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## **RESEARCH ARTICLE - ANTS**

Environmental Response of *Dinoponera lucida* Emery 1901 (Hymenoptera: Formicidae), an Endemic Threatened Species of the Atlantic Forest Central Corridor

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### Abstract

Endemic species of Atlantic Forest Central Corridor may have evolved under adverse climate conditions, and their response to modern climate change is unclear. The aim of this study is to evaluate the response of the endemic and endangered ant species Dinoponera lucida to biotics and abiotics factors based on three scales: ecological factors inside forest fragment, physical attributes of landscape and climatic variables of the assessed region. Data collection took place in a representative selection of forest fragments in the region where the species is distributed in. Pitfalls were used to collect samples and to assure the presence and absence of the species in the site. We also checked the abundance of food resources and applied a hemispherical photography technique to measure shading inside the fragment, in loco. Landscape attributes data and climatic predictors were collected through geoprocessing techniques. All predictors were associated with binary "presence" and "absence" data based applied in logistic models. There was no significant response to environmental aspects within the fragment or to landscape, but there was strong and peculiar response to climatic variables such as temperature and rainfall. Accordingly, D. lucida presents a restricted realized niche, a feature shared among many endangered species that can disappear due to displacement and to habitat loss caused by climate and environmental changes. This species presents all the criteria necessary to be considered as rare, which is a controversial subject with political implications for Espírito Santo state, and makes D. lucida the ideal target for urgent conservation strategies.

### Introduction

The Atlantic Forest biome is distributed along a vast extension of the Brazilian coast. Its territory covers tropical and subtropical regions and is considered one of the 35 hotspots (Myers et al., 2000; Mittermeier et al, 2004). Nowadays, this biome only counts on approximately 10% of its original distribution area (Silva & Castelleti, 2003, Ribeiro et al., 2009, Sloan et al., 2014). Deforestation has been causing environmental fragmentation that increases the number of habitat remnants and the distance between them, given the decrease in habitat-remnant sizes. Therefore, species adapted to conditions inside the forest are exposed to intense population decline (Bender et al., 1998; Ewers & Didham, 2007; Ewers & Banks-Leite, 2013).

One of the most threatened portions of this tropical forest is the Atlantic Forest Central Corridor (AFCC), a region under preservation priority that covers the south of Bahia state and the whole Espírito Santo state. It is responsible for the north-south connection of the Atlantic Forest and has a remarkable biological representation for the biome (Brasil, 2015).



Among endemic species in the AFCC, one finds the ant *Dinoponera lucida* Emery (1901), which belongs to the In-Danger category in the Red Book of the Brazilian Fauna Threatened with Extinction (ICMBio, 2018). This species presents low dispersion capacity and aggregated distribution pattern within fragments, nests with few individuals and distribution limited to Southern Bahia, Eastern Minas Gerais and Espírito Santo states (Campiolo et al, 2015; ICMBio, 2018, see Fig 1). This region faces intense deforestation pressure and landscape fragmentation, but *D. lucida* response to such environmental pressures remains poorly understood.

Assumingly, *D. lucida* is quite sensitive to the internal conditions of the fragment, to landscape structure and to regional climate, mainly to temperature and rainfall, given its biological features and degree of threat in their habitat. Therefore, the aim of this study was to evaluate *D. lucida* response to environmental components, to landscape attributes and to climatic variables - at local and regional scales. Our hypotheses were that the presence and abundance of *D. lucida* would be negatively influenced by the shortage of biomass as food supply and low shading within the forest fragment, small area of the fragment, irregular shape and high levels of isolation between fragments in the landscape, and extreme climatic conditions in their distribution region.

## **Material and Methods**

The spatial-ecological analysis was applied to assess *D. lucida* response to the environment at (a) microsite, (b) localities and (c) mesoscale levels, according to Leibold et al. (2004) recommendations. Its response to factors inside the fragment corresponds to the local level (= Microsite), and it was assessed in fragments in Espírito Santo state (Fig 1). The response to the landscape as a set of fragments corresponds to the landscape level (= Patches or Localities), which, together with the regional response analysis (= Mesoscale), covered the Southern Bahia and Espírito Santo states (Fig 1), where the response to climatic variables was also assessed.

Aligned pitfalls in 450 meters long linear transects emerged as an appropriate method to collect *D. lucida* representatives in the fragment, since it is expected that the ant does not have a homogeneous distribution, but presents aggregate patterns (Peixoto et al., 2010). Each transect had 10 pitfalls, which were separated 50 meters from each other. The number of transects used in each fragment was proportional to the logarithm of the fragment's area, which ranged from one to four transects. In total, there were 300 pitfalls distributed in 30 transects, on 10 forest fragments and conservation unities listed in Appendix 1-A. The transects were plotted between December and March, 2013.



Fig 1. Data sampling points of this study and current geographic distribution of D. lucida, according to ICMBio (2018).

Sampling was conducted under the permissions ICMBIO nº 35895-1 and nº 35947-1. The collected specimens were identified by the authors based on Lenhart et al. (2013), and were added to the collection at the Laboratory of Communities Ecology of Federal University of Viçosa.

**Microsites:** Species representatives, animal biomass (various invertebrates collected within the pitfalls, surrogate of food resources measure) and shading were recorded in 286 points distributed throughout the 10 fragments mentioned above (Appendix 1-A), since, from the 300 placed pitfalls, 14 were removed or lost. Pitfalls stayed on the ground for 48 hours to collect animal biomass. The contents found in the pitfalls were screened, dried in oven at 45 °C for 24 hours and weighed in Gehaka analytical scale (model AG200).

For shading measurement, hemispherical pictures of the canopy were taken with Fisheye 0.45 mm x 28 mm lens attached to a Nikon Coolpix 4500 4Mpx digital camera vertically positioned on the ground. They were analyzed in the Gap Light Analyzer software version 2.0 in order to calculate canopy opening, which was used as an indicator of environmental conditions in the forest.

Data about the presence and absence of the species were analyzed based on logistic regression models using quasibinomial distribution in R environment. Data were logtransformed in the model, because biomass data were too close to zero. This calculation was conducted in order to conform the data to the linearity of the independent variable and to the log odds (Saupe et al., 2015).

Landscape: *D. lucida* response to the physical attributes of the landscape (area, fragment shape and connectivity) was evaluated in 30 forest remnants in Bahia and Espírito Santo states (list in Appendix 1-B), where the presence or absence of the species were previously verified. Those physical attributes resulted from data provided by the *SOS Mata Atlântica* Foundation, at ratio 1:50,000 in the shapefile format, made available by the Laboratory of Image Processing and Geoprocessing (Lapig, 2015). Data analysis was performed in the free version (2.18) of the QGIS software in the Sirgas projection system 2000 UTM for zone 24.

Shape calculations of each fragment were based on the Pantton index adapted to metric units:  $If = P/[200.(\pi.At)^{0.5}]$ ,

wherein  $\mathbf{P}$  is the perimeter in meters and  $\mathbf{At}$  is the total area of the fragment in hectares. This index measures shape as a circularity deviation, so that the **If** value is close to 1.0 when the polygon is almost round-shaped, which increases depending on the complexity of its boundaries (Laurance & Yensen, 1991).

The connectivity analysis was performed through the visual classification of all remnants in a 10 km radius from the edge of each of the 30 assessed fragments. The area occupied by forest remnants around the fragment was calculated as the fraction of the total surrounding area within the 10 km radius. The Connectivity Index (Ic) expresses the proportion of forested area around the central remnant, which is used as connectivity indicator: zero (0) indicates complete isolation and one (1) indicates total connectivity (Simon et al., 2018, in print). The final model was based on logistic regression; the binary data of *D. lucida* presence in the fragments were related to landscape attributes by taking into consideration the quasibinomial distribution. The variable area was log-transformed, given its high variability.

**Regional scale:** Data from 19 climatic variables concerning temperature and rainfall were collected to analyze population response to climate (listed in Appendix 2). They were expressed in the raster format at 30-second resolution, released and made available at Worldclim (version 2.0) (Fick & Hijmans, 2017).

Climatic predictors of each point were remotely collected in the QGIS software (version 2.18) by using the Sirgas 2000 projection system. A previous selection of climatic variables was made through Principal Components Analysis (PCA) associated with the biological plausibility criteria and exclusion of variables in the correlation matrix, since climatic variables usually present strong collinearity. PCA was conducted in the factoMineR and factoextra Packages for R. The selected predictors were applied to the multivariate logistic regression model with quasibinomial distribution. After the significant predictors were selected, the Student's t-test was used to compare the means of each predictor in the presence and absence responses.

In all the analysis levels, presence records were assigned as 1 and absence records were assigned as 0.

Fig 1. Data sampling points of this study and current geographic distribution of D. lucida, according to ICMBio (2018).

Climatic predictor	p-value	Presence (1) $(n = 154)$	Absence (0) $(n = 96)$	Test t Student
Temperature Annual Range	5.76e-07	$\frac{XX}{\sigma_{x}}:14.47$ $\sigma_{x}: 0.466$ S <sup>2</sup> : 33.4539	$\frac{XX}{\sigma_{x}}: 14.91 \\ \sigma_{x}: 0.782 \\ S^{2}: 58.6843$	$\frac{\underline{X}\underline{X}_{1}}{\text{p-value}} = 2.855\text{e-}06$
Annual Mean Temperature	0.000135	$\frac{X:X}{\sigma_{x}: 1.103}$ S <sup>2</sup> : 187.232	$\frac{XX}{\sigma_{x}}: 22.59$ $\sigma_{x}: 1.846$ $S^{2}: 327.0748$	$\frac{XX}{p-value} = 6.672e-04$
Precipitation of Driest Month	0.004738	$\frac{XX}{\sigma_{x}}: 41.48$ $\sigma_{x}: 0.423$ $S^{2}: 27.5192$	$\frac{XX}{\sigma_{x}}: 41.54 \\ \sigma_{x}: 0.629 \\ S^{2}: 37.9489$	$\frac{XX}{p-value} = \frac{XX}{0}$



Fig 2. A-C: Significant Climatic Predictors according to Presence and Absence data.

#### Results

*D. lucida* representatives at the three levels were found to be: 48% of pitfalls inside the assessed fragments (local scale), 83% of the 30 assessed landscape fragments (landscape scale) and in 65% of the 250 analyzed points, at regional level. The presence of the species was verified in different vegetation formations of the Atlantic Forest, including the Dense Ombrophilous Forest, and its transition to Restinga, as well as in the Semidecidual Seasonal Forest.

Ant populations did not show significant response to environmental components inside the fragment (biomass (p-value = 0.1959) and shading (p-value = 0.1069)) or to landscape attributes (area (p-value = 0.336), shape (p-value = 0.655) and fragment connectivity (p-value = 0.827)).

However, based on the regional scale, *D. lucida* recorded significant responses to three climatic predictors. The assessed populations showed remarkable sensitivity to temperature and to minimum rainfall conditions. They mainly settled in regions presenting higher annual temperatures and lower thermal seasonality. The variance of the response stands out, since presence records flow close to the mean (low variance), whereas absence data show broader dispersed behavior (high variance) (Table 1; Fig 2).

## Discussion

## Local and landscape scales

Understanding the responses and behaviors of species at different scales is essential to model and predict their future (Leibold et al., 2004). Endemic species are mainly threatened by environmental changes, therefore, knowing their distribution and responses to microclimatic factors within the limits of their habitat is essential to the development of biologically valuable conservation interventions (Hannah et al., 2014).

*D. lucida* responses to ecological factors inside the fragment or to landscape structure were not verified in this study. Results suggested greater tolerance than the expected for food supply variations, which is probably due to its omnivorous and generalist eating behavior (Peixoto et al., 2010).

As the Atlantic Forest is a biome with a substantial variety of vegetal formations, *D. lucida* did not show response to the shading in the fragment. For ant communities diversity, shading inside the fragment is an overall important predictor, whereas, on the other hand, lack of response from these organisms to landscape structure in the Atlantic Forest (Santos et al., 2006), Caatinga (Gomes & Leal, 2010) and in the Amazon (Carvalho & Vasconcelos, 1999) seems to be common. Diversity patterns are observed in studies involving a whole set of ant species (Gascon et al., 1999; Schoereder et al., 2004; Bieber et al., 2006; Gomes et al., 2010), but such patterns cannot be seen when a single species is assessed. Assumingly, the response of these organisms to landscape is modulated by other, yet unknown, biological factors.

Except for the recent anthropogenic pressure on natural environments, the current landscape configuration results from past events closely related to climate. Resende et al. (2010) suggested that *D. lucida* is adapted to small forest remnants and, therefore, it might not respond to area fragmentation. This outcome may be an evolutionary inheritance of selective fragmentation pressures during the Pleistocene, which generated the high genetic and karyotypic diversity of the species (Mariano et al., 2004; Mariano et al., 2008; Santos et al., 2012; Simon et al., 2016).

The lack of *D. lucida* direct response to landscape found in this study may indicate that it is not the spatial configuration itself, but the factors derived from it. It should be noted that it may also derive from the study limitations, since the research was conducted in the region where the species is distributed in. Even though sampling within fragments provided a balanced proportion of presence and absence data, species representatives were found in most of the fragments and there were only a few records of species absence in the database. It takes substantial effort to assure species absence reliability. Presumably, increased absence records would allow detecting responses at regional levels, although it was not verified in this study.

In addition, it is important to highlight that, although data sampling has covered a considerable portion of the species current distribution, it may be possible to verify a different response to landscape configurations in the states of Bahia or Minas Gerais, where Atlantic Forest deforestation rates are still higher when compared to the state of Espírito Santo (SOS Mata Atlântica, 2018; SOS Mata Atlântica & INPE, 2019).

### Regional Scale: climate, niche and habitat

Ströher et al. (2019) emphasize the importance of climatic response patterns are becoming clearer on the Atlantic Forest history, even though the underlying mechanisms such as how the niche of each species affects the way they are influenced by the climate and vegetation

changes needs to be further investigated. The relation between the species and its environment throughout evolutionary time generates the ecological niche of the species itself. This niche corresponds to the environmental space available to the species, which is limited by environmental factors that determine species distribution in space (Whittaker et al., 1973). Beyond the biotic components, the niche of *D. lucida* depends on the combination of at least three climatic predictors, whereas the niche projection in physical space results in a limited region range which presents satisfactory environmental suitability.

Different from the expected, the annual rainfall volume does not influence *D. lucida* settlement. Rainfall was only significant in the driest month; however, the mean rainfall between sites presenting, or not, the species was the same. The mean itself can mask important biological effects in biological studies, so variance becomes informative of the ecological responses of the species. Based on variance, *D. lucida* gets close to the average to the values of rainfall in the driest month. The species does not tolerate locations drier or wetter than the average during the dry season.

Species tolerance to minimum rainfall values seems to restrict the species presence to Ombrophilous and Semidecidual forests, as water stress in the dry season gets more intense as the perennial forest turns deciduous (Saiter et al., 2015). In addition, given the species low tolerance to annual temperature variation, its populations prefer to settle in places presenting temperature variation close to the regional average. Climatic seasonality seems to be a determining factor for the species settlement in its own region, a fact that contributes to shape the geographic distribution of the species.

Range of geographic boundaries and suitable habitat availability are important dimensions of the hypervolumetric environmental space of the species, which is more important to face extinction threats than the tolerance of the species to limiting factors and its adaptation skills. The ability of the species to occupy its geographical space by filling its corresponding environmental space may be one of the most important aspects of its resistance to extinction (Saupe et al., 2015). This issue is a concern since potential distribution analysis predicts that the occurrence of the species tends to expand to its southern limits in a climate change scenario (Campiolo et al, 2015), where Espírito Santo becomes even more important and responsible for the preservation of this and other species with similar urgencies.

## Rarity, threat and implications

The region where *D. lucida* is currently distributed in, can be further reduced due to habitat loss caused by deforestation processes and also due to climatic and environmental changes (Campiolo et al, 2015). The Dense Ombrophilous and Semideciduous Seasonal forests are seriously threatened. Estimates point towards their intense geographic reduction due to climatic changes, which pose even more risks on highly vulnerable ecosystems (Follador, 2016).

Recently, *D. lucida* was inadvertently removed from the state of Espírito Santo list of watched out species for political and economic motivations, while it has evolved from vulnerable (VU) to endangered (EN) status in the Brazilian National List (ICMBIO, 2018). However, the aforementioned information shows that it has the profile that matches one of the seven definitions of rare species, since it is locally abundant in a specific and geographically limited habitat (Rabinowitz, 1981), presenting a high endogamy rate and consequently low genetic diversity within populations (Resende et al, 2010; Simon et al, 2016). These conditions are more threatening for social insects because of their reduced population effective size, even though a high number of individuals, suggests great abundance in ecological terms (Wilson, 1963; Chapman and Bourke, 2001).

The concept of rarity presented here takes into account small geographic range, narrow habitat specificity and small local population size (or effective size, for social insects) presented by Rabinowitz (1981), and can be applied to all endemic species in the AFCC, which have very similar evolutionary backgrounds (Carnaval et al., 2009; Alvarez-Presas et al., 2013), and regions with high levels of endemicity should receive full and unquestionable consideration (Fattorini, 2010).

The present results provide the basis for future predictions about the impacts of environmental changes on representatives of this species distributed in a very relevant biological portion of the Atlantic Forest. Therefore, the collected data are extremely important for those who plan biodiversity conservation strategies, since preservation actions developed for one specific endemic species can be extrapolated to several species that have evolved under similar environmental conditions.

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## **APPENDIX 1**: List of the sampling locations.

## A. 10 forest fragments at (a) microsite level.

Studied locations	Latitude	Longitude
Private Property in São Rafael	-19.378583	-40.437583
Fazenda Sayonara Private Reserve of Natural Heritage	-18.494583	-39.955267
Pau a Pique Private Reserve of Natural Heritage	-20.127964	-40.552563
Private Property in Regência	-19.633744	-39.881015
Duas Bocas Biological Reserve	-20.272617	-40.4797
Córrego do Veado Biological Reserve	-18.3703	-40.141567
Linda Lais Private Reserve of Natural Heritage	-19.952467	-40.752517
Sooretama Biological Reserve	-19.0446	-40.005583
Goytacazes National Forest	-19.435983	-40.0733
Augusto Ruschi Biological Reserve	-19.851683	-40.5633

APPENDIX 2: List of all 19 climate variables evaluated in this study.

Code	Variable name
BIO1	Annual Mean Temperature
BIO2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3	Isothermality (BIO2/BIO7) (* 100)
BIO4	Temperature Seasonality (standard deviation *100)
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7	Temperature Annual Range (BIO5-BIO6)
BIO8	Mean Temperature of Wettest Quarter
BIO9	Mean Temperature of Driest Quarter
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality (Coefficient of Variation)
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter
BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter

B. 30 forest fragments at (b) landscape and (c) mesoscale level.

Studied locations	Latitude	Longitude
Private Property in São Rafael	-19.378583	-40.437583
Fazenda Sayonara Private Reserve of Natural Heritage	-18.494583	-39.955267
Pau a Pique Private Reserve of Natural Heritage	-20.127964	-40.552563
Private Property in Regência	-19.633744	-39.881015
Duas Bocas Biological Reserve	-20.272617	-40.4797
Córrego do Veado Biological Reserve	-18.3703	-40.141567
Linda Lais Private Reserve of Natural Heritage	-19.952467	-40.752517
Sooretama Biological Reserve	-19.0446	-40.005583
Goytacazes National Forest	-19.435983	-40.0733
Augusto Ruschi Biological Reserve	-19.851683	-40.5633
Private Property in Guaratinga	-16.598277	-39.919411
Private Property in Itamaraju	-16.87	-39.65
Private Property in Prado	-17.3	-39.38
Private Property in Serra dos Aimorés	-17.831675	-40.279922
Private Property in Ibirapuã	-17.698916	-40.231192
Private Property in São Antônio do Canaã	-19.844816	-40.646719
Private Property in São João de Petrópolis	-19.804809	-40.672172
Private Property in Cariacica	-20.253568	-40.462747
Private Property in Viana	-20.406439	-40.461068
Private Property in Mathilde	-20.557557	-40.815533
Private Property in Marechal Floriano	-20.4	-40.66
Private Property in Domingos Martins	-20.35	-40.65
Goiapaba-Açu Municipal Park	-19.913928	-40.473891
David Victor Farina Natural Municipal Park	-19.930836	-40.12966
Morro do Aricanga Natural Municipal Park	-19.821565	-40.331407
Private Property in Mata Fria, Santa Maria de Jetibá	-19.990993	-40.797359
Vale Natural Reserve	-19.153167	-40.019667
Santa Lúcia Biological Station	-19.97325	-40.528667
São Lourenço Municipal Park	-19.928262	-40.608416
Embrapa Marilândia	-19.405004	-40.554187