UNIVERSIDADE FEDERAL DE UBERLÂNDIA
INSTITUTO DE BIOLOGIA

Programa de Pós-graduação em Ecologia, Conservação e Biodiversidade

VULNERABILIDADE DE FORMIGAS DO CERRADO AO AQUECIMENTO GLOBAL: INFLUÊNCIA DE CARACTERÍSTICAS MORFOLÓGICAS, COMPORTAMENTAIS E ECOLÓGICAS

LINO ABDELNOUR ZUANON

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Tese apresentada à Universidade Federal de Uberlândia, como parte das exigências para obtenção do título de Doutor em Ecologia, Conservação e Biodiversidade.

Orientador

Prof. Dr. Heraldo Luis de Vasconcelos

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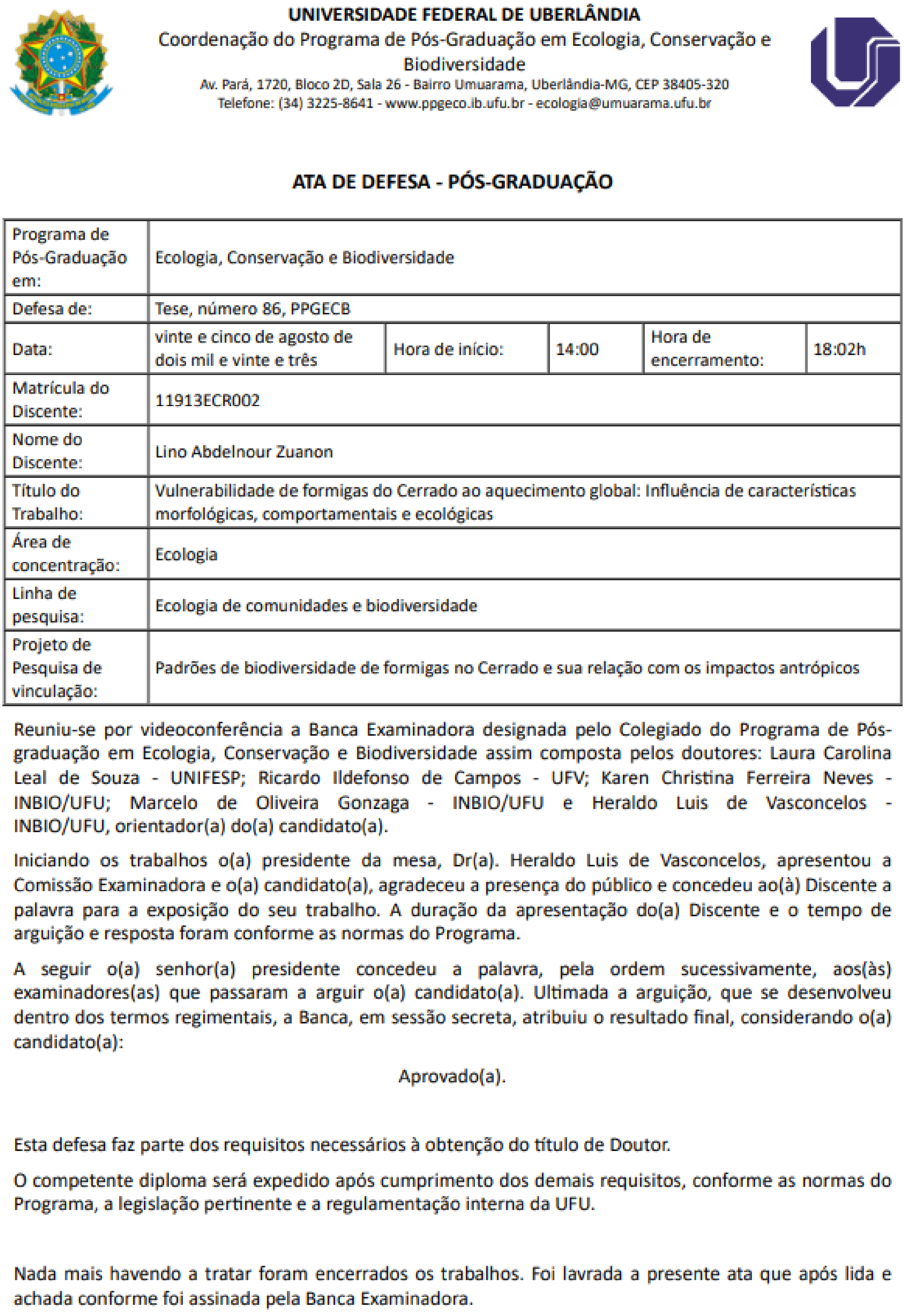

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ATA DE DEFESA - PÓS-GRADUAÇÃO

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Iniciando os trabalhos o(a) presidente da mesa, Dr(a). Heraldo Luis de Vasconcelos, apresentou a Comissão Examinadora e o(a) candidato(a), agradeceu a presença do público e concedeu ao(à) Discente a palavra para a exposição do seu trabalho. A duração da apresentação do(a) Discente e o tempo de arguição e resposta foram conforme as normas do Programa.

A seguir o(a) senhor(a) presidente concedeu a palavra, pela ordem sucessivamente, aos(às) examinadores(as) que passaram a arguir o(a) candidato(a). Ultimada a arguição, que se desenvolveu dentro dos termos regimentais, a Banca, em sessão secreta, atribuiu o resultado final, considerando o(a) candidato(a):

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A todos que direta ou indiretamente contribuíram para a minha formação e desenvolvimento dessa tese

- Aqui, finja que isso é uma semente.

- É uma pedra!

- Eu sei que é uma pedra, mas vamos fingir só por um minuto que é uma semente, tá? Vamos tentar imaginar... Olha essa árvore. Tudo que formou essa árvore enorme já estava dentro dessa sementinha pequenininha! Ela só precisou de um tempinho, um pouco de sol e chuva e VOILÁ!

-Essa pedra vai virar uma árvore?

-A semente! Tem que colaborar, tá?! Você pode até achar que não pode fazer muita coisa agora, mas isso é porque ainda não é uma árvore. Você só precisa ter paciência, ainda é uma semente.

-Mas é uma pedra...

Diálogo entre formigas – Vida de inseto (1998)

Sumário

Resumo geral

O aquecimento global é uma das maiores ameaças à conservação da biodiversidade. Embora a taxa de aquecimento seja mais alta em latitudes mais elevadas, entre os organismos ectotérmicos aqueles que estão sobre maior risco são os que habitam os trópicos, e nesse sentido é urgente entender mais sobre a biologia termal desses organismos. Em meu estudo explorei fatores ecológicos e comportamentais que podem ajudar a explicar a tolerância termal e vulnerabilidade ao aquecimento global de formigas do Cerrado. Primeiramente busquei responder se o tipo de habitat e sítio de nidificação ajudam a explicar a tolerância termal e vulnerabilidade de formigas ao aquecimento global. Para isso, testei a tolerância termal de 64 espécies presentes no solo ou vegetação de áreas de cerrado sentido restrito e floresta semidecídua. Ainda, medi a variação de temperatura entre esses ambientes e estratos. Meus resultados indicam que o tipo de vegetação (cerrado ou floresta) afeta a tolerância ao calor, mas não ao frio, enquanto o estrato de nidificação (solo ou arbóreo) afeta a tolerância ao calor e ao frio. Além disso, encontrei evidências de um forte sinal filogenético na resistência ao calor e na amplitude termal. Quanto a vulnerabilidade ao aquecimento global, encontrei que formigas em áreas de cerrado sentido restrito estão sob maior risco que formigas da floresta semidecídua. No cerrado, as formigas de solo podem ser mais vulneráveis ao aquecimento global que as formigas arbóreas, enquanto que em florestas as formigas arbóreas são as mais vulneráveis. Em segundo lugar, busquei determinar se existe plasticidade espacial e temporal na tolerância termal de formigas do Cerrado. Para isso, testei a tolerância termal de espécies generalistas de habitat que ocorrerem tanto no cerrado sentido restrito quanto na floresta semidecídua. Além disso, avaliei se a tolerância termal varia sazonalmente. Encontrei que a tolerância ao frio varia sazonalmente independentemente do tipo de vegetação ou sítio de nidificação da espécie de formiga. Também encontrei que a tolerância ao calor é maior para comunidades de formigas do cerrado sentido restrito do que as de floresta semidecídua. Finalmente, realizei um experimento de suplementação de carboidrato e proteína para formigas arbóreas com intuito de entender os efeitos da disponibilidade de nutrientes para essas formigas. Observei que tanto a disponibilidade de carboidratos quanto a de proteína aumentaram a riqueza de espécies e a abundância de formigas forrageando nas árvores. Entretanto, apenas a disponibilidade de açúcar aumentou a tolerância termal e o tamanho das colônias em ninhos artificiais. Ainda, a porcentagem de dano por herbívoros em folhas da árvore com maior disponibilidade de açúcar foi menor que em árvores onde não houve suplementação de açúcar. De forma geral, meu estudo reforça a ideia de que variações climáticas dentro e entre ambientes afetam a tolerância termal dos organismos ectotérmicos, indicando que estudos na escala local parecem ser fundamentais para avaliar os efeitos do aquecimento global sobre esses organismos.

Palavras-chave: Ecologia termal; Formicidae; Microclima; Limite termal mínimo; Limite termal máximo; Aclimatação, Sazonalidade; Ecologia nutricional, Dieta.

General abstract

Global warming is one of the biggest threats to biodiversity conservation. Although the rate of warming is higher at higher latitudes, it is the tropical ectothermic organisms that are the most risk . In this sense, it is urgent to understand more about the thermal biology of these organisms. In my study I explored some of the ecological and behavioral factors that may help explain the thermal tolerance and vulnerability to global warming of Neotropical savanna (Cerrado) ants. Firstly, I sought to understand the extent to which the type of habitat and nesting/foraging stratum help to explain the thermal tolerance and vulnerability to global warming of these ants. For this, I measured the thermal tolerance of 64 species present on the ground or in trees, both in of savanna (*cerrado sentido restrito*) and semi-deciduous forest areas, and measured the temperature variation in different vertical strata of these two habitats. The results suggest that vegetation type (savanna or forest) affects the heat but not the cold tolerance, whereas the nesting stratum (ground or arboreal) affects both the heat and cold tolerance. Furthermore, I found evidence of a strong phylogenetic signal in the heat and range of thermal tolerances. I also found evidence that savanna ants are more vulnerable to global warming than are ants from the semideciduous forest. Furthermore, in savannas, ground ants seem to be more vulnerable than arboreal ants, while the opposite is true in forests. Secondly, I evaluated if thermal tolerance is a plastic trait among Cerrado ants. For this, I measured the thermal tolerance of habitat generalist species that occurred both in savannas and forests. In addition, I evaluated if the thermal tolerance of species from a given habitat varies seasonally. I found that cold tolerance varies seasonally regardless of the type of vegetation or nesting/foraging stratum where the species occurred. I also found that savanna ants have a greater heat tolerance than those from the forest. Finally, during one year, I performed a carbohydrate and protein supplementation experiment for arboreal ants in order to understand the effects of nutrient availability for these ants. I found that both carbohydrate and protein availability increased the species richness and overall abundance of ants foraging in trees. However, only the supplementation of sugar increased the thermal tolerance and the size of colonies in artificial nests. Trees that received sugar presented lower levels of herbivore damage in their leaves than those from the remaining supplementation treatments. Overall, my study reinforces the idea that climatic variations within and between habitats affect the thermal tolerance of ectothermic organisms, indicating that studies at the local scale are essential to understand the effects of global warming on these organisms.

Keywords: Thermal ecology; Formicidae; Microclimate; Critical thermal minimum; Critical thermal maximum; Acclimation, Seasonality; Nutritional ecology, Diet.

Introdução geral

As mudanças climáticas, principalmente o aquecimento global, são uma das maiores ameaças à conservação da biodiversidade (Parmesan 2006). De forma geral, a temperatura dos ambientes está cada vez mais alta e variável, e estimativas indicam que a temperatura média global irá aumentar cerca de 1,5°C nas próximas duas décadas (Deutsch 2008, Stocker et al. 2013, IPCC 2021). Embora a taxa de aquecimento seja mais alta em latitudes mais elevadas, entre os organismos ectotérmicos, aqueles que estão sobre maior risco são os que habitam os trópicos (Huey et al. 2009, Diamond et al. 2011). Isso acontece porque a variação latitudinal no limite termal máximo dos ectotérmicos (temperatura em que se perde o controle muscular) não é tão grande quanto a variação latitudinal da temperatura do ar. Logo, espécies tropicais de ectotérmicos vivem em locais com temperaturas mais próximas de seus limites termais máximos e, assim, um pequeno aumento de temperatura já pode significar redução de fitness (Deutsch et al. 2008, Sunday et al. 2011). Considerando que os ectotérmicos representam boa parte da biodiversidade terrestre (Wilson 1992), e que a região tropical é a de maior biodiversidade do planeta (Gaston 1996, Willig et al. 2003), torna-se urgente entender a biologia termal desses organismos para poder avaliar as suas respostas frente às mudanças climáticas globais.

Particularmente, é importante entendermos até que ponto os limites termais das espécies tropicais se correlacionam com as condições climáticas do ambiente onde elas vivem, já que isto pode nos ajudar a melhor entender tanto os padrões biogeográficos de composição das comunidades, quanto também elaborar estratégias de conservação mais eficientes. Todos os ambientes terrestres são formados por uma variedade de microclimas relacionados à diferenças na vegetação, solo e topografia (Porter & Gates 1969, Potter et al. 2013), e esses microclimas correspondem às condições que os organismos lidam durante sua vida. Nesse sentido, o entendimento adequado da tolerância termal das espécies, especialmente de pequenos ectotérmicos, requer atenção na escala local (Potter et al. 2013). De acordo com a Teoria de Adaptação Termal (Kaspari et al. 2015), as espécies são fisiologicamente adaptadas à temperatura de seu ambiente. Visto que é altamente dispendioso energeticamente manter os limites térmicos acima do necessário (Angiletta 2009), a capacidade das espécies de tolerar o calor e o frio deve estar relacionada com a temperatura máxima e mínima a que as espécies estão expostas em seu habitat (Janzen 1967). De modo geral, a tolerância ao frio dos organismos segue o previsto pela Teoria da Adaptação Termal (Araújo et al. 2013), enquanto que a tolerância ao calor costuma ser melhor explicada pelo conservadorismo filogenético e assim ser menos influenciada por variações geográficas ou climáticas (Diamond & Chick 2018, Pintanel et al. 2019).

Entretanto, a maioria dos estudos que exploram a capacidade das espécies de tolerar o calor ou o frio consideram os limites termais como traços fixos e ignoram a possibilidade de plasticidade (Sasaki & Dam 2021). A plasticidade termal, que é a capacidade de um organismo de alterar seus limites térmicos superiores e inferiores, é uma possível adaptação fisiológica e um fator crítico de proteção das espécies contra os efeitos negativos da variação térmica, seja ela temporal ou espacial (Gunderson & Stillman 2015, Machekano et al. 2021). A plasticidade termal está amplamente presente entre os ectotérmicos, podendo variar tanto entre espécies quanto entre populações, e é influenciada por fatores como a frequência de temperaturas extremas, a sazonalidade ambiental (Gunderson & Stillman 2015, Rodrigues et al. 2021) ou até mesmo a dieta, uma vez que que o nível de consumo de açúcar e proteína está relacionado com a capacidade de insetos de resistir a temperaturas extremas (Andersen 2010, King et al.

2015, Bujan & Kaspari 2017). Algumas espécies, principalmente as mais generalistas, podem ocorrer em ambientes de estruturas altamente contrastantes, tendo que lidar com condições também variáveis de temperatura e disponibilidade de recursos, por exemplo. De forma semelhante, todos ambientes, mesmo que menos intensamente, estão sujeitos a variações sazonais de temperatura, e os organismos devem estar adaptados para lidar com esse tipo de flutuações. Sendo assim, entender o potencial de adaptação das espécies e até que ponto os organismos podem se aclimatar é fundamental para prever como elas podem responder ao aquecimento global (Rodrigues & Beldade 2020).

As formigas estão entre os organismos mais diversos e abundantes nos trópicos, fornecendo inúmeros serviços ecológicos e desempenhando um papel essencial no funcionamento dos ecossistemas (Hölldobler & Wilson 1990, Lach et al. 2010, Del Toro et al. 2012). De modo geral elas são organismos altamente termofílicos, porém com grande variação de tolerância termal entre grupos de espécies. Ainda, possuem hábitos alimentares diversos e estão presente nos diferentes estratos de praticamente todos os habitats terrestres (Andersen & Londsdale 1990, Hölldobler & Wilson 1990, Kaspari et al. 2015), o que as torna excelentes organismos modelo para estudos que exploram a relação entre tolerância termal, variação climática e plasticidade termal. Apesar de um crescente interesse no tema, a maior parte dos estudos com a tolerância termal de formigas foram conduzidos em regiões temperadas, enquanto ambientes tropicais, especialmente os não florestais, tem sido negligenciados.

Neste trabalho eu busquei entender alguns dos fatores que podem ajudar a explicar a tolerância termal de formigas em uma paisagem neotropical. Para isso, determinei o limite termal mínimo e máximo de espécies de formigas em diferentes tipos de vegetação (savana e floresta) e diferentes períodos do ano e relacionei essas medidas com características biológicas das espécies, como estrato de nidificação. Ainda, realizei um experimento de suplementação de nutrientes para formigas arbóreas na savana com objetivo de entender tanto os efeitos diretos da disponibilidade de recursos alimentares sobre a tolerância termal das espécies, como também os efeitos desses nutrientes na estrutura da comunidade de forma geral. Mais especificamente, tentei responder as seguintes questões: 1) O tipo de habitat e sítio de nidificação ajudam a explicar a tolerância termal e vulnerabilidade ao aquecimento global de formigas do Cerrado? 2) Existe plasticidade temporal e espacial na tolerância termal de formigas do Cerrado? 3) Quais os efeitos individuais e interativos da suplementação de açúcar e proteína na tolerância termal, posição trófica, estrutura da comunidade de formigas arbóreas e na interação entre formigas e plantas?

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CAPÍTULO 1

Thermal tolerance of ants in a Neotropical landscape: the importance of vegetation type and nesting site

Abstract

Considering that global temperatures are predicted to increase in the coming years, understanding the thermal tolerance of animals becomes crucial. This is especially true for tropical ectotherm organisms, an understudied group in the context of climate change, for which global warming is predicted to have the most deleterious consequences. Here, I evaluated the extent to which the thermal tolerance of a Neotropical ant community can be explained by habitat and microhabitat requirements and phylogenetic relatedness. For this, I compared the heat tolerance, the cold tolerance and the thermal tolerance range of ground-dwelling and arboreal ants from two contrasting adjoining habitats in the Cerrado: semideciduous forests and savannas. In addition, I compared the thermal tolerances of ants from different vertical strata (arboreal vs ground) and their susceptibility to global warming. On a community-wide basis, savanna ants were 0.9°C more tolerant to heat than ants from adjacent forests, while arboreal ants were 2.3°C more tolerant to heat and 1.6°C more resistant to cold than ground-dwelling ants. Overall, I found evidence that the habitat type (forest vs savanna) drives the heat tolerance, while vertical stratum (arboreal vs ground) drives the heat and cold tolerance of ants. Both tolerance to heat and the range of thermal tolerance presented a strong phylogenetic signal, whereas cold tolerance did not. In general, habitat and strata-related differences in thermal tolerance tracked those in microclimatic conditions, giving further support to the thermal adaptation hypothesis. Results also suggest an interactive effect between habitat and vertical strata on vulnerability to global warming, as in forests arboreal ants are more vulnerable than ground ones, whereas in savannas those living on ground are somewhat more vulnerable than those in trees Understanding how the projected increases in temperature can affect species interactions and community structure between contrasting types of habitats (and their microhabitats within) is essential to elaborate conservation measures that truly aim to protect species diversity, especially in the tropics. Although physiological measures like critical thermal limits are extremely useful, thermal ecology would be strongly benefited by works exploring the interaction between these traits and thermoregulatory behavioral responses.

Keywords: Insects, Ants, Thermal limits, Thermal heterogeneity, Microclimatic variation, Critical thermal maximum, Critical thermal minimum, Thermal physiology.

Introduction

Temperature is a key environmental factor, influencing the physiology and ecology of all organisms (Clarke 2017). Considering that global temperatures are predicted to increase in the coming years (Parmesan & Yohe 2003, Deutsch et al. 2008, IPCC 2021), understanding the thermal tolerance of animals becomes crucial. This is especially true for tropical ectotherm organisms, an understudied group in the context of climate change (Sheldon 2019), for which global warming is predicted to have the most deleterious consequences (Deutsch et al. 2008). Determining how species thermal tolerance is related to climatic conditions is imperative to develop conservation strategies in a rapidly changing world, and to improve our understanding of species distribution patterns at multiple scales.

All environments have a variety of microclimates determined by differences in vegetation, soil, and topography (Porter & Gates 1969, Potter et al. 2013). These microclimates correspond to what species truly experience during their lifespan in aspects of temperature variation, and it can be extremely contrasting with the surrounding macroclimate of the environment. In this sense, the understanding of species thermal tolerance requires attention to the local scale (Potter et al. 2013). The Thermal Adaptation Hypothesis (Kaspari et al. 2015) predicts that species are physiologically adapted to the temperature of their environment. It would be highly energetically costly to maintain thermal limits above what is needed (Angilletta 2009), therefore species capacity to tolerate heat and cold should be related to the maximum and minimum temperature that the species are exposed to in its habitat (Janzen 1967). While cold tolerance usually conforms to the Thermal Adaptation Hypothesis (Araújo et al. 2013), in general, heat tolerance shows phylogenetic conservatism and thus is less influenced by geographic or climatic variation (Diamond & Chick 2018, Pintanel et al. 2019).

Ants are one of the most diverse and abundant organisms in the tropics, providing numerous ecological services and playing an essential role in ecosystem functions (Holldobler & Wilson 1990, Lach et al. 2010, Del Toro et al. 2012). They are a highly thermophilic group that is present in the different strata of virtually every terrestrial habitat (Andersen & Londsdale 1990, Holldobler & Wilson 1990), which makes them excellent model organisms for studies exploring the relation between thermal tolerance and climatic variation along geographic gradients as well as across the vertical space (i.e. from the ground to the top of trees) (Bujan et al. 2020, Leahy et al. 2022). However, existing studies evaluating the influence of vertical climatic gradients on the thermal tolerance of ectotherms have been conducted exclusively in tropical forests (Scheffers et al. 2013), whose patterns of vertical thermal stratification can be quite contrasting to those found in more open habitats (Rey-Sánchez et al. 2016, Johnston et al. 2022), like grasslands and savannas which covers more than a quarter of the world’s land area (Bond 2019).

The aim of this study was to determine the extent to which the thermal tolerance of Neotropical ants varies between habitat types (woodland savanna vs semideciduous forest) and vertical strata (arboreal vs ground). I also evaluated the extent to which the thermal tolerance to heat and cold of is phylogenetically conserved. Since previous studies indicate that CT_{max} (Critical thermal maximum, the maximum temperature in which a species experience loss of coordinated muscle function or dies) is more related to microclimate gradients across vertical gradients than CT_{min} (Critical thermal minimum, the minimum temperature in which a species experience loss of coordinated muscle function or dies) (Bujan et al. 2020, Leahy et al. 2022), I expect that to differences in CT_{max}, but not in CT_{min} between arboreal and ground-dwelling species. Moreover, considering that ground temperatures are usually lower in forests than in more open habitats (Ewers & Bank-Leite 2013, De Frenne et al. 2019), I would also expect to find differences in the CT_{max} and CT_{min} between forest and savanna species.

Material and Methods

Study area

This study was conducted at the Reserva Ecológica do Panga (REP), a 404-ha reserve located 30km south of Uberlândia, Minas Gerais, Brazil (19° 10' S, 48° 23' W). The mean annual temperature of the region is 22ºC and the mean annual rainfall is 1,650 mm (Embrapa 1982). The REP is located within the Cerrado biome, which is characterized by a mosaic of vegetation types, including savannas, grasslands and forests (Cardoso et al. 2009). Ant sampling and thermal tolerance tests were performed in February 2022, whereas ambient temperature measures were performed in January and February of 2023. Sampling was conducted in adjacent woodland savanna (locally known as *cerrado sensu stricto*) and semideciduous forest areas. The latter has a relatively closed tree canopy (~20 m in height) whereas the woodland savanna has a much sparser tree cover, formed by trees usually not taller than 6 m in height (Cardoso et al. 2009).

Temperature measurements

For 32 days I measured habitat and stratum thermal variation by recording ambient air temperature at 10-minute intervals. For this, dataloggers (Kestrel model DROP D2) were simultaneously positioned on the ground and in trees of the savanna and forest around the same areas in which ants were sampled. The tree dataloggers were installed at the maximum height accessible by the ladder (6 meters high). In the savanna, this height corresponded to the canopy of the tree, while in the forest the datalogger was fixed to the main trunk of the tree, below the canopy. The dataloggers on the ground were positioned away from the base of the trees, so that it does not represent only the microclimate generated by the shade of a single tree canopy in the case of savannas. Dataloggers in forests were in the shade regardless of their distance to the base of trees because the canopy of this environment is much more closed than in savannas, making it difficult for direct sunlight to reach the ground.

Ant sampling

I sampled ants from savanna and forest vegetation using tuna baits that were placed on the ground (within petri dishes) or in trees on plastic vials attached with wire to the tree branches. Baited dishes/vials were collected after one hour or when recruitment was detected. I also performed active collection by placing opened plastic vials in front of individuals that were active close to baits, especially for species of solitary foraging. Considering that ants species differ on their activity schedule and aiming to collect as many species as possible, this sampling method was performed during the day and the night. I considered that arboreal ants from sampled trees 12m apart to be from different colonies, while for ground-dwelling ants I considered a minimum distance of 50 m between petri dishes or nest entrances to determine that ants tested were not from the same colony.

Thermal tolerance

In total, I tested the CT_{max} and CT_{min} of 64 species of ants. For half of these species, the tests were performed with ants from a single colony, whereas for the remaining species with ants from two to four different colonies. Colonies of eleven of the

64 ant species were found in both forest and savanna, and in this case colonies from both habitats were tested. CT_{max} measurements were performed using a Kasvi model K80-S01/02 Dry Bath, whereas for CT_{min} I used a Loccus model DB-HC Dry Bath. In each test 10 workers (for polymorphic or dimorphic species only minor workers were tested) of each species were placed individually in a 2 ml microcentrifuge vial sealed with a small cotton ball and placed randomly in the dry bath equipment. The initial temperature of the CT_{max} test was 36 degrees, which was increased by two degrees every 10 minutes of exposure until death or loss of muscle coordination in the workers, whereas the initial temperature of the CT_{min} was 16 degrees, which was decreased by two degrees every 10 minutes of exposure until death or loss of muscle coordination of the workers. Tests were carried out within a maximum period of up to five hours after the collection of the ants in the field. I considered the CT_{max} and CT_{min} of the species as the average temperature of death or permanent loss of muscle coordination of the 10 workers, and CT_{range} was calculated as $CT_{max} - CT_{min}$ for each species.

Phylogenetic signal in thermal tolerance

For the phylogenetic signal analysis, a phylogeny was constructed using ultraconserved elements from 357 ant species from the Cerrado biome (Neves et al. *in preparation*). Prior to conducting the analysis, the phylogenetic tree was pruned to include only 64 ant species relevant to the present study. I evaluated the degree of phylogenetic signal for three physiological traits of ants (CT_{max}, CT_{min} and $CT_{range)}$ using the Blomberg’s K statistic (Blomberg et al. 2003). This metric uses a Brownian motion model of trait evolution to evaluate whether the observed distribution of trait values differs from expectation. A K-value higher than 1 implies that related taxa resemble each other more than expected under a Brownian motion model of evolution (i.e., indicating a strong phylogenetic signal), whereas a K-value close to 0 indicates random expectation (i.e., no phylogenetic signal) (Blomberg et al. 2003).

Warming tolerance

I used the warming tolerance as an indicative of species' vulnerability to global warming, since it provides a estimate of how much the environment of an organism can warm before this organism reach physiological failure or death (Diamond et al. 2011). Warming tolerance was calculated as the CT_{max} of the species minus the maximum air temperature of the habitat and stratum in which the species was sampled. In this sense, a negative value of warming tolerance indicates that habitat temperature is beyond the species CT_{max} and should cause negative effects Considering that the temperature of the canopy of forests (where most of arboreal ants nest) can be very different from the understory, I calculated the warming tolerance of arboreal ants from forests in two ways: using the temperature of the datalogger positioned in the understory of the forest and using the temperature of the canopy of the adjacent savanna as an indicative of the temperature of the canopy in the forest.

Statistical analyses

Since it was not possible to install dataloggers on the canopy of the forests, the temperatures obtained on the vegetations between habitats were not comparable. In this sense, I evaluated the differences of temperature between habitats (forest and savanna) using only the temperature from the ground of these habitats, and evaluated the differences of temperature between stratum (ground and vegetation) separately for each habitat. For the savanna I used the temperature obtained from dataloggers on ground and canopy of this habitat, while for the forests I used the temperature from the ground and understory obtained from dataloggers in this habitat and the temperature of the canopy of the adjacente savanna as an indicative of the temperature on the canopy of the forest. For all comparisons I used linear mixed models, where the day was treated as a random fator.

I evaluated the effects of the habitat and stratum on the CT_{max}, CT_{min}, CT_{range} or warming tolerance) using type III, two-way ANOVAs and assuming a Gaussian error distribution. Since body size might have an influence on thermal tolerance, I also included body size (Weber´s Length, as based on measurements of 5-6 individuals per species) as covariate in these models, but since there was no effect of body size, I removed it from the models.

Body size measures were obtained from the database of the Laboratório de Ecologia de Insetos Sociais (LEIS), in the Universidade Federal de Uberlândia, which contain measures of the Weber's length (indirect measure of body size) of Cerrado ants' species. All values were composed by the mean between the measures of 5-6 individuals of the same areas in which thermal tolerance tests were performed. Ant species were classified as savanna or forest species and as arboreal or ground-dwelling based on the location of capture and nest observations of the individuals tested.

For the phylogenetic signal in thermal tolerance, the statistical significance of observed K-values was assessed through randomization tests that produced a null distribution of 999 K-values. To prune the phylogenetic tree, I used the "drop.tip" function available in the R package "ape" (Paradis & Schliep 2019). To investigate if the relation between thermal tolerance and habitat/stratum was dependent of species phylogeny, I tested for differences in species CT_{max} and CT_{min} between habitat and stratum while accounting for phylogenetic relatedness with a phylogenetic ANOVA using the function "phylANOVA" in the R package "phytools" (Revell 2012). Differences across habitats and across stratum were tested separately due to limitations of the analysis.

All model assumptions were checked by evaluating the plot of the residuals against the fitted values and the normal probability plot. All statistical analyses were performed in R v.4.1.1 (2021-08-10) using the packages "lmerTest" (Kuznetsova et al. 2017) "ape" (Paradis & Schliep 2019) and "phytools" (Revell 2012).

Results

Habitat and stratum differences in air temperature

There was a significant effect of the habitat type on the maximum ($F_{1,31}$=403.57, p<0.001) and minimum air temperature ($F_{1,31}$=12.14, p= 0.001) and on the temperature range ($F_{1,31}$= 405.68, p<0.001) at the ground. The maximum air temperature and temperature range at the ground was higher in the savanna than in the forest, while minimum air temperature was lower in the forest than in the savanna. In the savanna, there was a significant effect of the stratum on the maximum ($F_{1,31}$= 36.03, p<0.001) and minimum air temperature ($F_{1,31}$= 612.02, p<0.001) and on the temperature range ($F_{1,31}$= 22.18, p<0.001). In the savanna maximum air temperature and temperature range was higher in the ground than in the canopy, while the minimum air temperature was lower in the canopy. In the forest there was also a significant effect of stratum on the maximum ($F_{2,62}$= 218.07, p<0.001) and minimum air temperature ($F_{2,62}$= 88.46, p<0.001) and on the temperature range ($F_{2,62}$= 234.27, p<0.001). A posteriori pairwise comparisons revealed that maximum air temperature was higher in the canopy than in the understory and ground (Tukey test p<0.001), while there is no difference between the understory and the ground (Tukey test p= 0.191). The minimum air temperature was lower in the canopy than in the understory and ground (Tukey test p<0.001), and lower in the understory than in the ground (Tukey test p<0.001). The temperature range was higher in the canopy than on the understory and ground (Tukey test p<0.001), and higher in the understory than on the ground (Tukey test p= 0.042).

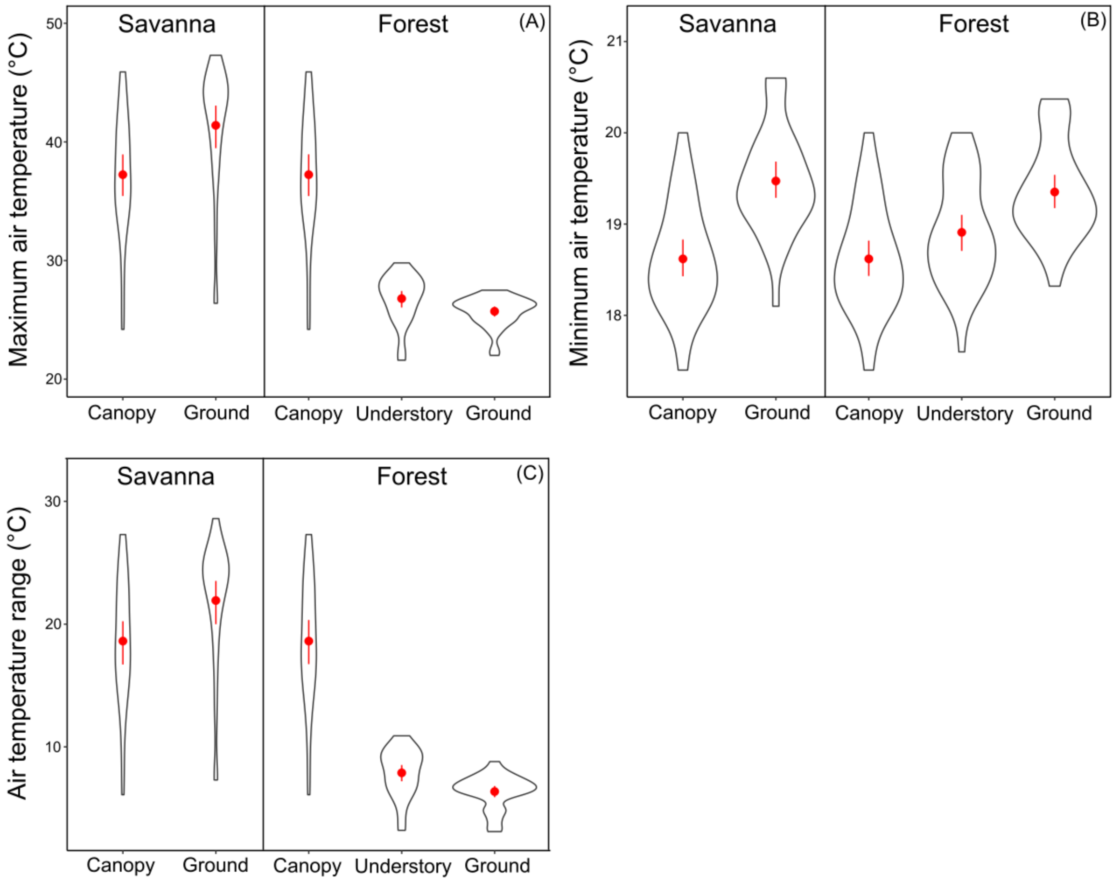

Figure 1. Variation in the maximum air temperature (A), minimum air temperature (B) and temperature range (C) in the arboreal and ground stratum of adjacent savanna and forest areas. Red dots indicates the mean of data points, while red line represent the standard deviation. The width of the curves (black lines) represents the frequency of data points.

Effect of habitat and stratum in thermal tolerance

There was a significant effect of habitat ($F_{1,71}$= 43.36, p<0.001) and stratum ($F_{1,71}$= 26.59, p<0.001) on CT_{max} and no interaction between these two factors ($F_{1,71}$=0.31,

$p = 0.575$) (Figure 2). On average, the ant species collected in the savanna presented a CT_{max} that was 1.8°C (95% CI: 1.1-2.5°C) higher than the forest species. Similarly, arboreal species presented a CT_{max} 1.9°C (95% CI: 1.4-2.4°C) higher than the ground-dwelling species. In the same way, there was an effect of habitat ($F_{1,71}$= 24.06, $p < 0.001$) and stratum ($F_{1,71}$= 25.01, $p <0.001$) on $CT_{range,}$ with no interaction between habitat and stratum ($F_{1,71}$= 0.075, p= 0.784) (Figure 2). The mean CT_{range} was 2.4°C (95% CI: 1.2-3.6°C) broader for the savanna than for the forest species, and 3.3°C (95% CI: 2.5-4.1°C) broader for arboreal than for ground-dwelling species. On the other hand, there was effect of stratum ($F_{1,71}$= 10.39, $p = 0.001$), but no effect of habitat ($F_{1,71}$= 3.08, $p = 0.083$) on CT_{min} and there was no interaction between habitat and stratum ($F_{1,71}$= 0.006, p= 0.935) (Figure 2). The mean CT_{min} was 1.4°C (95% CI: 0.9-1.9°C) lower for arboreal than for ground-dwelling species.

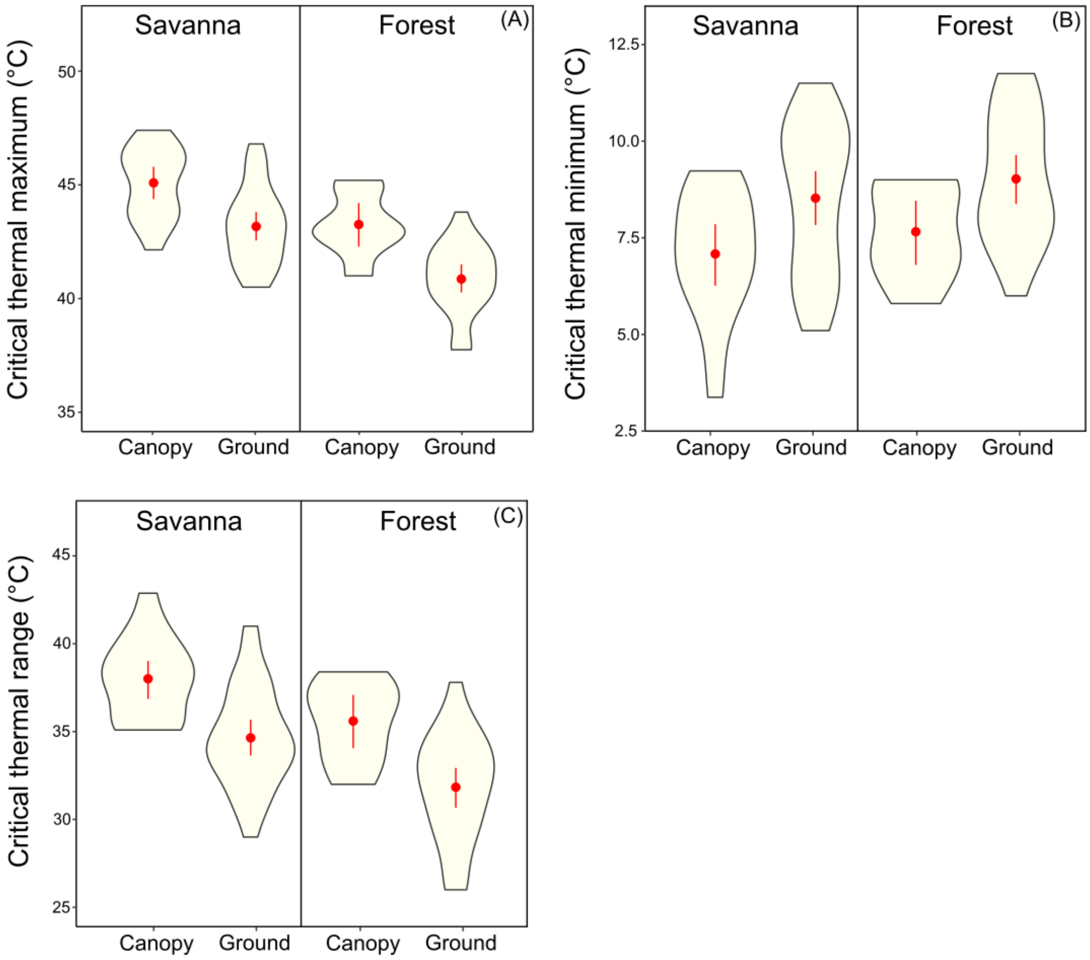

Figure 2. Variation in the CT_{max} (A), CT_{min} (B) and CT_{range} (C) of ant communities in relation to habitat type and vertical strata. Red dots indicates the mean of data points, while red line represent the standard deviation. The width of the curves (black lines) represents the frequency of data points.

Warming tolerance

There was a significant effect of habitat ($F_{1,71}$ = 1925.82, p<0.001) and stratum ($F_{1,71}$ = 60.56, p<0.001) on warming tolerance, and no interaction between these two factors ($F_{1,71}$=0.19, p= 0.66) in the model with temperature of the arboreal stratum based on dataloggers placed in the understory (Figure 3). The mean warming tolerance was 16.5°C (95% CI: 15.8-17.2°C) lower in savanna than in forest ants and 3.3°C (95% CI: 2.8-3.8°C) lower for ground-dwelling than for arboreal species.

In the model using the temperature of the canopy of savannas as an indicative of the temperature of the canopy of forests, I found a significant interaction between habitat and stratum ($F_{1,71}$= 468.35, p<0.001) on warming tolerance. A posteriori, pairwise comparisons revealed that, in general, species from savannas have a lower warming tolerance than species from forests (Tukey test p<0.001); however, there is no difference in the warming tolerance between arboreal species from savannas and arboreal species from forests (Tukey test p= 0.07), or between arboreal species from forests and ground-dwelling species from savannas (Tukey test p= 0.13) In savannas, ground-dwelling ants have lower warming tolerance than arboreal ants (Tukey test p<0.001), while in forests are the arboreal ants that have lower warming tolerance than the ground ants (Tukey test p<0.001)

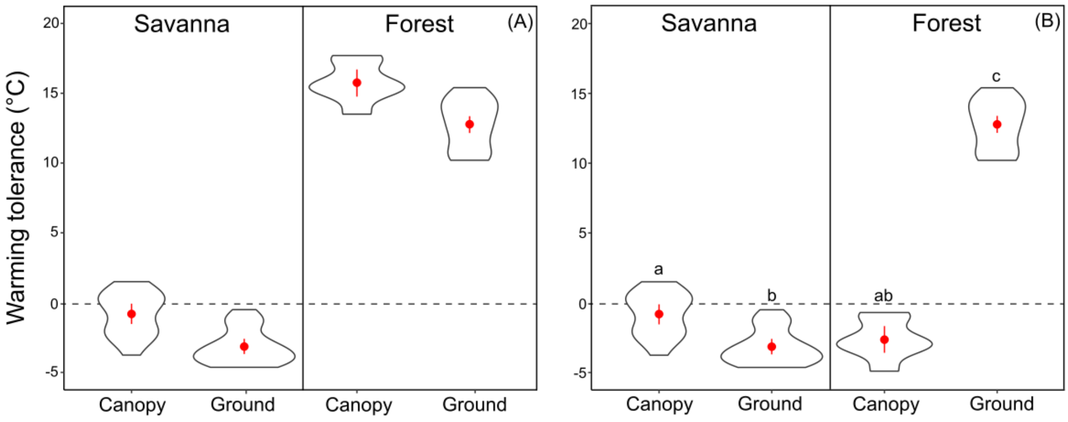

Figure 3. Variation in the warming tolerance of ant communities in relation to the type of habitat and nesting/foraging stratum using the temperature of the understory of the forest to arboreal ants from forests (A) and using the temperature of the savanna canopy as an indicative of temperature in the forest canopy (B). Red dots indicates the mean of data points, while red line represent the standard deviation. The width of the curves (black lines) represents the frequency of data points. The dashed line in 0°C represents the

potentially lethal threshold of temperature to ants. Different letters above the boxplot indicates significant differences among mean values in (B).

Phylogenetic signal

There was a significant phylogenetic signal in CT_{max}, CT_{min} and CT_{range} of the ant species, but the signal was stronger for CT_{max} and CT_{range} (Blomberg's K =0.78, p= 0.001 and K= 0.69, p= 0.001, respectively), than for CT_{min} (Blomberg's K = 0.41, p= 0.01). There was no difference in the effect of habitat and stratum on thermal tolerance of species whether accounting for phylogenetic relatedness or not. There was a significant effect of habitat (phylANOVA; $F_{1,63}$= 30.46, p<0.001) and stratum (phylANOVA; $F_{1,63}$= 22.52, p<0.001) on CT_{max}, while there was effect of stratum (phylANOVA; $F_{1,63}$= 12.70, p = 0.005), but no effect of habitat (phylANOVA; $F_{1,63}$= 2.11, p = 0.155) on CT_{min}

Discussion

The results of this study strongly suggest that ant species from woodland savannas, on average, present a greater resistance to heat (i.e., greater Ct_{max}) than those from adjacent semideciduous forest, whereas within both habitats arboreal species are more resistant than the ground-dwelling ones. On the other hand, tolerance to cold did not differ among ants from different habitats; however, it was greater among the arboreal than among the ground-dwelling species. Consequently, arboreal species seem to stand a wider range of temperatures than those that live on the ground.

In general, the observed differences in thermal tolerance between habitats and/or vertical stratum tracked those observed in the microclimatic conditions where the ants forage, giving further support to the Thermal adaptation hypothesis (Kaspari et al. 2015). In particular, I observed that the differences CT_{max} and CT_{range} of ants from different habitats matched the temperature variation between these same habitats, while there was no habitat- related change in CT_{min} even considering that savannas were slightly colder than forests. The differences in CT_{max}, CT_{min} and CT_{range} matched the variation of temperature between vertical strata in the forest when considering the canopy of this environment, but in the savanna it was only matched by the CT_{min}. In this sense, my results sugest that species in forests are buffered from thermal extremes when compared to species from savannas (Ewers & Bank-Leite 2013, De Frenne et al. 2019), in a similar way that ground-dwelling ants are when compared to arboreal ants in forest and, in a weaker way, in savannas.

Regardless of the habitat, arboreal ants tolerated a wider range of temperatures than ground-dwelling ants. In forests, the temperature variation between the soil and the understory (where dataloggers were positioned) was not enough to explain the difference in thermal tolerance of species between these strata. However, most of the arboreal ants on forests have their nests on the canopy, which is more exposed to the sun and much hotter and variable than the understory and the ground (Ruibal 1961, Kaspari et al. 2015). In savannas, on the other hand, arboreal ants were more tolerant to heat despite the ground being slightly hotter. It is possible that arboreal ants in savannas have a higher CT_{max} than ground-dwelling species because some behavioural responses to heat stress might be easier or only available in the ground (Tschinkel 1987, Chick et al. 2017), so arboreal ants would have to rely more on physiological adaptations to deal with the heat on the canopy. For example, most arboreal ants nest in cavities of branches that cannot be rapidly altered to facilitate thermoregulation as in the ground (altering nest chamber location and depth, for example) (Tschinkel 1987, Chick et al. 2017). In addition, the canopy usually is formed by linear paths of branches and lianas, while the ground presents a combination of grass, leaves, twigs, rocks and other structures forming a complex two-dimensional environment (Kaspari & Weiser 1999) that could offer more protection and options for ants to optimize locomotion minimizing risks of overheating, like making small pauses in the shade during foraging (Marsh 1985) or climbing up grass stalks to avoid the hot ground (Cerda 2000). Moreover, it is also possible that the higher CT_{max} of arboreal ants in savanna can be related to the strong phylogenetic signal we found in this trait. For example, species of the genus *Cephalotes* and *Pseudomyrmex*, which were the most heat tolerant in this study, are exclusively or almost exclusively arboreal, while Poneremorph species, which usually have low tolerance to heat (Nascimento et al. 2022), are much more abundant on the ground (Table S1). In this sense, the greater heat tolerance of arboreal ants in savannas could be more explained by niche conservatism than present microclimatic differences.

My results also suggests that ants from savannas are more vulnerable to global warming than forest ants, possibly with ground-dwelling species in savannas at the highest risk. This happens even that savanna ants are more heat tolerance because they experience temperatures very near from their CT_{max}, and in this sense even a small increase in air temperature could result in a negative effect on their fitness. The maximum temperature recorded in February in the savanna was potentially lethal for most species in this habitat, whether arboreal or ground-dwelling, which is concerning especially taking into account that temperatures are expected to rise in next years. It also indicates that for most species the physiological mechanisms are not enough to withstand the maximum temperature, and other behavioral mechanisms, as variations in time (diurnal vs nocturnal) and mode (cooler substrates) of foraging (Spicer et al. 2017, Stark et al.

2017, Garcia Robledo et al. 2018), might be important, especially in a highly thermally heterogeneous environment like a savanna.

When considering the temperature of the canopy of forests, my results indicates that arboreal ants in this environment are more vulnerable to global warming than ground-dwelling species, as also indicated by other studies (Diamond et al. 2012, Leahy et al. 2022). In addition, it indicates that arboreal ants from forest could be as vulnerable as ants in savannas. However, it is important to note that, for arboreal ants in forests, my results also suggests that the understory is a stratum with relatively low thermal risk to forage. Since many ants can change their local of foraging (Leahy et al. 2021) or even move their nests vertically to track more favorable conditions (Jones & Oldroy 2007), the exploration of the temperature gradients between the canopy and the ground might be one possible behavioral response that can buffer arboreal ants in forests from the impacts of global warming. In this scenario, forest ant species could be at advantage, especially those with more generalist nesting habits.

Exploring species-specific differences on thermal tolerance, focusing on traits of the most tolerant species and including natural and anthropogenic habitats with different structures on studies would be extremely important to better understand how the projected increases in temperature can affect species interactions and community structure in a rapidly changing world. Moreover, although physiological measures like critical thermal limits are very useful, they cannot explain alone the present and future response of species to thermal risks, and in this sense, studies exploring how thermoregulatory behaviors changes between species and habitats would also be extremely important.

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Supplemental Material

Table S1. List of ant species tested and their mean CT_{max}, CT_{min} and CT_{range} (± standard error).

Species	Habitat	Stratum	CT_{max}	CT_{min}	CT_{range}
Acromyrmex aspersus	savanna	ground	43 (± 1.4)	10.6 (± 1)	32.4
Anochetus targionii	forest	ground	39.8 (± 0.6)	10.8 (± 1)	29.0
Apterostigma sp. 03	forest	ground	38 (± 0)	11 (± 1.4)	27.0
Atta laevigatta	savanna	ground	42.9 (± 1.4)	9.4 (± 1.6)	33.5
Atta sexdens	savanna	ground	43 (± 1.1)	8.2 (± 0.6)	34.8
Azteca sp. 01	savanna	arboreal	43.8 (± 1.8)	6 (± 0)	37.8
Azteca sp. 02	forest	arboreal	43.4 (± 1)	7 (± 1.1)	36.4
Brachymyrmex cf. *aphidicola*	savanna	ground	44 (± 0)	7.2 (± 1)	36.8
Brachymyrmex cf. *aphidicola*	forest	ground	42.9 (± 1)	7.4 (± 1.3)	35.5
Camponotus atriceps	forest	arboreal	42.8 (± 1.4)	5.8 (± 1.8)	37.0
Camponotus balzani	savanna	arboreal	43.6 (± 0.8)	8.4 (± 0.8)	35.2
Camponotus blandus	savanna	ground	45.8 (± 2.6)	8.2 (± 0.6)	37.6
Camponotus bonariensis	savanna	arboreal	46.3 (± 0.7)	3.4 (± 1.9)	42.9
Camponotus lespesii	forest	ground	41.6 (± 0.8)	7.6 (± 1.8)	34.0
Camponotus melanoticus	savanna	ground	43.6 (± 1.1)	5.5 (± 1.8)	38.1
Camponotus melanoticus	forest	ground	42.5 (± 1.7)	6.9 (± 1.9)	35.6
Camponotus cf. sp. 79	forest	ground	42.2 (± 2)	8.2 (± 1.8)	34.0
Camponotus renggeri	savanna	ground	42.8 (± 1.7)	6.8 (± 2.5)	36.0
Camponotus senex	savanna	arboreal	46 (± 1.3)	6.7 (± 1.3)	39.3
Camponotus sericeiventris	savanna	arboreal	44.4 (± 1.3)	9 (± 1.7)	35.4
Camponotus sp. 77	forest	arboreal	41 (± 2.4)	9 (± 1.7)	32.0
Cephalotes atrattus	savanna	arboreal	46.4 (± 0.8)	7.8 (± 0.6)	38.6
Cephalotes depressus	savanna	arboreal	47.4 (± 1)	6.8 (± 1)	40.6
Cephalotes pusillus	savanna	arboreal	46.9 (± 1.6)	9.2 (± 1.5)	37.7
Crematogaster cf. *arcuata*	savanna	ground	46.1 (± 0.4)	5.1 (± 1.2)	41.0
Crematogaster cf. sp. 14	forest	arboreal	44.8 (± 1)	7.4 (± 1)	37.4
Cyphomyrmex rimosus	forest	ground	37.9 (± 1.3)	11.7 (± 1.9)	26.2
Dorymyrmex brunneus	savanna	ground	46 (± 0.9)	6 (± 0)	40.0
Ectatomma brunneum	savanna	ground	44.2 (± 0.6)	11 (± 1.3)	33.2
Ectatomma edentatum	forest	ground	42 (± 0.8)	7.7 (± 0.7)	34.3
Ectatomma opaciventris	savanna	ground	44.7 (± 1)	10.7 (± 1)	34.0
Ectatomma planidens	savanna	ground	43.6 (± 0.8)	8.9 (± 1)	34.7
Ectatomma tuberculatum	savanna	arboreal	44 (± 0)	8.9 (± 1)	35.1
Ectatomma tuberculatum	forest	arboreal	42.6 (± 1)	9 (± 1.1)	33.6
Forelius maranhaoensis	savanna	ground	44 (± 0)	10 (± 0)	34.0
Holcoponera striatula	savanna	ground	43.4 (± 1)	9.6 (± 1.3)	33.8
Holcoponera striatula	forest	ground	40.9 (± 1.5)	11.6 (± 3.7)	29.3
Hypoponera sp. 03	forest	ground	37.8 (± 1.2)	11.8 (± 1.9)	26.0

Labidus coecus	savanna	ground	41.9 (± 0.4)	6.1 (± 1.2)	35.8
Mycetomoellerius sp. 15	forest	ground	40 (± 0)	9 (± 1.4)	31.0
Mycetomoellerius dichrous	savanna	ground	42 (± 0)	10.8 (± 1)	31.2
Mycocepurus goeldii	savanna	ground	40.6 (± 2.1)	10 (± 1.1)	30.6
Neoponera marginata	savanna	ground	41.8 (± 0.6)	8.4 (± 0.8)	33.4
Neoponera marginata	forest	ground	42 (± 0)	10 (± 0)	32.0
Neoponera verenae	savanna	ground	41.8 (± 0.7)	9.5 (± 1.2)	32.2
Neoponera villosa	savanna	arboreal	42.1 (± 0.5)	6.3 (± 1.1)	35.9
Nomamyrmex esenbeckii	forest	ground	40.8 (± 1)	8.4 (± 0.8)	32.4
Nylanderia caeciliae	forest	ground	41.2 (± 1.3)	8.7 (± 1)	32.5
Odontomachus chelifer	forest	ground	40 (± 0)	8 (± 0)	32.0
Odontomachus meinerti	forest	ground	40 (± 0)	10.5 (± 1.3)	29.5
Pachycondyla harpax	savanna	ground	40.5 (± 1)	11.5 (± 1.9)	29.0
Pachycondyla harpax	forest	ground	40.8 (± 1.3)	8.9 (± 1.6)	31.8
Pachycondyla striata	forest	ground	42 (± 0)	9.6 (± 2.5)	32.4
Pheidole oxyops	savanna	ground	41.2 (± 1.7)	6 (± 0)	35.2
Pheidole oxyops	forest	ground	40.8 (± 1)	6.8 (± 2)	34.0
Pheidole sp. 122	forest	ground	40.2 (± 0.6)	10 (± 0)	30.2
Pheidole radoszkowskii	savanna	ground	41.4 (± 1.3)	7.1 (± 1.3)	34.4
Pheidole sp. 123	forest	ground	42 (± 0)	8 (± 0)	34.0
Pheidole cf. *triconstricta*	savanna	ground	43.5 (± 1)	10 (± 1.9)	33.5
Pheidole cf. *triconstricta*	forest	ground	41.5 (± 0.9)	7.7 (± 1)	33.8
Pheidole vafra	savanna	ground	41.8 (± 1.8)	6 (± 0)	35.8
Pseudomyrmex curacaensis	savanna	arboreal	45.6 (± 0.8)	7.8 (± 1.5)	37.8
Pseudomyrmex gracilis	savanna	arboreal	46.6 (± 1)	7.2 (± 1)	39.4
Pseudomyrmex unicolor	savanna	arboreal	46 (± 0)	7 (± 1.5)	39.0
Sericomyrmex mayri	forest	ground	40 (± 1.3)	10.3 (± 1)	29.7
Sericomyrmex scrobifer	savanna	ground	42.7 (± 1)	10.3 (±0.8)	32.3
Solenopsis nr *basalis*	savanna	arboreal	45.4 (± 1)	4.6 (± 1)	40.8
Solenopsis nr *basalis*	forest	arboreal	45.2 (± 1)	6.8 (± 1)	38.4
Solenopsis cf. *latastei*	savanna	ground	44.6 (± 1)	6.8 (± 1)	37.8
Solenopsis cf. *latastei*	forest	ground	43.8 (± 0.6)	6 (± 0)	37.8
Solenopsis substituta	savanna	ground	46.8 (± 1)	8.6 (± 1.3)	38.2
Tapinoma sp. 07	savanna	arboreal	43.3 (± 1.2)	5.8 (± 0.9)	37.5
Wasmannia auropunctata	savanna	ground	41 (± 1.4)	10.2 (± 1.5)	30.8
Wasmannia sigmoidea	savanna	arboreal	43.6 (± 0.8)	8.4 (± 0.8)	35.2
Wasmannia sigmoidea	forest	arboreal	43 (± 1.4)	8.6 (± 1.3)	34.4

CAPÍTULO 2

Seasonality affects cold tolerance whereas vegetation type affects the heat tolerance of ants in a Neotropical landscape

Abstract

Ectotherms organisms are exposed to different patterns of temperature variability across multiple scales, which can lead plasticity on their thermal tolerance. Understanding how much ectotherms can acclimate to temperature variability becomes essential to understand their distribution patterns and predict how they may respond to climate change. Here, I evaluated the extent to which the thermal tolerance of a Neotropical ant community varies temporally and spatially by comparing the heat tolerance (CT_{max}), cold tolerance (CT_{min}) and thermal tolerance range (CT_{range}) of the same ant species between different times of the year and between two contrasting habitat types (forests and savannas). On a community-wide basis, ants were 1.29°C more tolerant to cold in July, when temperatures were 20.3% lower than in February. Similarly, the range of thermal tolerances was 1.17°C wider in July when thermal daily variation was 27.4% greater than in February, whereas there was no significant variation in the $CT_{max.}$ Moreover, the effect of habitat type and vertical strata did not interact with the effect of seasonality on thermal tolerance, indicating that temporal plasticity in CT_{min} and CT_{range} are not restricted to a given habitat or foraging/nesting stratum. On the other hand, when comparing the thermal tolerance of habitat generalist species between the two habitat types where they occur, I found evidence that tolerance to heat is 0.88°C greater, on average, in the savanna than in the forest populations, whereas tolerance to cold did not differ significantly. Overall, the observed changes in thermal tolerance matched those in climatic conditions, and this suggests that even relatively modest variations in temperature can induce changes in the thermal tolerance of ants. This thermal plasticity, whether spatially or temporally, might give ants an ecological advantage, influencing not only their activity schedule and foraging performance, but potentially also the outcome of competitive and mutualistic interactions. In this sense, my results reinforce the notion that plasticity in thermal tolerance should be considered for more realistic predictions in studies that use niche modelling to understand changes in species distribution and vulnerability to global warming.

Keywords: Thermal limits, Acclimation, Thermal heterogeneity, Critical thermal maximum, Critical thermal minimum, Thermal physiology, Cerrado, Ants.

Introduction

Temperature is one of the most important abiotic factors influencing the growth, reproduction, and survival of organisms. This is especially true for ectotherms, which make them particularly vulnerable to climate change (Angilleta 2009, Buckley et al. 2013). Considering that most animals are ectotherms (Wilson 1992, Atkinson & Sibly 1997), and that global temperatures and temperature variability are predicted to change in the next years (Deutsch 2008, IPCC, 2021), understanding the extent to which these organisms can acclimate becomes essential to predict how they may respond to climate change.

Ectotherms have evolved innumerous traits to cope with thermal stress (Pincebourde & Casas 2019, Bishop et al. 2016, Roeder et al. 2021). Thermal tolerance plasticity, which can be one physiological adaptation to deal with ambient temperature variance (Gabriel et al. 2005), is the capacity of an organism for changing its upper and lower thermal limits and is a critical factor buffering species from the negative effects of thermal variation (Gunderson & Stillman 2015, Machekano et al. 2021). Although widely present among terrestrial and aquatic taxa of ectotherms, thermal tolerance plasticity varies across species and populations depending on environmental factors, such as temperature extremes and degree of thermal variation (Gunderson & Stillman 2015, Rodrigues & Beldade 2021).

Temperature can be spatially heterogeneous along geographical gradients of latitude or elevation, for example, but it can also change locally according to habitat specificities, such as vegetation type, canopy openness (Potter et al. 2013), and vertical strata (Yanoviak & Kaspari 2000, Leahy et al. 2021). Temporal thermal variation, on the other hand, occurs both daily and seasonally, with the extent of such variation being influenced by the structure (Renaud et al. 2011, Von Arx et al. 2012), and geographic position of the habitat (Sheldon & Tewksbury 2014). In this sense, organisms are exposed to different patterns of temperature variability across multiple spatial and temporal scales, which can lead to plasticity on their thermal tolerance. Understanding which factors can help explain this trait may help us to predict which species are more vulnerable to climate warming, as well as their evolutionary potential and distribution patterns (Sunday et al. 2012, García-Robledo et al. 2016, Barley et al. 2021).

In this study I evaluated the extent to which the thermal tolerance of a Neotropical ant community varies spatially and temporally. For this I compared the heat tolerance (hereafter CT_{max}), the cold tolerance (hereafter CT_{min}) and the thermal tolerance range (hereafter CT_{range}) of the same species between different times of the year (seasonal variation) and between two contrasting, adjoining habitat types (spatial variation). Since previous studies indicate the presence of thermal plasticity in ants (Angilletta et al. 2007, Diamond et al. 2017, Bujan et al. 2020b), I would expect that both the CT_{max} and the CT_{min} of our focal species would vary in response to seasonal and habitat-related variations in temperature.

Material and Methods

Study area

This study was conducted at the Reserva Ecológica do Panga (REP), a 404-ha reserve located 30km south of Uberlândia, Minas Gerais, Brazil (19° 10' S, 48° 23' O). The mean annual temperature of the region is 22ºC and the mean annual rainfall is 1,650 mm (Embrapa 1982). The REP is located within the Cerrado biome, which is characterized by a mosaic of vegetation types, including savannas, grasslands and forests (Cardoso et al. 2009). All ant sampling, ambient temperature measures and thermal tolerance tests were performed in February 2022 and in July 2022 on woodland savannas and forest areas (locally known as *cerrado sensu stricto* and *semideciduous forest*, respectively). The woodland savanna is characterized by a superior layer of trees (up to 6m) and shrubs, and a ground layer formed by grasses, herbs and small shrubs (Eiten 1972), while the forest is characterized by higher superior layer of trees (up to 20m) that composes a much more closed canopy than the savanna (Cardoso et al. 2009).

Temperature measurements

Temperature data of the focal months of my study was obtained from the nearest weather station from the REP (Uberlândia A507 station) on https://portal.inmet.gov.br/.

I measured habitat thermal variation by recording ambient temperature at a 10-minute interval with dataloggers (Kestrel model DROP D2) simultaneously positioned 1 m from the ground in savanna and forest vegetation for 12 days of 2022 in the same areas which ants were sampled.

Ant sampling

I sampled ants from savanna and forest vegetation using tuna baits that were placed on petri dishes above the soil for ground-dwelling ants and on plastic vials attached with wire to tree branches for arboreal ants. Baited dishes/vials were collected after one hour or when recruitment was detected. I also performed active collection by placing opened plastic vials in front of individuals that were active close to baits, especially for species of solitary foraging. Considering that ants species differ on their activity schedule and aiming to collect as many species as possible, this sampling method was performed during the day and the night. I considered that arboreal ants from trees separated by at least 12m apart were from different colonies, while for ground-dwelling ants I considered a minimum distance of 100m between petri dishes or nest entrances to determine that ants tested were not from the same colony. For seasonal variation on thermal tolerance, I tested 30 species represented by one colony each, while the other 14 species were represented by 2-5 colonies. For habitat variation on thermal tolerance, I tested the CT_{max} of 12 species represented by one colony per habitat and three species (*Ectatomma edentatum*, *Ectatomma tuberculatum* and *Pachycondyla harpax*) represented by 2-4 colonies per habitat, while for the CT_{min} I tested 11 species represented by one colony per habitat and four species (*Ectatomma edentatum*, *E. tuberculatum*, *Neoponera verenae* and *Pachycondyla harpax*) represented by 2-4 colonies per habitat.

Thermal tolerance

Measurements of CT_{max} were done using Kasvi model K80-S01/02 Dry Bath, whereas for CT_{min} I used Loccus model DB-HC Dry Bath. In each test 10 workers (for polymorphic or dimorphic species only minor workers were tested) of each species were placed individually in 2ml microcentrifuge vial sealed by cotton and placed randomly in the dry bath equipment. The initial temperature of the CT_{max} test was 36 degrees, which was increased by two degrees every 10 minutes of exposure until death or loss of muscle coordination in the workers, while the initial temperature of the CT_{min} was 16 degrees, which was decreased by two degrees every 10 minutes of exposure until death or loss of muscle coordination of the workers. Tests were carried out within a maximum period of up to five hours after the collection of the ants in the field. I considered the CT_{max} and CT_{min} of the species as the average temperature of death or permanent loss of muscle coordination of the 10 workers, and CT_{range} was calculated as $CT_{max} - CT_{min}$ for each specie.

Statistical analyses

I calculated the daily thermal variation in February and July as: (daily maximum temperature – daily minimum temperature) and compared the difference of temperature between months and between habitats using a Wilcoxon rank sum test. I compared the mean difference on the CT_{max}, CT_{min} and CT_{range} of the ant species using a paired Student's t-test and evaluated the effects of the habitat and stratum on the magnitude of the seasonal change of CT_{max}, CT_{min} and CT_{range} using type III, two-way ANOVAs with a Gaussian error distribution assumed. Model assumptions were checked by evaluating the plot of the residuals against the fitted values and the normal probability plot. Ant species were classed as savanna or forest species and arboreal or ground-dwelling based on the location of capture and nest observations of the individuals tested. The magnitude of seasonal change of each CT was calculated as the logarithm of the response ratio using the formula: log (CT determined in February / CT determined in July). I compared the mean difference on the CT_{max} and CT_{min} between species from savanna and forest using a paired Student's t-test. I used data from February (10 species for CT_{max}, nine species for CT_{min}), July (one species for CT_{max}, two species for CT_{min}) or the mean value between the two months (four species for CT_{max}, four especies for CT_{min}) when both were available to determine the same species' CT_{max} and CT_{min} on savannas and forest, but the CT of one species was always determined by data collected in the same period in a way that the only variation was the habitat in which the tested colony was collected. All statistical analyses were performed in R v.4.1.1 (2021-08-10) using the package “lmerTest” (Kuznetsova et al. 2017).

Results

Seasonal variation in habitat temperature

There was no difference on the mean maximum daily temperature between February (28.7°C) and July (27.9°C) (Wilcoxon rank sum test, W = 578, p = 0.101), whereas the mean minimum daily temperature was 4.1°C lower in July (16.1°C) than in February (20.2°C) (Wilcoxon rank sum test, W = 923.5, p < 0.001). The mean daily thermal variation was 3.2°C wider in July (11.7°C) than in February (8.5°C) (Wilcoxon rank sum test, W = 38.5, p < 0.001).

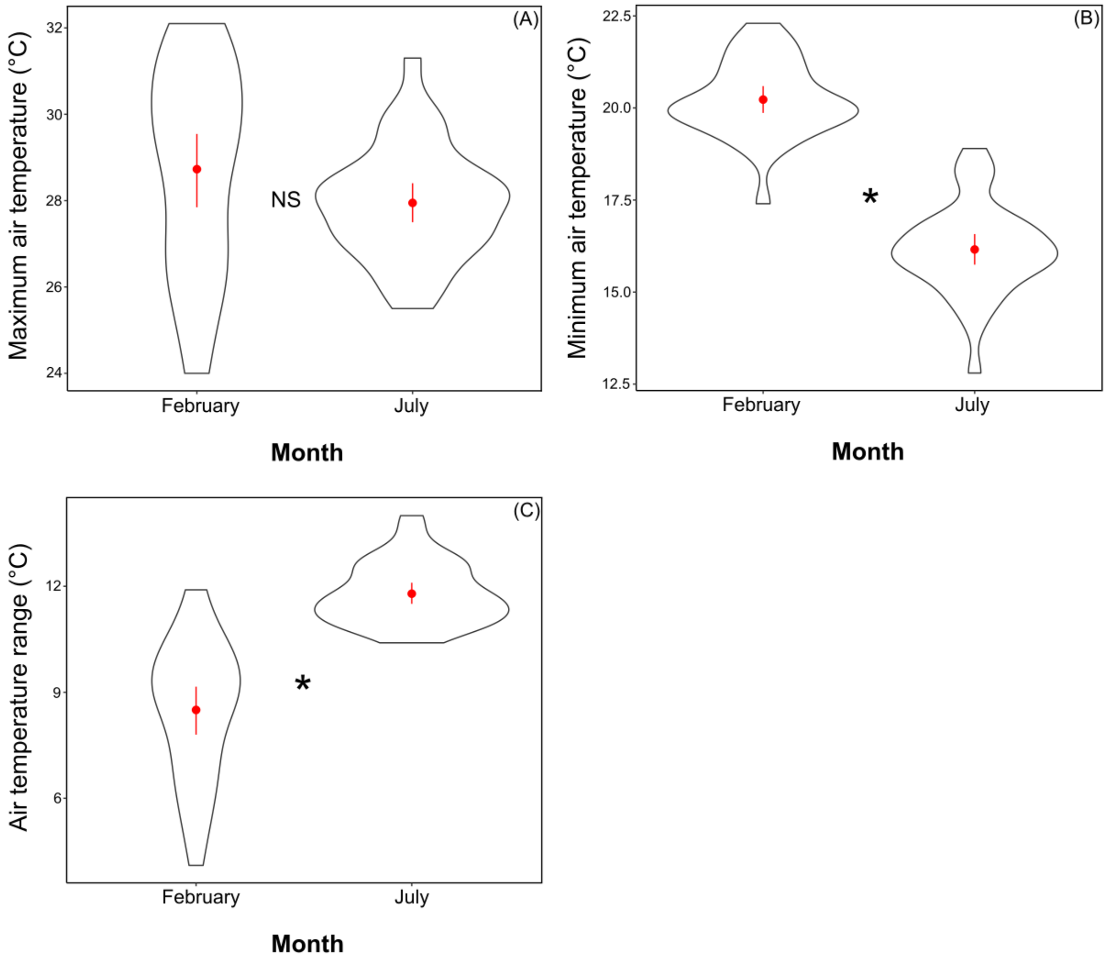

Figure 1. Variation in daily maximum temperature (A), minimum temperature (B) and thermal amplitude (C) between February and July of 2022. Red dots indicates the mean of data points, while red line represent the standard deviation. The width of the curves (black lines) represents the frequency of data points. An asterisk denotes a significant statistical difference between groups. NS = non-significant.

Seasonal variation in thermal tolerance

I measured the CT_{max}, CT_{min} and calculated the CT_{range} of 44 ant species from 21 genera in both February and July 2022. Among those, 31 species were collected in savannas and 16 in forests habitats (three species were collected both in savannas and forests) and 29 were collected in the vegetation while 15 were collected on the ground.

The CT_{max} of the sampled ant species did not differ significantly between February and July (paired t-test, t= 0.853, n = 44 species, p = 0.399) (Figure 2), whereas the CT_{min} was on average 1.29°C (95% CI: 0.992 to 1.591°C) lower in July than in February (t = 8.691, n = 44, p < 0.001) (Figure 2) and the CT_{range} 1.17°C (95% CI: 0.698 to 1.651), wider in July than in February (t = 4.973, n = 44, p < 0.001) (Figure 2).

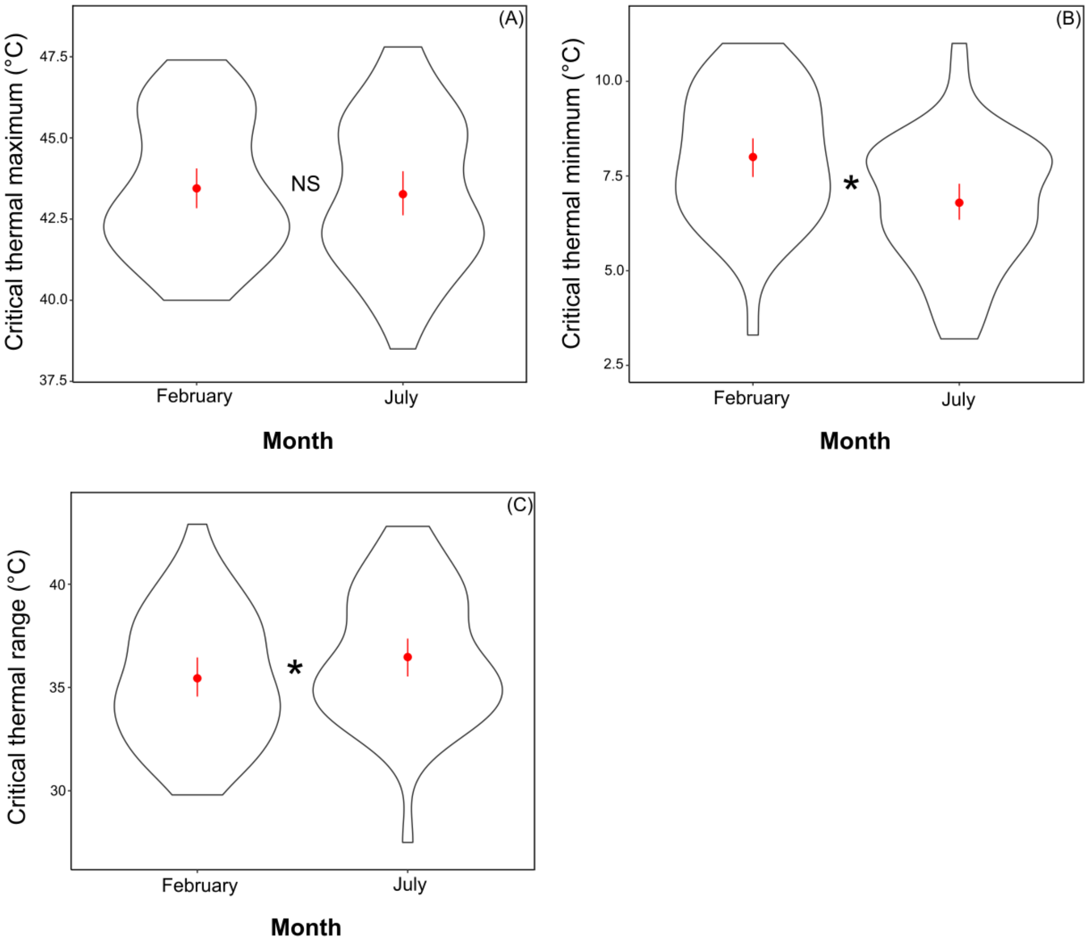

Figure 2. Seasonal variation in the CT_{max} (A), CT_{min} (B) and CT_{range} (C) of a Neotropical ant community (n = 44 species).. Red dots indicates the mean of data points, while red

line represent the standard deviation. The width of the curves (black lines) represents the frequency of data points. An asterisk denotes a significant statistical difference between groups. NS = non-significant.

I found no effect of seasonality on CT_{max} regardless of the habitat ($F_{1,43} = 0.28$, $p = 0.59$) or stratum in which the species was found ($F1_{1,43} = 1.03$, $p = 0.31$). The magnitude of the seasonal change in the CT_{min} was independent of the habitat ($F1_{1,43} = 0.06$, $p = 0.80$), but was stronger for ground-dwelling ants than it was for arboreal ants ($F_{1,43} = 4.95$, $p = 0.03$) (and there was no effect of interaction between habitat and stratum; $F_{1,43} = 0.48$, $p = 0.91$) (Figure 2). The magnitude of the change in the CT_{range} was independent of the habitat ($F_{1,43} = 0.10$, $p = 0.74$) and the stratum ($F_{1,43} = 0.71$, $p = 0.40$) (Figure 3) in which the ant species was found.

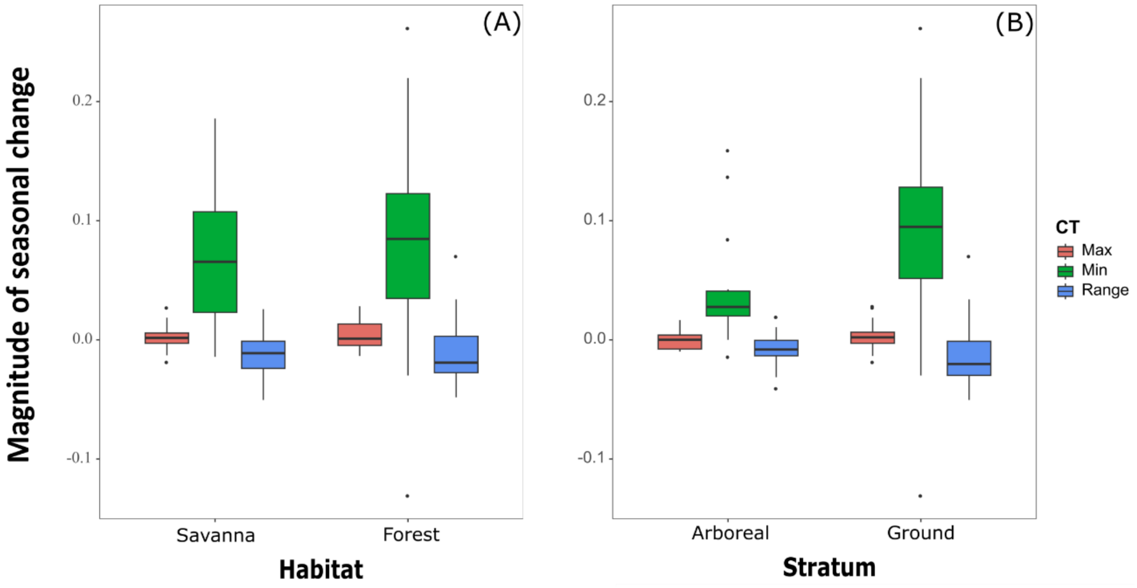

Figure 3. Magnitude of the seasonal change on CT_{max}, CT_{min} and CT_{range} according to habitat and stratum of ants (FA= Forest/Arboreal, FG= Forest/Ground-dwelling, SA= Savanna/Arboreal, SG= Savanna/Ground-dwelling)

Habitat variation in temperature

The maximum daily temperature was 7.6°C higher in the savanna (33.9°C) than in forest (26.3°C) (Wilcoxon rank sum test, W = 133, $p < 0.001$), while there was no difference in the mean minimum daily temperature between savannas (13.3°C) and forests (13.4°C) (Wilcoxon rank sum test, W = 68.5, p = 0.8624).

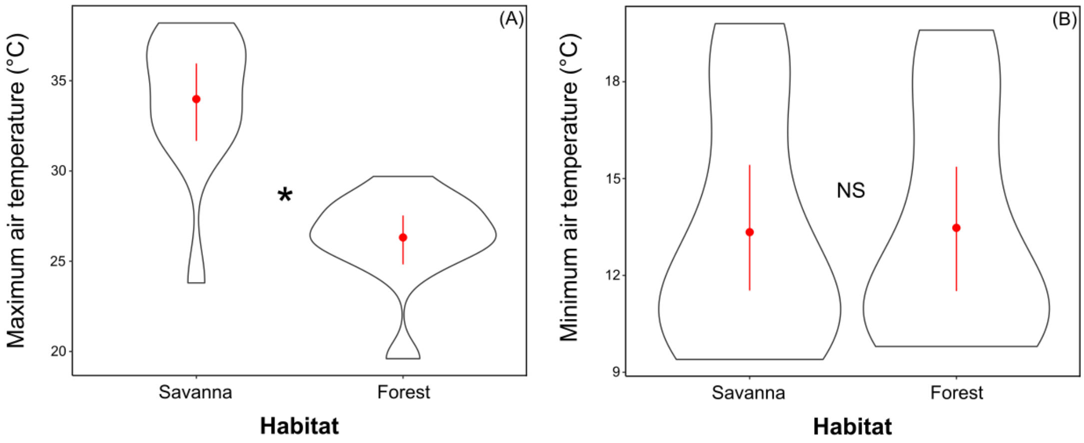

Figure 4. Variation on daily maximum air temperature (A) and minimum air temperature (B) between savanna and forest habitats. Red dots indicates the mean of data points, while red line represent the standard deviation. The width of the curves (black lines) represents the frequency of data points. An asterisk denotes a significant statistical difference between groups. NS = non-significant.

Habitat variation in thermal tolerance

I measured the CT_{max} of 15 ant species from 12 genera and the CT_{min} of 15 ant species from 10 genera in both the savanna and the forest habitats.

On average, the CT_{min} of the sampled species did not differ between habitats (paired t-test, t= 0.453, n = 15 species, p = 0.657), whereas the CT_{max} was on average 0.88°C (95% CI: 0.394 to 1.382°C) higher for ants in savanna than forest (paired t-test, t= 3.855, n = 15 species, p = 0.001) (Figure 5).

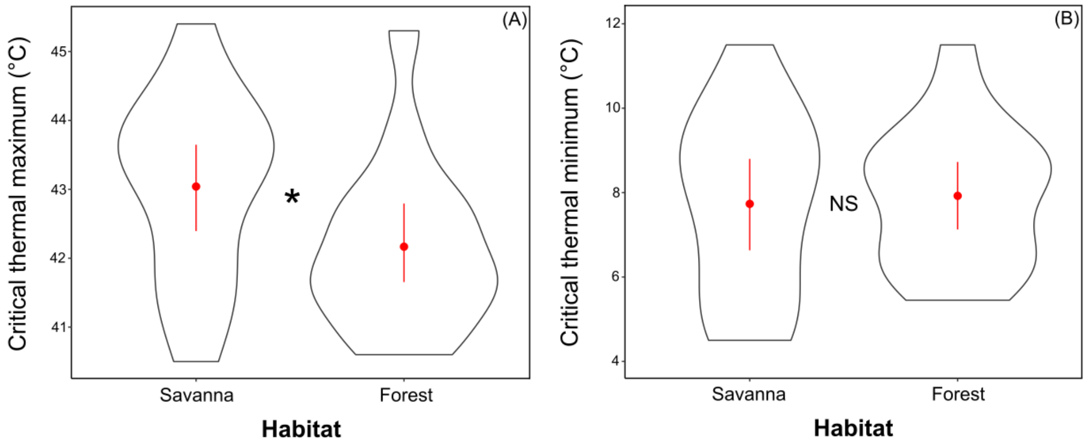

Figure 5. Differences in the CT_{max} (A) and CT_{min} (B) of the same ant species in different habitat types. Red dots indicates the mean of data points, while red line represent the standard deviation. The width of the curves (black lines) represents the frequency of data points An asterisk denotes a significant statistical difference between groups. NS = non-significant.

Discussion

The results of this study show that the thermal tolerance of Neotropical ants varies not only seasonally within the same habitat type, but also spatially between different habitats. However, while seasonally I found changes in CT_{min} and CT_{range}, but not in CT_{max}, spatially I found differences in CT_{max} but not in CT_{min}. Furthermore, I found that the community-wide seasonal change in thermal tolerance to cold was comparatively greater than the between habit change in thermal tolerance (1.29 vs 0.88°C), a finding which is in agreement with previous ones indicating that in ectotherms (Araujo *et al.*, 2013), including insects (Hoffman et al. 2005, Chanthy et al. 2012, Coulin et al. 2019), there is often greater plasticity in the CT_{min} than in the CT_{max}.

Overall, the observed changes in thermal tolerance matched those in climatic conditions. Although the response of individual species was somewhat variable, on a community-wide basis ants presented greater cold tolerance in July, when temperatures were 20.3% lower than in February. Similarly, the range of thermal tolerances was wider in July when thermal daily variation was 27.4% greater than in February, whereas I did not find a significant variation in the CT_{max}, which might be related with the fact that the maximum daily temperatures of February and July were very similar. On the other hand, when I compared the thermal tolerance of habitat generalist species between the two habitat types where they occur, I found evidence that tolerance to heat is greater, on average, in the savanna than in the forest populations, whereas tolerance to cold did not differ significantly. These findings are also consistent with the thermal measurements of the environments, which showed significant differences in the maximum but not in the minimum daily temperatures between the savanna and forest. It is important to note that the CT_{min} of colonies between savanna and forest were variable, although the changes across species were not congruent. In this sense, it suggests that factors others than habitat type might be important to determine an intercolony variation in the cold tolerance of ants, such as diet (Bujan & Kaspari 2017) or time of activity (Garcia-Robledo et al. 2018).

Seasonal variation in heat tolerance has been demonstrated previously in a study with ants from the temperate zone of North America (Bujan et al. 2020b), where the seasonal variation in temperature is comparatively much higher than in the savanna-dominated landscape of central Brazil. This suggests that even relatively modest variations in temperature (as observed in the present study) can induce changes in the thermal tolerance of ants. Interestingly, and contrary to expectations, the effect of habitat type and vertical strata did not interact with the effect of seasonality on thermal tolerance, indicating that temporal plasticity in CT_{min} and CT_{range} are not restricted to a given habitat or foraging/nesting stratum. This increase in the ability to resist to cold might give ants an ecological advantage in cooler months, influencing not only their activity schedule and foraging performance (Cerda et al. 1997, Albrecht & Gotelli 2001, Coulin et al. 2019), but potentially also the outcome of competitive (Wittman et al. 2010) and mutualistic interactions (Tamashiro et al. 2019).

On the other hand, the ability to tolerate more heat in savannas may increase the chances that an ant species will be able to establish a nest, forage and compete in an environment where exposure to high temperatures is a major risk. However, it is noteworthy that the CT_{max} of ant populations from savannas was, on average, only 0.88°C degrees higher than that of the forest populations, while the daily maximum temperature was on average 7.6°C degrees higher in the savanna than in the forest. Thus, this difference in the CT_{max} alone is not enough to explain why habitat generalist ant species manage to occupy such different thermal environments. It is possible that along with an increase in thermal tolerance there are behavioral mechanisms that allow these ants to cope with the high temperatures of the savanna habitat. For instance, there is evidence that the increased consumption of carbohydrates, can generate a short-term increase in the upper thermal limit (Gibbs et al. 1997, Chown & Nicholson 2004, Bujan & Kaspari 2017). In addition, ants can change their diel foraging schedules (as to avoid the hottest periods of the day) (Cerda et al 1997, Yela et al. 2020), the places where they forage (seeking to forage in more shaded areas) (Meisel 2006), or even the architecture of their nests (deeper nests on warmer habitats) (Yela et al. 2020, Sankovitz & Purcell 2021).

The design of my study does not allow identifying whether the observed differences in heat tolerance between savanna and forest populations are entirely due to plasticity or whether they could be related to evolutionary changes. Studies with ants of the genus *Temnothorax* have shown, through garden experiments, that differences in heat tolerance between rural and urban populations separated by several kilometers are due to rapid evolutionary changes (Diamond et al. 2017). However, contrary to these studies, the populations studied here were from adjoining habitats (maximum distance of 700 m), which probably allows a continuous gene flow between them making this type of process more difficult.

Evidence of spatial variation in the thermal tolerance of ants has been also found in studies conducted along elevational (Bishop et al. 2016) or latitudinal gradients (Bujan et al. 2020a), as well as – similarly as done in here - in studies that evaluated small scale thermal gradients (Boyles et al. 2009, Boyle et al. 2020). In this sense, my results reinforce the notion that plasticity in thermal tolerance should be considered in studies that use niche modelling to predict changes in species distribution and vulnerability to global warming. Also, the spatial variation in CT_{max} suggests that habitat generalist species may have a physiological advantage to succeed in a warming world. My study has a community approach, but it is probable that the pattern I found was determined by some species and not all of them. In this way, studies at species level would be important to better understand which factor or factors, other than diet (Gibbs et al. 1997, Chown & Nicholson 2004, Bujan & Kaspari 2017), influence the capacity of different ant species to change their thermal limits. These studies could be fundamental to elucidate the mechanisms behind patterns such as the contextuality of niche partitioning (Grevé et al. 2019), or more specifically how the intraspecific variation in the thermal tolerance of ants can help explain species activity schedule and community dynamics between thermally contrasting habitats. Moreover, considering that temperature can be a determining factor in mutualistic interactions involving ants (Fitzpatrick et al. 2014, Tamashiro et al. 2019), it would also be important to investigate whether temporal fluctuations in the balance of interactions may be related to spatial and temporal variations in the thermal tolerance of ants.

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Supplemental Material

Table S1. List of ant species tested in this study and their mean CT_{max}, CT_{min} and CT_{range} (± standard error) between months. (H= habitat in which the species was collected, St= stratum in which the species was collected, S= savanna, F= forest, S/F = savanna and forest, A= arboreal, G= ground; CT’s without standard error are mean values between savanna and forest colonies)

			February			July		
Species	H	St	CT_{max}	CT_{min}	CT_{range}	CT_{max}	CT_{min}	CT_{range}
Atta laevigatta	S	G	43 (± 1.6)	9.3 (± 1.5)	33.7	43.6 (± 0.8)	8 (± 0)	35.6
Atta sexdens	S	G	43 (± 1)	8.2 (± 0.6)	34.8	41.2 (± 1)	8.4 (± 0.8)	32.8
Azteca sp. 01	S	A	43.8 (± 1.7)	6 (± 0)	37.8	44.8 (± 1)	6.2 (± 1.4)	38.6
Brachymyrmex cf. *aphidicola*	S/F	G	43.4	7.3	36.1	41.8	7.8	34
Camponotus atriceps	F	A	42.8 (± 1.3)	5.8 (± 1.7)	37	41.2 (± 1.9)	5.8 (± 2.5)	35.4
Camponotus blandus	S	G	45.8 (± 2.5)	8.2 (± 0.6)	37.6	45.8 (± 1.7)	8 (± 2.1)	37.8
Camponotus bonariensis	S	A	46.2 (± 0.6)	3.3 (± 1.8)	42.9	45.6 (± 1.5)	3.2 (± 2.1)	42.4
Camponotus lespesii	F	G	41.6 (± 0.8)	7.6 (± 1.7)	34	40.2 (± 1.1)	6.4 (± 2.4)	33.8
Camponotus melanoticus	F	G	42.5 (± 1.7)	6.9 (± 1.8)	35.6	43.2 (± 1.9)	3.8 (± 2.7)	39.4
Camponotus renggeri	S	G	42.8 (± 1.6)	6.8 (± 2.5)	36	42.6 (± 1.6)	6.8 (± 2.6)	35.8
Camponotus senex	S	A	46 (± 1.2)	6.7 (± 1.3)	39.3	45.4 (± 1.4)	6.1 (± 1.2)	39.3
Camponotus sericeiventris	S	A	44.4 (± 1.2)	9 (± 1.6)	35.4	44.2 (± 1.1)	8.2 (± 1.4)	36
Cephalotes atrattus	S	A	46.4 (± 0.8)	7.8 (± 0.6)	38.6	47.4 (± 0.9)	7.4 (± 0.9)	40
Cephalotes depressus	S	A	47.4 (± 0.9)	6.8 (± 1.0)	40.6	47.4 (± 1.3)	5.6 (± 1.5)	41.8
Cephalotes pusillus	S	A	46.9 (± 1.5)	9.2 (± 1.5)	37.7	47.8 (± 0.6)	6.4 (± 0.8)	41.4
Crematogaster cf. *arcuata*	S	G	46.1 (± 0.4)	5.1 (± 1.2)	41	46.2 (± 0.6)	3.4 (± 0.9)	42.8
Crematogaster cf. sp14	F	A	44.8 (± 1)	7.4 (± 0.9)	37.4	45.6 (± 0.8)	5.4 (± 1.3)	40.2
Dorymyrmex brunneus	S	G	46 (± 0.9)	6 (± 0)	40	45.4 (± 2.1)	4.8 (± 1)	40.6
Ectatomma brunneum	S	G	44.1 (± 0.5)	11 (± 1.3)	33.1	44 (± 0)	8 (± 0)	36
Ectatomma edentatum	F	G	42 (± 0.7)	7.7 (± 0.7)	34.3	42 (± 0)	6.2 (± 0.6)	35.8
Ectatomma opaciventris	S	G	44.6 (± 1)	10.6 (± 1)	34	45 (± 1)	8.2 (± 0.6)	36.8
Ectatomma tuberculatum	F	A	42.6 (± 0.9)	9 (± 1)	33.6	43.4 (± 0.9)	8.2 (± 0.7)	35.2
Holcoponera striatula	S	G	43.4 (± 0.9)	9.6 (± 1.2)	33.8	42.4 (± 0.8)	8.6 (± 0.9)	33.8
Labidus coecus	S	G	41.9 (± 0.3)	6.1 (± 1.1)	35.8	39.4 (± 0.9)	5.4 (± 1.6)	34
Mycetomoellerius dichrous	S	G	42 (± 0)	10.8 (± 1)	31.2	42 (± 0)	10 (± 0)	32
Mycocepurus goeldii	S	G	40.5 (± 2.1)	10 (± 1.1)	30.5	41.8 (± 0.6)	8.6 (± 0.9)	33.2
Neoponera marginata	F	G	42 (± 0)	10 (± 0)	32	41.8 (± 0.6)	8 (± 0.8)	33.8
Neoponera verenae	S	G	41.7 (± 0.7)	9.7 (± 0.7)	31.95	42 (± 0)	8 (± 0)	34
Neoponera villosa	S	A	42.1 (± 0.5)	6.2 (± 1)	35.9	42 (± 0)	6 (± 0)	36
Nylanderia caeciliae	F	G	41.2 (± 1.2)	8.6 (± 0.9)	32.6	40 (± 0)	7.8 (± 0.6)	32.2
Odontomachus chelifer	F	G	40 (± 0)	8 (± 0)	32	40.3 (± 0.8)	6.3 (± 0.8)	34
Pachycondyla harpax	F	G	40.7 (± 1.3)	8.9 (± 1.5)	31.8	40.4 (± 0.8)	6.8 (± 1)	33.6
Pachycondyla striata	F	G	42 (± 0)	9.6 (± 2.5)	32.4	41.8 (± 0.6)	6.2 (± 0.6)	35.6
Pheidole oxyops	S/F	G	41	6.4	34.6	42	4.05	37.95
Pheidole radoszkowskii	S	G	41.4 (± 1.3)	7 (± 1.3)	34.4	40.8 (± 1.3)	4.6 (± 0.9)	36.2
Pseudomymex curacaensis	S	A	45.6 (± 0.8)	7.8 (± 1.4)	37.8	45.2 (± 1.3)	7.3 (± 2)	37.9
Pseudomyrmex gracilis	S	A	46.6 (± 0.9)	7.2 (± 1)	39.4	45.3 (± 1.4)	6.8 (± 1)	38.5
Sericomyrmex mayri	F	G	40 (± 1.3)	10.2 (± 1)	29.8	38.5 (± 1)	11 (± 2)	27.5
Sericomyrmex scrobifer	S	G	42.6 (± 1)	10.3 (± 0.8)	32.3	42.2 (± 0.6)	8.1 (± 1.2)	34.1
Solenopsis basa	S/F	A	45.3	5.7	39.6	45.4	5.3	40.1
Solenopsis prox latastei	S	G	44.6 (± 0.9)	6.8 (± 1)	37.8	44.2 (± 0.6)	4.6 (± 0.9)	39.6
Solenopsis substituta	S	G	46.8 (± 1)	8.6 (± 1.3)	38.2	46.2 (± 0.6)	7 (± 1)	39.2
Wasmannia auropunctata	S	G	41 (± 1.4)	10.2 (± 1.4)	30.8	42.8 (± 1.0)	8.2 (± 1.4)	34.6
Wasmannia sigmoidea	F	A	43 (± 1.4)	8.6 (± 1.3)	34.4	43.4 (± 0.9)	8 (± 0)	35.4

Table S2. List of ant species tested in this study and their mean CT_{max} (± standard error) between habitats. (CT_{max} without standard error are mean values between results of February and July)

	CT_{max}	
Species	Savanna	Forest
Acromyrmex subterraneus	42 (± 0.9)	41.5 (± 0.8)
Atta laevigatta	43 (± 1.6)	42.8 (± 1.0)
Brachymyrmex cff. *aphidicola*	43.7	41.5
Camponotus melanoticus	43.6 (± 1.1)	42.5 (± 1.7)
Ectatomma edentatum	43.8 (± 0.6)	42 (± 0)
Ectatomma tuberculatum	44 (± 0)	42.6 (± 0.9)
Holcoponera striatula	43.4 (± 0.9)	40.8 (± 1.4)
Neoponera marginata	41.8 (± 0.6)	42 (± 0)
Pachycondyla harpax	40.5 (± 1)	40.7 (± 1.3)
Pheidole oxyops	41.6	41.4
Pheidole radoszkowskii	41.1	40.5
Pheidole triconstricta	43.5 (± 1)	41.5 (± 0.8)
Solenopsis basa	45.4	45.3
Solenopsis cff. *latastei*	44.6 (± 0.9)	43.8 (± 0.6)
Wasmannia sigmoidea	43.6 (± 0.8)	43 (± 1.4)

Table S3. List of ant species tested in this study and their mean CT_{min} (± standard error) between habitats. (CT_{min} without standard error are mean values between results of February and July)

	CT_{min}	
Species	Savanna	Forest
Brachymyrmex cff. *aphidicola*	6.4	8.7
Camponotus melanoticus	5.5 (± 1.7)	6.9 (± 1.8)
Ectatomma edentatum	7.6 (± 1.2)	6.2 (± 0.6)
Ectatomma tuberculatum	8.9 (± 1)	9 (± 1)
Holcoponera striatula	9.6 (± 1.2)	11.5 (± 3.7)
Neoponera marginata	8.4 (± 0.8)	10 (± 0)
Neoponera verenae	8.8 (± 0.7)	8.6 (± 1.7)
Nomamyrmex esenbeckii	10 (± 0)	8.4 (± 0.8)
Pachycondyla harpax	11.5 (± 1.9)	8.9 (± 1.5)
Pheidole oxyops	5	5.45
Pheidole radoszkowskii	4.6 (± 0.9)	6.4 (± 0.8)
Pheidole triconstricta	10 (± 1.8)	7.7 (± 0.9)
Solenopsis basa	4.5	6.5
Solenopsis cff. *latastei*	6.8 (± 1)	6 (± 0)
Wasmannia sigmoidea	8.4 (± 0.8)	8.6 (± 1.3)

CAPÍTULO 3

Nutrient supplementation to arboreal ants: effects on trophic position, thermal tolerance, community structure and the interaction with the host-tree

Abstract

Carbohydrates and proteins are essential to maintain the basic functions of animals. During one-year we conducted a factorial experiment to determine the influence of carbohydrate (sucrose) and protein supplementation on the thermal tolerance, trophic position, overall abundance, species richness and composition, and on the strength of the protective effects of arboreal ants on their host tree (*Caryocar brasiliense*). Using *Azteca* ants as model we found evidence of dietary and thermal plasticity among arboreal ants as colonies supplied with protein increased their trophic level relative to colonies that received no protein. Colonies that received sucrose increased their thermal tolerance on average by 1.5°C over a six-month period, whereas those that did not receive sucrose did not change their thermal tolerance. Overall ant abundance was lower in control trees than in those that received any nutrient addition treatment. Species richness was also lower in control trees, but those receiving sucrose presented more species than those receiving only protein. There was greater similarity in species composition between the trees that received sucrose than between these and those receiving only protein or just water as control. Trees whose ant colonies received sucrose presented lower levels of leaf damage than those that did not. Overall, these results indicate that food resources can modulate the population and community ecology of arboreal ants as well as their interaction with the host trees. Interestingly, although arboreal ants are thought to be N-limited, it was the supplementation of sucrose – not protein - that elicited most of the responses.

Keywords: Nutritional ecology; Ecological Stoichiometry; Cerrado; Formicidae; Diet; Nitrogen; Sucrose

Introduction

Nutrition influences every aspect of an animal´s life, either directly by building the components of the organisms and affecting their performance, or indirectly by influencing the interaction between species and the environment at multiple scales (Simpson & Raubenheimer 2012). Rather than simply maximizing food intake, consumers must regulate foraging in a way that it matches the optimal mixture of macronutrients and micronutrients required for survival (Simpson & Raubenheimer 2000, Feldhaaar 2014), and this becomes even more challenging when considering that nutrient availability varies between and within habitats (Elser & Sterner 2002). Nutritional interactions between organisms and the environment might influence species physiology and behaviour, working as a bottom-up force that alters species relations and the structure of biological communities (Raubenheimer *et al.* 2009, Bujan & Kaspari 2017, Raubenheimer & Simpsons 2018).

Carbohydrates and proteins are essential compounds that act in a complementary way to maintain basic functions of animals, such as growth, survival, and reproduction (Tilman et al. 1982, Simpson & Raubenheimer 1993). The availability of carbohydrates and protein depends on the type of food resource, which in turn varies temporally and spatially. For example, for organisms in the canopy of tropical forests there is often a greater availability of carbohydrates than of proteins (Yanoviak & Kaspari 2000). According to the Compensation Hypothesis (Kaspari & Yanoviak 2001, Davidson, 2005) the attractiveness and utility of a food resource to a given organism is conditional to its availability in the environment in question. Thus, for consumers in the forest canopy, protein may be more attractive than the abundant carbohydrate (Takahashi et al. 2019; Law et al. 2020).

Ants are one of the most ubiquitous and diverse group of arthropods in the canopy of tropical forests and savannas (Hölldobler & Wilson 1990). In general, arboreal ants have a carbohydrate-based diet that follows the high availability of sugary plant and insect exudates in the canopy (Blüthgen et al. 2003, Davidson et al. 2003, Rico-Gray & Oliveira 2007). However, most arboreal ants are omnivorous (Davidson et al. 2003, Davidson et al. 2004, Ribeiro et al. 2019) whose foraging behavior and trophic level can vary according to availability of different food sources in the environment (Tsang et al. 2020). Although some studies suggests that ants are more constrained by protein sources in the canopy, and therefore more attracted to it (Yanoviak & Kaspari 2000; Ribeiro et al. 2019), there is also evidence that in fact the most limiting resource for arboreal ants are carbohydrates (Lasmar et al. 2023). It has been suggested that if selective pressures across ants’ evolutionary history favored species that feed on the most abundant resource, then carbohydrates could be a limiting nutrient for arboreal ants even considering its high abundance in the canopy (Lasmar et al. 2023).

Carbohydrates fuel the more energetically costly activities of the colonies and is related to the maintenance of the workers' body functions, whereas protein is fundamental for the growth and development of larvae and pupae (Blüthgen & Feldhaar 2010). In this sense, both nutrients act in complementary ways to ants’ colony growth and survival, which ultimately could affect species interactions and the structure of communities (Rowles & Silverman 2009, Kaspari et al. 2012). Moreover, both carbohydrates and protein can interact and influence numerous other aspects of an ant´s life. For example, carbohydrates can provide energy for thermoregulation (Chown & Nicholson 2004) and increase evaporative cooling effects (Gibbs et al. 1997), while protein can influence the production of heat shock proteins, which are essential to cope with thermal stress (Andersen et al. 2010, King & McRae 2015). Therefore, nutrition can alter the critical thermal maximum (CT_{max}) of ant workers (Bujan & Kaspari 2017) which in turn can affect their foraging schedules, disrupt transitive hierarchies (Cerda et al. 1997, 1998) and/or affect their mutualistic interactions with plants (Fitzpatrick et al. 2014).

Many species of arboreal ants nest or forage on plants that have extrafloral nectaries (non-reproductive organs and plant tissues that produce carbohydrate-rich nectar, EFNs hereafter). This interaction can be considered mutualistic when ants prey on herbivores and thus increase plant fitness, while benefiting from nesting and food resources (Rico-Gray & Oliveira 2007). Several non-exclusive hypotheses have been proposed to explain why ants defend EFN-producing plants. The Fuel for Foraging Hypothesis (Caroll & Janzen 1973, Davidson et al. 1988, Grover et al. 2007) proposes that the ant visitors are fueled by carbohydrates provided in the EFNs, increasing ant´s foraging and aggressiveness. The Ownership Hypothesis (Janzen 1969, Davidson et al. 1988) proposes that a valuable resource elicits ownership behavior, so that ants would defend the plant against its natural enemies while protecting and dominating the food resource. Lastly, the Deficit Hypothesis (Ness et al. 2009) proposes that the nutritional imbalances of EFNs (high C:N) increases the ants' need for protein, increasing the chance that they attack an herbivore on the plant.

Although there are studies showing how the availability of carbohydrates alters the foraging behavior and aggressiveness of ants (Kay et al. 2010, Pacelhe et al. 2019), ultimately benefiting the plant (Kost & Heil 2005, González-Teuber et al. 2012), only a few studies have explored how protein availability (and its interaction with carbohydrates) affects the outcome of the interaction between ants and plants (e.g. Passos & Leal 2019). Furthermore, relatively few studies have evaluated how nutrient supplementation affects the thermal tolerances and trophic position of individual colonies as well as the structure of the ant communities foraging on trees.

Here we evaluated the influence of nutrient supplementation on the ecology of arboreal ants. For this, we performed a one-year long field experiment in which carbohydrate (sucrose) and protein were supplied to ants associated with an EFN-producing savanna tree species. We addressed four questions. At the level of individual ant colonies we asked: (1) What are the individual and combined effects of carbohydrate and protein supplementation on the thermal tolerance of arboreal ants? We expected that thermal tolerance would vary among colonies subject to different nutrient supplementation treatments, since carbohydrates and protein are known to influence the thermal tolerances of insects in different ways (Andersen et al. 2010, King & McRae 2015). In addition, (2) we evaluated what are the individual and combined effects of carbohydrate and protein supplementation on the trophic position of arboreal ants? According to the Compensation Hypothesis, which states that animals tend to forage in a way to correct nutritional imbalances (Kaspari & Yanoviak 2001, Davidson 2005), we expected that colonies supplemented with the most limiting resource would have the greatest change in their trophic positions.

At the community level we asked: (3) What are the individual and combined effects of carbohydrate and protein supplementation on the overall abundance, species richness, and composition of arboreal ants on trees? Given that competition over food resource is a strong structuring force among arboreal ant communities (Savolainen et al. 1988, Parr & Gibb 2010, Cerda et al. 2013, Camarota et al. 2018), we would expect that both protein and sucrose would generate an increase in the overall abundance and species richness of ants on trees. Furthermore, considering that the attractiveness of food resources varies between species (Ribeiro et al. 2019), we also expected that species composition would be different across the nutrient supplementation treatments. Finally, we asked: (4) Does nutrient supplementation affects the protective effects of ants against the herbivores of their host tree (level of foliar damage)? We expected that, if the Ownership Hypothesis (Janzen 1969, Davidson et al. 1988) is correct, then colonies receiving complementary food resources (i.e., both carbohydrate and protein) would better protect their host trees. On the other hand, if the Deficit Hypothesis (Ness et al. 2009) is correct, then the supplementation of protein should diminish the ants' need for protein and therefore their predatory activities. In this sense, the most protected trees would be those in which colonies receive carbohydrates only. Similarly, if the Fuel for Foraging Hypothesis (Caroll & Janzen 1973, Davidson et al. 1988, Grover et al. 2007) is correct, access to carbohydrates would fuel foraging and aggressive behavior of ants, increasing protection on trees supplemented with carbohydrates.

Material and Methods

Study area

This study was conducted at the Reserva Ecológica do Panga, a 404-ha reserve located 30 km south of Uberlândia, Minas Gerais, Brazil (19° 10' S, 48° 23' W). The mean annual temperature of the region is 22°C and the mean annual rainfall is 1,650 mm. All observations and experiments were performed on *Caryocar brasiliense* trees located within an area of approximately five hectares covered by the dominant vegetation type of the reserve (woodland savanna, locally known as *cerrado sensu stricto*), and which is characterized by a superior layer of trees and shrubs and a ground layer formed by grasses, herbs and small shrubs (Eiten 1972). *Caryocar brasiliense* is a common and widespread species in the savannas of central Brazil which produces EFNs on its leaves and inflorescences.

Supplementation experiment

We selected and marked 67 medium sized (3-4m in height) *C. brasiliense* trees that were separated from each other by at least 12 m. This distance was large enough to ensure that ants from a given experimental tree were not foraging in another tree. In fact, foraging by predominantly arboreal species was largely restricted to the host tree. This was because the savanna where our experiment took place was relatively open and therefore there was very little connectivity between the crowns of different trees (Powell et al. 2011). Each of the selected trees received one of the following nutrient supplementation treatments (1) carbohydrate only (20% water solution of sucrose) (n = 17 trees); (2) protein only (20% water solution containing equal parts of isolate whey protein, calcium caseinate and egg white powder) (n = 16) (3) carbohydrate and protein (mixture containing equal parts of the carbohydrate and protein solutions) (n = 19) and (4) control trees (water only) (n = 15). The amounts of carbohydrate and protein added to the water solutions are similar to those used in previous studies (Ribeiro et al. 2019, Kaspari et al. 2012) and were chosen because they mimic the concentration of these two elements in natural food sources (Bluthgen et al. 2004, Dussutour & Simpson 2008). A total of six sealed plastic cups (6 cm high, 5 cm in diameter), with access holes (five 6-mm diameter holes, drilled on the sides of each cup), were wired onto the branches of each experimental tree (Figure 1). Each cup was filled with 60 ml of water, sugar, protein or sugar and protein solution. A piece of folded tissue paper in the cup served both as a feeding platform and a structure that ants could use to escape the liquid if they fell in. Solutions and tissue platforms were changed weekly. The experiment had a duration of one year (August 2020 - July 2021).

Figure 1. Supplementation cup of protein treatment (20% water solution containing equal parts of isolate whey protein, calcium caseinate and egg white powder) with recruitment of ants of the genus *Azteca* on the feeding platform of folded tissue paper.

Critical thermal maximum (CT_{max})

To evaluate the influence of the nutrient supplementation treatments on ant´s resistance to heat, we measured the CT_{max} of 21 colonies of an unidentified *Azteca* species (hereafter *Azteca*) just prior and again six months after the beginning of the experiment. The CT_{max} of the ants was determined using a dry bath equipment (Kasvi Dry Bath model K80-S01 / 02). In each test 20 workers of the same colony were placed individually in Eppendorf tubes sealed by cotton and placed randomly in the dry bath equipment. The initial temperature of the test was 36°C, which was increased by two degrees every 10 minutes of exposure until death or permanent loss of muscle coordination in the workers. Tests were carried out within a maximum period of up to four hours after the collection of the ants in the field. We considered the CT_{max} of the species as the average temperature of death or permanent loss of muscle coordination of the 20 workers.

Stable isotope analyses

We determined the isotopic signature ($\delta^{15}N$) and the carbon and nitrogen ratios (C:N ratio) of ants from 18 *Azteca* colonies. For this, we collected approximately 20 workers from each colony 10 months after the beginning of the experiment. We removed the gaster of each worker during sample preparation to avoid the effect from recently ingested food items on the analysis (Bluthgen et al. 2003, Tillberg et al. 2006). Ant samples were dried in an oven at 60°C for 48 h and then crushed with an agate mortar and pestle. The dried samples were put into small tin capsules in precisely weighed amounts (1.25-1.5mg) then molded into a spherical shape, put on ELISE dishes, and sent to the University of California Stable Isotope Facility, in Davis, California, USA, for analysis. The results were expressed in delta notation per thousand, with an internationally acknowledged standard as reference.

Sampling of the ant fauna

To estimate the abundance and diversity of arboreal ants in the experimental trees (n = 67), two observers counted the number and determined the identity of the ants foraging on the main trunk and branches of each tree for a period of two minutes during the morning (7:00 am – 9:30 am), and again in the afternoon (12:00 am – 2:30 pm), and evening (7:00 pm – 9:30 pm) of the same day. This was done once in May 2021, and again one month later by the same two observers. When necessary, ant specimens were collected and stored in alcohol for later identification in the laboratory. Voucher of all species collected are deposited at the Zoological Collection from the Federal University of Uberlândia (UFU) in Uberlândia, Brazil.

Artificial nests

We also assessed the effect of nutrient supplementation on the occupation of artificial wooden nests by arboreal ants. For this we wired six nests to the branches of about half of the experimental trees (32 of the 67 trees). The nests were made of bamboo (~ 100 mm in length and 10 mm of diameter). Three of the bamboo nests had an opening of 6 mm^2 and the other three an opening of 8 mm^2, which were the most used nest entrance sizes in an experiment conducted earlier (Powell et al. 2011). The bamboo nests were installed in October 2020 and removed in August 2021. Each nest was sealed with adhesive tape and transported to the laboratory, where they were opened and the identity and number of ants was recorded, as well as the presence of eggs, larvae, pupae and alates.

Herbivory measurements

We estimated the level of leaf damage on 59 of the 67 experimental trees. For this, one branch from each tree from was randomly selected and 10-14 newly initiated leaves (with no signs of damage) from this branch were marked using plastic-coated wires placed around the leaf petiole. This was done in September 2020 when *C. brasiliense* was flushing new leaves. After 90 days, we collected the marked leaves and produced a scanned image of each one. The area damaged by chewing insects and the total area of each leaf was determined using the ImageJ software (Rasband 2013).

Statistical analyses

We analyzed the individual and interactive effects of the nutrient supplementation treatments on the isotopic signature ($\delta^{15}N$) and on the carbon to nitrogen ratio (C:N) of *Azteca* ant workers using two-way ANOVAs, in which the predictor variables were carbohydrate (with or without) and protein (with or without). The same model was used to evaluate the magnitude of change (the effect size) of the CT_{max} of the *Azteca* ants. The magnitude of change was calculated as the logarithm of the response ratio using the formula: log (CT_{max} after supplementation / CT_{max} before supplementation). Similarly, two-way ANOVAs were employed to evaluate the effects of nutrient supplementation on the species richness and overall abundance (i.e., the abundance of all species combined) of ants foraging on trees as well on the proportion of artificial nests colonized by ants and the abundance of ants in nests. Finally, a two-way Anova was run to evaluate effects on leaf herbivore damage in *C. brasiliense* trees. When a significant interaction between the effects of carbohydrate supplementation and protein supplementation was detected, we performed a posteriori, pairwise multiple comparisons among individual treatments using the Tukey method.

Data on ant abundance was log (x +1) transformed prior to the analyses to meet the assumption of data normality and homoscedasticity. Similarly, data on leaf herbivory was arcsine square root transformed prior to the analysis. A Gaussian error distribution was assumed in most models, except the one on species richness in which a quasi-Poisson model was run, and the one on the proportion of artificial nests colonized by ants in which a binomial distribution was assumed. Model assumptions were checked by evaluating the plot of the residuals against the fitted values and the normal probability plot. Analyses were performed in R v.4.1.1 (R Core team 2022), using the packages “car” (Fox & Weisberg 2020) and “emmeans” (Russell 2021).

To evaluate the differences in ant species composition among trees subject to different nutrient addition treatments we performed a two-way cluster analysis. For this, we first built a matrix containing information about the total number of trees (from each nutrient addition treatment) in which each ant species was recorded. The analysis was run in PCORD 7.0 (MJM Software Design, Gleneden Beach, Oregon, USA) using the Bray–Curtis index of similarity (with individual species data relativized by the species total) and the group average linkage method (Peck 2010). In this analysis, we used only data for those species that occurred in at least three of the 67 trees sampled, since the occurrence of a rare species in a giving treatment could be more related to its low abundance in the community than its nutritional requirements.

Results

Effects on the trophic position and thermal tolerance of Azteca

Protein supplementation presented a significant effect on the δ15N and C:N ratio of the *Azteca* colonies, whereas the supplementation of carbohydrate (sucrose) did not have an effect (Fig. 2). Colonies that received protein (i.e., only protein or protein together with carbohydrate) presented, on average, a δ15N 4% greater and a C:N ratio 7.2% lower than those that did not. Conversely, while sucrose supplementation affected the critical thermal maxima (CT_{max}) of *Azteca*, protein did not have an effect (Fig. 3). On average, colonies receiving sucrose (sucrose only or sucrose together with protein) increased their CT$_{max}$ in 1.59°C, whereas the mean absolute change in the CT$_{max}$ of the colonies that did not receive sucrose was close to zero (- 0.022◦C).

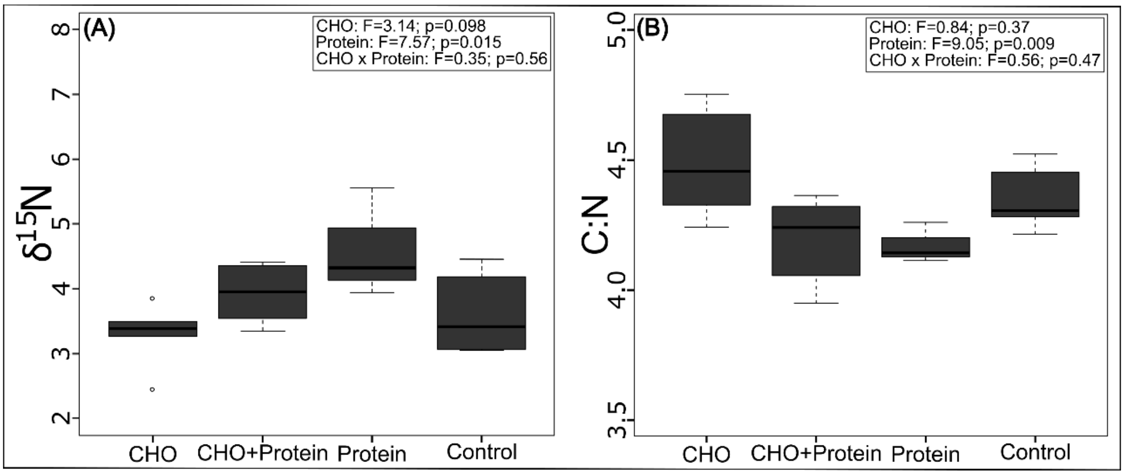

Figure 2. Effects of carbohydrate and protein supplementation on the δ15N (A) and the carbon to nitrogen ratio (B) of Azteca sp. colonies.

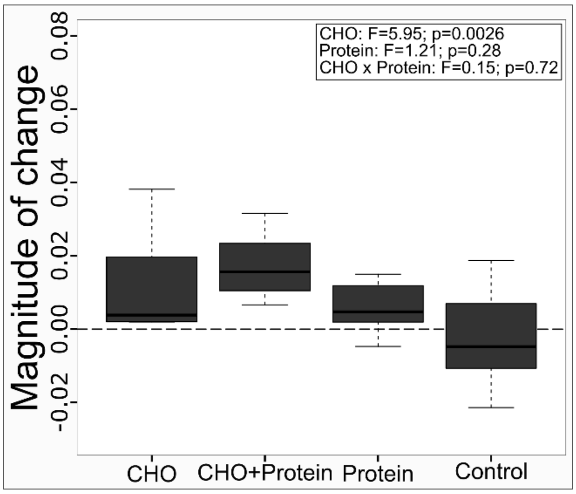

Figure 3. Effect of carbohydrate and protein supplementation on the thermal tolerance of *Azteca* sp. Values represent the magnitude of the difference (effect size) in the CT$_{max}$ of ant workers prior and six months after the beginning of the experiment.

Effects on overall ant abundance, species richness and composition

There was a significant interaction between the effects of carbohydrate and protein supplementation on ant species richness per tree. Control trees had fewer ant species than those that received carbohydrate, protein, or both (Tukey test, $p \leq 0.008$). In addition, there was a difference between those that received only carbohydrate or only protein, with the former presenting more species than the latter (Tukey test, $p = 0.042$) (Figure 4). Similarly, there was a significant interaction between the effects of carbohydrate and protein on the overall abundance of foraging ants per tree. Overall ant abundance in control trees was significantly lower than in trees from all the remaining treatments (Tukey test, $p \leq 0.006$) (Figure 4).

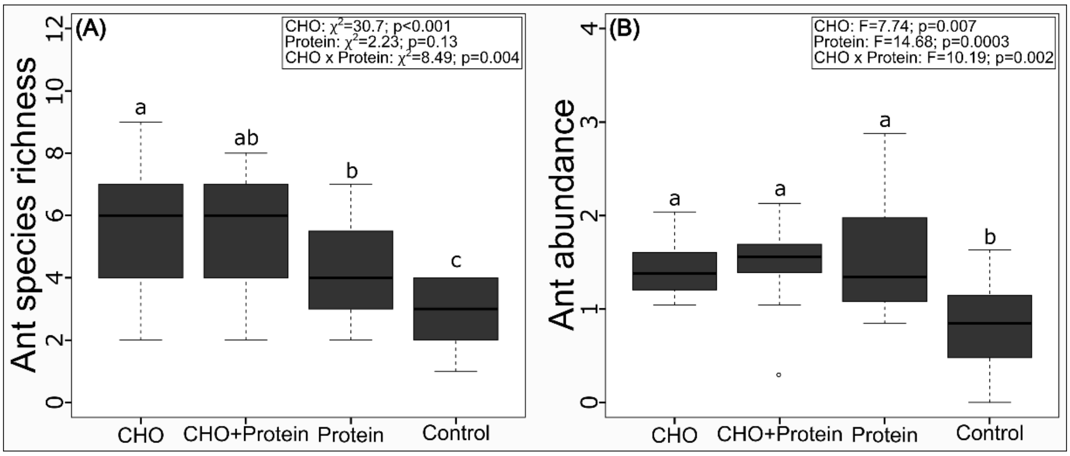

Figure 4. Effects of carbohydrate and protein supplementation on the species richness (A) and overall abundance (B) of ants (log transformed) foraging in *Caryocar brasiliense* trees. Since there was a significant interaction between the main effects a posteriori multiple comparison test was performed, and thus different letters above box plots indicate significant differences among mean values.

We recorded a total of 43 ant species from 19 genera in the 67 experimental trees (Table S1). Twenty-three species were rare being recorded in just one or two trees. Excluding the rare species, we found that the similarity in species composition was comparatively greater between trees that received sucrose only and those that received sucrose and protein than between these two and those that received protein only or the control trees (Figure 5). Five species (*Azteca* sp. 1, *Camponotus senex*, *C. bonariensis*, *Pseudomyrmex gracilis*, *P. curacaensis* and *Tapinoma* sp. 7) were relatively widespread being found in trees from any treatment. *Brachymyrmex* nr. *aphidicola*, *Camponotus melanoticus*, *C. fastigatus*, *Dorymymrex* sp. 10, *Ectatomma tuberculatum* and *Pheidole radoszkozwski*, were found mainly in trees receiving sucrose (alone or in combination with protein) whereas *Atta laevigata*, *Camponotus blandus*, *Camponotus substitutus*, *Neoponera villosa* and *Solenopsis basalis* in trees that received protein (alone or in combination with sucrose) (Figure 5).

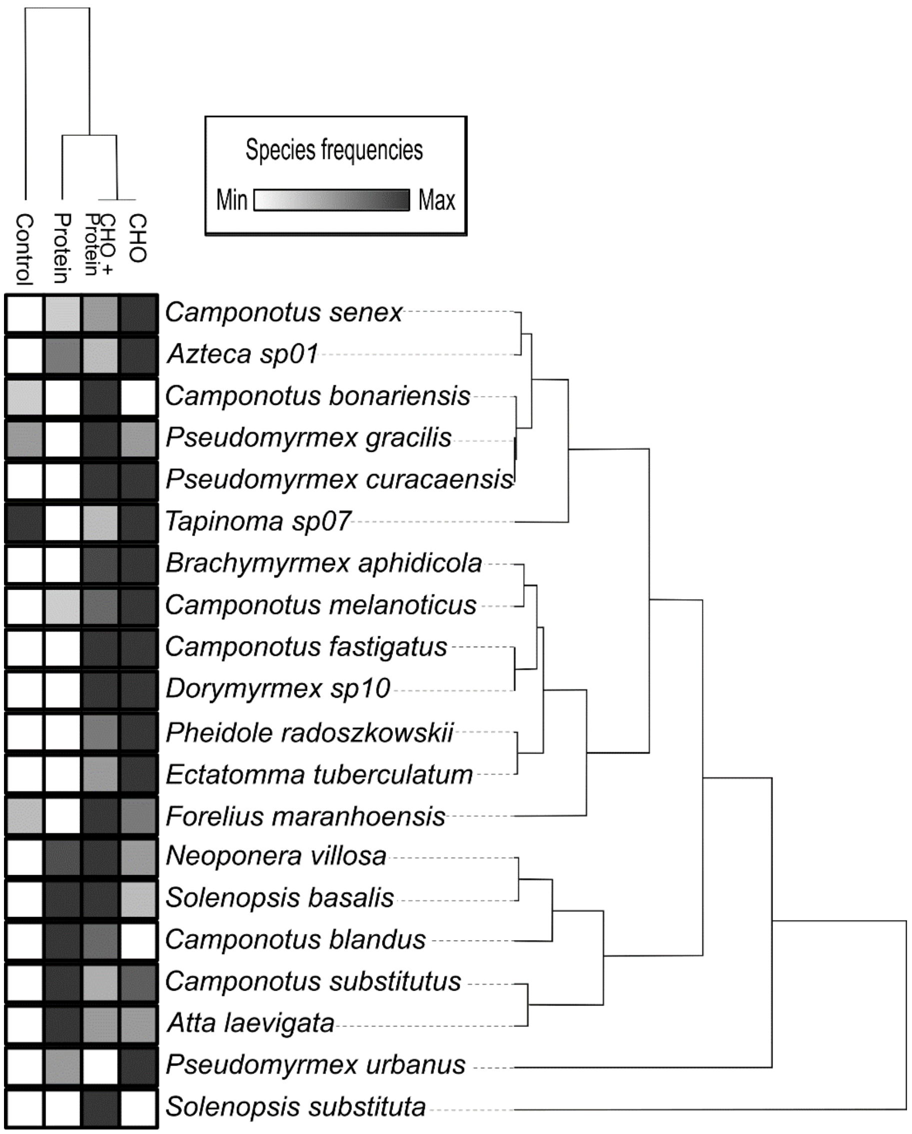

Figure 5. Two-way cluster dendrogram showing the relative frequency of different ant species in trees subject to different nutrient supplementation treatments.

Colonization of the artificial nests

From 192 artificial nests, 44 (22.9%) were colonized by a total of four species of ants: *Camponotus senex, Camponotus melanoticus, Camponotus bonariensis* and *Pseudomymex gracilis*. From these 44 occupied nests, six had only ant workers, and 38 contained workers and ant brood (eggs, larvae and/or pupae). Neither carbohydrate nor protein supplementation affected the proportion of artificial nests colonized by ants (Figure 6). However, nests in tree supplemented with carbohydrates had significantly more ant workers per nest than those in trees without carbohydrates, whereas protein supplementation did not affect the abundance of ants in the artificial nests (Figure 6).

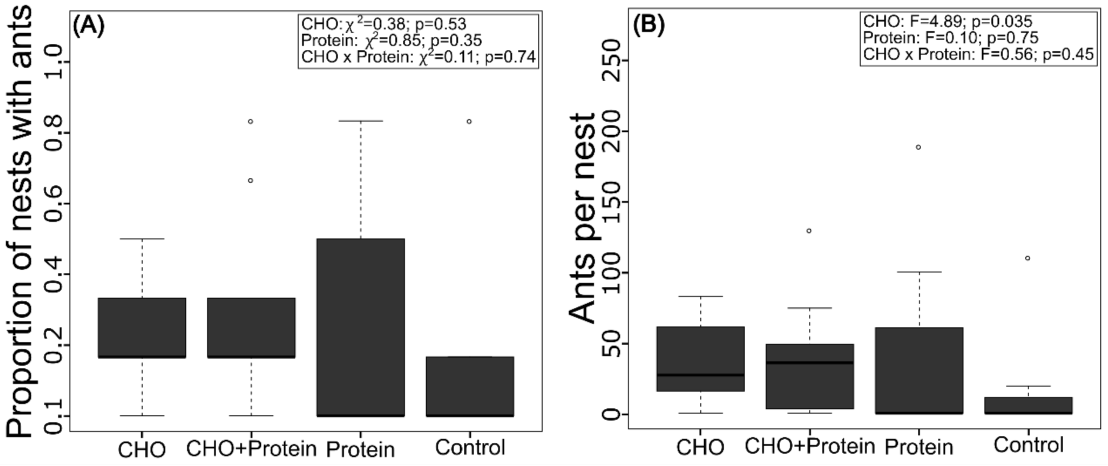

Figure 6. Effects of carbohydrate and protein supplementation on the proportion of artificial nests colonized by ants (A) and the overall abundance of ants in the colonized nests (B).

Leaf herbivory

Damage by leaf chewing insects was 1.5 times lower in trees that received carbohydrates than in those that did not, whereas leaf damage in trees receiving protein was not significantly different from those that did not (Figure 7).

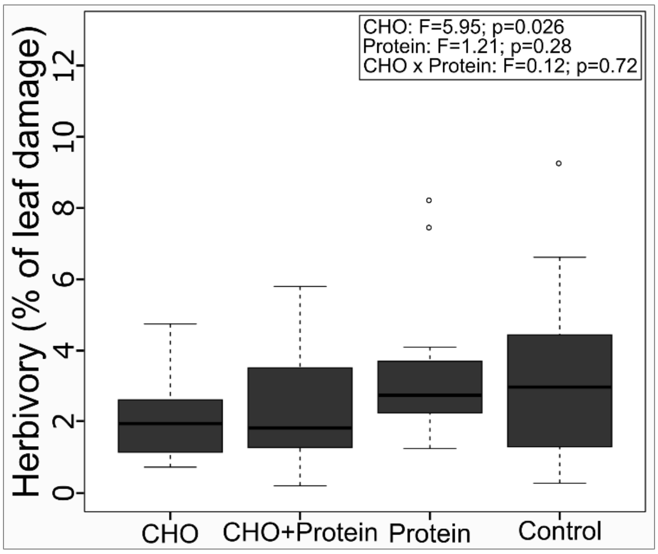

Figure 7. Indirect effect of carbohydrate and protein supplementation on the amount of leaf damage by chewing insects in *Caryocar brasiliense*.

Discussion

Colony level effects

The results of this study showed that colonies of *Azteca* that received protein had a lower C:N ratio and a higher δ15N than those that were not supplemented with protein. These findings reinforce the notion that omnivorous ants, such as *Azteca*, have some degree of dietary plasticity, depending on the relative availability of different nutrients in the environment (Tsang et al. 2020, Duyck et al. 2011). Ants of the genus *Azteca* are numerically and behaviorally dominant, with polydomous nests that commonly are distributed through numerous branches of the same plant (Dejean et al. 2009, Koch et al. 2016, Dejean et al. 2018). They are usually associated with coccoid Hemipteras in the vegetation from which they obtain carbohydrate-rich honeydew (Davidson et al. 2003, Johnson et al. 2001), which may help to explain why only the experimental supplementation protein -- but not that of sucrose – affected the trophic level of *Azteca*.

In addition, we found evidence of thermal plasticity in *Azteca* since colonies increased their CT_{max} after receiving sucrose for six months. These results support early findings with laboratory colonies of *Azteca chartifex* (Bujan & Kaspari 2017), which showed that *Azteca* support higher temperatures when fed with carbohydrates. The observed increase in the CT_{max} of *Azteca* colonies can be explained because, in insects, carbohydrates is stored in the hemolymph as disaccharides (Thompson 2003), and thus can be used to generate ATP (Suarez et al. 1993), which is essential to cope with thermal stress (Sokolova 2013). In addition, carbohydrates can also be stored as glycogen in muscle and fat bodies (Sacktor 1970), and this can generate ATP and metabolic water, and increase evaporative cooling effects through the releasing of bound water, thus improving desiccation resistance (Gibbs et al. 1997). Irrespective of the exact mechanism, it is clear that a carbohydrate-rich diet can help arboreal ants to expand their thermal niche and tolerate higher temperatures, potentially increasing foraging performance and competitive ability [cf. Bujan & Kaspari 2017]. On the other hand, although protein is necessary to the production of heat shock proteins (Andersen et al. 2010, King & McRae 2015), a higher content of protein over carbohydrates on an insect diet is also related to reduced performance and increased mortality (Lee et al. 2008, Makalkov et al. 2008, Dussutour & Simpson 2009, Kay et al. 2012), since the elimination of nitrogenous waste products is highly costly (Lee et al. 2008, Kay et al. 2012) and can increase physiological stress (O´Donnell 2008).

Community level effects

Relative to control trees, trees receiving carbohydrate and/or protein had more ant species and more ant workers foraging on their leaves and branches. Surprisingly however, and in contrast to a similar study that involved the supplementation of these same nutrients to arboreal ants over the short term (7 days) (Ribeiro et al. 2019), we found that trees receiving only sucrose had more species than those receiving only protein. Nevertheless, in agreement with this same study (Ribeiro et al. 2019), we found significant variation in species composition between trees subject to different nutrient addition treatments, reinforcing the view that different ant species present different levels of attractiveness to carbohydrates or protein sources. In fact, predatory arboreal ants like *Neoponera villosa* and *Solenopsis basalis* (Zuanon, 2018 – unpublished data) were more much frequent in trees supplemented with protein than in those receiving sucrose only.

Contrary to expected, the nutrient addition treatments did not affect the rate of occupancy of the artificial nests placed in the experimental trees. However, nests in trees that were supplemented with carbohydrates had more ants than those that did not receive carbohydrates. This result is in line with a laboratory study in which *Cephalotes pusillus* colonies provided with a diet rich in extrafloral nectar had five times more individuals per colony as compared to control colonies (Byk & Del-Claro 2011). One possibility is that the increased availability of carbohydrates on trees has reduced the mortality of ant workers (Groover et al. 2007, Kay et al. 2012), ultimately increasing colony size. The fact that protein supplementation did not elicit a similar response can perhaps be explained because all the species (three *Camponotus* and one *Pseudomyrmex*) that colonized the artificial nests can overcome their N-deficiencies by means of endosymbiotic bacteria (Russell et al. 2009, Hu et al. 2018)

Effect on the host tree

We detected significant lower levels of leaf damage in trees that received sucrose, whereas protein supplementation did not have an effect. These results lend some support to the Fuel for Foraging Hypothesis, since according to this hypothesis carbohydrates can fuel more metabolically expensive behavior, increasing aggression and improving foraging performance by ants, which ultimately can lead to an increase in the protection of the host plants (Caroll & Janzen 1973, Groover et al. 2007). Carbohydrate supplementation also increased the number of ant workers occupying the artificial nests, and thus this may well have enhanced the level of protection provided by ants, given that larger colonies are more effective in defending their partner plants against herbivores than smaller ones (Rocha et al. 1992). Similarly, it is relatively well known that some ant species are more aggressive towards insect herbivores than others (eg. Rico-Gray & Thien 1989, Del-Claro & Marquis 2015), and in this sense the larger number of ant species foraging in trees supplemented with carbohydrates may have increased the chances that the more aggressive ants were present in these trees (Yachi & Loreau 1999). It is also possible that carbohydrate supplementation, by increasing the CT_{max} of arboreal ants, may have allowed these ants to forage in hotter periods (when otherwise they would probably be not active), thus increasing the chances of encountering potential herbivores and ultimately benefiting the host plant (Fitzpatrick et al. 2014). Finally, it is important to mention that herbivory levels were very low among all surveyed trees, and in this sense, it is likely that the differences in leaf damage we detected may not have a significant influence on plant fitness. However, considering that herbivory pressure can vary temporally and spatially (Fagundes et al. 2017, Vasconcelos et al. 2020) and that ant-plant mutualisms are highly facultative (Bronstein et al. 2006), the relevance of carbohydrate availability to the strength of ant plant interactions may be more important in other contexts, like just after an intense fire (Vasconcelos et al. 2020) or across the different ontogenetic stages of the host-plants (Duyck et al. 2011).

Conclusions

Overall, the results of this study indicates that the availability of food resources can modulate the population and community ecology of arboreal ants as well as the mutualistic interaction between these ants and their host trees. The supplementation of carbohydrate as well as of protein increased the number of ant workers and the number of ant species foraging on trees. However, and although arboreal ants are thought to be N-limited (Rico-Gray & Oliveira 2007, Blüthgen & Fiedler 2004, Nepi et al. 2012), it was the supplementation of sucrose – not protein – that elicited most of the responses. Only sucrose-supplemented colonies had an increase in their thermal tolerance, and trees with more carbohydrate available were visited by more ant species, had larger colonies and were better protected than those that did not receive carbohydrates. In this sense, our results give further support for the idea that carbohydrates can be more important than protein for arboreal ants despite the higher availability of sources of carbohydrate than of protein in the tree canopy (Lasmar et al. 2023).

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Supplemental Material

Table S1: List of the ant species recorded in this study in trees supplemented or not with carbohydrate (CHO) or protein. Numbers represent the number of trees in which the species was recorded.

	Supplementation treatment				
Species	CHO*	Protein	CHO*+Protein	Water	Total of trees
Camponotus senex	15	9	11	7	42
Camponotus bonariensis	7	7	11	8	33
Brachymyrmex nr. *aphidicola*	13	2	12	2	29
Pseudomyrmex gracilis	7	6	8	7	28
Azteca sp. 1	8	7	6	5	26
Neoponera villosa	4	7	8	0	19
Pseudomyrmex curacaensis	5	4	5	4	18
Camponotus melanoticus	8	2	6	0	16
Solenopsis basalis	2	6	6	0	14
Camponotus substitutus	4	5	2	0	11
Tapinoma sp. 7	4	1	2	4	11
Camponotus blandus	0	4	3	0	7
Forelius maranhaoensis	2	0	3	1	6
Pheidole radoszkowskii	3	0	2	0	5
Atta laevigata	1	2	1	0	4
Camponotus fastigatus	2	0	2	0	4
Dorymyrmex sp. 10	2	0	2	0	4
Ectatomma tuberculatum	2	0	1	0	3
Pseudomyrmex urbanus	2	1	0	0	3
Solenopsis substitute	0	0	3	0	3
Pheidole oxyops	1	1	1	0	3
Cephalotes eduarduli	1	1	0	0	2
Cephalotes pusillus	0	0	1	1	2
Pheidole vafra	1	0	1	0	2
Brachymyrmex nr. *fiebrigi*	0	0	1	0	1
Camponotus arboreus	0	0	1	0	1
Camponotus balzani	0	0	1	0	1
Camponotus cingulatus	1	0	0	0	1
Cephalotes depressus	1	0	0	0	1
Cephalotes persimilis	0	1	0	0	1
Crematogaster nr. *arcuata*	1	0	0	0	1
Ectatomma edentatum	0	0	1	0	1
Myrmelachista nodigera	1	0	0	0	1
Nesomyrmex spininodis	0	0	1	0	1
Pheidole nr. *mapinguar*	0	0	0	1	1

Pheidole fracticeps	0	0	1	0	1
Solenopsis pollux	0	1	0	0	1
Solenopsis nr. *latastei*	0	0	1	0	1
Wasmannia sigmoidea	0	1	0	0	1

Discussão geral

Neste estudo obtive resultados que indicam que a tolerância termal de formigas do Cerrado pode ser explicada em parte por diferenças de habitat, mais especificamente pelo tipo de vegetação (cerrado sentindo restrito ou floresta semidecidual) e estrato de forrageio e/ou nidificação (solo ou vegetação) das espécies. Além disso, encontrei evidências de que existe um efeito interativo do tipo de habitat e estrato de nidificação/forrageamento na vulnerabilidade ao aquecimento global de formigas do Cerrado, com espécies de solo sendo mais vulneráveis em áreas de cerrado sentido restrito, enquanto que em áreas de floresta semidecídua as espécies mais vulneráveis são as de formigas arbóreas. Meus resultados também sugerem que a tolerância termal é um traço plástico tanto temporalmente quanto espacialmente, variando de acordo com a sazonalidade do ambiente e também entre populações de espécies generalistas que ocorrem em áreas de cerrado e floresta. Finalmente, o presente estudo indica que mudanças na dieta, particularmente o maior consumo de carboidratos, aumentam a resistência ao calor das espécies de formiga e são um mecanismo em potencial para lidar o estresse termal. Além disso, observei que a disponibilidade de recursos alimentares pode afetar a posição trófica, a estrutura da comunidade de formigas arborícolas e a interação destas com a árvore hospedeira. Os principais resultados, conclusões e implicações obtidas nesse estudo estão sumarizadas na tabela 1.

Tabela 1. Sumário dos principais resultados desse estudo

Tema de estudo	Principais resultados	Conclusões e implicações
Fatores que influenciam a tolerância termal de formigas (Capítulo 1)	Tipo de vegetação (cerrado ou floresta) afeta a tolerância ao calor, mas não ao frio	Diferenças na tolerância termal relacionadas ao ambiente ou estrato acompanharam as

	Estrato de nidificação (solo ou arbóreo) afeta a tolerância ao calor e ao frio Forte sinal filogenético na resistência ao calor e na amplitude termal	variações nas condições microclimáticas, dando suporte à Teoria da adaptação termal
Vulnerabilidade ao aquecimento global de formigas do Cerrado (Capítulo 1)	Formigas em áreas de cerrado sentido restrito são mais vulneráveis ao aquecimento global que formigas da floresta semidecídua No cerrado, formigas de solo podem ser mais vulneráveis ao aquecimento global que formigas arbóreas, enquanto que Em florestas, formigas arbóreas são mais vulneráveis que as formigas de solo	A vulnerabilidade ao aquecimento global de formigas pode ser diferente entre espécies de ambientes adjacentes, ou até mesmo entre estratos do mesmo ambiente. Estudos em escala local são fundamentais para prever as respostas das espécies aos aumentos de temperatura
Plasticidade termal de formigas (Capítulo 2)	A tolerância ao frio varia sazonalmente independente do tipo de vegetação ou sítio de nidificação da espécie de formiga A tolerância ao calor é maior para populações de formigas em áreas de cerrado sentido restrito que em florestas semidecíduas	Mesmo pequenas variações sazonais de temperatura podem afetar a tolerância ao frio de espécies de formigas A plasticidade na tolerância termal de espécies generalistas de habitats pode ser uma

		vantagem importante perante o aquecimento global
Disponibilidade de recursos alimentares para formigas arbóreas (Capítulo 3)	A suplementação de carboidrato e proteína aumenta a abundância e riqueza de formigas forrageando nas árvores A suplementação de carboidrato aumenta a tolerância termal e o tamanho de colônia de formigas árboreas. Árvores cujas formigas receberam um suprimento de carboidratos são melhor protegidas contra herbívoros	A disponibilidade de recursos alimentares, especialmente carboidratos, pode modular a ecologia das populações de formigas arbóreas, assim como a interação mutualística entre essas formigas e as árvores onde forrageiam.

De modo geral, encontrei que a tolerância termal das espécies de formigas está relacionada com a temperatura de seus habitats, assim como o proposto pela Teoria da adaptação termal (Kaspari et al. 2015). Esse padrão foi constatado tanto comparando espécies diferentes entre tipos de vegetação e estratos, como também para populações da mesma espécie entre ambientes de cerrado e floresta. De forma semelhante, encontrei que a tolerância ao frio acompanha a diminuição de temperatura que ocorre nos meses mais frios do ano, variando sazonalmente independentemente do tipo de habitat e estrato de nidificação/forrageamento.

Tendo em vista a origem florestal das formigas de cerrado (Vasconcelos et al. 2018, Andersen & Vasconcelos 2022), muitas espécies são compartilhadas entre esses dois ambientes. Dessa forma, é possível que as diferença de tolerância termal que encontrei entre comunidades florestais e de cerrado esteja relacionada não apenas com diferenças na composição de espécies mas também com a plasticidade na tolerância termal das espécies que ocorrem em ambos os habitats. Já no caso das diferença entre comunidades arbóreas e de solo, esta tende a ser explicada unicamente por diferenças na composição de espécies, já que há forte estratificação vertical na composição dessas comunidades. Considerando que encontrei um forte sinal filogenético na tolerância ao calor e na amplitude termal das espécies de formigas, é possível que a diferença de tolerância termal entre estratos seja uma consequência do conservadorismo filogenético entre as espécies que evoluíram diferentes hábitos de nidificação. De fato, espécies exclusivamente arbóreas, como as do gênero *Pseudomyrmex* e *Cephalotes,* são altamente tolerantes ao calor, e enquanto as Poneromorfas, que são bem mais comuns no solo do que na vegetação, possuem comparativamente uma menor tolerância ao calor.

De modo geral, as formigas mais vulneráveis ao aquecimento global são as espécies de solo em áreas de cerrado sentido restrito. Considerando as condições de temperatura que formigas arbóreas têm que lidar no dossel das florestas, a vulnerabilidade dessas espécies até chega a ser semelhante à de espécies de cerrado. Entretanto, o *buffer* termal fornecido pelo dossel fechado da floresta gera condições mais amenas tanto no sub-bosque como no solo, com potencial para que as espécies florestais arbóreas lidem com o aquecimento global através de comportamentos de mudança de ninho e local de forrageio, por exemplo. Por outro lado, o dossel do cerrado sentido restrito é muito mais aberto que o de florestas, fazendo com que mais luminosidade chegue abaixo da copa das árvores e também no solo. Assim, é provável que no cerrado as formigas arbóreas não sejam capazes de minimizar os efeitos do aquecimento global simplesmente mudando seus hábitos de forrageamento ou nidificação. Nesse sentido, trabalhos que explorem como o comportamento pode ajudar as formigas arbóreas e de solo a lidar com o aumento de temperatura seriam de extrema importância. Particularmente, seria interessante investigar os traços e mecanismos específicos que podem facilitar a migração vertical de formigas em diferentes ambientes, e avaliar o impacto da transição de espécies entre solo e vegetação na estrutura das comunidades.

Finalmente, através de um experimento em que manipulei a disponibilidade de carboidratos e proteínas para formigas arbóreas, encontrei evidências que reforçam a noção de que o consumo de carboidrato é um mecanismo termoregulatório com potencial para ajudar as formigas a evitar os efeitos negativos do aquecimento. Tendo em vista que as fontes de açúcar para formigas são mais abundantes na vegetação que no solo (Yanoviak & Kaspari 2000, Davidson et al. 2003), é possível que o maior consumo de carboidratos como forma de aumentar a tolerância termal seja um mecanismo mais importante para formigas arbóreas do que as de solo. No caso do cerrado, a baixa disponibilidade de açúcar no solo é particularmente importante se considerarmos que formigas de solo podem ser mais vulneráveis ao aquecimento que as arbóreas. Nesse sentido, estudos que busquem entender como o efeito do açúcar e outros nutrientes variam de acordo com características ecológicas de diferentes espécies seriam de alto valor para prever a resposta dos ectotérmicos ao aquecimento global.

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