ESCOLA DE CIÊNCIAS DA SAÚDE E DA VIDA

Progress Report

Genomic Analyses: Jaguar Individuals that are part of the Iberá Reintroduction Project

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Data Collection: As of February 2025, 18 complete genomes have been sequenced from jaguars that are part of the Iberá reintroduction project. This includes all the founders of this population and some of their offspring. All the genomes were sequenced using the Illumina platform (at a minimum mean depth of coverage of 10x) and subsequently mapped onto our current jaguar reference genome. The same procedure has been conducted by our research group for jaguars from multiple regions, allowing in-depth comparisons of their levels of diversity, patterns of genome-wide homozygosity (which is an accurate measure of inbreeding), among other features which are relevant to investigate population-level evolution. Our current dataset comprises 76 complete jaguar genomes from diverse regions (e.g. all Brazilian biomes in which the species still occurs), including the 18 individuals from the Iberá project.

It is noteworthy that whole-genome sequencing provides the ultimate level of genetic information to investigate population-level processes and to empower conservation assessment and management decisions. At present, very few wildlife species worldwide are assessed and managed routinely using whole-genome sequences, given the cost and complexity of generating and analyzing such data, but this is an approach that will likely be employed soon for multiple endangered organisms for which the resources are available. It is therefore relevant that jaguars are among the first wild cats for which such an approach is being used, and that the Iberá project is the first for which whole-genome sequences will be generated and analyzed for all available individuals. Such an effort in data collection will provide unprecedented information on the evolution of a jaguar population in the wild, and allow finely-tuned management decisions to be made on the basis of in-depth genomic information.

Analyses: Multiple types of analyses can be performed with whole-genome data. Some of them (e.g., tracking the history of individual genomic segments and inferring the action of natural selection across the genome) will become feasible when a larger sample size, and multiple generations of this population, can be analyzed. At present, our analyses are focusing on measuring levels of genetic diversity and patterns of genome-wide homozygosity in the founders of this population as well as the offspring that have been sampled so far. An initial measure of diversity is genome-wide heterozygosity, which is the frequency of heterozygous sites across the genome. This provides an accurate assessment of mean diversity across each individual's genome, which can be compared across individuals. Another important measure is the number and length of Runs of Homozygosity (ROHs), which are homozygous blocks observed in the genome. Such blocks are formed when individuals bearing the same segment

mate, i.e., they derive from inbreeding episodes in the history of the population. Since those blocks are broken up by recombination over time, longer blocks can be inferred to derive from recent inbreeding, whereas shorter blocks are derived from more ancient inbreeding. Human-induced inbreeding, since it is recent, tends to induce long ROHs, whereas shorter ROHs are generally due to a longer history of population bottlenecks or small effective population sizes over time. Therefore, by analyzing the number and size of ROHs in genomes from multiple individuals, representing different populations with different demographic histories, one can investigate the impact of inbreeding on these sampled populations, which is very relevant in the context of conservation assessment and planning. A third approach that also provides relevant information is to map the spatial distribution of variation (e.g., Single-Nucleotide Variants – SNVs) across individual genomes, and to compare individuals within and among populations. In the case of the Iberá population, we can compared individuals that are directly related and observe changes in the presence of ROHs (homozygous blocks, i.e., regions devoid of variation) across generations. This allows the direct monitoring of diversity over time, and can be performed for every family that is part of the project, enabling management decisions with respect to subsequent matings, introduction of additional individuals, among others.

Results and Discussion: The results obtained so far indicate that the Iberá individuals bear similar levels of genomic diversity to those sampled in healthy Brazilian populations (Figure 1). The only individuals that bear lower levels of diversity (heterozygosity) are two founders, Tobuna and Naro. The same result is observed in the analysis of Runs of Homozygosity (ROHs). The overall number and length of ROHs observed so far in Iberá animals is similar to, or lower than, those observed in wild Brazilian populations, including those still bearing large jaguar populations, such as the Amazon. This is due to the fact that Iberá animals derive from an admixed background, formed by animals originating from different regions, a process that tends to increase genetic diversity and decrease the occurrence of ROHs. Such a pattern should be monitored carefully and continuously as the population grows and evolves, including the assessment of evolutionary forces acting upon this initial background of high diversity, followed by subsequent rounds of breeding among founders and locally born animals. Again, the only two individuals bearing a higher incidence of ROHs are Tobuna and Naro, two founders. This is not a problem, since such ROHs can be broken up by mating these individuals to unrelated ones, as was the case of Tobuna (Figure 2). In this analysis of SNV density, one can see the large ROHs present in Tobuna's genome (likely due to inbreeding in captivity), which are no longer present in her daughter Tania's genome, since it is likely that Tania's father (unsampled) was unrelated to Tobuna. The same type of analysis can be performed from every breeding pair and its o□spring, as exemplified by Figures 2-5. In those analyses, one can see cases in which ROHs present in parents disappear in the o□spring, while others are formed if the parents carry the same haplotype (DNA sequence) in a given region, which is a normal process in populations. Outbred populations tend to have a lower presence of ROHs, and more regions displaying high diversity (warmer colors in these heat maps). Therefore, the current sample of Iberá individuals presents a consistent pattern of mostly high diversity across the genome, as expected from an outbred population. Such a pattern should be continuously monitored over the next generations, aiming to sample as many individuals as possible to fully represent their genetic make-up.

Figures

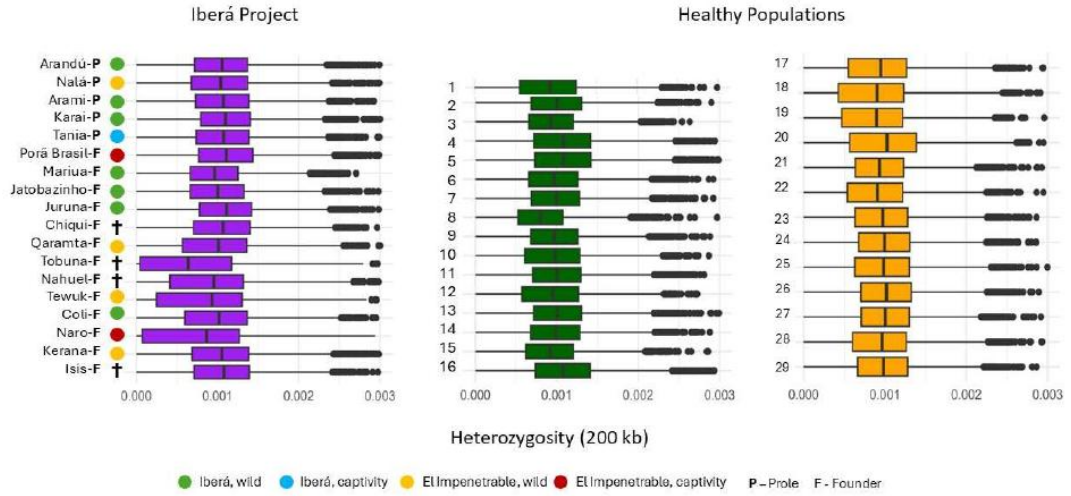

Figure 1. Boxplots depicting genome-wide levels of heterozygosity of all the individuals from the Iberá project (left panel) whose genomes have been sequenced so far. For comparisons, they are shown next to panels depicting individuals from genetically health populations sampled in the Amazon (green) and Cerrado (orange) biomes. For each individual, the central bar represents the mean, the box limits represent the 25% and 75% quartiles, the line represents the range, and the dots represent outliers.

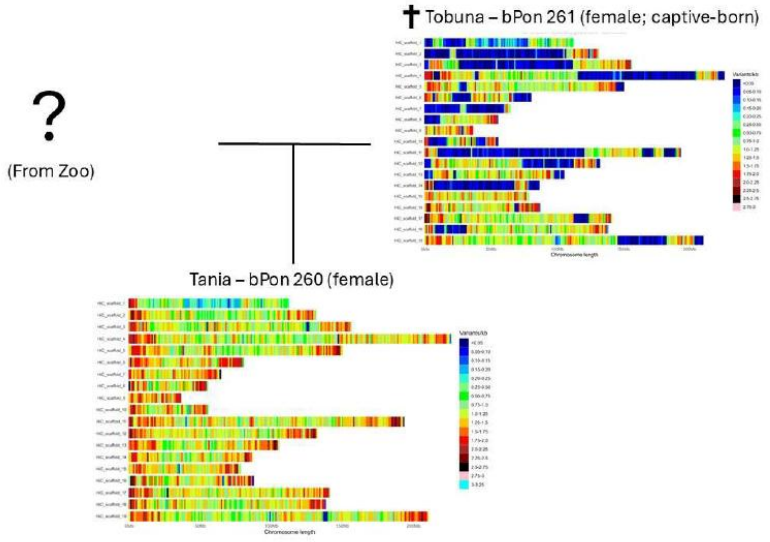

Figure 2. SNV (Single-nucleotide variant) density plot for individual jaguar genomes. Each panel depicts a single individual's genome, with each bar representing one chromosome. The heatmap colors indicate the density of SNVs per 1000 bases (1 kb) of the genome (see internal legend): warmer colors indicate more diverse regions, whereas blocks of the coldest colors indicate ROHs (Runs of Homozygosity). This graph depicts the genomes of an inbred captive-born female and her daughter.

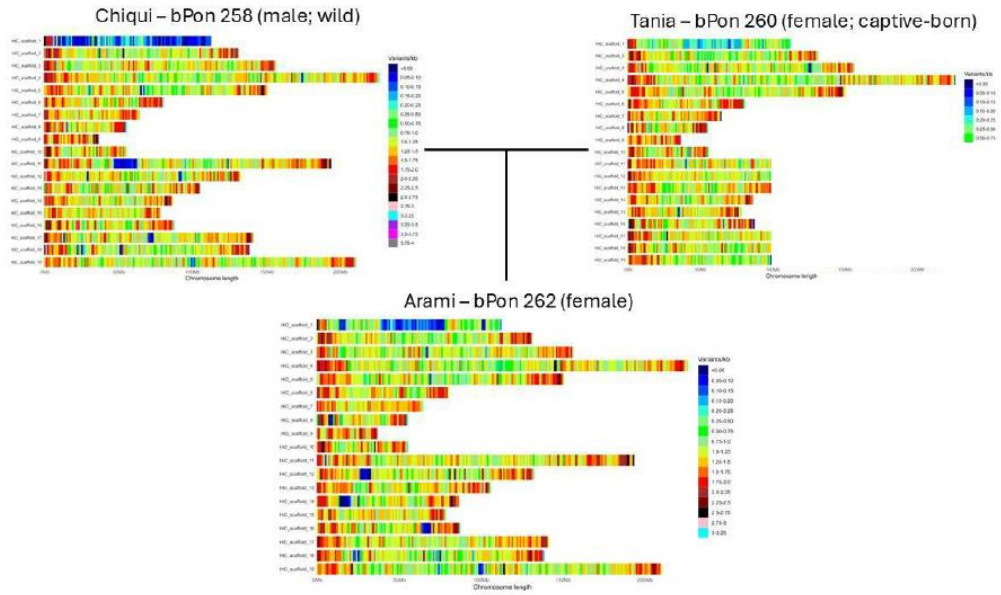

Figure 3. SNV (Single-nucleotide variant) density plot for individual jaguar genomes. Each panel depicts a single individual's genome, with each bar representing one chromosome. The heatmap colors indicate the density of SNVs per 1000 bases (1 kb) of the genome (see internal legend): warmer colors indicate more diverse regions, whereas blocks of the coldest colors indicate ROHs (Runs of Homozygosity). The top bar represents the X chromosome, which bears almost no diversity in males, since it is hemizygous. This graph depicts the genomes of a mating couple and their daughter.

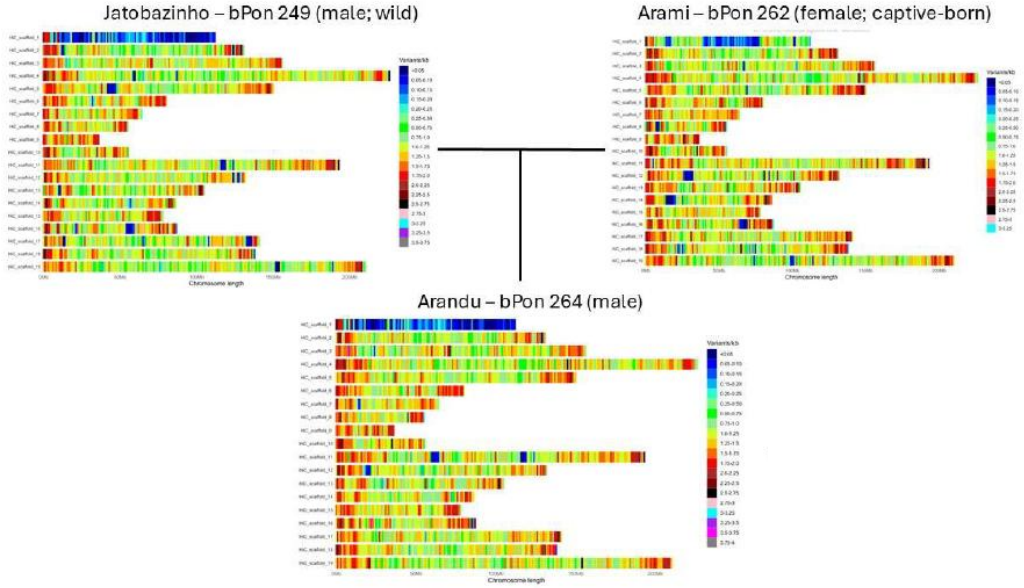

Figure 4. SNV (Single-nucleotide variant) density plot for individual jaguar genomes. Each panel depicts a single individual's genome, with each bar representing one chromosome. The heatmap colors indicate the density of SNVs per 1000 bases (1 kb) of the genome (see internal legend): warmer colors indicate more diverse regions, whereas blocks of the coldest colors indicate ROHs (Runs of Homozygosity). The top bar represents the X chromosome, which bears

almost no diversity in males, since it is hemizygous. This graph depicts the genomes of a mating couple and their son.

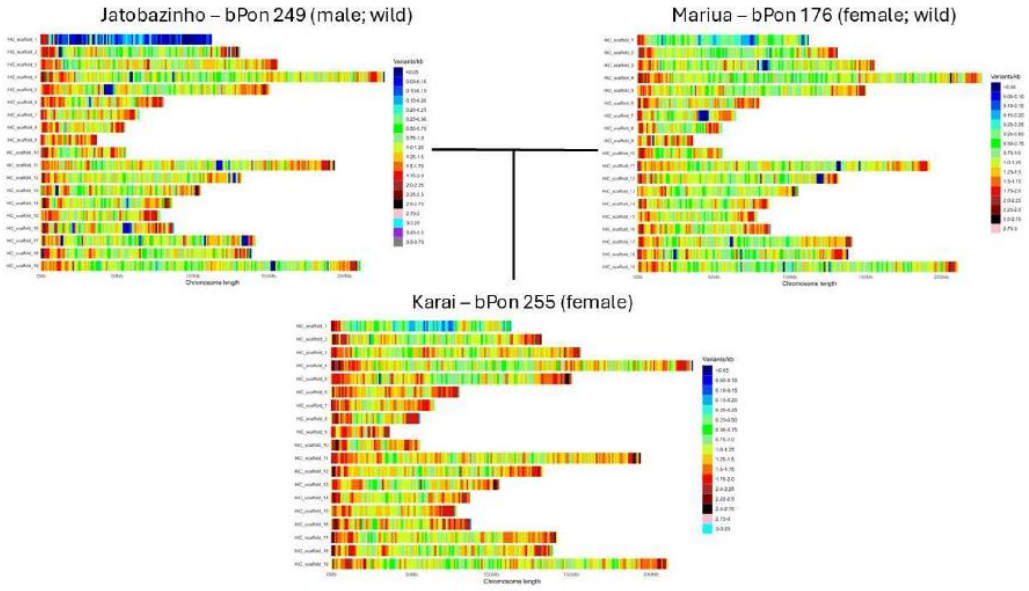

Figure 5. SNV (Single-nucleotide variant) density plot for individual jaguar genomes. Each panel depicts a single individual's genome, with each bar representing one chromosome. The heatmap colors indicate the density of SNVs per 1000 bases (1 kb) of the genome (see internal legend): warmer colors indicate more diverse regions, whereas blocks of the coldest colors indicate ROHs (Runs of Homozygosity). The top bar represents the X chromosome, which bears almost no diversity in males, since it is hemizygous. This graph depicts the genomes of a mating couple and their daughter.

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