**Are climate change impacts on lizard genetic diversity mediated by thermal tolerances?**

**Introduction**

The genetic diversity of populations is deeply affected by demographic factors such as population size (Kimura 1979; Leffler et al. 2012; Hague and Routman 2016)⁠ and dispersion (Tigano and Friesen 2016)⁠. Esses aspectos demográficos, por sua vez, são afetados por condições ambientais cujo efeito é modulado por características próprias das espécies, como a fisiologia (Huey, 1991; Walther et al., 2002; Kearney & Porter, 2009). Por exemplo, o metabolismo dos organismos ectotérmicos depende de temperaturas ambientais, que portanto afetam sua capacidade locomotora (Miles, 2004) e consequentemente sua capacidade de forrageio e fuga de predadores, atividades essenciais para sobrevivência, crescimento e reprodução (Porter et al., 1973; Adolph, 1990; Adolph & Porter, 1993). Temperaturas ambientais também podem afetar o tempo em que animais ectotérmicos estão ativos a cada dia (Grant & Dunham, 1988; Adolph & Porter, 1993; Sinervo & Adolph, 1994) e o tempo de atividade restringe a quantidade de energia que indivíduos podem adquirir e alocar para crescimento, manutenção e reprodução (Porter et al., 1973; Sinervo & Adolph, 1994; Kearney & Porter, 2009). Variações demográficas decorrentes desses processos ecofisiológicos podem, portanto, afetar a diversidade genética de populações e respectivas viabilidades populacionais.

Conservation of genetic diversity is crucial for the persistance of species and populations in the long term (cite), since diversity confers resillience to environmental change (Frankham, 2005) and diseases (King & Lively, 2012). Genetic diversity is also important for the functioning of ecosystems and the services hey provide (Faith et al., 2010; Srivastava, Cadotte, Macdonald, Marushia, & Mirotchnick, 2012; Rosauer et al., 2016), factors directly linked to human well being (cite). Therefore, we must understand how this diversity originates so we can better conserve the necessary conditions for its maintenance, as well as preserve the evolutionary and biogeographical history contained in species genetic material (Purvis, Agapow, Gittleman, & Mace, 2000; Davies Jonathan & Buckley, 2011).

The Cerrado savanna, in central Brazil, is the most diverse savanna in the world, with a very high proportion of its species occurring nowhere else (Oliveira and Marquis 2002)⁠. This diversity is threatened not only by fires, but by deforestation used to make way for pastures and plantations (Ratter et al. 1997)⁠, and climate change, which has been linked to population declines and species extirpations all around the globe (Bellard et al. 2012)⁠. A large percentage of South American biodiversity originated during the Quaternary period (from 2.58 million years ago to the present day), a period with intense climatic changes, which deeply affected vegetation dynamics and species diversification in the continent (Mayle 2004; Carnaval et al. 2009; Werneck 2011; Collevatti et al. 2012; Werneck et al. 2012; Ledo and Colli 2017; Costa et al. 2018)⁠⁠ (but see Melo et al. 2016). Cycles of expansion and contraction of forested areas isolated enclaves of open vegetation and the species associated with those (Van der Hammen 1974; Absy and Hammen 1976; Hammen and Absy 1994; Mayle 2004)⁠. This isolation might have led to local adaptations which influenced their current genetic diversity. Current climate change is happening much more rapidly, possibly leading to great loss of biodiversity (Sinervo et al. 2010)⁠. Are enclaves stable (Trabalho da Fernanda e da Marcella)?

Species and populations respond differently to temperature changes depending on their thermal tolerance, that is, in which temperature ranges they are better able to perform activities important for their survival and reproduction (Porter et al. 1973)⁠. Populations experiencing thermal conditions inside their ideal ranges will have higher reproductive rates (Adolph and Porter 1993)⁠, which leads to increased genetic diversity (Zamudio et al. 2016)⁠. That is especially evident in animals whose metabolism is very influenced by environmental temperatures, such as reptiles and insects. This makes those animals excellent models to study the subject and identify patterns that can be tested in other kinds of organisms.

My goal is to investigate if the genetic diversity of lizard populations is determined by the interaction of their thermal physiology with temperatures they experienced during their evolutionary history. My hypothesis is that populations that experienced climates inside their thermal tolerances for longer during the Quaternary period achieved higher reproductive performance and consequently, have higher genetic diversity in the present day. I will then investigate what consequences future climate change might bring to the genetic diversity of those animals, using the information learned from the climate changes in the past.

Hypothesis:

* Genetic diversity and demographic performance correlate with ecophysiological performance
* Populations at the ecotone are less diverse and had worse ecophysiological and demographic performance then those at core or enclave sites
* Future climate change will deteriorate ecophysiological conditions, leading to loss of genetic diversity

**Methods**

As a part of an ongoing collaboration involving Universidade de Brasília, Instituto Nacional de Pesquisas da Amazônia and University of California Santa Cruz, we have obtained tissue samples and thermal tolerance data for at least 20 species of lizards in the following locations: Brasília, Distrito Federal (15.7998°S, 47.8645°W), Nova Xavantina (14.6644°S, 52.3585°W), Gaúcha do Norte (12.9656°S, 53.5636°W) and Alta Floresta (9.8765°S, 56.0855°W) at Mato Grosso state; Lagoa da Confusão (10.9201°S, 50.1833°W) and Pium (10.4428°S, 49.1819°W) at Tocantins state; Canindé de São Francisco (9.6419°S, 37.7878°W) at Sergipe state and Parque Nacional dos Campos Amazônicos (8.4553°S, 61.1283°W) at Amazonas state. This dataset includes sites in the core of the Cerrado, in the border of the Cerrado with the Amazon forest and on Cerrado enclaves inside the Amazon Forest. I will sample an additional enclave site in Boa Vista (2.819444°N, 60.671389°W), Roraima state. I will evaluate candidate species from the data set and pick species that occur in the Cerrado core, border and enclaves to perform the study.

Tissue samples will be used to establish the phylogenetic relationships between populations, estimate the time since they diverged, quantify the amount of genetic diversity in each one, and estimate population sizes and migration rates at the Mid-Holocene (MH, 6000 years ago), Last Interglacial (LIG, 130000 years ago) and Last Glacial Maximum (LGM, 22000 years ago). The genetic diversity of each population will be quantified through the number of cytochrome b polymorphic sites, number of haplotypes and haplotype diversity (Santos, Nogueira, Giugliano, & Colli, 2014). Time of divergence between populations will be estimated using the standard mitochondrial DNA divergence rate for lizards (2% per million years (Thorpe, Leadbeater, & Pook, 2005) with 1% standard deviation and relaxed molecular clock (Santos et al., 2014).

Data on thermal preferences were obtained by placing lizards in MDF (Medium Density Fiberboard) thermal gradients, measuring 100 cm in length, 15 cm width e 30 cm height, open at the top with a 2 cm layer of sand and vermiculite at the bottom. The termal gradient (aproximately from 20°C to 50°C) was established by positioning a 60 W incandescent lamp at one site and an ice pack at the opposite side (Paranjpe et al. 2013). Animals free to choose their preferred temperatures for one hour, with temperature sensors attached to their body (type T, 1-mm diameter thermocouples, Omega Engineering) connected to a datalogger which records temperatures measured at the sensors every minute (Eltek® 1000 Series Squirrel Meter Data Logger 64K, 10 Channel 1001WD). The temperature measures during the first ten minutes of the experiment were excluded, since animals were acclimating to the set up (Paranjpe et al. 2013)⁠. The thermal gradient data will be used to determine the range of preferred temperatures of each population, defined as the central 90% range of temperatures registered in the datalogger, averaged among all individuals of each population (Caetano et al. 2019)⁠. I will use a simple ANOVA to test if thermal tolerance limits differ significantly between populations. For lineages which had not yet originated in any of those past times, the thermal preferences of the most recent common ancestor will be reconstructed through Markov Chain Monte Carlo phylogenetic interpolation (Pagel et al. 2004)⁠. All procedures were approved by the ethics in animal use committee of University of Brasília (Process: 33786/2016).

For all spatial analysis described next, I will use WorldClim (Fick and Hijmans 2017)⁠ global climate surfaces (minimum, maximum and average monthly temperatures and total monthly precipitation), for the present (1961-1990), MH, LGM, as well as for the years 2050 and 2070 under carbon emission scenarios RCP 4.5 and RCP 8.5. The same variables for LIG will be obtained from (Otto-Bliesner et al. 2006)⁠. I will also create surfaces of thermally constrained time of activity for each population, considering their thermal tolerances estimated as above, under climate conditions at the present, past and future scenarios described above. For that, I will estimate daily variation in air temperature as a sinusoid ranging from maximum to minimum air temperatures, then account for how much time those temperatures fall inside the species thermal tolerance range (Caetano et al. 2019; Sinervo et al. 2010)⁠, using R package Mapinguari (Caetano et al. 2019)⁠. These estimates will be capped by day length at each location, estimated at each site using Corripio’s method (Corripio 2003)⁠. I will also calculate a vegetation index using dynamic global vegetation models (Sitch et al. 2008)⁠ for all present, past and future scenarios described above.

I will establish circles of 10, 50, 100 and 200 km radius around the point coordinates of focus populations, from which I will extract averages and variances of environmental, ecophysiological and vegetation variables from the present and past surfaces described above. Those values will then be used as predictors of present genetic diversity, using the Random Forest models. I will repeat the modeling using values extracted from each circle size and compare model accuracy to establish which option generates the best predictors. The Random Forest algorithm was chosen due to its robustness to the high levels of collinearity expected from the choice of predictors (James et al. 2013)⁠. I will also include matrices of geographical and phylogenetic distances as covariates to control for those sources of autocorrelation. The accuracy of the models will be assessed through True Skill Statistics (TSS) derived from cross-validation with 30% of the original data set, which will be set aside before model fitting. The importance of predictors will be evaluated by the average Increase in Node Impurity (INI) resulting from their removal from decision trees (James et al. 2013)⁠. The same framework will be applied to models of estimates of population size and migration rates for MH, LIG and LGM, using only predictors for the corresponding time.

How to include the temporal effects on future projections?

I hope the results of this study help guiding efforts to conserve South American biodiversity, by identifying priority areas for conservation, raising awareness of climate change impacts on biodiversity, and hopefully help make the case for the reduction of carbon emissions. I will make the data and statistical analysis used publicly available, to facilitate the replication of this methodology for other regions of the world and other groups of animals and plants.

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I just read your proposal. The main goal is very interesting and appealing, but we need to improve the proposal to make it stronger.

in general, we need to very precisely describing the objectives, which are the main questions that we aim to address here, why they are important, and how you are going to answer them (material and methods).

so, general advice, be much more precise about everything. much more detailed. and skip all the issues that are not related to your proposal, like the amazon!

I would suggest to follow this structure:

Genetic diversity is a key variable that allows populations to adapt to global changes. Examples. what is genetic diversity. why genetic diversity is important. how some populations are in danger because they have few genetic diversity, examples.

Climatic cycles have been pushing species to adapt to the changing conditions. Since 3 million years ago, climate was dominated by glacial-interglacial cycles. blabla. in South America, rainforest and savanas changed their distribution... blabla. examples. In this context, ectotherm species, such as lizards, needed to adapt or perish.

Genetic diversity is linked to Pleistocene refugia (climatically stable areas are more diverse genetically than instable areas). So, past climatic changes are key factors explaining current genetic diversity.

But how current ectotherms are going to deal with the ongoing fast temperature warming?

set 3 main objectives, clearly! be extremely precise about what you want to answer, which data you need to answer it, which methods are you applying, and which results you expect.

and try to relate all with climate change, and why lizards are important and key species in their ecosystems because they are food for other species, control insects, etc.

I have some small edits in your texts.

about your cv, best put your publications first (main author). And it would be better to have at least one out before submitting this!

I hope that my suggestions make sense for you,

take the ones that you think are interesting to re-write the proposal,

all the best,

and sorry for the late answer!