Climate changes effects on the genetic structure of a savanna tree species.

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Background and aims: Climatic fluctuations can act as main agents in species diversification and extinction processes, directly impacting Earth's biota. The present study explores the relationship between climatic variations and genetic structure of *Eugenia dysenterica*, a Brazilian savanna tree species.

Methods: A fuzzy set based integrative analysis called HaploVectors was used in order to analize variables correlation with haplotypes turnover and divergence.

Key Results: Analyses revealed that the genetic differentiation in *Eugenia dysenterica* southeast populations can be explained by past pluviosity variations and mid-Holocene suitability.

Conclusions: The present study shows that climatic variations impact the microevolution of a *E.dysenterica*, supporting that human influence on future climate is likely to affect the genetic structure of this and other Cerrado plants.

Keywords Cerrado, haplovectors, microevolution, genetic structure, climate changes, pluviosity, savanna, phylogeography.

BACKGROUND AND AIMS

Climatic fluctuations are an important part of Earth history, they can act as main agents in species diversification and extinction processes, directly shaping life as we know it (WELTER et al., 2003; HOORN et al., 2010; KOZAK; WIENS, 2010). When it comes to climatic variation glacial and interglacial phases stand out among historical processes. Recent studies exploring the influences of glaciation fluctuations on biotic structure and diversity show a huge range of outcomes, as modifications in geographic distribution, reproduction, and growth, impacting life on a micro and macro scale (DAVIS; SHAW, 2001; HOFFMANN; SGRÓ, 2011; ORDONEZ; SVENNING, 2017).Ordonez e Svenning (2017), for example, demonstrate new selection pressures derived from climate variations in the Quaternary period, while Davis e Shaw (2001) points out how the genetic structure of a Brazilian plant has changed due to climate provoked range modifications in the same period. The Quaternary is especially studied due to the fact that it was the last glaciation period, so recent that we still are in its interglacial phase, which allows a more precise evaluation of its impacts on the current species and populations dynamics (COMES; KADEREIT, 1998).

Most of the processes responsible for population structure and dynamics leave imprints in the genome, DNA analysis is a good alternative for disentangling species changes over time and space (AVISE, 2000). This approach allows us to elucidate evolutionary and ecological mechanisms and also processes responsible for a species current situation (SOUZA et al., 2017; KIJEWSKI et al., 2018). Phy-

logeographic studies are being a fundamental tool in this area, enabling reconstructions of species demographic history and placing genetic structure in time and space (AVISE, 2000; KNOWLES; MADDISON, 2002). By gathering the genetic information obtained from phylogeographic studies with modeled climatic variables for distinct periods of time we can draw a picture of the impact climatic variations exercise over species evolution, and then make better predictions of how future fluctuations may affect life (RUZZANTE et al., 2008; DINIZ-FILHO et al., 2018).

The Brazilian savanna (Cerrado) has been the focus of many genetic and environmental studies (TELLES et al., 2003; DINIZ-FILHO et al., 2016; BOAVENTURA-NOVAES et al., 2018). Cerrado has a history marked by range expansions and contractions; during drier periods Cerrado tended to expand over the forest domain, while during wetter periods the savanna retracted and forests dominated (SALGADO-LABOURIAU et al., 1997; OLIVEIRA; MARQUIS, 2002; PESSENDA et al., 2010). Therefore, changes in precipitation were important variables in the dynamics of Cerrado. As part of Cerrado vegetation, dynamics of *Eugenia dysenterica* populations also changed through time. Lima et al. (2017) pointed out variations in the suitable area for this species. However, a historical refugium seems to have maintained the general stability of the group, being those stable areas responsible for the high genetic diversity of the species (DINIZ-FILHO et al., 2016; LIMA et al., 2017). Despite these conclusions about *E.dysenterica* demographic history, there seems to be a genetic differentiation that can not be explained by the refugia hypothesis alone. Southern populations present distinct haplotypes and also have less genetic diversity compared to the others (LIMA et al., 2017; DINIZ-FILHO et al., 2016; BOAVENTURA-NOVAES et al., 2018). Lima et al. (2017) suggested that local conditions in the Quaternary period may have functioned as a watershed for this population. Diniz-Filho et al. (2016) support the hypothesis proposed by Lima et al. (2017) pointing out that besides climatic variations the actual genetic diversity

in the southern population might have been maintained by stable areas functioning as alleles matrices. However, these hypothesis over how Quaternary climate changes and climatic suitability influences the genetic spatial structure in *E.dysenterica* have not yet been tested.

Adopting an approach recently proposed by Duarte et al. (2019), the following study aims to evaluate the effects of climatic variables and climate changes during the Quaternary period on *E.dysenterica* genetic spatial structure focusing on the evolutionary relationship among its haplotypes. Based on previous studies we expect phylogenetic relationships among *E.dysenterica* haplotypes to be explained by fluctuations in suitability, since this parameter uses climate and other abiotic and biotic features to be generated (DINIZ-FILHO et al., 2016; LIMA et al., 2017). Last, but not less important, we aim to understand how climate changes impact the distribution of natural populations of *E.dysenterica* in the Cerrado, helping the construction of future predictions and conservation actions for this biome.

METHODS

Genetic data and climatic suitability

Our study uses data provided by Lima et al. (2017). Authors collected 333 individuals of *E.dysenterica* distributed across 23 localities in the Cerrado biome (Fig.1). Four regions from chloroplast DNA were sequenced and concatenated for statistical analysis. They also modeled the climatic suitability values for *E.dysenterica* in the Last Glacial Maximum (LGM; 21ka), Mid-Holocene (6ka) and Present-day periods. Here, we used this data added to other databases to explore unanswered questions, in a way, giving continuity to Limas study

Data compilation

Climatic variables were extracted from WorldClim (http://www.worldclim.org/), exception for evapotranspiration and pH, which were collected from the SPEI database (http://spei.csic.es/) and Harmonized World Soil Database (http://www.fao.org/soilsportal) respectively. The evapotranspiration
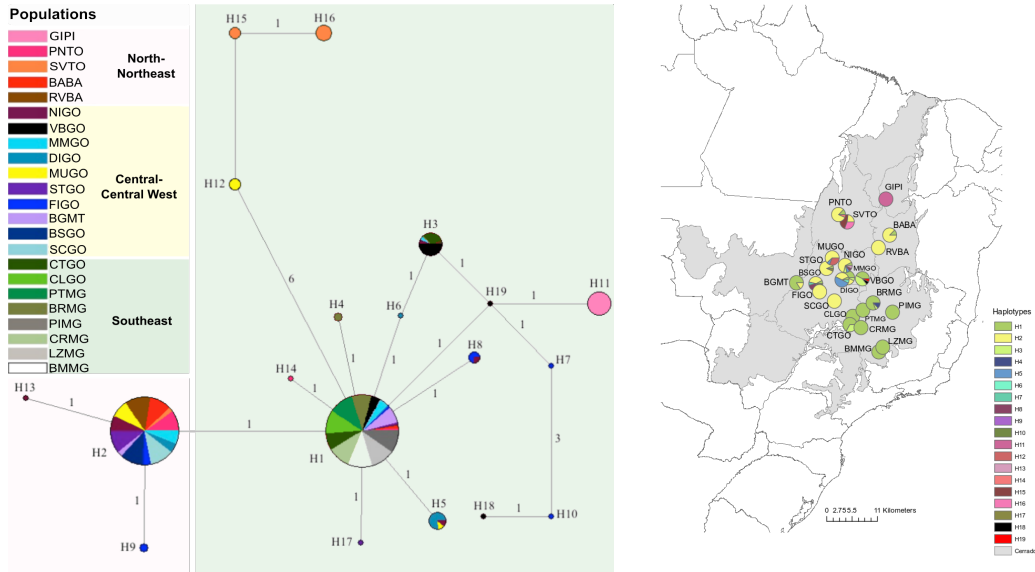
Figure 1: The map presents the geographic distribution of *E.dysenterica* studied populations and haplotypes, while the network shows the evolutive relationship among haplotypes. Font: Lima et al. (2017)

data, after being downloaded, was selected to fit the coordinates which were the closest to this study ones. Cells containing unrealistic values were excluded. Finally, an average of the values was made for each coordinate, which included data from January 1950 until July 2018, we used these average values in our analysis. When it comes to the Worldclim data we downloaded all models of the bioclimatic variables at 5 minutes resolution for the Last Glacial Maximum, Mid Holocene and Present. An average was made from the Global Climate Models (GCMs) available in Worldclim for the Mid Holocene and Last Glacial Maximum.

Analysis
To test the hypothesis of a correlation between genetic distribution and climatic variables we used the function HaploVectors in the package GenVectors in R (available at https://github.com/vanderleidebastiani/Gen -/Vectors). This function explores not only the genetic turnover, but also the genetic divergence between (meta)populations. In other words it analyzes beyond the amount of (meta)population's shared alleles, focusing on the degree of divergence among them.

Statistically speaking GenVectors is a fuzzy set based integrative analysis. It allows us to

extract haplotypic eigenvectors from our data and then perform null model-based tests in order to test the influence of environmental, biogeographic or spatial data on the haplotypic distribution and evolutionary distance between haplotypes. The null model tests used are designed to see if a given environmental or biogeographic factor can explain the genetic turnover among sets of populations, and if that is true verify its relation with the genetic divergence among the population sets. (DUARTE et al., 2019) We also calculated a Pearson correlation between the variables, aiming to test the correspondence among them.

RESULTS

Haplovectors
The analysis exploring the relationship between climatic variables and the genetic structure of *E.dysenterica* is shown in Table 1. The haplovector 1 is responsible for explaining 21% of the variation found, while Haplovector 2 explains 14%.

The Last Glacial Maximum shows significant values for suitability ($P_{turnover} = 0.001$), temperature seasonality ($P_{turnover} = 0.002$), and precipitation ($P_{turnover} = 0.004$; $P_{divergence} = 0.042$) in haplovector 2. As we can see, only

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Table 1: Results obtained by null model tests implemented in HaploVectors with climatic variables (temperature seasonality TS and precipitation) and suitability of LGM, mid-Holocene at present-day, and pH and evapotranspiration of present-day.

Model	LGM			Mid-Holocene			Present-day		
	F_{obs}	$P_{turnover}$	$P_{divergence}$	F_{obs}	$P_{turnover}$	$P_{divergence}$	F_{obs}	$P_{turnover}$	$P_{divergence}$
Haplovector 1 (21%)									
Suitability	0.018	0.412	0.923	0.003	0.204	0.085	0.012	0.935	0.903
TS	0.119	0.160	0.962	0.035	0.186	0.968	0.117	0.041	0.935
Precipitation	0.015	0.349	0.952	0.003	0.595	0.975	0.138	0.087	0.541
Latitude	-	-	-	-	-	-	>0.001	0.893	0.989
Longitude	-	-	-	-	-	-	0.095	0.094	0.961
pH	-	-	-	-	-	-	0.003	0.423	0.098
Evapotranspiration	-	-	-	-	-	-	0.038	0.593	0.944
Haplovector 2 (14%)									
Suitability	0.224	0.001	0.082	0.005	0.074	0.036	0.007	0.382	0.403
TS	0.923	0.002	0.103	0.174	0.014	0.184	0.253	0.006	0.093
Precipitation	0.108	0.004	0.042	0.081	0.013	0.043	0.093	0.117	0.362
Latitude	-	-	-	-	-	-	0.003	0.107	0.068
Longitude	-	-	-	-	-	-	0.208	0.007	0.104
pH	-	-	-	-	-	-	0.003	0.839	0.805
Evapotranspiration	-	-	-	-	-	-	0.153	0.218	0.046

precipitation has significative value for both haplotype distribution and evolutionary relationship, while the others influences only haplotypes distribution.

In the Mid-Holocene the same variables (suitability, TS and precipitation) show significant results in Haplovector 2. However, suitability was significant for haplotype structure instead of the haplotype distribution ($P_{divergence}$ = 0.036 ; $P_{turnover}$ = 0.074)For the Present-day TS showed significant value for Haplovectors 1 and 2 ($P_{turnover}$ = 0.041 ; $P_{turnover}$ = 0.006, respectively) while Longitude showed significance in Haplovector 2 ($P_{turnover}$ = 0.007).

Therefore only the influences of precipitation and suitability on *E.dysenterica* haplotype structure were mediated by evolutionary divergences among haplotypes. As precipitation is the only variable that presents significant $P_{divergence}$ at all the past values, it is the one that better explains the relationships between the species haplotypes.

Pearson correlation test

The Pearson analysis shows a high negative correlation between pluviosity and temperature in Present and Mid Holocene (r = -0.72 and r = -0.51 respectively) and a moderate in LGM (r = -0.49). Although the Haplovectors test has not pointed temperature as significant in shaping genetic features, understanding the relationship between pluviosity and temperature may elucidate questions about how precipitation dynamics work.
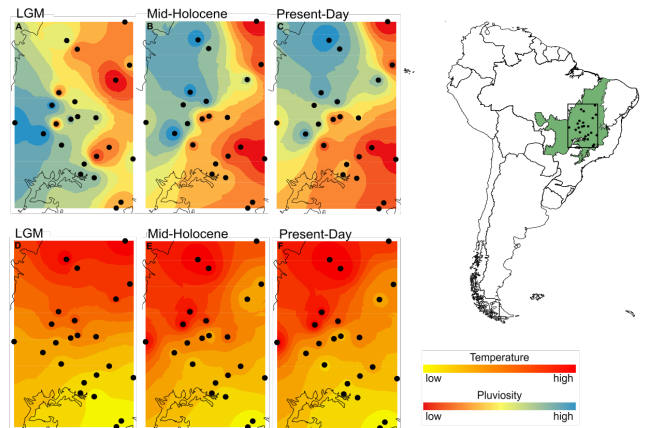

Figure 2: The South America map points out the sample region, demarcated in the rectangle, the green area corresponds to Cerrado original area. Maps A-C represents pluviosity in the Last Glacial Maximum, mid-Holocene and Present while maps D-F represent temperature at the same time periods. Black dots refer to *E.dysenterica* populations in the area.

Pluviosity and Temperature

The temperature maps show that the north-western region had a significant increase in precipitation and temperature through time (Fig.2). While south-eastern populations were submitted to a significant decrease in pluvios-
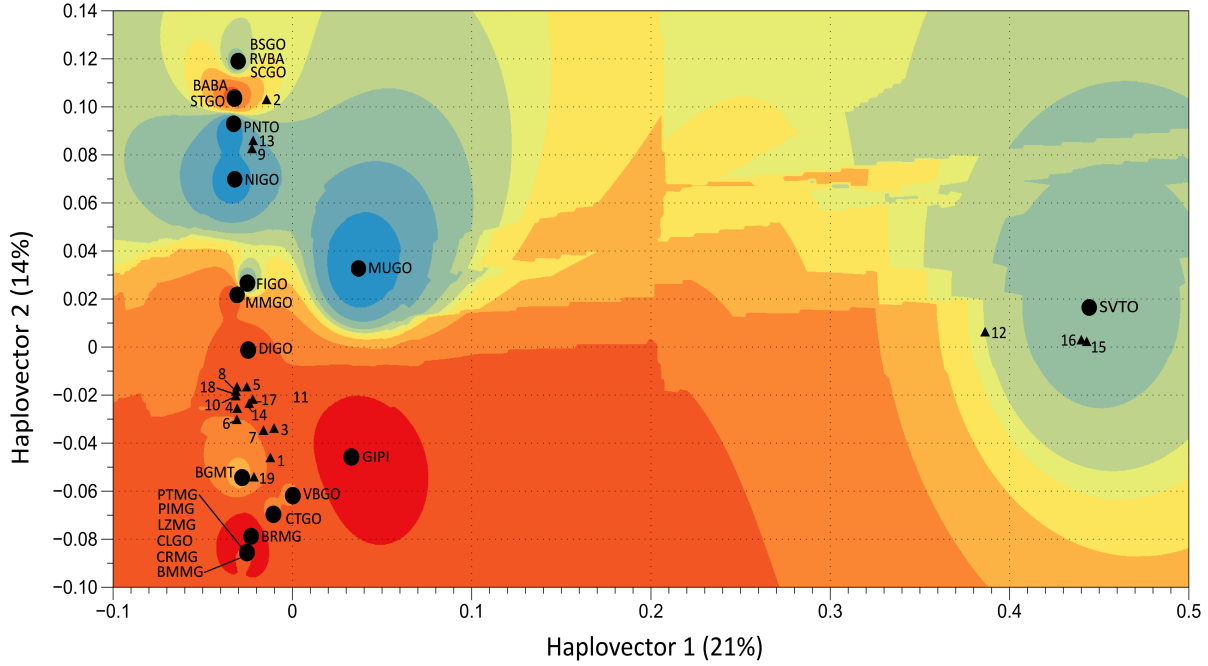
Figure 3: The plot shows the relationship among populations and haplotypes of *E.dysenterica*, through an haplovectores analysis. The y-axis is haplovector 2, and the x-axis haplovector 1, and the colors represent the pluviosity pattern in the mid-Holocene period according to Fig.2 map B.

ity, and a minor increase in temperature.

Haplovectors and pluviosity

Most of the differences between populations and haplotypes can be seen vertically in the plot (Fig.3). The bottom half populations are mostly drier than upper ones, as we can see through the predominance of reddish tones. They also relate to a greater diversity of haplotypes, upper half populations show less genetic diversity, being restricted to a small set of haplotypes: 2, 9 and 13. These differences are geographically reflected since southern populations are mainly located at the bottom of the graph and upper ones tend to the north. In relation to Haplovector 1, we emphasize STVO location at the extreme right of the graph, probably due to the number of haplotypes mutations and the connections between haplotype 12, 15 and 16 (Fig.1).

DISCUSSION

If we analyze Lima et al. (2017) haplotype network we can see that the separation between groups also separates the two most common haplotypes: H2 and H1, however, it is not possible to make conclusions about which factors shaped this diversification. Through the haplovectors approach, we were able to infer that past pluviosity and the mid-Holocene suitability influenced the segregation of the two main haplotypes clusters, impacting the evolutive relationship among *E.dysenterica* haplotypes and shaping its microevolution. The formation of these two distinct population clusters corroborates with Diniz-Filho et al. (2016) and Lima et al. (2017) results, demonstrating the genetic difference between southeastern and northwest populations. Populations clusters vicariance can be seen in Lima et al. (2017) work as a complex dinamics, work as a complex dinamics, in which the predominance of populations in the north or south alternates according to the time period. Although she used time periods before the LGM for this analysis its plausible to assume that this alternated dynamics remained and, at least since the LGM, has been driven by pluviosity variations. This, added to the fact that H1 and H2 have diverged in the Pleistocene period (LIMA et al., 2017), indicate a recent genetic pattern, presumably no fully consolidated. Therefore

future changes in the environment, mainly in pluviosity, could be extremely influential in the species genetic relationships yet to come.

Even though the Pearson analysis found a significant correlation between precipitation and temperature, which lead us to expect both of them to present significant evolutive indexes, temperature does not show any significance. However, both of them show significant values for $P_{turnover}$, justifying the correlation between them and indicating that during punctual periods haplotype distribution can also be explained by the temperature patterns. Despite this the present study use only a selected division of climatic periods, where the oldest data corresponds to the LGM. Literature suggests that past events and other punctual climate variations were also important on Cerrado vegetation distribution (SALGADO-LABOURIAU et al., 1997; OLIVEIRA; MAR-QUIS, 2002; PESSENDA et al., 2010) by excluding these data our study might be concealing important variations for evolutionary relations, what may end up generating false negatives correlations. Hence the association of the present work with studies using different approaches might be fundamental in order to better explain the obtained results and consequently to understand climate variations impacts on Cerrado organisms.

Studies from Diniz-Filho et al. (2016) and Lima et al. (2017) point out population stability as the main responsible for $E.dysenterica$ genetic diversity. They show that suitable conditions for the species have only established in the southwest after the LGM. They also demonstrate that the northeast has maintained itself suitable throughout this period. Our study complements these data, showing that besides stability influence in genetic diversity the main responsible for the genetic structure of the haplotypes is the variation in pluviosity, with a minor influence of suitability changes on the evolutionary relationships of the mid-Holocene. The southeast pluviosity loss after the LGM seems to have an inverse correlation with suitability values, yet the increase of pluviosity in the northeast does not show any relationship with suitability at all. This lack of correlation between suitability and plu-

viosity is corroborated by the Pearson analysis result and might be explained by the influence of other factors that counterbalanced the negative impact pluviosity rise would exert, allowing the permanence of good suitability in the northeast region. The increase in temperature might be a good explanation, due to its strong negative correlation with pluviosity. This would explain how the genetic diversity in the populations of the northwest remained, despite the variation of one of the main agents responsible for its structuring. Corroborating the hypothesis that genetic diversification in Eugenia was based on the presence of stable areas through time, and contextualizing pluviosity in this scenario (DINIZ-FILHO et al., 2016; LIMA et al., 2017).

The area which covers $E.dysenterica$ range also includes other Cerrado species, as Dimorphandra mollis (SOUZA et al., 2017) and Handroanthus ochraceus (VITORINO et al., 2018). D.mollis distribution is mainly in the south of $E.dysenterica$ range, while H.ochraceus distribution is a little more comprehensive than $E.dysenterica$. It is interesting to notice that these species genetic structuration may also be related to pluviosity changes in this area. Souza et al. (2017) found a relation between D.mollis decrease in suitability at the LGM and its genetic pattern, as we can see in our study (Fig.2) the LGM was a period of increased dryness in the south, which might be related with the suitability loss that restricted the species distribution, since precipitation was one of the variables used in the suitability calculation. The opposite happened with H.ochraceus showing a slight expansion of this species in the LGM, probably due to its preferences for dry and hot conditions (VITORINO et al., 2018). Based on that we can speculate that pluviosities changes influenced not only Eugenia but also other trees from Cerrado, maybe even being responsible for the main patterns of genetic structure in the biome. Therefore, anthropogenic impacts in future climate have a great chance of significatively changing not only the genetic structure and diversity of $E.dysenterica$ but also from many other Cerrado trees.

Our study shows the importance of

historical climate fluctuations in shaping *E.dysenterica* genetic structure. Understand the influence of climate on species genetics is important to make accurate predictions of how future climate changes may affect the organisms. These predictions help the conservation of Cerrado and its surrounding biomes, which include the Amazon forest, Atlantic forest, Caatinga, and Pantanal (OLIVEIRA; MARQUIS, 2002; COSTA, 2003). The further exploration of our data may elucidate even more the agents responsible for shaping *E.dysenterica* genetic diversity, the negative correlation between fires and pluviosity (GARDNER, 2006; ALVARADO et al., 2017), for example, might be an indication that fire regimes also affect *E.dysenterica*s microevolution, and that they too are important for Cerrado conservation.

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References

ALVARADO, S. T. et al. Drivers of fire occurrence in a mountainous brazilian cerrado savanna: Tracking long-term fire regimes using remote sensing. **Ecological indicators**, Elsevier, v. 78, p. 270–281, 2017.

AVISE, J. Phylogeography: the history and formation of species harvard univ. **Press, Cambridge, MA**, 2000.

BOAVENTURA-NOVAES, C. R. et al. Genetic drift and uniform selection shape evolution of most traits in Eugenia dysenterica DC. (Myrtaceae). **Tree Genetics and Genomes**, Springer Verlag, v. 14, n. 5, 10 2018. ISSN 16142950.

COMES, H. P.; KADEREIT, J. W. The effect of quaternary climatic changes on plant distribution and evolution. **Trends in plant science**, Elsevier, v. 3, n. 11, p. 432–438, 1998.

COSTA, L. P. The historical bridge between the amazon and the atlantic forest of brazil: a study of molecular phylogeography with small mammals. **Journal of Biogeography**, Wiley Online Library, v. 30, n. 1, p. 71–86, 2003.

DAVIS, M. B.; SHAW, R. G. **Range shifts and adaptive responses to quaternary climate change**. 2001. 673–679 p.

DINIZ-FILHO, J. A. F. et al. **Overcoming the worst of both worlds: integrating climate change and habitat loss into spatial conservation planning of genetic diversity in the Brazilian Cerrado**. [S.l.]: Springer Netherlands, 2018.

DINIZ-FILHO, J. A. F. et al. Spatial autocorrelation analysis and ecological niche modelling allows inference of range dynamics driving the population genetic structure of a Neotropical savanna tree. **Journal of Biogeography**, 2016. ISSN 13652699.

DUARTE, L. et al. Genvectors: an integrative analytical tool for spatial genetics. **bioRxiv**, Cold Spring Harbor Laboratory, p. 330761, 2019.

GARDNER, T. A. Tree–grass coexistence in the brazilian cerrado: demographic consequences of environmental instability. **Journal of Biogeography**, Wiley Online Library, v. 33, n. 3, p. 448–463, 2006.

HOFFMANN, A. A.; SGRÓ, C. M. **Climate change and evolutionary adaptation**. 2011. 479–485 p.

HOORN, C. et al. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. **science**, American Association for the Advancement of Science, v. 330, n. 6006, p. 927–931, 2010.

KIJEWSKI, T. et al. **Random forest assessment of correlation between environmental factors and genetic differentiation of populations: Case of marine mussels Mytilus**. 2018.

KNOWLES, L. L.; MADDISON, W. P. Statistical phylogeography. **Molecular Ecology**, Wiley Online Library, v. 11, n. 12, p. 2623–2635, 2002.

KOZAK, K. H.; WIENS, J. J. Accelerated rates of climatic-niche evolution underlie rapid species diversification. **Ecology Letters**, Wiley Online Library, v. 13, n. 11, p. 1378–1389, 2010.

LIMA, J. S. et al. Demographic stability and high historical connectivity explain the diversity of a savanna tree species in the Quaternary. **Annals of botany**, 2017. ISSN 10958290.

OLIVEIRA, P. S.; MARQUIS, R. J. **The cerrados of Brazil: ecology and natural history of a neotropical savanna**. [S.l.]: Columbia University Press, 2002.

ORDONEZ, A.; SVENNING, J. C. Consistent role of Quaternary climate change in shaping current plant functional diversity patterns across European plant orders. **Scientific Reports**, Nature Publishing Group, v. 7, 2 2017. ISSN 20452322.

PESSENDA, L. C. R. et al. Late Pleistocene and Holocene vegetation changes in northeastern Brazil determined from carbon isotopes and charcoal records in soils. **Palaeogeography, Palaeoclimatology, Palaeoecology**, 2010. ISSN 00310182.

RUZZANTE, D. E. et al. Climate control on ancestral population dynamics: insight from patagonian fish phylogeography. **Molecular ecology**, Wiley Online Library, v. 17, n. 9, p. 2234–2244, 2008.

SALGADO-LABOURIAU, M. et al. Late quaternary vegetational and climatic changes in cerrado and palm swamp from central brazil. **Palaeogeography, Palaeoclimatology, Palaeoecology**, Elsevier, v. 128, n. 1-4, p. 215–226, 1997.

SOUZA, H. A. V. E. et al. A large historical refugium explains spatial patterns of genetic diversity in a Neotropical savanna tree species. **Annals of Botany**, 2017. ISSN 10958290.

TELLES, M. P. D. C. et al. Genetic diversity and population structure of Eugenia dysenterica DC. ("cagaiteira" - Myrtaceae) in Central Brazil: Spatial analysis and implications for conservation and management. **Conservation Genetics**, 2003. ISSN 15660621.

VITORINO, L. C. et al. Demographical expansion of Handroanthus ochraceus in the Cerrado during the Quaternary: Implications for the genetic diversity of Neotropical trees. **Biological Journal of the Linnean Society**, 2018. ISSN 10958312.

WELTER, S. et al. Fingerprints of global warming on wild animals and plants. **Nature**, v. 421, n. 6918, p. 54–57, 1 2003.