Conservation and Landscape Genetics of Lowland Tapir (*Tapirus terrestris*) in a Periodically Flooded Amazonian Forest, Brazil

Final report to the Rufford Foundation

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Introduction

The Amazon forest represents one of the last terrestrial frontiers in Biology. The richness of species and the complexity of the interactions continue to baffle and intrigue scientists. On the same token, the wealth of natural resources, from water to minerals to oil, has made the biome a central theme in South American development policy and plans. A fundamental question that me posed is how can these resources be sustainably used to improve livelihoods in the Amazon region, the countries that harbour the biome, and the world at large?

Figure 1. Two lowland tapirs in a wildlife refuge in Manaus, Brazil. (Photo: A. Gonçalves da Silva)

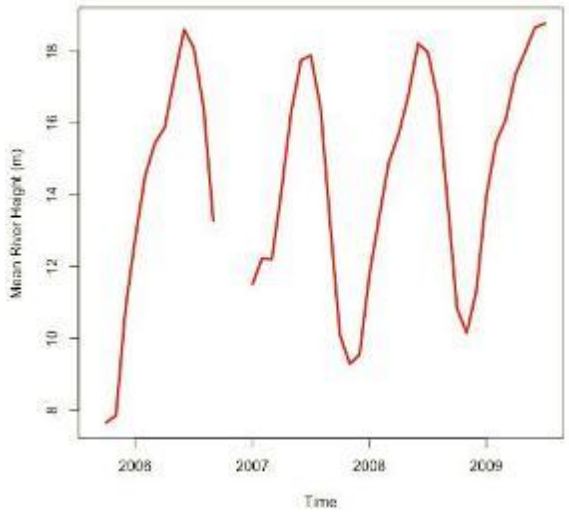

Figure 2. Hydrological cycle of the Jaú River from 2005-09. (Source: Brazilian National Water Institute

The answer to that question is still a matter of hot debate. Currently, at the international level, discussions are taking place to evaluate the success of the UN Convention on Biological Diversity (CBD), and set new goals for the coming 40 years. Most will argue that the CBD has had only moderate success, particularly when compared to the Convention on the Trade of Endangered Species (CITES) and the Convention on Migratory Species (CMS). However, while the CBD is not widely known for achieving substantive success in the protection of species, as is CITES with elephants and CMS with migratory birds, for instance, it has played a crucial role in strengthening Environment Ministries within the countries that harbour the Amazon. This has given these Ministries prominence in the media and clout in intra-government negotiations about policies and budgets.

The increased participation of Environment Ministries in government has led to a demand for in-depth scientific information on which to base decisions. The Ministries and the government have become increasingly interested and reliant on conservation scientists for high quality information, and training in appropriate techniques. In Brazil, for instance, the Ministry of the Environment has attended workshops, symposiums and conferences

held by professional societies (e.g., the Brazilian Society for the Advancement of Science) outlining potential policy decisions, and requesting feedback from the scientific community. The Ministry's policy enforcement agencies (IBAMA and the Chico Mendes Biodiversity Institute) have organized workshops and meetings, where scientists and NGO's are invited to lead discussions, to develop action plans for specific species, or to train personnel in appropriate biodiversity monitoring techniques. Much of this progress is due to the Brazilian government's commitments to the CBD 2010 Biodiversity targets.

One of the main issues in the Amazon biome is the construction of hydroelectric dams to meet current and future energy needs for sustained development. More than 70 are currently planned for the Brazilian Amazon, some of the effects are seen with the first major dam (Balbina), and we will certainly see them happening again with the Belo Montes damn which has just received government approval (http://news.bbc.co.uk/2/hi/8633786.stm), in spite of strong protests from members of the civil society.

In this context, we proposed to explore lowland tapir's (*Tapirus terrestris*; Figure 1) use of habitat in a relatively pristine region of the Amazon basin, which is subjected to periodic upheaval due to flooding (Figure 2). Our goal is to understand how different elements of the landscape, including the dynamic changes caused by the flooding, contribute to the species' population dynamics, and thus to its survival. This information is essential for constructing landscape models in which distinct development options can be examined, and the potential effect on the species survival measured.

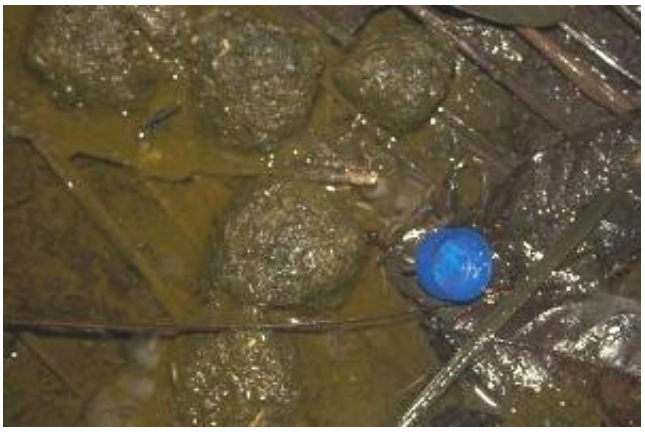

Figure 3. Tapir dung found in the study site. (Photo: A. Gonçalves da Silva)

Furthermore, it will allow us deeper insight into the effects of the flood/drought cycle, which is thought to be a feature that cause constant and drastic transformation of the landscape. And, hopefully, some insight into what happens when this cycle is interrupted by dams. In doing so, informed decisions can be made about potential development pathways. Albeit at a much smaller scale, the process is akin to what the Intergovernmental Panel on Climate Change (IPCC) has done for climate, and what the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES – UN Environment Programme) hopes to achieve for biodiversity.

The lowland tapir is an ideal species for this study because of: (*1*) its size, which makes it the largest Neotropical herbivore, and thus plays an essential role in the biome's ecological structure and function; (*2*) its close relationship with water, which makes it particularly susceptible to the flood/drought regime in the region –

in fact, members of the local community, we have found, say that the tapir will disappear to the "centre of the Earth" during the flood season, and re-appears at the river's banks in the drought; and (*3*) the species' has a relatively large and well coordinated group of scientists and interested people working for its conservation under the umbrella of the IUCN/SSC Tapir Specialist Group, in which the data collected for this project will be disseminated and incorporated into the national action plans for the species throughout its range.

Methods

Traditionally, such a study would be conducted by radio-telemetry of captured animals. However, this approach would be logistically and technically challenging in the dense Amazon forest, would only allow us to track a handful of animals, and would not afford insights about the long-term interaction between tapirs and the flood/drought cycle. For these reasons, we decided on a genetic-based approach, with samples obtained from fresh dung found by researchers and field assistants walking on and off trails (Figure 3). The distribution of genetic variation in relation to landscape features can then be used to understand the interaction between species and landscape.

Figure 4. The *recreio* continuing on its way after delivering us to the mouth of the Jaú River. (Photo: A. Gonçalves da Silva)

The approach makes use of the inheritance properties of DNA, in which one is more likely to share genetic variants with closely related individuals than with the population at large, and the geographic property of breeding, in which one is more likely to reproduce with a geographically proximate mate than with one that is further away, or separated by some geographical feature that restricts movement. With these two properties, we can estimate the probability that two geographically separate dung samples come from the same individual, or from related individuals. These measures of genetic relationships can then be examined within a geographical context (i.e., in a Geographical Information System, or GIS, database) to ascertain how landscape features might be influencing the mating pattern of the species', and consequently, its population ecology and evolution.

As a first step, it was necessary to develop the genetic tools to assess genetic variation. In this regard, we decided to use microsatellite markers, which are the same used in forensic sciences to determine paternity in humans, or whether an individual was present at a crime scene, and afford a number of variants (alleles) at

Figure 5. Carlos "Tripa" Abraão using his tapir whistle. (Photo: A. Gonçalves da Silva))

each locus and a number of loci throughout the genome. The combination of variants across a number of loci within an individual forms its genotype, which is akin to a fingerprint, and can be a powerful tool to distinguish individuals. In total, we have developed 13 species-specific loci (Gonçalves da Silva *et al.* 2009), 10 of which were extensively tested in a captive population of lowland tapirs in Argentina (Gonçalves da Silva *et al.* in press). We have also developed appropriate tools to assess variation at a mitochondrial DNA locus, which has the particular property of being maternally inherited, and will thus provide us with information about female-specific interactions. As a second step, we are developing an extensive GIS database with landscape features likely to affect tapir movement and location, such as presence of fruiting trees of interest (e.g., the patauá, *Jessina bataua*, an oil palm species that produces a fruit the tapir is very attracted to, or the goiaba-deanta, *Bellucia grossularioides*, the so-called tapir guava), and incorporates the flooding regime.

Achievements

Logistically, this study has been extremely challenging. The study site is about 220km northwest of Manaus, the largest city in the Brazilian Amazon. It is only reachable by an 18h ride in a *recreio*, a common mode of transport in the Amazon River (Figure 3), and then another 3-4h ride on a small boat with an outboard engine. At the site, a research station has been built by collaboration between IBAMA, the park's management authority, and the *Fundação Vitória Amazônica* (FVA), a local NGO and one of our partner's in this study. The research station has minimal facilities for cooking, and hooks for hanging our hammocks, but no electricity. Our partnership with FVA has allowed us access to the research station, and an introduction to the small local community of about five families that lives by the station. This introduction led to Carlos "Tripa" Abraão, who we trained as our field assistant and whose canoe we used to reach potential sampling localities (Figure 5).

Through our partnership with FVA, we also met the Chico Mendes Biodiversity Institute officials responsible for the park, with whom we were able to establish a rapport, and obtain assistance on several occasions, as well as the necessary sampling permits. Finally, through FVA we met Dr. Izeni Farias (Federal University of the Amazon – UFAM), who is the curator of the University's genetic samples collection. As such, all samples collected in this study are being deposited in this collection, and a sub-sample is being removed for analysis pertaining to this study. This means the samples are publicly available, and can be used by other interested researchers.

Figure 6. Geographic location of samples (*N* =47) along the Jaú River. Satellite image taken during the dry season.

In total, we have carried out 150 days of sampling between May 2008 and November 2009, collecting 47 fresh dung samples (Figure 6). While this number seems relatively small, it represents one of the most thorough and intensive tapir sampling efforts in the Central Amazon to date. Furthermore, it would not have been possible to radio-collar and follow as many individuals in the same time frame. At the moment, we have 34 samples already in lab. The final 13 are waiting the CITES export permit (already awarded, but not delivered) to be shipped to the lab. Of the samples in the lab, we have conducted preliminary tests to ascertain their quality and our ability to recover genetic data from them. The results have been extremely promising for both the microsatellite and the mitochondrial data (Figure 7). Moreover, a preliminary assessment of the mitochondrial data suggests large amounts of genetic variation, with five variants being identified in eight examined samples. These variants suggest at least two distinct lineages, which are not exclusive to one side or the other of the river's banks. At this early stage, it is hard to attribute any further meaning to these observations aside from the exciting prospects about the insights that the high levels of genetic variation observed so far, and the fact that there are separate lineages, will afford once the dataset is complete.

Therefore, at this stage, we have stopped sampling and have changed our focus to the lab for the collection of the genetic data. We are still awaiting the final samples to arrive, in order to process them all together, and reduce costs and duplication of effort. In the future, we may need to do additional sampling for

continued monitoring and refinement of the genetic information. However, for now, we believe we will be able to answer some of our questions with the data at hand.

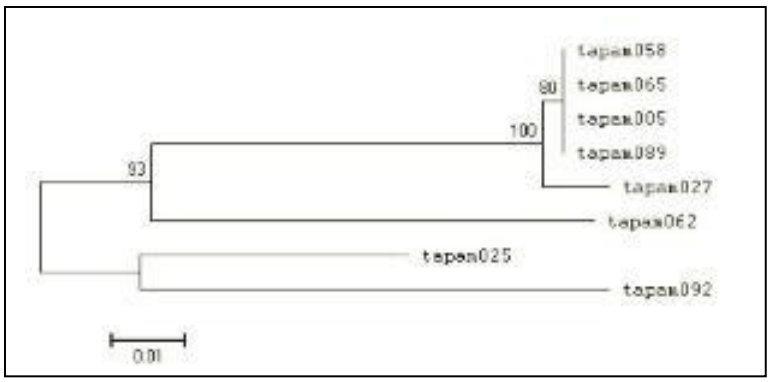

Figure 7. Genetic tree (NJ) showing the relationship from 255bp of the mitochondrial DNA control region from eight samples of lowland tapir from the Jaú National Park. Numbers over branches indicate node support values from 1000 bootstrap replicates.

On other fronts, the project has made headway in promoting the methods employed in this study and the conservation of tapirs. In this regard, we have made several presentations for the students of the graduate program in Biodiversity at the UFAM, to the graduate program in Ecology of the National Institute for Amazonian Research (INPA), and to the students of the Biological Dynamics of Forest Fragments Project (Smithsonian Tropical Research Institute and INPA).

These have caught the attention of two Master's students (Adriana Barcelos and Gabriela Medeiros). Adriana is studying tapirs in the northern part of the Brazilian Amazon, in the state of Roraima, examining seed dispersal. She has volunteered here time for this project, and has expressed interest in pursuing tapir conservation using genetic tools as a doctoral candidate. Gabriela is being co-advised by me, and Eduardo Venticinque (Wildlife Conservation Society and UFAM), and is applying the same tools and methods of this study to answer similar questions in an area just upstream from the Amazon's largest dam (Balbina). Gabriela's study is particularly interesting because she will examine the effect of loss of the flooding regime due to the Balbina Hydroelectric Dam, which created a permanently flooded landscape with numerous islands. Thus, her results have the potential to contribute to our interpretation of the population patterns that we uncover. Their interest in working with me has no doubt been driven by the work you support.

I have also been active in with the IUCN/SSC Tapir Specialist Group (TSG), in particular with the Tapir Conservation Newsletter. Because 2010 is the year of Biodiversity and we are expecting a milestone meeting of Conference of the Parties to the Convention on Biological Diversity, I am organizing a two part series for our Newsletter addressing (1) the expectations that surround this meeting, in particular relating to achieving the 2010 Biodiversity Targets, and (2) the major outcomes and identified paths forward after the conclusion of the meeting. Within the Group, I am also involved with the organization of the next International Tapir Symposium, to be held in Malaysia in 2011. I am looking forward to presenting the results of the Amazon work to the tapir conservation and scientific community.

In collaboration with researchers of the TSG and UBC, I have analyzed the Argentinean captive population of lowland tapirs using molecular markers to make recommendations to improve the local breeding program. This study has already been accepted for publication in the Journal of Heredity (Gonçalves da Silva *et al.* in press), and the results and recommendations have been presented to the breeding program managing authority. Finally, along with Mike Zavada (East Tennessee State University), I have signed a contract with Nova Scientific Publishing, Inc. to edit a book on tapir ecology, evolution and conservation (to be published in 2011). We aim to produce a comprehensive compendium of current knowledge on tapirs targeted to advanced high school and undergraduate students. Our hope is that such a volume will help increase public awareness and attract young and enthusiastic students to biology and conservation. I intend to include information relating to the results obtained by our analysis of the Jaú tapir population.

Challenges

In spite of this progress, we are not where we would like to be. We have had two major setbacks that have delayed our progress:

1. The shipping of the first batch of samples, which confusion between the courier, the Brazilian customs, and myself, caused the samples to sit at customs for almost a month. Fortunately, the issues were resolved, and laboratory work was able to commence. We do not foresee the same problem for the second batch of samples.

2. Our original protocol for extracting DNA, developed from samples collected in a captive setting, has not worked as well on the wild caught samples. Further optimization has produced improved results, however it requires a piece of equipment that has been in back order for the past three months, and we only just received it.

3. Adriane Morais, our principal person in Manaus, was offered a permanent position with the Amazon State Government, which meant we were without a local person dedicated to the project for a number of months. This position was eventually filled by Adriana Barcelos, who has been essential in the liaising with Carlos Abraão at the field-site, organizing the deposit of the samples at the collection at UFAM, and shipping the subsamples to the lab.

4. Brazilian officials responsible for CITES permitting are currently on strike, setting our work back, as we await for the delivery of the permit that has already been awarded.

Opportunities

In the short-term, we see three different opportunities where our project can have an impact:

- Obtaining samples in the Amazon is extremely challenging due to the lack of adequate logistical support, and the environmental conditions of extreme heat and humidity. Our results show that is possible to sample dung, and recover DNA in quantity and quality sufficient for most analyses. Therefore, we can work with the Chico Mendes Biodiversity Institute and FVA, who are both partners in a monitoring program within the Jaú National Park, and in other areas, to collect genetic samples not only of tapir, but other species as well. The genetic data could be used to estimate numbers of individuals, sex ratio, movement patterns, and other biological information of relevance. To this effect, we are currently working on a manuscript to publish the methods in a peer-reviewed journal, and also train other personnel of the relevant organizations in the collection and preservation of the material. One community member (Carlos Abrão), and two students (Adriana Barcelos and Gabriela Medeiros) are already trained, and at least two members of the IUCN/SSC Tapir Specialist Group are also starting to use the same techniques. This will allow for a larger breadth of monitoring options, and for higher quality of the collected data.

- A national action plan for lowland tapir is currently being developed in Brazil. The expertise and data generated by this project will be used to inform the action plan, in particular in relation to amount of genetic variation to expect in pristine conditions (even with some level of subsistence hunting), habitat use of tapirs in the region of the Jaú River, and around the Balbina Dam Reservoir (and what that means for other areas), and the effects of the flooding regime.

- Methods and techniques to combine landscape and population data into a temporally dynamic model in which one simulate different scenarios are still relatively new, in particular for wild species. Our project hopes to pioneer some of these techniques, and assist in setting some of the standards for organizations such as IPBES.

Financial report

In total, we were awarded £4,074.00. Below, we break down expenses under each category of the original budget (Table 1).

Table 1. Break down of expenses

Category	Requested (£)	Spent (£)
Transport	3,610.00	2,819.60
Field Equipment & Supplies	464.00	467.19
Lab Supplies	0.0	787.16
Total		**4,073.95**

With the change in location, £1,300.00 that was originally budgeted for hiring a car was instead employed in obtaining additional genetic information, because we did not have as many samples as we originally thought. The additional genetic marker, the mitochondrial DNA, which was not included in the original proposal, but r, will give us more information per sample, in particular, information pertaining to female dispersal and movement patterns. All receipts are filed with the University of British Columbia accounting office.

Acknowledgments

The work would not have been possible without the valuable help and assistance of Gonçalo Ferraz, Adriane Morais, Fundação Vitória Amazônica, especially Sérgio Borges and Simone Iwanaga, Instituto Chico Mendes para a Biodiversidade, and the University of British Columbia. Samples were collected under permits ICMBio/SISBIO no. 17173-1 and 17173-2 and deposited in the genetics tissue collection at UFAM. Sub-samples for analyses were exported under CITES export permits 106529 and 108698, and imported under Canada Food Inspection Agency permit no. A-2009-01966-6.

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On behalf of the Amazon tapir conservation team, thank you for your support!

The Amazon Tapir Conservation Field Team. Anders Gonçalves da Silva, Carlos "Tripa" Abrão, Adriana Barcelos, and Carlos André Nogueira (from left to right).