

A Preliminary Study of the Genetic Diversity of Pygmy Marmoset *Callithrix pygmaea* (Primates: Cebidae: Callithrichinae) using Short Sequence Repeats (SSR)

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A PRELIMINARY STUDY OF THE GENETIC DIVERSITY OF PYGMY MARMOSET *CALLITHRIX PYGMAEA* (PRIMATES: CEBIDAE: CALLITHRICHINAE) USING SHORT SEQUENCE REPEATS (SSR)

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Introduction

Genetic diversity is a major concern in conservation biology, the loss of genetic diversity is often associated with a reduction in reproductive fitness and a population decrease. Genetic diversity is required for populations to evolve and adapt to environmental changes that in present times are more frequent and more rapid due to anthropogenic factors (Frankham et al. 2003).

Primates play a fundamental role in the dynamics of tropical ecosystems. The pygmy marmoset *Callithrix pygmaea* is the smallest primate species in Ecuador and shows a high degree of specialization in habitat and diet (de la Torre et al. 2009). This specialization combined with the increase of human activities in tropical rainforests could drive pygmy marmoset populations to genetic bottle necks with the subsequent loss of genetic diversity. Almost nothing, however, is known about the genetic diversity of this species so we began a pilot study to evaluate the genetic diversity of 3 wild groups of pygmy marmosets in one population located on the margins of the Aguari-co River, in northeastern Ecuador. We developed a non-invasive protocol to obtain DNA samples from feces to characterize the genetic diversity of the groups as a first and necessary step in the implementation of a program to evaluate human impact on the genetic diversity of pygmy marmosets and other Ecuadorian primate species.

Study area and subjects

The San Pablo population is located at the margins of the Aguari-co river (0°16'27"S, 76°25'29"W) (Fig 1). This area has varzea forest and is seasonally flooded by white-waters rivers. Three groups (P1, P2, P4), in this population have been monitored since the year 2000 and were the study subjects (de la Torre et al. 2009). Group size varied from 5 to 7 individuals during the sampling period. Groups P1 (5 individuals) and P2 (6 individuals) had the most distant home ranges, separated by open areas, houses and several plantations (closest linear distance between P1 and P2 home ranges: 250 m). Groups P1 and P4 (7 individuals) had a disturbed, secondary forest connecting their home range areas (closest linear distance between P1 and P4 home ranges: 165 m). Finally, between the home range areas of groups P2 and P4 there are open areas with some trees but no houses (closest linear distance between P2 and P4 home ranges: 200 m).

Methods

Collection of fecal samples

Fecal samples were collected from September 2008 through February 2009 from these three groups. Large leaves of banana (*Musa paradisiaca*) and heliconia (*Heliconia* spp.) were placed very early in the morning under the feeding tree of a group before the marmosets began their daily activities. Animals were observed by one field worker in periods of 3 hours from 0600 to 0900 and from 1500 to 1800 (local time). The leaves were checked continuously during the observation periods. Due to the collection methods, we could not accurately address a particular fecal sample to a given animal in a group; however, based on our observations, we are confident that the collected feces belonged to at least 3 different animals in each group. Collected feces were stored on filter paper and placed in a flask with a desiccant (silica gel). The closed flasks with the samples were dried with controlled sunlight (avoiding drastic rises of temperature) during one or two days to reduce the probability of contamination of the feces with fungi. The samples were later transported to Quito and placed in a freezer at -20°C .

Genetic analyses

DNA from the samples was obtained using the QIAamp DNA Stool Mini Kit (QIAGEN). Polymerase chain reaction technique was used to amplify nDNA microsatellites; we tested primers established by Nievergelt et al. (1998) for *Callithrix jacchus*. The PCR amplification was

performed in a 25 μL reaction volume which include buffer 1X, 1.5mM of MgCl_2 , 0.2 μM of dNTP's, 0.24 μM of each primer, 0.5U of Taq polymerase (Invitrogen) and 5ng of sampled DNA. The amplification program consisted of an initial denaturation of 3 min at 95°C ; 40 cycles of denaturation for 1 min at 94°C , annealing for 1 min at optimum temperature (Table 1) and an elongation during 1 min at 72°C ; and a final elongation at 72°C during 5 min. Polyacrylamide 6% and urea 5M gels were used to separate DNA bands.

Results

Forty two fecal samples were collected from the three groups; 23 samples from group P4, 12 samples from group P2 and 7 samples from group P1. Five of the nine loci tested (CJ-1, CJ-7, CJ-11, CJ-12, and CJ-15) showed a higher number of amplified samples (>10 amplified samples per primer) (Table 1) and were used for a preliminary analysis of the number of alleles in each loci. DNA samples from group P4 showed various alleles at the five loci tested, samples from group P2 showed alleles at 3 loci, and samples from group P1 showed alleles at four loci (samples from this group showed fixed alleles at 2 loci) (Fig 2).

Discussion

Although the number of samples for each group was small (P1 $n = 7$; P2 $n = 12$; and P4 $n = 23$) and the number of loci analyzed was also low (5 loci), the results evidence the

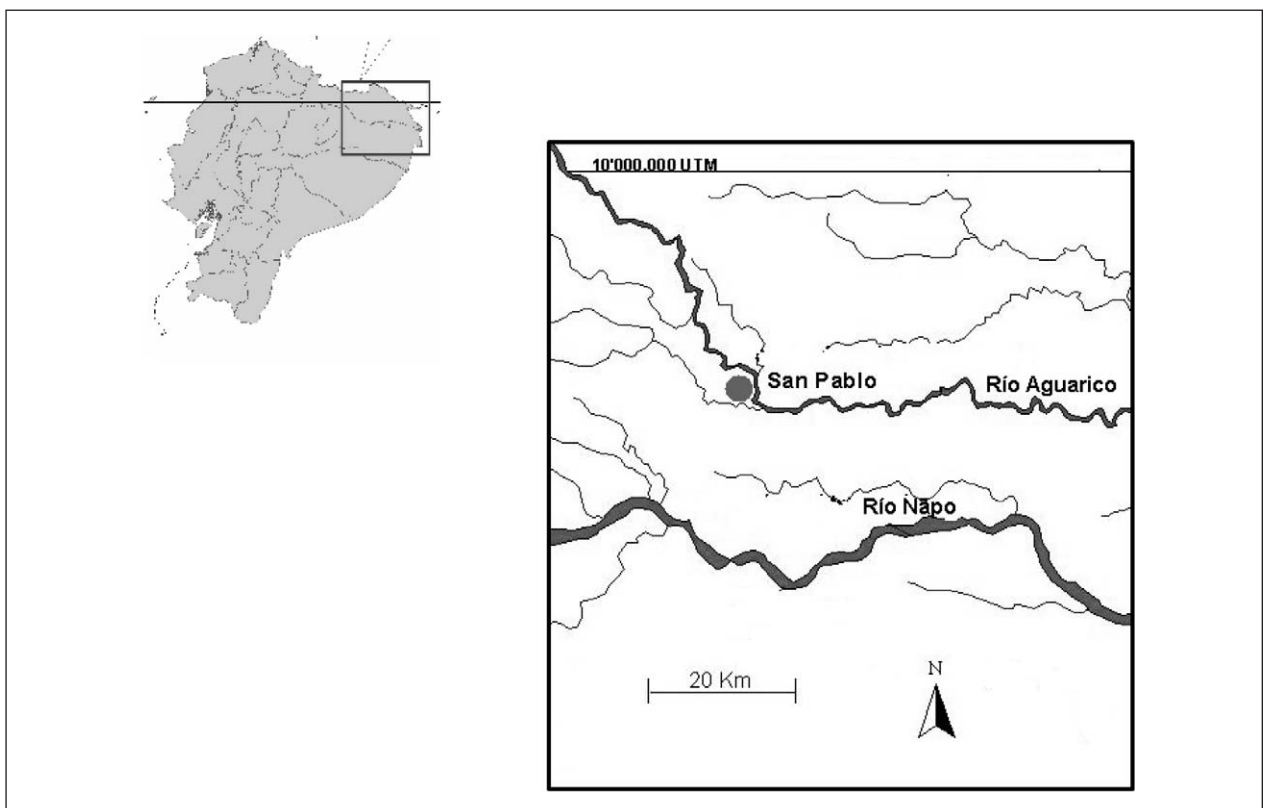


Figure 1. Location of the San Pablo population in Ecuadorian Amazonia

feasibility of inter-specific use of microsatellite primers to evaluate genetic variability of related primate species (Clisson et al. 2000, Nievergelt et al. 1998). The results also allow us to differentiate several alleles in some of the tested loci. The fixed alleles found in groups P1 and P2 could be an artefact of the collection methods (we could not address fecal samples to particular individuals) and the small sample sizes for these groups, making it possible that more than one sample belonged to the same individual. However, they could also suggest a reduced genetic variability in groups affected by strong habitat fragmentation (Frankham et al. 2003). We are now collecting more samples to identify different genotypes in each group to be able to assess genetic variability in this and other populations of pygmy marmosets.

The conservation of biodiversity is critical in tropical forests of the Amazon. The annual deforestation rate in Ecuador is estimated at around 200,000 ha and most of it takes place in the Amazon region, which is also affected by oil exploitation and mining (Ministerio del Ambiente et al. 2001, SENPLADES 2007). Molecular genetics is a tool to evaluate the impact of human activities on the populations of species inhabiting these areas, such as the pygmy

Table 1. Annealing temperature, number of amplified samples and product size range in base pairs (bp) for the nine loci analyzed in *Callithrix pygmaea*

Primer	Annealing temperature (°C)	# Amplified samples	Product size range in base pairs (bp)
CJ-1	58	11	128 - 176
CJ-6	58	0	-
CJ-7	50	13	119 - 127
CJ-10	50	4	217 - 243
CJ-11	50	14	106 - 142
CJ-12	58.4	27	132 - 168
CJ-13	50	0	-
CJ-14	56.2	9	156 - 184
CJ-15	50	15	124 - 138

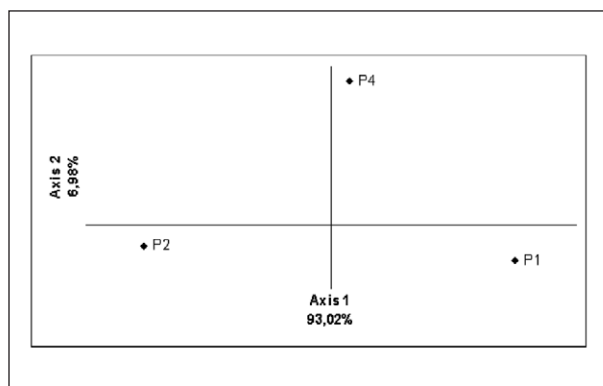


Figure 2. Allele frequencies at the five loci in the pygmy marmoset groups P1, P2, and P4 (numbers in the X axis correspond to the base pairs)

marmoset. Sensitive genetic markers, such as microsatellites, have the power to detect reductions in heterozygosity and allelic diversity in small and fragmented populations (Frankham et al. 2003) and provide useful information for conservation planning. This is the first study of this type for pygmy marmosets in Ecuador and elsewhere; our results suggest that non-invasive methods to collect DNA for the evaluation of the genetic diversity can be used even for small species such as pygmy marmosets. Our next steps in this research will be to improve the collection methods in the field (to reduce DNA damage, increase sample size and identify genotypes) and include more populations in the genetic analyses.

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