the Gamboa locality distinct from the combined Panama West and Soberania localities. The assignment plot is depicted in Figure 2. GENECLASS2 analysis correctly assigned 78% of individuals to their sampling localities (when 3 populations were assumed). When two populations were assumed, assignment success increased to 88.1% of individuals as did the quality index ($k = 3$: 62.06% $k = 2$: 80.77%), which represents the mean value of individual assignment scores (Piry et al. 2004). Both STRUCTURE and GENECLASS2 recovered localities across the Chagres as distinct populations, but did not always recover two distinct populations when comparing localities across the Panama Canal. GENELAND on the other hand, clearly delineated three populations with minimal variance in the posterior probabilities of population estimation over multiple runs. The location of the riverine barriers under study were largely consistent with the population limits delineated by GENELAND (Figure 4).

DISCUSSION

The results suggest that both the Chagres River and the Panama Canal have contributed to population genetic structure in *S. geoffroyi* inhabiting the Panama Canal watershed. Although the sampling regime is limited, the results provide good, albeit preliminary, evidence of differentiation across two riverine barriers. Analyses of F-statistics, haplotypic data and output from Bayesian assignment algorithms are collectively consistent with the Chagres River playing a role in relatively strong population differentiation, especially considering the small geographic distances between sampling localities (~ 6km). All analyses except STRUCTURE and GENECLASS2, indicate that there is detectable population differentiation among sampling localities across the Panama Canal. As expected, the level of differentiation was smaller across the Panama Canal than across the Chagres River.

There were possible limitations imposed by the sampling regime for interpreting

differentiation of sampling localities across the Panama Canal. In particular, the wider geographic sampling at the Panama West locality may cause additional allelic and haplotypic variation to be sampled. Analyses of microsatellite data suggest that this is not the case. Although the number of haplotypes is larger in the Panama West locality, it was Gamboa which had the greatest nucleotide diversity. However, because of these differences in sampling regime, the results of this study should be interpreted as preliminary evidence. More generally, the limited number of sampling localities also underscores the need for caution absent broader geographic sampling. Previous studies investigating the role of riverine barriers have found discrepant results when sampling at different localities along riverine barriers (e.g. Patton et al. 1994).

Differentiation discepancies according to marker type — The degree of differentiation among sampling localities inferred using mitochondrial data was larger that that calculated with microsatellite genotypes. This was true across both the Chagres River and the Panama Canal. The AMOVA conducted on both genetic data sets indicated that a greater proportion (~ three times) of the among group variance was explained by mitochondrial sequence differences, as expected for a uniparentally inherited marker.

Number of Distinct Populations using Bayesian Clustering — Although the results from STRUCTURE and GENECLASS2 suggest two populations in the data set, Bayesian algorithms have been reported to perform poorly at detecting populations with low differentiation (Waples & Gaggiotti 2006). Moreover, the creators of STRUCTURE caution that the large parameter space complicates the selection of *k* (Pritchard et al. 2000). This situation seems applicable to the current study as evidenced by the overlapping variances of Pr(X | k) for two and three populations (Figure 3). On the other hand, GENELAND consistently identified three populations and the geographic projection of population membership probabilities coincided with the aproximate location of both putative barriers under study. These results underscore the variability of *k* estimates from different population clustering algorithms and suggest that future researchers should use multiple methods (Rowe & Beebee 2007) and evaluate results in light of the biological significance to the study species (Pritchard et al. 2000).

Riverine Barriers in the Panama Canal Watershed — The Chagres River has been associated with genetic structure in at least one other species. Lampert at al. (2003) showed that the Chagres River formed a barrier to dispersal of túngara frogs as indicated by isolation by distance patterns calculated using microsatellite markers. Evidence that the Panama Canal has affected gene flow in a multitude of species is more abundant. Meyer at al. (2009) showed that bat populations inhabiting the islands created upon the flooding of Gatún Lake had lower genetic diversity and higher genetic differentiation than mainland populations, according to their dispersal abilities. Studies of freshwater fish suggest that distinct species assemblages existed on either side of the Cordillera Central on the Atlantic and Pacific Slopes of the Isthmus (Meek & Hildebrand 1916, Smith et al. 2004) and now exist in the same communities as a consequence of the aquatic connection provided by the Canal (Smith et al. 2004). Thus, the Panama Canal has affected population structure in a variety of taxa, increasing gene flow in aquatic species and restricting it in some terrestrial species.

Demographic evidence also supports the idea of reduced gene flow in terrestrial species as a consequence of the creation of the Panama Canal. Intensive studies on Barro Colorado Island

(BCI) have shown that multiple bird species have become locally extinct, most likely as a cause of the limited dispersal across the Canal (Robinson, 1999). There is also demographic evidence that the canal had significant effects on *S. geoffroyi* populations: in Barro Colorado Island, the tamarin population has seen decline, as observational (Enders 1939, Eisenberg and Thorington 1973) and census data (Wright et al. 2000) suggests. While habitat conversion (from secondary to primary forest) has been suggested as a cause of the decline of tamarins on BCI, the results of this study and those cited above suggest that the absence of dispersal and gene flow may have played a part in this demographic change.

Conservation Implications — The lower differentiation across the Panama Canal suggested by this study points to only modest structure. However, in the absence of migrants these populations may diverge in the future, as has happened more clearly across the Chagres. In fact, divergence may be hastened across the Panama Canal, due to decreasing habitat availability west of the Panama Canal. In contrast, the Chagres headwaters are < 25km from both Soberanía and Gamboa populations and well forested. This may insure that over time gene flow will persist across, i.e. around, this barrier, which will likely not be the case for the Panama Canal. This study adds to the growing body of literature on the effects of recent anthropogenic barriers on population structure and genetic diversity (Piertney et al. 1998, Epps et al. 2005, Smith et al. 2004). The results of this study highlight the utility of the Panama Canal Watershed as an ideal testing ground for questions of population structure. Moreover, the proximity of natural protected areas to two rapidly growing population centers (Rompré et al. 2008) provide challenges for species conservation, but ample opportunities for conservation-oriented biological research on a number of tropical species. It is hoped that the current study will stimulate such research.

Acknowledgments

I would like to thank: The Natural Authority of the Environment of the Republic of Panama (ANAM) for authorization to conduct this project; the Michigan State University Museum for graciously providing the loan of specimens and authorizing their sampling; R. Lessnau, C. R. Laughter, M. E. Martin and D. J. Smith who generously provided their expertise in trapping and handling animals; M. Tejada-Gonzalez and A. J. Sosa-Bartuano for invaluable assistance in the field and a wealth knowledge of Panamanian natural history; E. A. Lacey, C. Moritz, J. S. Brashares, provided critical feedback on the manuscript; The Smithsonian Tropical Research Institute for providing a unique community of researchers and facilities to support research. This work was funded by the Museum of Vertebrate Zoology and Department of Integrative Biology, the American Society of Mammalogists, Sigma Xi and NSF DDIG grant 0608467. SLDM was supported by the Ford Foundation Predoctoral Fellowship, the Chancellor's Opportunity Fellowship from UC Berkeley and a University of California Dissertation Year Fellowship.

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Table 1. Mitochondrial DNA primers.

Primer Name	5'-3' sequence	Reference
SCJ5	TTGGTTATGTAATTAGTGC	(Cropp et al. 1999)
SCJ1	GAGCGAGAATACTAGTAGAAG	(Cropp et al. 1999)
SGDL1-F	GCACACGACTACCAAGCAAGATTATGA	This study
SGDL1-R	GGGTGGGGTGGGGACCAAGA	This study

Table 2. Diversity statistics for mitochondrial sequence data. **N** indicates number of individuals sequenced in each population. Standard deviation is for sampling and stochastic processes.

Population	N	Number of Haplotypes	π Nucleotide diversity	h Haplotype diversity
Panama West	17	7	0.00361 ± 0.00068	0.797 ± 0.067
Gamboa	22	2	0.00509 ± 0.00146	0.529 ± 0.045
Soberanía	14	3	0.00146 ± 0.00028	0.648 ± 0.081

Table 3. Per-population microsatellite characteristics. **N:** Number of individuals typed. **Na:** Number of alleles loci used in this study. **Ar:** Allelic Richness. **Par:** Private allelic richness. **Ho:** Observed heterozygosity. **He:** Expected heterozygosity. Population means ± SD for each are presented.

Population	Locus	N	Ta (ºC)	Na	Ar	Par	Ho	He	UHe
Gamboa	Mean	19		2.86 ± 1.21	2.35 ± 0.57	0.19 ± 0.16	0.55 ±0.21	0.50 ±0.13	0.52 ±0.14
	SB7	19	54	5	3.0636	0.3341	0.421	0.652	0.670
	SB8	19	54	2	1.8387	0.2213	0.421	0.332	0.341
	SB19	19	54	2	1.9801	0.0003	0.579	0.478	0.491
	SB38	19	54	2	1.8763	0.0959	0.474	0.361	0.371
	Ceb10	19	52	4	3.1310	0.2419	0.947	0.680	0.698
	Ceb11	19	52	3	2.5717	0.4081	0.684	0.532	0.546
	Ceb128	19	52	2	1.9913	0.0080	0.316	0.499	0.512
Panama West	Mean	22		4.29 ± 1.80	2.85 ± 0.74	0.51 ± 0.55	0.54 ± 0.21	0.60 ± 0.14	0.61 ± 0.14
	SB7	22	54	5	3.2074	0.5249	0.455	0.658	0.673
	SB8	22	54	7	3.3217	1.3316	0.591	0.684	0.700
	SB19	21	54	2	1.9914	0.0012	0.524	0.500	0.512
	SB38	22	54	6	4.0426	1.2063	0.955	0.785	0.803
	Ceb10	22	52	3	2.7673	0.0774	0.500	0.624	0.638
	Ceb11	22	52	4	2.6181	0.2420	0.455	0.567	0.580
	Ceb128	22	52	3	1.9836	0.1592	0.273	0.361	0.369
Soberania	Mean	18		4.14 ± 2.34	2.87 ± 0.99	0.48 ± 0.58	0.61 ± 0.19	0.57 ± 0.16	0.58 ± 0.17
	SB7	17	54	8	4.2180	1.4753	0.882	0.782	0.806
	SB8	16	54	6	3.6544	1.0413	0.750	0.689	0.712
	SB19	17	54	2	1.9356	0.0001	0.588	0.415	0.428
	SB38	18	54	5	3.7931	0.4310	0.722	0.748	0.770
	Ceb10	18	52	4	2.5491	0.4001	0.444	0.451	0.463
	Ceb11	18	52	2	1.9584	0.0142	0.333	0.444	0.457
	Ceb128	17	52	2	1.9544	0.0004	0.529	0.438	0.451

Table 4. Pairwise comparisons for F statistics using mtDNA and microsatellite data as calculated by ARLEQUIN. Microsatellite data are above the diagonal and shaded. Statistically significant differences at $p < 0.01$ and $p < 0.001$ level are denoted by one and two stars (*), respectively. P values calculated based on 10,100 permutations.

Population	Panama West	Gamboa	Soberanía
Panama West		0.13616**	0.06428**
Gamboa	0.37476**		0.13247**
Soberanía	0.13090*	0.42510**	

Table 5. Analysis of molecular variance for microsatellites and mitochondrial (in parentheses) data. Based on 10,100 permutations ($P < 0.0001$).

Source of Variation	d.f.	Sum of Squares	Variance Components	Percentage of Variation
Among Populations	2 (2)	22.95 (18.43)	0.24 (0.48)	11.11 (34.28)
Within Populations	115 (50)	223.70 (45.83)	1.95 (0.92)	88.89 (65.72)
Total	117 (52)	246.703 (64.26)	2.19 (1.39)	
Fixation Index		FST: 0.11 (0.34)		

Table 6. Michigan State University Museum specimens sampled for this study.

Catalog #	Species	Sex	Year Collected	Specific Locality	Latitude	Longitude
MR.22872	*Saguinus geoffroyi*	M	1973	Cerro Cama	9.01667	-79.90000
MR.22874	*Saguinus geoffroyi*	M	1973	Vicinity of La Chorrera	8.88333	-79.78333
MR.22878	*Saguinus geoffroyi*	F	1973	15 km W of Balboa	8.95000	-79.70306
MR.22875	*Saguinus geoffroyi*	M	1973	15 km W of Balboa	8.95000	-79.70306
MR.22947	*Saguinus geoffroyi*	M	1973	6.5 km NW of Balboa	8.98197	-79.61785
MR.22885	*Saguinus geoffroyi*	M	1973	5 km NE of Arraijan	8.96598	-79.63393
MR.22891	*Saguinus geoffroyi*	M	1973	3.3 km NE of Arraijan	8.97110	-79.62878
MR.22889	*Saguinus geoffroyi*	F	1973	2.5 km NE of Arraijan	8.95000	-79.58635
MR.22998	*Saguinus geoffroyi*	F	1974	3 km W of Balboa	8.95000	-79.56816
MR.22989	*Saguinus geoffroyi*	M	1974	8.5 km W of Balboa	8.95000	-79.64850
MR.22935	*Saguinus geoffroyi*	F	1973	6 km SW of Balboa	8.92924	-79.61707
MR.22994	*Saguinus geoffroyi*	F	1974	8.5 km WSW of Balboa	8.92762	-79.58917
MR.22923	*Saguinus geoffroyi*	M	1973	3.5 km SW of Balboa	8.89885	-79.61811
MR.22963	*Saguinus geoffroyi*	F	1973	4 km ESE of Arraijan	8.91164	-79.60525
MR.22907	*Saguinus geoffroyi*	M	1973	9 km W of Balboa	8.99156	-79.60846
MR.22902	*Saguinus geoffroyi*	F	1973	9 km E of Arraijan	8.95000	-79.61363
MR.22949	*Saguinus geoffroyi*	F	1973	4 km E of Arraijan	8.93616	-79.61640
MR.22934	*Saguinus geoffroyi*	M	1973	8 km SW of Balboa	8.95865	-79.62900
MR.22895	*Saguinus geoffroyi*	F	1973	7 km E of Arraijan	8.96730	-79.60800
MR.22985	*Saguinus geoffroyi*	F	1973	5 km ENE of Arraijan	8.95000	-79.64396
MR.22980	*Saguinus geoffroyi*	F	1973	2.5 km ENE of Arraijan	8.92059	-79.63807
MR.22915	*Saguinus geoffroyi*	F	1973	6 km WSW of Balboa	8.95000	-79.59395

Figure 1. Sampling localities in the Panama Canal watershed. Inset shows the haplotype parsimony network generated by TCS, with haplotypes color-coded to correspond with sampling localities.

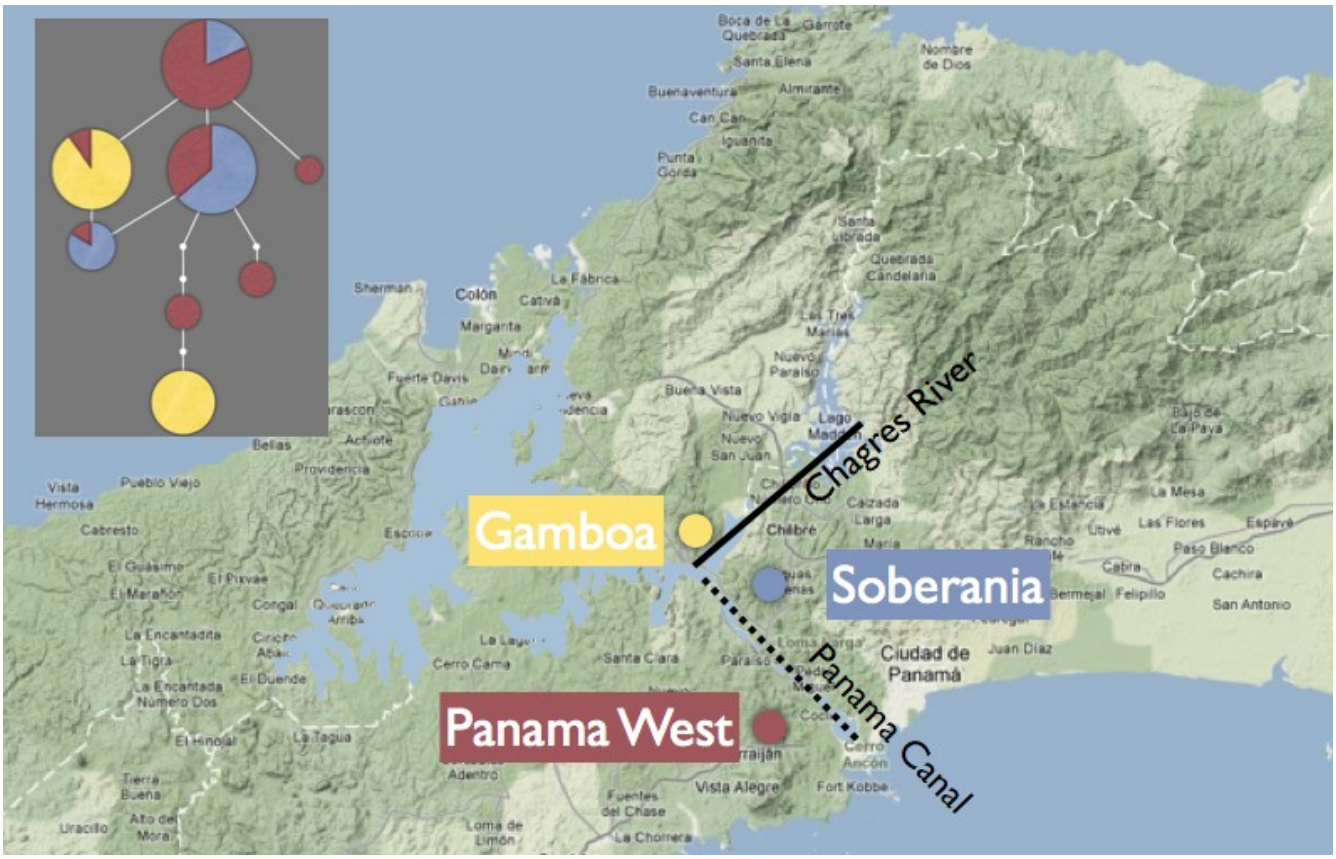


Figure 2. Map depicting Panama Canal zone before the construction of the Canal. Mitochondrial haplotype frequency charts are plotted at sampling localities which are labelled as in Figure 1.

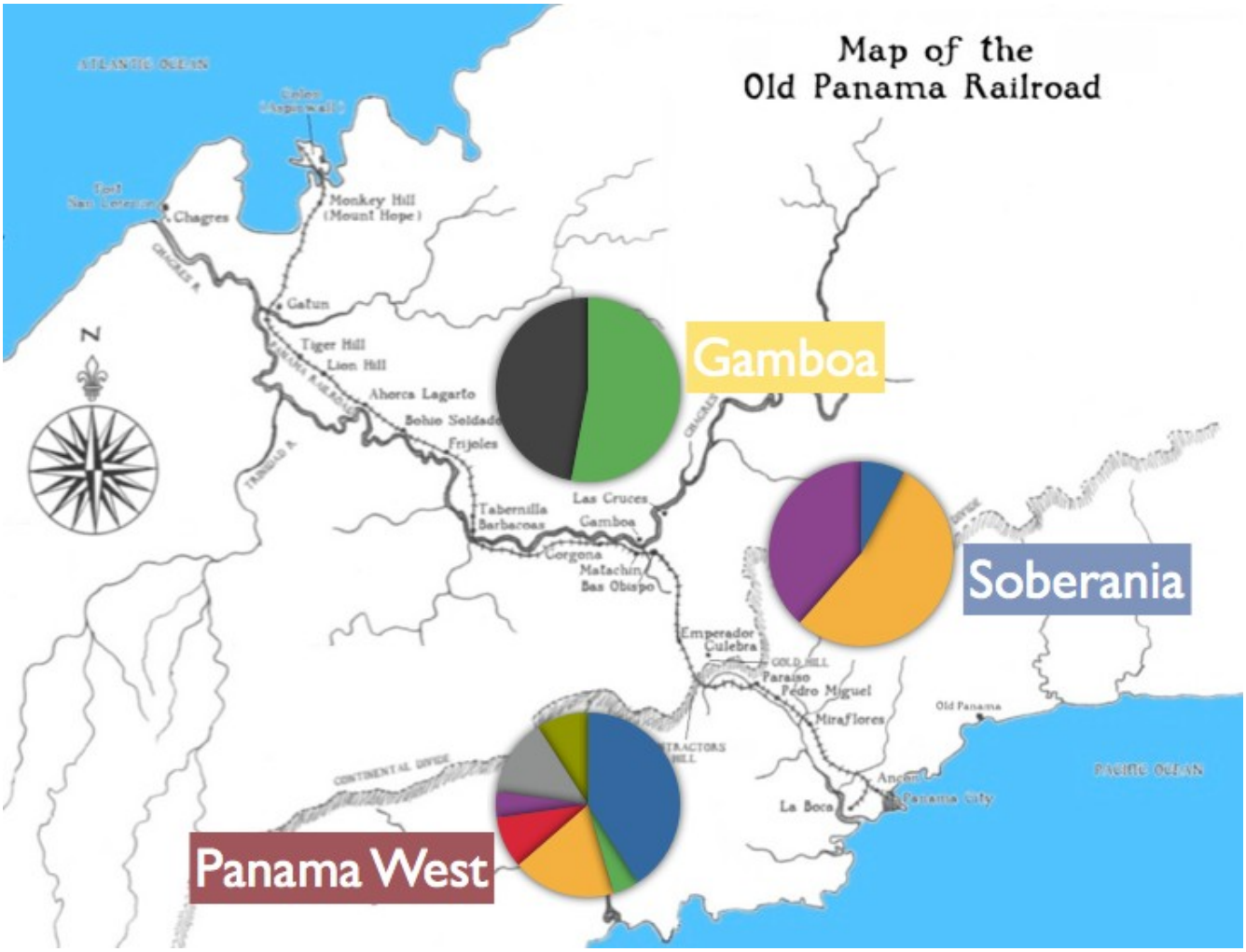


Figure 3. Posterior probability of each *k* estimate of STRUCTURE. Error bars are the variance of the posterior probability estimate. Inset is the bar plot for the most likely number of populations (*k* = 2), which shows the fractional assignment probability to each individual to the clusters inferred by STRUCTURE.

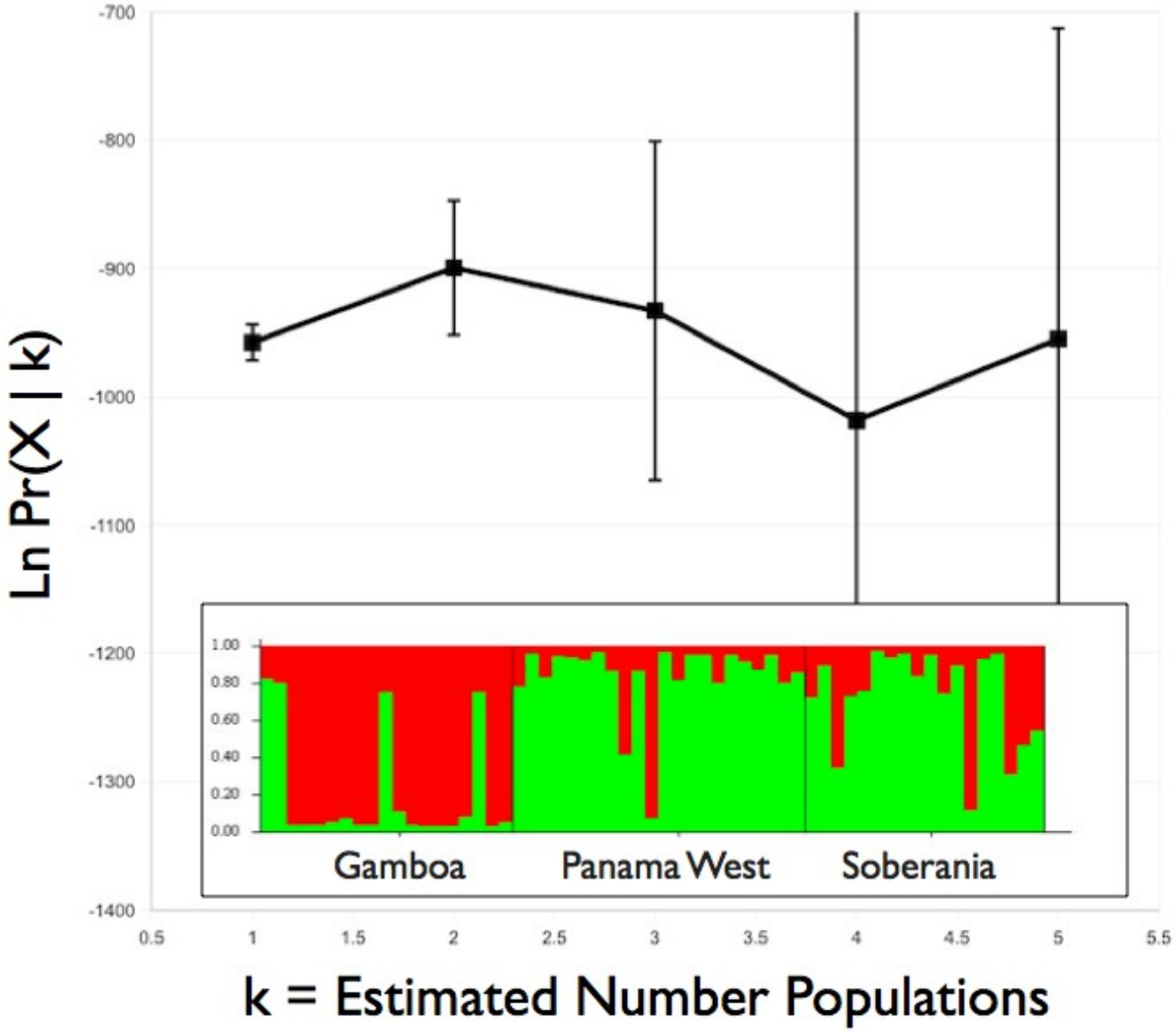
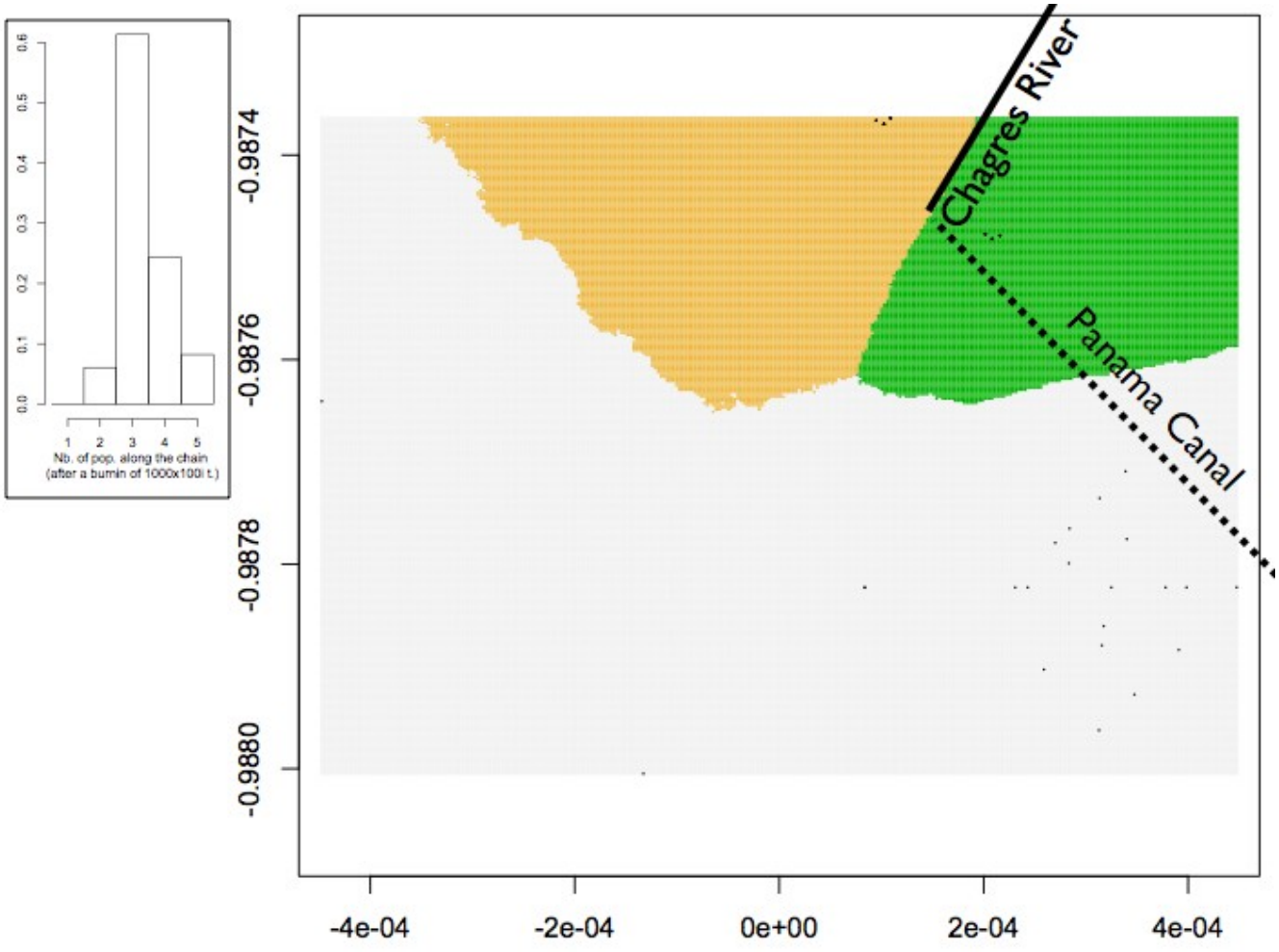


Figure 4. Map of population membership as calculated by GENELAND. Pixels are colored according to the modal posterior probability of population membership. The approximate location of Panama Canal and the Chagres River (drawn using georeferenced satellite images) are indicated in black. The inset shows the density of the estimate of k (number of populations) along the Markov chain.



CHAPTER 3

Male Cooperation in Polyandrous Geoffroy's Tamarins (*Saguinus geoffroyi*): Kinship and Paternity

ABSTRACT

Male cooperation in reproduction is rare in nature. Given that males are typically expected to be reproductive competitors, the adaptive outcomes of this form of cooperation are puzzling. Tamarins (*Saguinus*) exhibit cooperative polyandry, where multiple adult group males mate with a single breeding female and subsequently cooperate to rear her young. I examine the indirect and current direct fitness consequences of male cooperation in Geoffroy's tamarin (*Saguinus geoffroyi*) using data from a multi-year field study in central Panama. Demographic data and genetic analyses of paternity and relatedness suggest that male groupmates exhibit high relatedness values and often share paternity over multi-year associations. Although some groups had only one male sire, no statistically significant differences in testicular volume were found among males which sired young and those that did not. However, large variability in testicular volume among male groupmates may indicate a role for sperm competition in this system. The high proportion of adult male groupmates related at $r > 0.25$ in all groups examined, suggests a pattern which may be consistent with fraternal cooperative polyandry in this species. In sum, this study provides evidence for the joint role of indirect and direct fitness benefits in male-male cooperation in tamarins and provides an example that provides novel insight into the nature of cooperative male reproductive partnerships.

INTRODUCTION

Male cooperation in breeding is rare among animals. Typically males are expected to compete for mating opportunities (Bateman 1948, Trivers 1974). Nevertheless, males in a number of species cooperate to gain access to females and mating opportunities. Male lions form coalitions to oust resident males and mate with the females in the pride (Packer et al. 1991). Manakins (DuVal 2007, McDonald & Potts 1994) and wild turkeys (Krakauer 2005) engage in elaborate cooperative courtship displays in order to attract females. Dolphins form male alliances to "herd" potentially fertile females (Connor et al. 1992). Although rare, male reproductive cooperation occurs in a diverse array of taxa, raising questions regarding the adaptive consequences of this suite of behaviors.

One form of male reproductive cooperation occurs in cooperative polyandrous systems (Faaborg & Patterson 1981) in which two or more males mate with a single female and cooperate to take care of her young. This breeding system is rare, having been reported for a single fish (Kohda et al. 2009), a handful of avian taxa (Faaborg et al. 1995, Heinsohn et al. 2007, Goldizen et al. 2000) and, among mammals, tamarins (Goldizen 1989) and specific human societies in the Himalayan highlands (Crook & Crook 1988). Potential adaptive explanations for this form of reproductive cooperation include the classic hypotheses formulated to explain cooperative breeding: (a) **Indirect Fitness Benefits:** individuals cooperate because the recipients are individuals that share some proportion of their genes and, thus, indirectly increase the helper's fitness (Hamilton 1964); (b) **Current Direct Fitness Benefits:** individuals cooperate because they obtain a direct fitness payoff in a variety of ways such as increased survival, reproduction or access to resources (c) **Future Direct Fitness Benefits:** individuals cooperate because of a

delayed benefit that will increase fitness such as reproductive opportunities following inheritance of a territory or breeding position.

Tamarins (*Saguinus:* Callithrichinae) are small (ca. 300-550g) neotropical primates that live in multi-male, multi-female groups (Rylands et al. 1993). Cooperation is pervasive in these animals, in which group members help in territory defense, foraging and predator avoidance (Caine & Weldon 1987). A single breeding female mates with two or more males to produce fraternal twins; all males subsequently contribute to caring for the infants, providing every type of care except lactation. Tamarin males show low levels of aggression (Goldizen 1989), expend much energy on affiliative behavior (mostly in the form of grooming) and have been reported to share carrying tasks roughly equally (Terborgh & Goldizen 1985). The cost of this care, particularly the cost of carrying infants, is high (Rylands et al. 1993). Though the high costs of this behavior seem to be shared, it is not clear whether the benefits are likewise allocated. The relative paucity of genetic studies of the breeding system of Callitrichines (Rylands et al. 1993, but see Huck et al. 2005) has prevented the elucidation of what fitness benefits play a role in shaping this behavior.

Here I use demographic, morphological, behavioral and genetic data from a multi-year study of Geoffroy's tamarin (*Saguinus geoffroyi*) to describe the genetic relatedness among adult tamarin males and to test hypotheses regarding the adaptive benefits of reproductive cooperation among male groupmates. Specifically, I test the following predictions: (1) male groupmates are more related than random expectation, a prerequisite for indirect fitness and (2) males in a group share paternity. The future direct fitness benefits hypothesis will not be tested in the current study, owing to its short duration relative to tamarin lifespan. Nonetheless, the examination of these initial hypotheses in tamarins should yield insights into the nature of adaptive benefits of cooperative polyandry and male reproductive cooperation.

Methods

Study Sites— The study was conducted at two field sites in the vicinity of Soberanía National Park, in the provinces of Panama and Colón, Republic of Panama: (a) The Soberanía field site (100 ha) was located within the park boundary close to Camino de Plantación (9.076º, -79.659º) and consisted of mature secondary growth forest, including a cacao plantation abandoned for over 70 years. (b) The Gamboa field site (88 ha) was located just outside the park boundaries, in and around the rural town of Gamboa, Colón Province (9.118º, -79.698º). The site consisted of 22.04 ha section of disturbed secondary forest in the center of the town with gallery forests and backyard trees interspersed between private residences and roads. The sites were separated by 6 km and experienced similar climatic regimes: annual rainfall averaged mm,with over 80% of annual rain falling in the wet season from Mid-April to Mid-December (Panama Canal Authority; Smithsonian Tropical Research Institute).

Capture, Handling and Release — Tamarins were captured using hand-activated live traps baited with bananas (Garber et al. 1993) or by blow-darting (BioJect, Blowguns Northwest, Richland WA) individuals with tranquilizer darts (Pnueu-Dart, Williamsport, PA). Individuals from the Soberanía population were only captured in traps and were not anesthetized. To prevent excessive stress, handling time was limited to < 15 min and manipulations were constrained to marking and sampling hair (see Handling, Marking and Genetic Sample Collection). Soberanía individuals were released immediately at the capture site. Gamboa population adults were captured using blow-

darting; infants and juveniles were trapped because they were too small to safely dart. In both trapping and darting, I anesthetized Gamboa individuals using Ketamine (7.5mg/kg) and Zoletil (3.75 mg/kg Vibrac SA, Carros Cedex, France). To minimize potential injuries, individuals were darted at feeding stations that were eye-level above the ground. Animals were followed by two field assistants with a mesh net to catch individuals which strayed from the feeding station. Gamboa individuals were handled for 48 ± 14 min and were placed in a pet kennel for 3.67 ± 2.13 hrs until fully recovered. Respiration, heart rate and internal body temperature of anesthetized individuals were measured throughout handling procedures to monitor animal condition. All capture and handling procedures were approved by the UC Berkeley Institutional Animal Care and Use Committee and followed the guidelines of the American Society of Mammalogists (Gannon & Sikes 2007).

Morphological Measurements — In order to assess if there were morphological differences in male groupmates related to breeding status, a variety of measurements were collected from animals in the Gamboa population (anesthetized animals). Mass was measured using a 1 kg spring-loaded precision scale (Pesola AG, Baar Switzerland). Body length, tail length, head and chest circumference were measured using a flexible cloth tape measure accurate to 0.5 cm. Canine length, knee-heel length, biparietal diameter, cheek width, testis length and testis width were obtained using digital calipers (Mitutoyo America, Aurora, IL). Correlations among morphological measures were examined using a Spearman correlation coefficient among all pairwise combinations. Morphological variables were examined using a Shapiro-Francia normality test and a F test for equal variances. Values were then compared using a Student's or Welch t-test or a Wilcox Rank Sum test, as appropriate. If morphological variables were correlated, the variables were included in a linear regression and the residuals were tested. Testicular volume was calculated using the formula $V = 1/6\ \pi W^2 L$ (Abbott & Hearn 1978). In order to examine variance among males testes size within groups, the deviation from mean intragroup testicular volume – standardized by intragroup mean testicular volume – was compared using two-sample tests as described above. All analyses were conducted using the statistical program R (R Development Core Team, 2005).

Marking and Genetic Sample Collection — Individuals were marked for visual identification with ball-link chain collars with colored beads or (for individuals which had not attained full adult body size) by shaving portions of the tail in distinct patterns. For permanent identification I inserted a PIT chip (Biomedic Data Systems, Seaford, DE) subcutaneously between the shoulder blades of all individuals. To facilitate locating and monitoring groups in the field, I attached radio collars (RI-2DM, Holohil Systems Ltd, Ontario Canada) using a ball-link chain to one individual in each of four groups (2 in each study population). The mass of the radiotransmitters was 20g, which represented < 4.5% of adult body mass.

Genetic Sampling — Previous genetic work in tamarins has revealed that fraternal tamarin twins exhibit bone marrow chimerism as a result of the exchange of hematopoietic and lymphatic cells in-utero (Benirschke et al. 1962). This results in identical genetic profiles for both twins when using blood or blood-derived tissues (Signer et al. 2000). The use of leucocyte-poor genetic samples such as hair allows individual genotypes to be obtained (Signer et al., 2000; Huck et al., 2005), thus enabling analyses of parentage and relatedness central to testing hypotheses of this

study. To examine the possible influence of chimerism, genetic samples from three different tissue types were taken: (a) Hair samples were plucked from the base of the tail and saved in coin envelopes and stored dry. (b) Buccal cell samples were collected with a sterile Dacron swab and stored in RNA*later* and frozen -20ºC until extraction. (c) Ear tissue was collected from the pinnae using surgical scissors and stored in RNA*later* and frozen -20ºC until extraction. The multiple sample types were genotyped to corroborate the results obtained from hair samples.

Demographic Data — Individuals were captured in Soberanía in May-July 2005 and June-July 2006. Subsequently, group compositions were censused weekly in Soberanía from May 2006 - May 2007. I captured individuals and conducted a full census of individuals in Gamboa during March - July 2008 and November-December 2009. Breeding females were identified on the basis of morphological measurements of genitals and nipples and the presence of milk in nipples. Individuals under 300 gr were considered infants that had been born in the group they were first identified.

DNA Extraction and Microsatellite Genotyping — DNA was extracted from all sample types using a Qiagen DNA Micro Kit (Qiagen, Valencia, CA). DNA yield was quantified using a NanoDrop Spectrophotometer (Thermo Fisher Scientific, , MA). Seven polymorphic microsatellite loci (Table 2), originally developed for other New Word primates (Bohle & Zischler 2002, Escobar-Paramo 2000), were amplified oABI 2720 (Applied Biosystems, Foster City, CA)or BioRad iCycler (Bio-Rad, Hercules, CA) thermocyclers using fluorescently labelled primers. Cycling conditions were 94ºC for 4 min; 94ºC for 1 min, Ta (see Table 2) for 1 min, 72ºC for 75 s, repeated 35 times; 72ºC for 10 min. Polymerase Chain Reaction volume was 10 µL with: 40 ng of genomic DNA, 1µL of 10X PCR Buffer (Biosystems, Foster City, CA), 2.5mM $MgCl_2$, 0.8µL 10 mg/mL BSA, 0.4 mM of each DNTP, 3pm of each primer and 0.5 U of Taq polymerase (Invitrogen, Carlsbad, CA). I added 1µL of PCR product to 8.8 µL of formamide with 0.2 µL of GeneScan 500-LIZ size standard (ABI, Foster City, CA) to genotype samples in an ABI 3730 automated sequencer (ABI, Foster City, CA). I scored genotypes manually using Genemapper 4.0 (ABI, Foster City, CA).

The low DNA copy number in hair samples required special handling to ensure reliability of genotypes. First, all hair samples utilized were plucked manually to ensure that follicles were present. Hair samples were extracted in a dedicated room were no high copy genetic material or PCR product were present. PCR preparation occurred in a separate room with no amplified PCR products allowed. Negative controls were used at the sampling, extraction, amplification and genotyping stages; reactions showing evidence of contamination in the negative controls were discarded. Amplification was replicated using alternate tissue types (buccal and ear) when available, to validate results obtained using hair samples. Samples showing homozygous genotypes were genotyped at least 2 times to confirm they were true homozygotes.

Analysis of Genetic Data — Microsatellite loci were tested for deviations from Hardy-Weinberg equilibrium and examined for the presence of null alleles using the program G ENEPOP 4.0 (Rousset 2008). Locus specific data and indices of molecular diversity were calculated in C ERVUS 3.0.3 (Kallinowski et al. 2007). Genetic data were analyzed separately for each population.

Paternity was assigned using the likelihood-based method of C ERVUS 3.0.3 (Kalinowski et al. 2007). Calculations of relatedness values were conducted using the programs K INSHIP 1.3.1 and

RELATEDNESS 5.0.8 (Queller & Goodnight 1989). Young were divided into two categories: infants and juveniles. Infants could be readily assigned a mother by morphological, behavioral and demographic criteria. In contrast, because older juveniles were not associated with a specific lactating female, it was necessary to assign both parents via genetic analyses. For infants, paternity was assigned when a known mother and a sire were assigned as the most likely parents at the 95% (strict) confidence level with no more than one allelic mismatch. Since maternity of juveniles was not known from other data, a search for the most likely parent pair was conducted in CERVUS. Criteria for parent pair assignment for juveniles followed that of infants.

To assess kinship among males, the mean pairwise relatedness value for adult male groupmates was compared to the mean pairwise relatedness of: (a) all individuals in the population, or background, (b) all population males, (c) random pairs and trios of males and (d) known parent-offspring relationships. Relatedness values for each category were tested for normality using a Shapiro-Francia normality test and a F test was used to test for equal variances. Values were then compared using a Student's or Welch t-test or a Wilcox Rank Sum test, as appropriate.

RESULTS

Captures — A total of 36 individuals were captured, marked and sampled. These individuals were resident in 6 social groups (Table 1), as determined by the consistent spatial and temporal association of the same individuals during all observations and their aggressive interactions with other groups. Overall, 92% of group members were captured and all individuals in the Gamboa population were captured.

Adult Male Groupmate Characteristics — Soberanía had an average of 2 ± 1 adult males per group (n=3), while Gamboa had an average of 3 ± 1 adult males per group (n=3). These averages include only captured individuals (see Figure 3). Capture and census data from Soberanía indicated that adult males remained in the same group continuously throughout the study period from May 2006 to May 2007 (n=3) and that adult male composition of one group was the same at initial capture in June 2005. Capture data from Gamboa similarly revealed that adult male composition was the same in both sample periods (March 2008 and December 2009).

Kinship among Adult Male Groupmates — Calculations of kinship among male groupmates were limited to groups with near-complete sampling, i.e. no more than one unsampled adult (n=5). Mean ($\pm$ SE) relatedness values among adult males within groups were $r = 0.45 \pm 0.29$ in Soberanía (n=6) and $r = 0.28 \pm 0.13$ in Gamboa (n=9). Mean relatedness among adult male groupmates was significantly different from the mean relatedness among all adult males in Gamboa, but not in Soberanía (Gamboa: $t = 2.1711$, $df = 63$, $p = 0.0337$, Soberania: $W = 70.5$, $p = 0.1874$). Likewise, mean relatedness among adult male groupmates was significantly different from the background relatedness among all adult population members in Gamboa, but not in Soberanía (Gamboa: $W = 1179.5$, $p = 0.04423$, Soberania: $W = 261.5$, $p = 0.0868$). The background relatedness was not statistically significantly different from zero (Gamboa: $t = -0.1247$, $df = 170$, $p = 0.901$ Soberania: $t = -0.3905$, $df = 152$, $p = 0.6967$). The mean relatedness among all males in each population was also not statistically different from zero (Gamboa: $t = -0.7247$, $df = 54$, $p = 0.4718$ Soberania: $t = 0.2326$, $df = 44$, $p = 0.8171$). Sample sizes and mean relatedness for groups of individuals of known relationships are presented for

comparison in Figure 1. Mean relatedness of adult male groupmates was not different from the mean relatedness between known mother-offspring pairs (Gamboa: $t = 2.1737$, $df = 11.173$, $p = 0.05207$, Soberania: $t = -0.2658$, $df = 1.251$, $p = 0.8275$). All groups were composed of a high proportion (> 67%) of related males ($r > 0.25$) and all groups which were completely sampled contained at least two highly related males (Figure 2). Collectively these results are consistent with high relatedness among adult male groupmates at the level expected for half-sibling or higher.

Paternity — Behavioral observations in Gamboa revealed that all adult males within a social group copulated with the breeding female (n = 7 copulations in 2 groups). Paternity analysis assigned fathers to a total of 15 individuals in 5 groups. All nine offspring which were sampled as infants were assigned with high confidence (95%) to a male in the group.

A parent pair was assigned with high confidence to 6 juveniles. Group structure and/or membership when juveniles were conceived was not known. All juveniles except one (5/6), were assigned to the breeding female of the group in which they resided during the study period. The remaining juvenile was assigned a mother in a neighboring group. Half of the juveniles (3/6) were sired by a male that resided in the same group as the juvenile during the study period, the remaining juveniles had sires that resided in neighboring groups at the time of the study. Because group compositions at the time of conception of juveniles were not known, inferences about intragroup vs. extragroup paternity cannot be made.

For groups where multi-year information was present (n=4), the patterns of paternity over time suggest that more than one male sires offspring in most groups, with one clear exception in group LC where one male sired 3 young in 3 separate breeding seasons (Figure 4). Taking into consideration all groups and years for which offspring were assigned 40% of groups (n=5) had only one sire for all offspring. Groups with more than one sire (60%) had an average of 2.3 ± 0.58 males siring offspring (n=3 groups).

Overall, 60% of adult males sired at least one offspring when pooling both populations (Gamboa: 42.8% n = 7 males; Soberanía: 66.7% n = 6 males). The mean ($\pm$ SD) percentage of adult males obtaining paternity within a group was $68.7 \pm 30.2\%$ (n=5 groups).

Figure 3 presents a diagram that summarizes group membership during sampling periods and indicates all relationships inferred by genetic analyses. A summary of maternity and paternity assignment can be found in Table 3.

Morphological Measurements — Body mass and body length were the only morphological variables that were statistically significantly correlated with each other ($S = 36.652$, $rho=0.69$ $p = 0.038$). Paired comparisons found no statistically significant differences in morphological variables between adult males who sired offspring and those that did not. Males which were sires had testicular volume which was, on average, 33% larger than non sires in their group (Figure 5a), but this difference was not statistically significant: the standardized difference from mean intragroup testicular volume was not significantly different among sires and non-sires ($t = 1.3669$, $df = 1.993$, $p = 0.3054$). However, the standardized difference in testicular volume among male groupmates was large; the male with the largest testicular volume in each group had testes that were statistically significantly larger than other groupmates ($t = 3.6464$, $df = 3.895$, $p = 0.02287$, Figure 5b).

Discussion

The results of this study confirm the occurrence cooperative polyandry in a non-human mammal. Analyses of genetic relatedness indicated that adult male groupmates were more related than expected based on background levels of relatedness in the population. Paternity analysis across multiple years suggested that adult male groupmates distribute paternity among themselves, although in some groups there was only one sire. Geoffroy's tamarin males appear to accrue both direct and indirect fitness benefits in this arrangement.

Kinship — Tamarin groups in the study population always contained related adult males, which suggests that relatedness among male groupmates is an important component of the social structure of this species. There are only two other studies in tamarins (*Saguinus*) that have used genetic data to study the breeding systems of wild tamarins. An re-analysis of the data in Suarez (2007 unpubl thesis) and Huck et al. (2005) shows that in these two congeneric species, adult male groupmates were related in every group studied. In captivity, only closely related *S. oedipus* (sister to *S. geoffroyi*) males can be successfully induced to live in the same group (Price & McGrew 1991). The same result was found for experimentally formed marmoset (subfamilial to *S. geoffroyi*) groups in captivity (Schaffner & French 2004). These data strongly suggest that relatedness is the norm for cooperatively breeding tamarin males.

Paternity — Although important, relatedness does not appear to be the only benefit maintaining male cooperation. Behavioral and genetic evidence suggest that tamarin males also share direct fitness benefits. Adult male groupmates in virtually every study of wild tamarins have been shown to mate with the breeding female (*S. fuscicollis:* Terborgh and Goldizen 1985, Goldizen et al. 1996, *S. mystax:* Huck et al. 2005, *S. labiatus*: Suarez 2007 unpubl thesis) and this appears to be widespread in the Callitrichinae (Sussman & Garber 1987). As found in previous studies, I report evidence of a single male dominating paternity in some groups. However, in a majority of groups infants and/or juveniles were sired by other group males. While group composition when those juveniles were sired was not known, it suggests that males tradeoff at least part of their reproductive success over multiple breeding attempts. The only published study with comparable data set suggests a similar outcome.

Huck at al. (2005) found evidence that one male was responsible for siring all young in some groups of *S. mystax*, but likewise found instances where other males sired young; up to a 33% of offspring in a group. Additionally, Huck et al. (2005) provide robust evidence that a twin pair may be sired by different fathers, as was found in a separate unpublished study on *S. labiatus* (Suarez, 2007 unpubl thesis). Collectively these data suggest that tamarin males may trade off paternity across multiple years, but sometimes within the same litter.

In order to accrue both direct and indirect fitness benefits male partnerships must be somewhat stable over multiple breeding attempts. Capture and census data from this study indicated that co-breeding male pairs/trios were stable in two separate populations throughout the study period. Although these data are limited to 2 yrs (and in one case 3 years), available evidence from other *Saguinus* species suggests that male partnerships are long (4 to 8+ yrs: Garber et al. 1993) and that males frequently migrate in pairs (Garber et al. 1993) or migrate into the same groups (Huck at al. 2005, Huck et al. 2004).

While the limited temporal scope of available studies (Huck et al. 2005, Suarez 2007 unpubl. thesis, this study) may overestimate differences in reproductive success among males, it is

evident that some males are more successful than others. The reasons for this asymmetry are currently unknown, as body size measurements (Garber et al. 1993), behavioral dominance (Goldizen 1989) and hormonal levels (Huck at al. 2005b) do not appear to play a role. The current study also failed to detect differences in morphological measurements between sires and non-sires, including testicular volume. However, statistical analyses revealed that one male in each group had testicular volume which was statistically significantly larger than other male groupmates. Sires may have not had the largest testes size in their groups due to a mismatch between the timing of collection of morphological measurements and the timing of conception of assigned offspring, as tamarins undergo marked seasonal changes in testes size (Garber at al. 1996). Tamarins have very large testes for their body mass (Harcourt et al. 1995) and exhibit multiple male mating, suggesting that sperm competition may be a way that males compete post-copulation. Future studies of wild tamarins should incorporate comparisons of indirect and direct measures of sperm competition to paternity – while accounting for reproductive timing – to test this hypothesis.

Implications for the evolution of male-male cooperation — The genetic data presented here suggest that co-breeding tamarin males appear to accrue both direct and indirect fitness benefits. This result is timely in light of recent research which highlights the relative importance of indirect and direct fitness benefits in cooperative societies (Dickinson 2004, Canestrari et al. 2005, Clutton-Brock 2002). In contrast, in the particular case of males who engage in cooperation in reproduction, other species seem to accrue either direct or indirect fitness benefits. In manakins, there appear to be no immediate direct fitness benefits for males, instead subordinate cooperators appear to benefit from inheritance of an alpha breeding postion in the future (McDonald & Potts 1994, DuVal 2007). Male wild turkeys appear to rely completely on indirect fitness benefits in their cooperative courtship (Krakauer 2005). However, in male lion coalitions indirect fitness benefits drive cooperation in small coalitions, but larger coalitions of unrelated individuals appear to share paternity (Packer et al. 1991). Thus, tamarins appear to be distinctive in that males may gain both indirect and direct fitness benefits.

The specific case of cooperative polyandry also brings to light differences, but also parallels in different taxa. Galapagos hawks form cooperatively breeding assemblages of many unrelated males, which apparently have equal probability of siring young and randomly distribute paternity (Faaborg et al. 1995). *Eclectus* parrot males are not related, but instead gain direct benefits from paternity (Heinsohn et al. 2007). Interestingly, and in similar fashion to the current study, within a single breeding attempt one male usually dominates paternity and additional males gain direct fitness benefits in future breeding attempts (Heinsohn et al. 2007). The only other mammal reported to be cooperatively polyandrous presents some striking similarities to tamarins. Human inhabitants of some areas in Asia, particularly Tibet, have been reported to practice fraternal cooperative polyandry (Crook & Crook 1988, Smith 1998).

The observation that cooperatively polyandrous males in birds are usually not related suggests that mammals experience different selective pressures in establishing cooperative polyandry. One possibility is that, due to internal gestation and lactation by females, the costs for males (shared reproduction and paternal care) are high and may require direct and indirect fitness benefits to stabilize cooperation. Cooperative polyandry may also be related to the high costs of infant rearing due to environmental conditions and/or infant physiology as an evolutionary model suggests (Chao, 1997). In accordance with this hypothesis, observations of lone female-male

tamarin pairs raising young in the wild are rare (Goldizen 1988, but see Windfelder, 2000 for possible case), but tamarins readily reproduce in monogamous pairs in captivity, where animals have ample food supply and short travel distances.

In sum, the results of this study suggest that tamarin males residing in a group may gain both indirect and direct fitness benefits in partnerships that may last for multiple breeding seasons. A high proportion of males in every group studied were related at the level expected for brothers ($r > 0.25$), a pattern consistent with fraternal cooperative polyandry. Tamarins are within an evolutionarily diverse group (ca. 40 species) of primates, the Callitrichinae, which share many of the characteristics of tamarins (polyandry, male parental care, twinning). As this study shows, the Callitrichinae offer fertile ground for future comparative work to understand the adaptive benefits and evolution of cooperative male parental care in cooperative societies.

Acknowledgments

I would like to thank: the Natural Authority of the Environment of the Republic of Panama (ANAM) and the US Fish and Wildlife Service for authorization to conduct this research. R. Lessnau, C. R. Laughter, M. E. Martin, D.V.M. and D. J. Smith, D.V.M. who generously provided their expertise in trapping and handling animals. E. A. Lacey, C. Moritz, J. S. Brashares provided critical feedback on the manuscript. The Smithsonian Tropical Research Institute for providing a unique community of researchers and facilities to support research. R. Graziani, E. López Vdovenko, S. Sánchez Navarro, M. Tejada Gonzalez and A. J. Sosa Bartuano for invaluable assistance in the field. A. Varma who donated his considerable photographic skills. AW Goldizen and PA Garber's groundbreaking studies inspired this projected. This work was funded by the Museum of Vertebrate Zoology and Department of Integrative Biology, the American Society of Mammalogists, Sigma Xi Grants in Aid of Research and an NSF DDIG grant #0608467. SLDM was supported by a Ford Foundation Predoctoral Fellowship, a Chancellor's Opportunity Fellowship and a UC Dissertation-Year Fellowship from UC Berkeley.

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Table 1. Group compositions in Gamboa and Soberania. *An adult female emigrated from group PH to group BA in the early stages of the 2008 season; numbers in parentheses indicate composition before the emigration. † Group CT and AG were not completely sampled: 2 and 1 adult individuals, respectively, evaded capture.

	Gamboa			Soberania		
	BA	LC	PH	NJ	CT	AG
Individuals	3(2)*	7	7(8)*	6	7	6
Adult Males	2	3	4	2	2†	2†
Adult Females	1(0)*	1	1(0)*	2	1†	1†
Juveniles	0	1F	1F	2M	0	1F
Infants	0	1F/1M	1M/1F	0/2F	1M 1F	1M

Table 2. Locus specific information for microsatellite loci used in paternity and relatedness analyses for each population. **Na:** Number of alleles loci used in this study. **Ar:** Allelic Richness. **Ho:** Observed heterozygosity. **He:** Expected heterozygosity. **NE-I:** Non-exclusion probability of identity. **NE-S:** Non-exclusion probability of sibling identity. **HWE Prob:** Hardy-Weinberg deviation test chi-square probability.

Gamboa:

Locus	Ta (ºC)	Na	Ar	Ho	He	UHe	NE-I	NE-S	HWE Prob
SB7	54	5	3.0636	0.421	0.652	0.670	0.186	0.470	0.212
SB8	54	2	1.8387	0.421	0.332	0.341	0.501	0.709	0.245
SB19	54	2	1.9801	0.579	0.478	0.491	0.387	0.608	0.356
SB38	54	2	1.8763	0.474	0.361	0.371	0.473	0.688	0.176
Ceb10	52	4	3.1310	0.947	0.680	0.698	0.163	0.451	0.018
Ceb11	52	3	2.5717	0.684	0.532	0.546	0.277	0.553	0.417
Ceb128	52	2	1.9913	0.316	0.499	0.512	0.376	0.595	0.110

Soberanía:

Locus	Ta (ºC)	Na	Ar	Ho	He	UHe	NE-I	NE-S	HWE Prob
SB7	54	8	4.2180	0.882	0.782	0.806	0.076	0.378	0.201
SB8	54	6	3.6544	0.750	0.689	0.712	0.129	0.438	0.205
SB19	54	2	1.9356	0.588	0.415	0.428	0.428	0.649	0.086
SB38	54	5	3.7931	0.722	0.748	0.770	0.101	0.401	0.110
Ceb10	52	4	2.5491	0.444	0.451	0.463	0.331	0.608	0.304
Ceb11	52	2	1.9584	0.333	0.444	0.457	0.407	0.630	0.289
Ceb128	52	2	1.9544	0.529	0.438	0.451	0.412	0.634	0.388

Table 3. Mothers and fathers assigned to all offspring sampled in both study populations. Maternity was assigned using behavioral, demographic and genetic criteria. Sires were assigned based on genetic analyses of paternity using multilocus microsatellite genotyping. Birth years of juveniles are marked with a star (*) to indicate that these are estimated and group composition was not known during conception.

Offspring	**Year of Birth**	**Population**	**Mother**	**Father**
NJ Infant 1	2006	Soberania	NJ Breeding Female	NJ Male 2
NJ Infant 2	2006	Soberania	NJ Breeding Female	NJ Male 2
NJ Juvenile 1	2004*	Soberania	NJ Breeding Female	AG Male 2
NJ Juvenile 2	2004*	Soberania	NJ Breeding Female	AG Male 2
AG Infant 1	2006	Soberania	AG Breeding Female	AG Male 1
AG Juvenile 1	2005*	Soberania	AG Breeding Female	AG Male 2
CT Infant 1	2006	Soberania	CT Breeding Female	CT Male 2
CT Infant 2	2006	Soberania	CT Breeding Female	CT Male 2
LC Juvenile	2007*	Gamboa	PH Breeding Female	LC Male 1
LC Infant 1	2008	Gamboa	LC Breeding Female	LC Male 1
LC Infant 2	2009	Gamboa	LC Breeding Female	LC Male 1
PH Juvenile 1	2007*	Gamboa	PH Breeding Female	LC Male 1
PH Infant 1	2008	Gamboa	PH Breeding Female	PH Male 1
PH Infant 2	2009	Gamboa	PH Breeding Female	PH Male 4

Figure 1. Mean pairwise relatedness for different relationships of interest. Number of pairwise values is noted in parentheses (N). Data from 3 groups in each of 2 populations. Statistically significant comparisons are denoted with black bars and stars (* $p < 0.05$, ** $p < 0.01$).

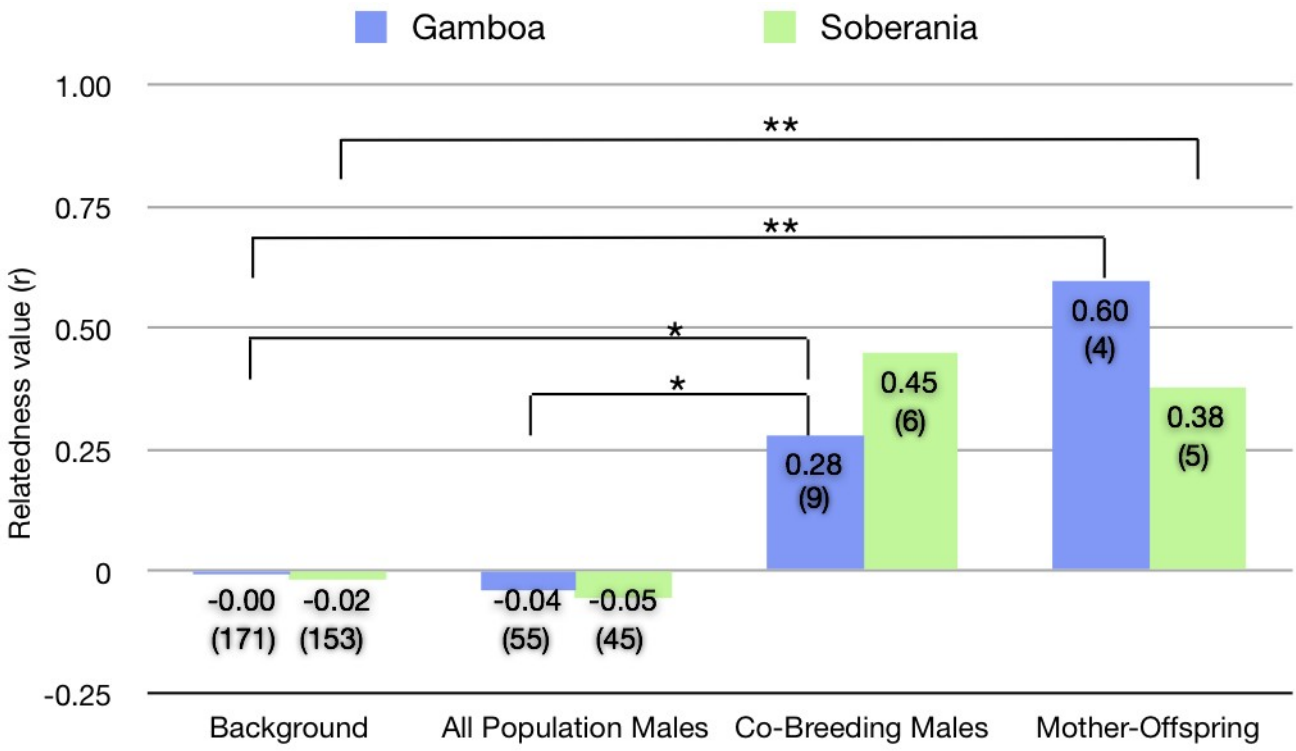


Figure 2. Proportion of adult males in study groups related at r > 0.25. Data from five groups in two populations. Number of males shown below group name. Relatedness values calculated from seven polymorphic microsatellite loci.

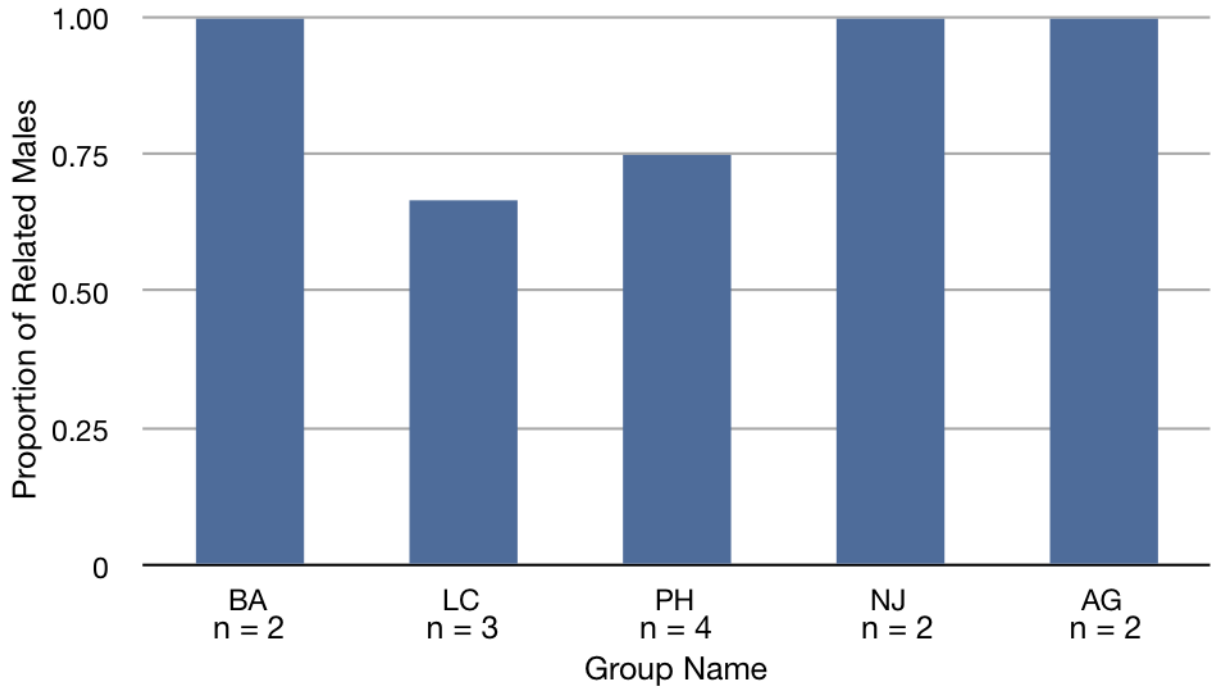


Figure 3. Group structure of study groups. Fathers identified by genetic analyses are noted in parentheses. Mothers are the breeding female of each group, unless otherwise noted in parentheses. Solid lines between adult malendicate relatedness at r = 0.5, dashed lines between males indicate relatedness at r >0.25. The asterisk indicates the emigration of an adult female from a group where she was originally captured to a group where she attained the breeding position.

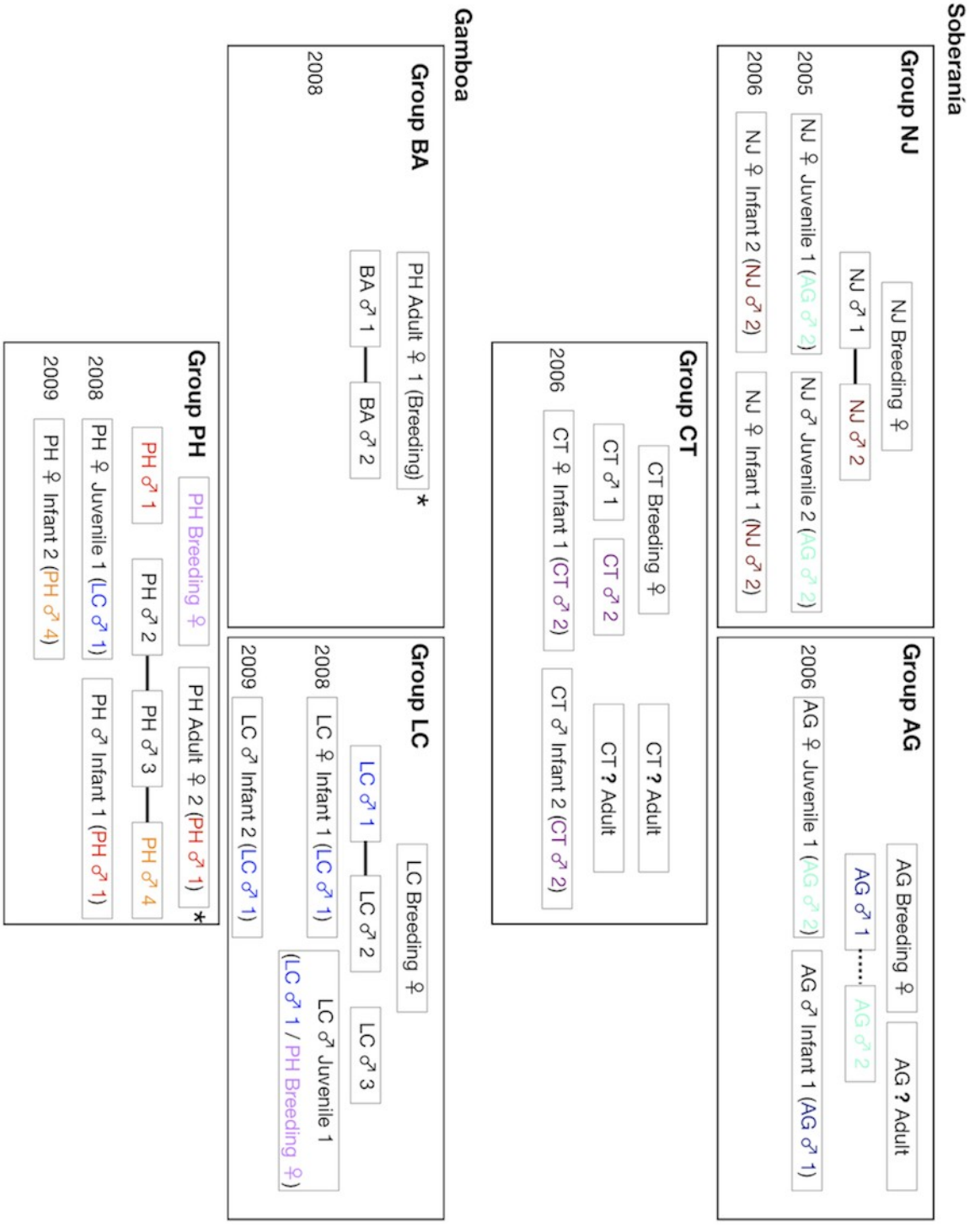


Figure 4. Paternity of males in groups where multi-year data were available. The proportion of adult male groupmates (at the time of capture) related at r > 0.25 and mean relatedness among groupmates is noted beside bar graphs. The asterisk (*) denotes the likely birth year of offspring captured as juveniles, which were born when group composition was not known. Juveniles represented in the bar charts in the groups where they were captured.

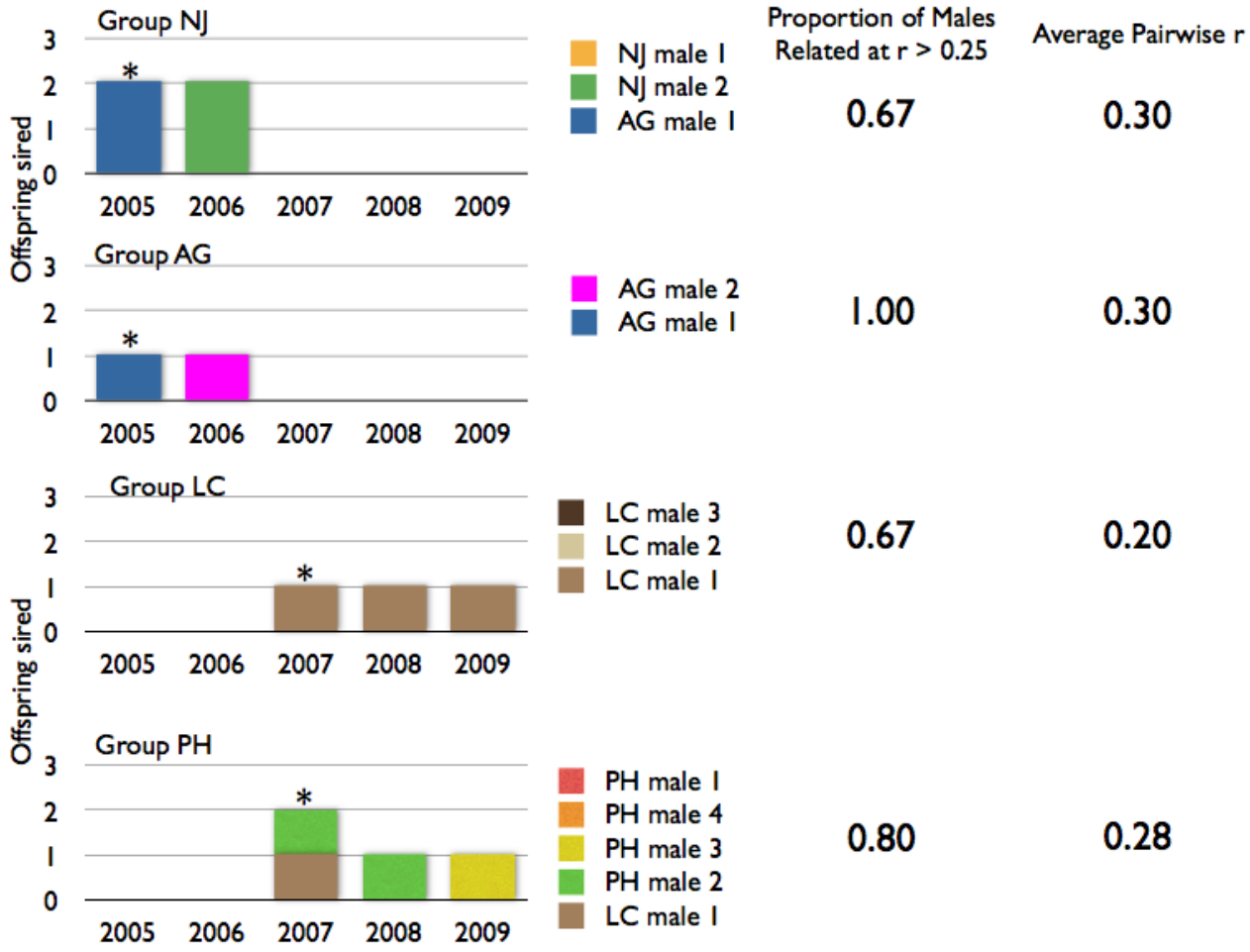
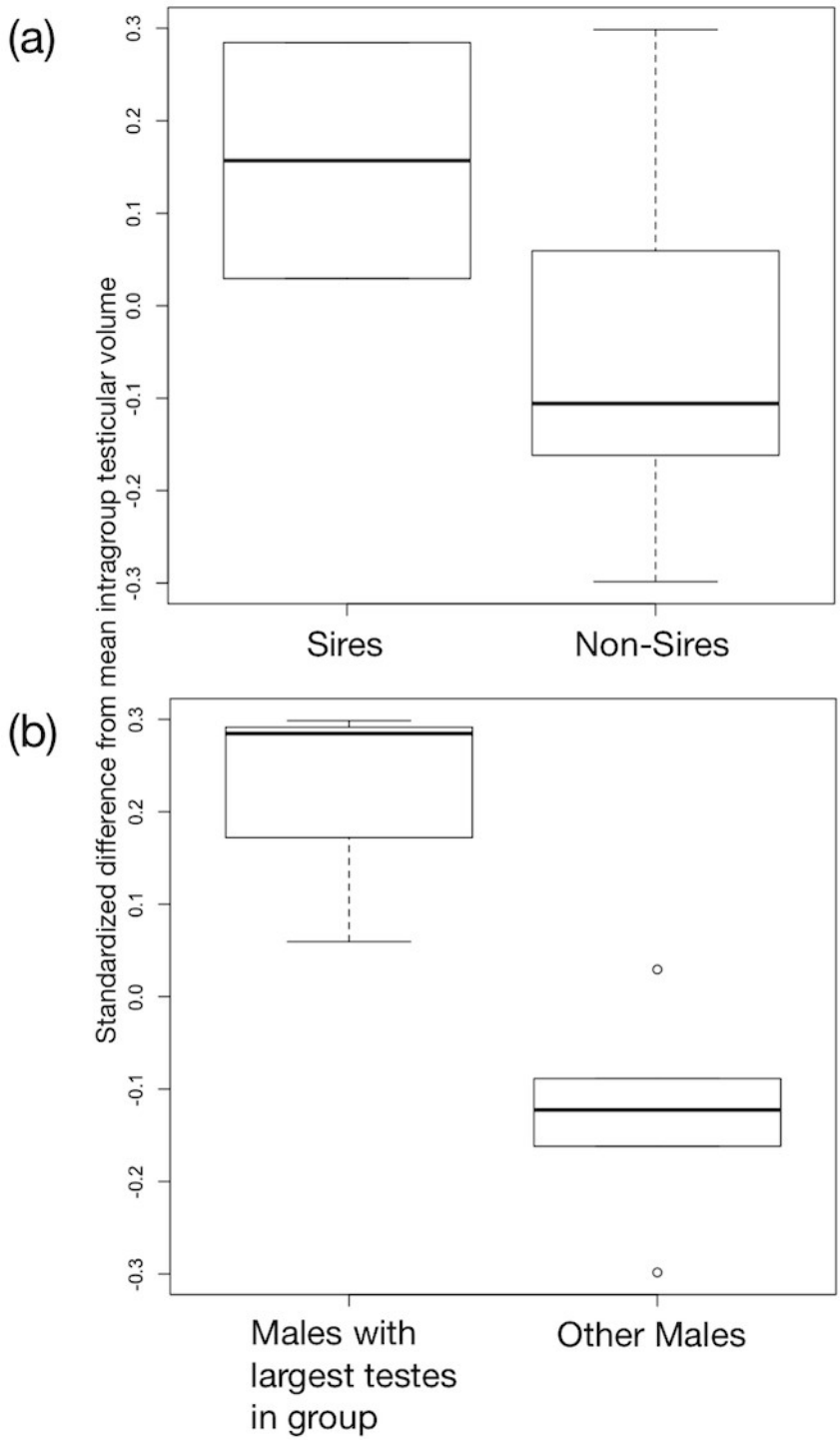


Figure 5. Differences in testicular volume among males, quantified as standardized difference from intragroup mean testicular volume. (a) Testicular volume in males assigned and sires vs. non-sires. (b) Testicular volume in males with the largest relative testicular volume in their group vs. all other group males. Difference is statistically significant at $p < 0.05$.



Appendix 1

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