"EFFECTS OF HABITAT FRAGMENTATION ON MOVEMENT PATTERNS AND DISPERSAL OF SILVERY BROWN TAMARINS (*Saguinus leucopus*) IN THE MUNICIPALITY OF NORCASIA, COLOMBIA

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ABSTRACT

Changes in landscape characteristics due to habitat fragmentation have been identified as major determinants of animal movement patterns and dispersal. Habitat fragmentation can restrict the dispersal of individuals between populations and cause populations to experience a reduction in their genetic diversity and an increase in the genetic differentiation between populations, both of which ultimately can cause the local extinction of the species inhabiting the landscape. Although deforestation and fragmentation of habitat are considered the main threats to primate survival, more research is needed to explore the effects of habitat fragmentation on primate population structure. This project used genetic data to evaluate how heterogeneous and fragmented landscapes affect the movement and dispersal behavior of silvery brown tamarins (*Saguinus leucopus*), an endemic and endangered primate species from Colombia. The results of this project will not only provide an empirical understanding of how habitat features affect tamarin movement and population persistence, but will also contribute to identifying the ecological requirements for habitat corridors to connect isolated populations of this species. These data will hopefully provide important information to the development and implementation of a successful conservation plan for these critically endangered primates, as well as for other species inhabiting the Andean region of Colombia.

INTRODUCTION

Primates and their habitats have been strongly affected by human activities in most tropical ecosystems (Strier, 1997). Activities such as hunting, selective logging, and cattle ranching, among others, have lead to the deforestation and fragmentation of primate habitat and are considered the main threats to their survival (Cowlishaw and Dunbar, 2000). Habitat fragmentation decreases total habitat area, increases the isolation of patches, reduces functional connectivity between patches, and can restrict individual dispersal or gene flow among patches (Noss et al., 2006), which can cause a reduction in population size. With restricted gene flow, habitat fragmentation leads to changes in microevolutionary processes that include greater levels of inbreeding, loss of genetic diversity within fragments and an increase in the genetic differentiation among fragments (Frankham et al., 2002).

Of the 31 primates species in Colombia, 27 suffer from ongoing habitat fragmentation, and, of these species, six are considered endangered by the IUCN. *Saguinus leucopus*, the silvery brown tamarin, is an endemic primate of Colombia listed as an endangered species by the IUCN due to the serious reduction of its population over the last three generations as a result of ongoing threats that include deforestation and live capture for pet trade (Morales-Jiménez et al., 2008). Its small distribution is restricted to the Andean region of Colombia, an area facing an especially high impact of human activity and intensive colonization. Cattle ranching, logging, expansion of agriculture, mining and dam building all characterize this area and cause a continuous process of loss, reduction and fragmentation of tamarin habitat (Defler, 2003). About 85% of the tropical wet forest within the tamarin's geographic range has disappeared (Etter et al., 2005), and about 80% of the remaining forest is in different successional states. Moreover, cattle ranching occupy about 80% of the tamarin's distribution, and most of that area is nearly deforested (Etter, 1997; Etter et al., 2005; Kattan et al., 1996). To date, this species only occurs in one official protected area; however, silvery-brown tamarins only are found in a small portion of that area, which, as a whole, suffers anthropogenic disturbance (Roncancio et al., 2009). Urgent conservation efforts are needed to protect the silvery-brown tamarin and other species whose distributions are restricted to the Andean Region of Colombia.

Due to the fact that habitat loss is the most important factor that affects primate diversity in Colombia, and especially silvery brown tamarins, it is not only important to prevent such habitat destruction, but also to understand how gene flow, population structure, genetic diversity and consequently species survival is being affected by such habitat transformation. This project evaluated how heterogeneous, human modified landscapes affect silvery brown tamarin movement and dispersal behavior, in order to understand how habitat fragmentation affects their gene flow and spatial distribution of genetic variation. The project to date has finished all the data collection in the field and is currently conducting both the landscape and genetic analysis to specifically examine the spatial distribution of genetic variation of silvery brown tamarins populations and identify what environmental factors favor or their dispersal and in turn the connectivity between populations of this species.

METHODS

Field component:

The project was initially proposed to be conducted in the Municipality of Norcasia, Caldas, an area characterized by a heterogeneous landscape composed of a mosaic of pastures, gallery forests and secondary forest in different successional states. However, as we started collecting data in the field and analyzed land use change over time, we realized that the degree of habitat fragmentation in Caldas was too recent, and that the scale at which we were going to sample was to small to have a significant representation of the genetic diversity of the species. Consequently, in order to evaluate the effect of habitat fragmentation on tamarin dispersal, we expanded our sampling scheme to a bigger landscape that represents the most human modified range of the species, and that has fragments of forest that have been isolated for more than 5 decades. Additionally, to complement our sampling effort, we signed a Memorandum of Understanding between the University of Texas at Austin and the Universidad de Antioquia (UdeA) in Colombia. Through this agreement, we are currently collaborating with the Mammalian Genetics Lab at UdeA and have augmented the sample size of our research to ~100 individuals from 8 distinct tamarin populations. This MOU will also provide further opportunities for Colombian biologists to do internships at UT Austin and for students from UT to undertake research in Colombia.

Sample collection:

We collected DNA samples 53 individuals from 12 social groups and 5 different populations in Caldas and Antioquia using multi-compartment hand-activated traps baited with ripe bananas following methodologies developed by Watsa (2012) (Figure 1). For each individual captured, we collected both hair and tissue samples as well as data on morphometrics, general health assessment, weight and sex and dental casts. Hair samples were plucked from the base of the tail, and saved in envelopes and stored dry, while ear tissue were collected from the pinnae using biopsy punches and stored in RNA*later,* a nucleic acid preservation buffer.

Figure 1. Trapping event in one of the sampled locations, a) tamarin trap with captured individuals inside and b) trapping procedure with one individual

Of all the groups sampled we were able to capture, ~80% of all individuals of each group. Although, we attempted to trap all individuals of each group, in some cases in order to reduce self-inflicted injuries caused by long duration of individuals in the trap, this did not happen. This was the case, especially for adults and subadults individuals who were carrying infants. Nonetheless, these individuals that never enter the trapped, remained near the trap and were successfully reunited with the rest of the group after these were released. The trapped groups and bating areas were baited for three days after the capture procedure, and in all cases, social groups and individuals were seen together and with no injuries. No mortalities associated with trapping were observed. We recorded the date, time, group composition, sex of individuals and GPS location at the time of sample collection, where each trapping was done.

<u>Landscape classification</u>

We collected GPS data of the different habitats in the landscape of study and partially classified the landscape (Figure 2). We collected at least 25 ground reference points of the different habitats and used as a reference to classify Landsat 7 TM satellite imagery from the USGS using Maximum Likelihood methods. We have so far explored different landscape classification schemes based on the available habitats for tamarin and after evidencing tamarins moving through different and unexpected substrates (i.e. scrubland and grassland). Furthermore we are also currently validating the accuracy of the classification with a land cover classification provided by the Colombian government (Instituto Agustin Codazzi and IDEAM). This data has allowed us to evidence that tamarins move over substrates that have previously not been considered as suitable habitat (i.e. fences, electric poles, shurblands and pastures). This information can help us better identify those habitat types that potentially favor tamarin movement, and thus identify potential habitat corridors. This data will be taken into account to test whether there is an observable relationship between how populations are being structured and the landscape characteristic.

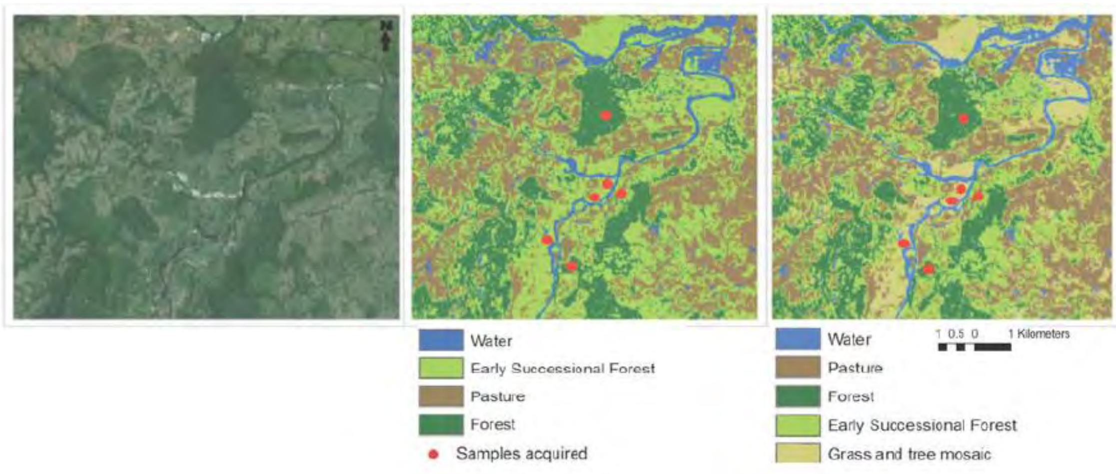

Figure 2. Satellite image and landscape cover classification with associated sampling locations in the Municipality of Norcasia and Dorada, Caldas

DNA extraction and analysis:

We extracted genomic DNA from all samples using Qiagen DNA Micro kits (Qiagen, Valencia, CA). We initially proposed to genotype 12 different autosomal microsatellite markers that have already been used for population genetic studies of other Callitrichids. However, since we will just be looking at a small set of molecular markers, comprising only a small portion of the genome, we decided to use a next generation sequencing techniques that provide the opportunity to assay thousands of markers found throughout the genome. This approach offers a much more thorough view of the genetic variation present in our sample allowing us to make higher resolution inferences about population history and gene flow.

We have successfully standardized the laboratory techniques to build genomic libraries and identify thousands of potential makers distributed throughout the genome of the silvery brown tamarin in the Primate Molecular Ecology and Evolution Laboratory and Genomic Sequencing and Analysis Facility at the University of Texas at Austin. This is one of the first studies to analyze single nucleotide polymorphism (SNP) markers in a large number of individuals from a New World primate. This approach will augment the molecular data currently available for New World primates and will advance the field of primatology by demonstrating the broad utility of modern molecular techniques to identify novel, informative molecular markers in a non-model taxon.

Landscape Genetic Analysis:

We are currently analyzing the genetic data to quantify the neutral genetic variability and infer the spatial structure in genetic variation. We will use a Bayesian model to identify the number of genetic clusters among the samples and determine whether tamarin social groups living in different fragments of forest are genetically distinct. This analysis will also allow the detection of possible gene flow among forest patches as a result of current and past dispersal. Additionally we will examine the genetic structure of *S. leucopus* with an analysis of molecular variance and test for population differentiation by performing pairwise tests for differentiation using F-statistics. Furthermore, the effect of landscape features on tamarin dispersal will be evaluated using causal modeling and analyses of resistance surfaces in CIRCUITSCAPE (McRae & Shah 2009). For the former, simple and partial Mantel tests will be conducted to examine the correlation between genetic distance and both Euclidean distances (straight line distances on map) and resistance distances between sampling sites that incorporate aspects of landscape heterogeneity by taking into account how easy it is for a tamarin to transverse each habitat type. CIRCUITSCAPE will be used to calculate the effective resistance between sampling sites on a resistance surface (land cover map classified by a resistance value) using circuit theory. This approach is desired when inferring the effect of landscape on gene flow over multiple generations, as it takes into account all the possible paths across a landscape, rather than assuming that gene flow is related to the total cost along a single optimal path. Different resistance surfaces will be used with varying resistance costs for each habitat class to evaluate whether each habitat type facilitates, impedes or has no effect on gene flow.

Environmental Workshops:

Involving the local community of the Municipality of Norcasia is a priority for this project, as a means to raise and create awareness of the threats silvery brown tamarins and their habitats are facing. This project involved all stakeholders by developing conservation workshops in the local schools to familiarize the community and general public with these primates and the risk of extinction they face. We have successfully built a strong relationship with the local community and cattle ranching farm owners, which have showed their interest in participating in the project. Throughout the project, we have developed 5 conservation workshops in the local school (Figure 4 and 5) and conducted informal meetings and interviews with the community (Figure 6 and 7). We have been able to raise awareness of the threats and degree of endemicity of the silvery brown tamarin, its role in the ecosystem and in the community's livelihoods. Moreover, we have been able to develop a close relationship with the owners of two of the cattle ranching farms where we worked and we have explored the feasibility of implementing alternative cattle ranching strategies (e.g. silvopastoral systems with live fences) that benefit both the beef production and the silvery-brown tamarin habitat (Figure 8and 9). Finally, we have had constant communications with the Environmental State Corporation, CORPOCALDAS and have sent trimestral reports of the project activities. They have continually shown great interest in the project results, as they are currently planning a project on habitat corridor implementation. We believe, that once we have finished analyzing the samples, these results will contribute significantly to the implementation and design of these corridors in the area.

Figure 4. Environmental workshops conducted in the local community school “Institución Educativa Buenavista Posprimaria La Habana” in the Municipality of Norcasia.

Figure 5. Coloring and activity and posters distributed to the kids attending the conservation workshops.

Figure 6. Informal interviews conducted to the local community.

Figure 7. Banners of the project at the entrance of the community and in the banks of the La Miel River “Welcome to La Miel River, home of the silvery brown tamarin”.

Figure 8. Ponchos and mugs given to the managers of the cattle ranching farms with the message “Lets protect the tamarin, is UNIQUE and OURS”

Figure 9. Informal meeting held with the owners of one of the cattle ranching farms.

DISCUSION

One of the main difficulties we encountered while developing this project was the long periods of time required for trapping tamarins. Although we had anticipated that this would be time consuming for some groups, and thus we have prepared accordingly (i.e., use playbacks and caller animals), in some localities none of these solutions were available or effective. Although we have successfully trapped various tamarin groups, we realized that for certain groups the baiting process can take more than 3 months without success, especially for groups inhabiting conserved forest. Due to the fact that many studies have documented that tamarin aversion to bait can be reduced by employing a decoy or caller individual next to the trapping site in a similar compartment, we employed a caller individual lent by CORPOCALDAS who manages the tamarin rescue center in Caldas. During our various attempts in using a caller animal, we observed that while adult individuals attracted the wild tamarins to the baiting platform, they represented a threat and consequently they vocalize and display aggressively towards the caller and never ate the bananas. On the contrary, using a juvenile or infant as a caller gave the most success and after a couple of weeks of exposure wild tamarins were being trapped. However, since CORPOCALDAS' mission is to reintroduce captive tamarins into the wild, it was not very easy to have access to young tamarins as they are less imprinted to humans and therefore the target for release programs. When we used a caller animal no physical interaction between the caller and the wild tamarins was observed, and the caller did not shown any health problems.

Another unforeseen difficulty we encountered at the beginning of the project was the lack of support by some of the community members. Although we conducted an informal meeting with the community to familiarize them with the project objectives, methods and team members, after a couple of months of being in the field collecting data some members of the community thought our trapping methods were causing the death of the tamarins, and thus they had a negative perception of our project. After realizing this, we did another meeting that was attended by the above mention people, and we were able to clarify any misunderstandings and thanks to the support given by some of the community leaders, our project had a 100% approval. Although we managed to meet a couple of times with the community, the number of people that participated in some of these meetings was quite low. Community members of our study site have no steady jobs and as such they plan their lives on a daily basis. Consequently, it was sometimes hard to plan ahead of time the meetings and have high attendance rates. Even the community leaders, which were very interested in the project, did not assist some of our meetings if that same day someone offered them a job. One solution we found to deal with this problem was to convene our meetings the same day as the meetings developed by the company that manages the hydroelectric in the area, which informally employs 80% of the community and requires a mandatory attendance to the meetings. We expect that as the project continues developing, we are able to bring together all of the people who are interested in the project and that are fully committed without relying in the hydroelectric meetings.

We believe that to date the most important outcomes of our project are: 1) we have successfully trapped and sampled 53 individuals from 12 social groups and 5 different populations in Caldas and Antioquia; 2) we have built a strong relationship with the local community and cattle ranching farm owners, which have showed their interest in participating in the project; 3) we have provided opportunities for 3 Colombian undergraduate students to develop short-term research projects. Students have gained training in field primatology methodologies, and have acquire theoretical background in primatology and conservation; and 4) our preelimary results have contributed to the revision of the current action plans that seek to preserve silvery-brown tamarins and integrate the local community. Although our project has not been completed and we still don't have the accurate information to identify what environmental factors favor or restrict silvery-brown tamarin dispersal and thus habitat corridors for the species, we have established a strong relationship with the Environmental State Corporation, CORPOCALDAS. Once we have finished collecting and analyzing the samples, these results will contribute significantly to the implementation and design of these corridors in the area.

The next steps we will take in this project will be to finish identifying genetic markers in all of the samples and start conducting the population genetic and demographic analyses. This information will later be used to do all the geographic information system analyses to identify the influence of landscape on gene flow. This information will aid in the identification of environmental factors that favor or restrict silvery-brown tamarin dispersal and thus habitat corridors for the species. Once the project is completed, we will be able to test different biological corridor configurations for ability to augment gene flow between isolated tamarin populations and explore whether several potential biological corridor configurations across the landscape might achieve connectivity among populations in a preliminary, qualitative analysis. These results then will be provided to CORPOCALDAS, in order to inform science-based management recommendations for the conservation of silvery brown tamarins and CORPOCALDAS' biological corridor construction program and management of matrix habitats. Moreover, we will publish the results on our project in international and national peer reviewed journals (i.e. International Journal of Primatology, Conservation Genetics, Conservation Biology) as well as through meetings and conferences, in order to share the gained knowledge with other researches working in different areas and with different taxa.