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MARIANNE AZEVEDO SILVA

ANTS, BIODIVERSITY AND LATITUDINAL GRADIENT: INVESTIGATING INTERACTIONS WITH PLANTS, FUNCTIONAL VARIATION AND GENETIC DIVERSITY IN THE CERRADO SAVANNA

FORMIGAS, BIODIVERSIDADE E GRADIENTE LATITUDINAL: INVESTIGANDO INTERAÇÕES COM PLANTAS, VARIAÇÃO FUNCIONAL E DIVERSIDADE GENÉTICA NO CERRADO

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“You are capable of more than you know. Choose a goal that seems right for you and strive to be the best, however hard the path. Aim high. Behave honorably. Prepare to be alone at times, and to endure failure. Persist! The world needs all you can give.”

E. O. Wilson

“Você é capaz de mais do que imagina. Escolha um objetivo que pareça certo para você e se esforce para ser o melhor, por mais difícil que seja o caminho. Mire alto. Comporte-se com honra. Prepare-se para ficar sozinho às vezes e para suportar o fracasso. Persista! O mundo precisa de tudo o que você pode dar.”

E. O. Wilson (tradução livre)

RESUMO

Biodiversidade é de importância central nas ciências, podendo ser investigada sob diferentes perspectivas e níveis de organização. Um dos padrões mais consistentes da distribuição da biodiversidade no planeta é o gradiente latitudinal de diversidade (GLD), segundo o qual a diversidade biológica aumenta dos polos em direção ao equador. Alguns grupos podem apresentar padrões inversos ao GLD, como é o caso das formigas no Cerrado brasileiro. No Cerrado, as formigas apresentam uma alta diversidade. Isso porque, na vegetação do Cerrado, formigas encontram sítios de nidificação e uma alta incidência de recursos líquidos açucarados, oriundos, principalmente, de nectários extraflorais (NEFs) e insetos trofobiontes. Recentemente, tem sido demonstrado que a diversidade de espécies de formigas apresenta um GLD inverso no Cerrado, aumentando com o aumento da latitude. No entanto, outros níveis de organização biológica ainda não foram explorados neste gradiente. Nesta tese tivemos como objetivo geral investigar a biodiversidade de formigas em diferentes escalas da organização biológica ao longo de um gradiente latitudinal de Cerrado. Também tivemos como objetivo compreender fatores que potencialmente moldam esses níveis de diversidade. No **Capítulo 1**, nós avaliamos como fatores bióticos e abióticos – latitude, heterogeneidade ambiental, recursos, e comunidades de plantas e formigas – influenciam a frequência de interações formiga-planta na vegetação do Cerrado. Baseado em modelagem de equações estruturadas, nós encontramos que a proporção de plantas com formigas no Cerrado é indireta e negativamente relacionada com a latitude. Heterogeneidade ambiental e comunidade de plantas também tiveram um efeito negativo nas interações formiga-planta. Por outro lado, recursos açucarados de NEFs e hemípteros trofobiontes, e a comunidade de formigas afetaram positivamente as interações. No **Capítulo 2**, nós investigamos a variação funcional de uma formiga dominante, *Camponotus crassus*, ao logo do gradiente latitudinal de Cerrado. Nós caracterizamos a pilosidade do mesosoma e o tamanho do corpo de operárias, e avaliamos suas relações com a heterogeneidade ambiental, os recursos açucarados e as comunidades de plantas e formigas. Nós também avaliamos possíveis correlações entre variações morfológicas e genéticas entre localidades. Nossos resultados sugerem que a disponibilidade de recursos líquidos açucarados teve o maior efeito sobre a pilosidade de *C. crassus*, mas não no tamanho do corpo. Além disso, a pilosidade de *C. crassus* não covariou com a variação genética, sugerindo uma possível plasticidade fenotípica nesta espécie. No **Capítulo 3**, nós avaliamos a diversidade genética (DG) de *C. crassus* ao longo do gradiente latitudinal de Cerrado e potenciais fatores que influenciam essa diversidade, incluindo a comunidade de formigas, heterogeneidade ambiental, a disponibilidade de recursos líquidos, e a demografia de *C. crassus*. Nós encontramos que a DG de *C. crassus* apresenta um GLD inverso, sendo maior quanto maior a latitude. Nós encontramos ainda que precipitação e disponibilidade de recursos estão negativa e positivamente relacionadas com a DG de *C. crassus*, respectivamente. Com os resultados obtidos nesta tese, nós avançamos no conhecimento sobre a contexto-dependência das interações formiga-planta, variação funcional intraespecífica, e diversidade genética de formigas no Cerrado. O estudo traz importantes contribuições para o conhecimento sobre o GLD e os fatores que moldam a biodiversidade em formigas nessa savana neotropical.

ABSTRACT

Biodiversity is a central concept in science and can be investigated from various viewpoints and levels of organization. One of the most consistent patterns of biodiversity distribution on the planet is the latitudinal gradient of diversity (LGD), which states that biological diversity increases from the poles towards the equator. Some groups may exhibit inverse patterns relative to the LGD, such as ants in the Brazilian Cerrado savanna. In the Cerrado, ants are highly diverse and dominant on foliage, where they can find nesting sites and nutritious sugary food resources, mainly from extrafloral nectaries (EFNs) and honeydew-producing insects. Recently, it has been reported that ant species diversity follows an inverse LGD in the Cerrado, increasing with latitude. However, other levels of biological organization along this gradient have not been explored yet. Here, we investigate ant biodiversity at different biological scales along a latitudinal gradient in the Cerrado. Additionally, we aimed to understand the factors that potentially shape these levels of diversity. In **Chapter 1**, we assessed how biotic and abiotic factors – latitude, environmental heterogeneity, resources, and plant and ant communities – influence the frequency of ant-plant interactions in Cerrado vegetation. Based on structured equation modelling, we found that the proportion of plants with ants in the Cerrado is indirectly and negatively related to latitude. Environmental heterogeneity and plant community also had a negative effect on ant-plant interactions. On the other hand, sugary resources from EFNs and hemipteran trophobionts, along with the ant community, positively affected the interactions. In **Chapter 2**, we examined the functional variation of a dominant ant, *Camponotus crassus,* along the latitudinal gradient of Cerrado. We characterized the mesosoma pilosity and body size of workers and evaluated their relationships with environmental heterogeneity, resources, and plant and ant communities. We also assessed possible correlations between morphological and genetic variation among sampling sites. Our results suggest that the availability of sugary liquid resources had the most significant effect on *C. crassus* pilosity, but not on body size. Additionally, *C. crassus* pilosity did not covary with genetic variation, implying potential phenotypic plasticity in this species. In **Chapter 3**, we assessed the genetic diversity (GD) of *C. crassus* along the latitudinal gradient of Cerrado and the potential factors influencing this diversity, including the ant community, environmental heterogeneity, resource availability, and *C. crassus* demography. We found that *C. crassus* GD exhibits an inverse LGD, being higher at higher latitudes. We also found that precipitation and resource availability are negatively and positively associated with *C. crassus* GD, respectively. The results of this thesis represent a

step further in our understanding of context-dependent ant-plant interactions, intraspecific functional variation, and genetic diversity of ants in the Cerrado. Our study adds to the knowledge of LGD and the factors shaping biodiversity in the neotropical Cerrado savanna.

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INTRODUÇÃO

Biodiversidade, termo inicialmente cunhado por E. O. Wilson (1988), é de importância central nas ciências biológicas, mas também com impactos diretos política e socialmente (Diáz e Malhi 2022). Em sua mais recente definição, biodiversidade é descrita como “A variabilidade entre os organismos vivos de todas as origens, incluindo terrestres, marinhos e de outros ecossistemas aquáticos, bem como os complexos ecológicos dos quais fazem parte. Isso inclui variação em atributos genéticos, fenotípicos, filogenéticos e funcionais, bem como mudanças em abundância e distribuição no tempo e no espaço dentro e entre as espécies, comunidades biológicas e ecossistemas” (tradução livre do conceito cunhado pelo Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services – IPBES – e disponível em https://www.cbd.int/convention/articles/?a=cbd-02). Assim, biodiversidade pode ser entendida como um conceito multidimensional, dentro e entre espécies e em diferentes escalas (Diáz e Malhi 2022). Pode ser, portanto, investigada sob diferentes perspectivas (genética, taxonômica, filogenética, funcional) e níveis de organização biológica (organismos, populações, comunidades e ecossistemas) (Colwell 2009; Diáz e Malhi 2022).

A biodiversidade não está uniformemente distribuída na superfície terrestre, podendo a biosfera ser considerada um mosaico complexo de conjuntos variáveis e descontínuos de genes, espécies, comunidades e ecossistemas (Colwell 2009). Um dos padrões mais antigos e consistentes da distribuição da vida no planeta é o gradiente latitudinal de diversidade (GLD), segundo o qual a diversidade biológica aumenta da região dos pólos em direção ao equador (Pianka 1966; Rosenzweig 1995; Gaston 2000; Willig et al. 2003). Mesmo que o GLD seja um padrão evidente, ainda não há consenso sobre os mecanismos que o geram (Brown 2014). Sendo assim, diversas hipóteses envolvendo mecanismos biológicos e não biológicos têm sido sugeridas (Willig et al. 2003). Sem recorrer a processos ecológicos ou evolutivos, segundo a hipótese da restrição geométrica, espécies em um domínio (planeta Terra) com bordas limitantes (pólos) se arranjam aleatoriamente de forma a originar um pico de diversidade no meio do domínio (equador) (Colwell e Hurtt 1994). Já as hipóteses que invocam mecanismos biológicos, podem ser categorizadas em três grupos (Mittelbach et al. 2007). O primeiro refere-se às hipóteses ecológicas, dentre as quais destacam-se duas frentes: (1) uma que credita o GLD ao aumento da produtividade primária em ambientes mais próximos ao equador, os quais podem sustentar mais indivíduos de diferentes espécies (Currie 1991; Allen et al. 2002), (2) e outra que credita o GLD à segregação de nichos (espécies em regiões tropicais apresentam adaptações mais especializadas às condições abióticas e às interações bióticas, permitindo que o uso do recurso seja mais finamente distribuído entre diferentes espécies, o que favoreceria a coexistências das mesmas (ver Brown 2014). O segundo grupo de hipóteses para o GLD, refere-se às hipóteses históricas, as quais sugerem que os trópicos tiveram mais tempo para acumular espécies que as zonas temperadas, uma vez que são mais antigos e sofreram menos mudanças climáticas ao longo da história do planeta (Pianka 1966; Mittelbach et al. 2007). Por fim, o terceiro grupo de hipóteses para a origem do GLD é o de hipóteses evolutivas, as quais se referem às maiores taxas de diversificação com a diminuição da latitude (Rosenzweig 1995). A este último grupo, inclui-se a hipótese da velocidade evolutiva (Rohde 1992), segundo a qual devido aos efeitos cinéticos da maior incidência de energia (temperatura) com a diminuição da latitude, maior é a taxa de mutação do DNA e menor o tempo de geração de indivíduos. Consequentemente, maior é a taxa de divergência genética entre populações e maior a taxa de especiação nas comunidades (Rohde 1992). O GLD é congruente para diversos grupos taxonômicos (como mamíferos, insetos e plantas), independentemente de habitat (marinhos ou terrestres), forma de dispersão (ativa ou passiva) ou regulação da temperatura corporal (endo ou ectotérmicos) (Hillebrand 2004). Este gradiente também pode ser verificado em diferentes escalas e contextos geográficos (diferentes continentes e oceanos; hemisfério norte e sul) e em distintos períodos de tempo (tanto recentes quanto há milhões de anos) (Willig et al. 2003; Hillebrand 2004). No entanto, alguns grupos podem apresentar exceções ao GLD (incluindo extensão latitudinal menor que 10°, espécies parasitas e flora aquática), e padrões inversos ao mesmo, como é o caso das formigas no cerrado brasileiro (Vasconcelos et al. 2018).

Formigas fazem parte de um dos grupos de insetos mais abundantes e numericamente dominantes do planeta (Hölldobler e Wilson 1990), somando cerca de 20 quatrilhões de indivíduos na Terra, os quais correspondem a cerca de 12 megatoneladas em biomassa de carbono (Schultheiss et al 2022). Esse alto número e biomassa fazem das formigas animais cruciais para o funcionamento dos ecossistemas, com papéis ecológicos diversos e importantes, como controle biológico, dispersão de sementes, proteção de plantas e ciclagem de nutrientes (Del-Toro et al. 2012). No cerrado, as formigas (principalmente as arbóreas) apresentam uma alta diversidade quando comparadas a outras savanas tropicais do mundo (Campos et al. 2011). O cerrado é considerado um dos *hotspots* de biodiversidade (Myers et al. 2000) e corresponde, originalmente, a cerca de 26% do território brasileiro (Vieira et al. 2022). Sendo formado por um mosaico de fitofisionomias vegetais (Oliveira-Filho e Ratter 2002), a heterogeneidade espacial do cerrado é apontada como um dos fatores que contribuem para a alta diversidade de formigas neste bioma (Ribas et al. 2003). Além disso, dois principais fatores explicam a ubiquidade das formigas na vegetação do cerrado. Primeiramente, diversas espécies de plantas no cerrado apresentam sítios de nidificação para as formigas, seja em galhos naturalmente ocos ou estruturas ocas formadas a partir da atividade, por exemplo, de insetos galhadores e besouros (Morais 1980; Oliveira e Freitas 2004; Schoereder et al. 2010). Segundo, no cerrado há uma alta incidência de recursos líquidos açucarados, oriundos, principalmente, de nectários extraflorais (NEFs) e de insetos trofobiontes (Oliveira e Leitão-Filho 1987; Oliveira e Oliveira-Filho 1991; Del-Claro & Oliveira 2000; Oliveira e Freitas 2004). Plantas com NEFs chegam a corresponder a 30% das espécies arbóreas do cerrado, sendo essas estruturas secretoras encontradas principalmente nas famílias Mimosaceae, Bignoniaceae e Vochysiaceae (Oliveira e Freitas 2004). De forma análoga, insetos trofobiontes podem ocorrer em aproximadamente 30% das espécies de plantas nas comunidades de cerrado (Lopes 1995). Diversas espécies de formigas tendem a apresentar comportamento agressivo e atacam insetos herbívoros que se aproximam dos recursos disponíveis nas plantas (ninhos e/ou alimentos), levando à proteção das plantas contra herbivoria e aumentando a sobrevivência de trofobiontes ao ataque de predadores (Oliveira et al. 1987; Oliveira e Brandão 1991; Oliveira 1997; Del-Claro e Oliveira 2000). Assim, em alguns sistemas, a interação entre formigas e plantas no cerrado pode ser considerada um mutualismo defensivo (Rico-Gray e Oliveira 2007; Bronstein 2015). Além das interações protetivas, formigas também podem atuar como dispersoras secundárias de sementes no cerrado, as quais tem sua germinação aumentada pela remoção da polpa e arilo e pelo transporte promovido pelas formigas até os ninhos, locais comumente ricos em nutrientes (Leal e Oliveira 1998; Christianini et al. 2007; Magalhães et al. 2018). Dada a abundância e papéis ecológicos, o cerrado torna-se um ambiente ideal para investigação não apenas de formigas, mas também das interações entre esses insetos eusociais e as plantas (Oliveira e Marquis 2002; Oliveira e Freitas 2004).

Recentemente, tem sido demonstrado que a diversidade de espécies de formigas apresenta um gradiente latitudinal inverso no cerrado, com a riqueza de espécies aumentando com o aumento da latitude (Vasconcelos et al. 2018). Não apenas a diversidade de espécies tem mostrado esse padrão, como também as redes de interações formiga-planta: redes maiores, com maior diversidade de interações e dissimilaridade também ocorrem em maiores latitudes (Dáttillo e Vasconcelos 2019). Em ambos os casos, o aumento da diversidade está amplamente relacionado com o aumento da produtividade primária e da precipitação (Vasconcelos et al. 2018; Dáttillo e Vasconcelos 2019). Além disso, gradientes latitudinais também têm sido reportados para a estratificação vertical de ocorrência de formigas no cerrado, com a dissimilaridade entre estratos diminuindo quanto maior a latitude (Vasconcelos et al. 2023). Dessa forma, a diversidade de espécies de formigas, em diferentes escalas (i.e. local e de estratificação vertical), bem como as redes de interações com plantas, já foram investigadas ao longo em gradiente latitudinal de cerrado. No entanto, outros níveis de organização biológica ainda não foram explorados no gradiente, sendo cruciais para o entendimento dos padrões de distribuição da biodiversidade desse grupo de insetos tão importante para o cerrado.

Através de dados de campo e coletas padronizadas em sete localidades, este trabalho tem como objetivo geral investigar a biodiversidade de formigas ao longo de um gradiente latitudinal de cerrado, em diferentes escalas da organização biológica: interações formiga-planta, funcional e genética. Também tivemos como objetivo compreender os fatores que potencialmente moldam essas diversidades (especificamente, heterogeneidade ambiental, comunidades de formigas e plantas, e disponibilidade de recursos).

No primeiro capítulo, nós avaliamos a contexto-dependência das interações formiga-planta e como a heterogeneidade ambiental, disponibilidade de recursos e comunidades interagentes moldam a frequência de plantas com formigas no cerrado. Usando modelos de equações estruturadas (Grace et al. 2015), nós buscamos estabelecer relações de causalidade, bem como os efeitos diretos e indiretos das variáveis analisadas sobre as interações formiga-planta.

No segundo capítulo, nós investigamos a variação funcional intraespecífica de uma espécie de formiga abundante na folhagem do Cerrado, *Camponotus crassus* Mayr (Formicinae; Oliveira e Freitas 2004; Calixto et al. 2021). Nós descrevemos a variação da pilosidade e tamanho do corpo nesta espécie e relacionamos essas características com a heterogeneidade ambiental, comunidades de plantas e formigas, e disponibilidade de recursos líquidos na vegetação. Também avaliamos a potencial covariação morfológica e genética na espécie, buscando compreender se os padrões observados são fruto de plasticidade fenotípica ou adaptação.

No terceiro capítulo, nós avaliamos como a diversidade genética intraespecífica de *C. crassus* varia ao longo do gradiente latitudinal de cerrado. Nós também analisamos potenciais fatores que moldam essa variação genética, incluindo a heterogeneidade ambiental, comunidade de formigas, disponibilidade de recursos, e dados demográficos de *C. crassus*. Com os resultados gerados nessa tese, nós avançamos no conhecimento dos padrões e fatores que influenciam a biodiversidade de formigas do Cerrado em diferentes níveis da organização biológica e numa escala geográfica ampla, trazendo importantes contribuições para diferentes áreas do conhecimento.

CAPÍTULO 1

UNVEILING CONTEXT-DEPENDENCE IN A TROPICAL SAVANNA: DIRECT AND INDIRECT EFFECTS OF ENVIRONMENTAL HETEROGENEITY, RESOURCES, AND COMMUNITY TRAITS ON ANT-PLANT INTERACTIONS

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ABSTRACT

Mutualisms are ubiquitous in nature and play a central role in the dynamic, diversity, and resilience of biological systems. Mutualisms are subjected to context-dependence, being influenced by multiple factors such as environmental conditions, resource availability, community and organism traits. Ant-plant interactions are commonly highlighted as models for understanding mutualism. Here, we evaluated how major abiotic and biotic parameters – *latitude*, *environmental heterogeneity*, *resource availability*, and *ant and plant communities* – drive the frequency of ant-plant interactions in Brazilian Cerrado savanna. We used a structural equation modelling (SEM) framework to access causality between variables and their relative direct and indirect effects on ant-plant interactions. We observed a total of 3,345 plants sampled in 34 transects in 7 localities distributed along a latitudinal gradient in the Cerrado. The proportion of plants with ants were indirectly and negatively associated with *latitude*. *Environmental heterogeneity* and *plant community* had a negative overall effect on ant-plant interactions. On the other hand, plant-derived *resources* (extrafloral nectaries, hemipteran trophobionts) and *ant communities* were positively associated with the proportion of plants with ants in Cerrado vegetation. Ant abundance, ant species richness, and ant co-occurrence on foliage were the major drivers of ant-plant interactions in Cerrado. Our study highlights a causality network between multiple factors influencing ant-plant interactions and is a step forward in the understanding of these prominent interactions in the tropics. We also open new avenues of investigation that could be greatly improved with the use of SEM framework.

INTRODUCTION

Defined as “all mutually beneficial, interspecific interactions, regardless of their specificity, intimacy or evolutionary history” (Bronstein 2015), mutualisms may represent 19% to 50% of the interactions within a community (Stone and Roberts 1991). With almost all organisms on the planet being direct or indirectly involved in a mutualism (Bronstein 2015), this interaction plays a central role in the dynamic, diversity, functionality and resilience of biological systems (Holland et al. 2005; Bronstein 2015; Chomicki et al. 2019; Stone 2020). As any other type of interspecific interactions, mutualisms are subjected to context-dependence, which occurs when ecological relationships change in sign (-, 0, +) or magnitude (strong or weak) in response to the ecological, spatiotemporal and observational conditions under which they were reported (Chamberlain et al. 2014; Catford et al. 2022). Mutualisms frequently involve bidirectional energy flow and are commonly affected by the presence of additional species, which make them more prone to vary spatially and temporally when compared to other types of interactions (Chamberlain et al. 2014).

Different factors may lead to context-dependence in mutualisms. Environmental conditions are fairly recognized to promote substantial variability in the strength of multiple interactions (Liu and Gaines 2022), and abiotic changes were already reported to have the more dramatic effects on mutualisms (Chamberlain et al. 2014). Local community diversity also affects consistency of mutualisms because increasing partner abundance also increases partner options (Dunkley et al. 2020). As a result, in general, the higher the community diversity, the higher the opportunities of conflict and of decreasing the strength or occurrence of mutualisms (Thrall et al. 2007). Resource availability also shapes mutualism, mainly those interactions that involve resource exchange (Hoek et al. 2016; Pringle 2016). For instance, less-beneficial interactions may weaken resource-supplying mutualists when resources are limited (Palmer et al. 2015), whereas competitive exclusion of subordinate species may occur when resources are abundant (Hoek et al. 2016). Finally, changes in species behavior, morphology and genetics and the presence of third-party species may also modulate mutualisms in nature (Chamberlain et al. 2014; Hoeksema and Bruna 2015; Palmer et al 2015). Given these multiple contextual factors, it is imperative to simultaneously investigate all aspects potentially affecting mutualism and clarify their relative importance for the interaction (Hoeksema and Bruna 2015). Such an approach should increase our understanding and predictive power to forecast the outcomes of mutualisms (Catford et al. 2022).

Ant-plant interactions are commonly highlighted as models to understand mutualism (Rico-Gray and Oliveira 2007; Bronstein 2015). The ubiquity of ant-plant interaction is mainly attributed to ant traits, including high abundance (10% - 15% of total terrestrial animal biomass), broad ecological tolerance, social organization, territoriality, and predominantly omnivorous diet, which make these small insects ideal partners for foraging on highly dispersed plant-derived resources (Bentley 1977; Beattie and Hughes 2002). Characterized as a bidirectional (when plants absorb nutrients from ant wastes) or unidirectional consumer resource mutualism (Holland et al. 2005), plants benefit from ants' presence on their surface whenever these insects deter herbivores and, as a consequence, increase plant growth and reproductive success (Koptur 1992; Beattie and Hughes 2002; Oliveira and Freitas 2004; Chamberlain and Holland 2009). On the other hand, ants benefit from plant rewards, which include food resources (sugary secretion from extra-floral nectaries, EFN, and food bodies; Figure 1A), and shelter (by nesting in specialized plant structures known as domatia, or by nesting opportunistically in insect gall cavities, base of leaves, or hollowed out stems; Figure 1C) (Koptur 2005; Beattie and Hughes 2002). Resource-based ant-plant interactions can also be mediated indirectly by trophobiont insects (such as sap-sucking hemipterans and lepidopteran caterpillars; Figure 1B) that produce nectar-like liquids that attract aggressive ants, which in turn can benefit the plant by deterring herbivores (Carroll and Janzen 1973; Koptur 2005). Given that ant-plant protective mutualisms are always conditioned by a third-party (plant damage by herbivores) for benefits to occur, these interactions are more prone to be context-dependent when compared to other types of mutualism (Bronstein and Barbosa 2002).

Ant-plant protective interactions occur when requirements of resource availability and ant defensive ability are met (Koptur 2005; Chamberlain and Holland 2009; Díaz-Castelazo et al. 2017). Therefore, plant-derived liquid rewards play a central role in attracting ants to forage on foliage (Díaz-Castelazo et al. 2017), with the presence of EFNs near reproductive structure and non-vascularized EFNs increasing the effect of ant defense (Leal et al. 2023). Presence of trophobionts may also reduce herbivore damage to meristems, favoring vegetative growth (Oliveira and Del-Claro 2005). Ant identity (which reflects foraging, recruitment, aggressiveness, and competitive behaviors), numerical dominance, and level of intimacy with plants are also determinant in ant-plant interactions (Ribas and Schoereder 2004; Rosumek et al. 2009; Sendoya et al. 2009; Leal and Peixoto 2017; Díaz-Castelazo et al. 2017; Leal et al. 2023). Moreover, ant-plant interactions are indirectly associated with latitude (Chamberlain and Holland 2009), environmental heterogeneity (Ribas et al. 2003) and some other environmental conditions. For instance, ant presence on leaves is directly associated EFN-activity, which in turn is affected by water availability, soil nutrients and primary productivity (Yamawo 2017). Contrastingly, decreasing rainfall leads to an increase in ant foraging activity on foliage (Leal and Peixoto 2017). Finally, ant and plant community characteristics, such as ant species richness (Chamberlain and Holland 2009) and plant life history (annual or perennial; Trager et al. 2010), also account for the magnitude of the effect of ant-plant interactions. Given their pervasiveness in tropical environments, it is important to unveil the direct and indirect effects of biotic and abiotic factors on ant-plant interactions across large spatial scales (Rico-Gray and Oliveira 2007; Díaz-Castelazo et al. 2004; Díaz-Castelazo et al. 2017).

The Brazilian Cerrado savanna, a world’s hotspot (Myers et al. 2000; Oliveira and Marquis 2002), is the ideal scenario to investigate ant-plant interactions at a large spatial scale. First, Cerrado presents high arboreal ant species diversity (Campos et al. 2011). Secondly, there is a high occurrence of sugary liquid rewards in Cerrado vegetation (Oliveira and Freitas 2004). Plants bearing EFNs may correspond to more than 30% of arboreal species in Cerrado, mostly in the plant families Mimosaceae, Bignoniaceae and Vochysiaceae (see Oliveira and Freitas 2004). Similarly, trophobionts may occur in approximately 30% of the plant species in Cerrado communities (Lopes 1995). Ant nests are also commonly found in different plant species (e.g. Morais 1980), promoting the presence of ants on Cerrado foliage. Third, Cerrado presents high herbivore activity, mainly during the wet season (Marquis et al. 2002). Fourth, Cerrado presents a wide distribution that covers nearly 26% of Brazilian territory (Oliveira-Filho and Ratter 2002; Vieira et al. 2022) (Figure 1D). Finally, Vasconcelos et al. (2018) reported an inverse latitudinal gradient in Cerrado ant communities, which could possibly account for variation of ant-plant interactions along this gradient.

This study evaluates the context-dependence of the presence of ants on Cerrado foliage. We investigate how the following sources of variation – *latitude*, *environmental heterogeneity*, *resource availability*, and *ant and plant communities* – drive the frequency of ant-plant interactions in Cerrado.

Our study is based on the following assumptions and/or facts: (i) interspecific interactions modulate ecological and evolutionary processes (Bronstein 2015), and can be mediated by multiple contextual (abiotic and biotic) factors (Chamberlain et al. 2014; Hoeksema and Bruna 2015); (ii) field-based, large spatial scale studies on context-dependence of interspecific interactions are lacking (Chamberlain et al. 2014; Hoeksema and Bruna 2015;

Catford et al. 2022); (iii) contextual factors can be embedded in a complex network of direct and indirect relationships (Liu and Gaines 2022).

We used a structural equation modelling framework to access causality between variables, and the relative direct and indirect effects of each conceptual variable in ant-plant interactions (Grace et al. 2015). Given that ant presence on plants require resource availability (Chamberlain and Holland 2009), we hypothesized that EFNs and hemipteran trophobionts will be the major drivers mediating ant foraging on Cerrado foliage. Additionally, since habitat structure (vegetation physiognomy) and abiotic factor (e.g., climate, soil nutrients) are commonly pointed out as factors modulating context-dependence in interspecific interactions (Liu and Gaines 2022), we also hypothesized that environmental heterogeneity and its correlates (plant community and latitude) will indirectly impact ant presence on Cerrado foliage.

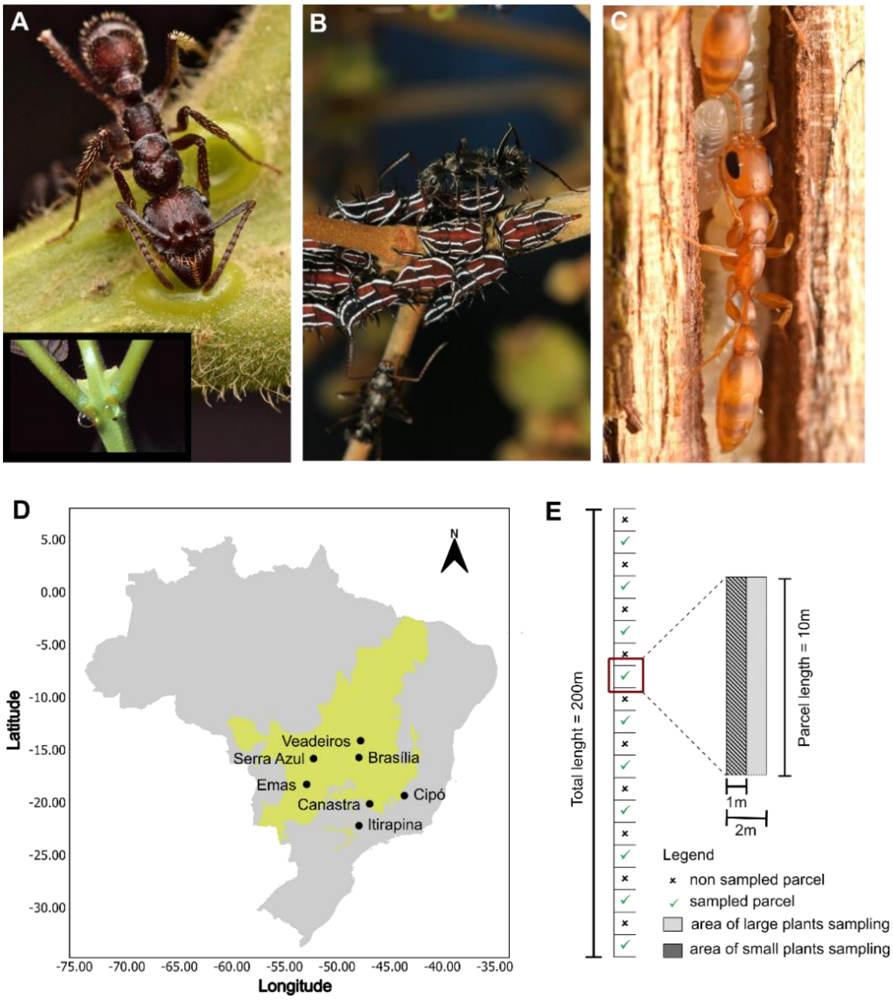


Figure 1. Examples of the main resources used by ants on Cerrado vegetation: (A) *Ectatomma* feeding on extrafloral nectary (Credit: P. Hönle); accumulated extrafloral nectar produced by glands of *Qualea grandiflora* is shown in detail (Credit: Paulo S. Oliveira), (B) *Camponotus* attending honeydew-producing treehoppers (Credit: S. F. Sendoya), and (C) *Pseudomyrmex* nesting inside a hollow branch of a plant (Credit: P. Hönle). (D) Brazil (in grey) with the distribution of Cerrado savanna in green. Black circles indicate sampling site localities. (E) Sampling scheme in each transect.

MATERIAL AND METHODS

Sampling sites

This study was carried out in seven reserves along the Cerrado distribution in Brazil (Figure 1D): Estação Ecológica de Itirapina (state of São Paulo), Parque Nacional Serra da Canastra, Parque Nacional Serra do Cipó (both in the state of Minas Gerais), Parque Nacional das Emas, Parque Nacional Chapada dos Veadeiros (both in the state of Goiás), Parque Nacional de Brasília (in Distrito Federal), and Parque Estadual Serra Azul (state of Mato Grosso).

Given that the *cerrados* comprise a mosaic of vegetation physiognomies, ranging from open grasslands to forest-like vegetation (Oliveira-Filho and Ratter 2002), samplings in all localities were conducted in the same Cerrado physiognomy – Cerrado *sensu stricto*. This physiognomy in characterized by the prevalence of herbaceous vegetation, with trees and shrubs often 3-8m tall, totalizing more than 30% crown cover (Oliveira-Filho and Ratter 2002). Cerrado has a seasonal climate, with a very strong dry season during southern winter (Oliveira-Filho and Ratter 2002). Along our study sites, mean annual temperature ranged from 17.8°C (Parque Nacional Serra da Canastra) to 23.5°C (Parque Estadual Serra Azul) (data from WorldClim). Annual rainfall ranged from 1371 mm (Estação Ecológica de Itirapina) to 1818 mm (Parque Nacional Chapada dos Veadeiros) (data from WorldClim). Because vegetative growth and extrafloral nectary activity are higher during the wet season (Díaz-Castelazo et al. 2004; Silva and Oliveira 2010), as well as herbivore activity (Marquis et al. 2002), fieldwork in all localities was conducted in the morning during the warm/wet season, from November 2012 to April 2013.

Sampling design

In each sampling site, we established five transects of 200 m, at least 1 km apart from each other. The geographic coordinates (latitude and longitude) of transects were recorded using a global positioning system (GPSmap 60CSx, Garmin International Inc., Olathe, KS, USA, WGS 1984 UTM Zone 23S). Transects were divided into 20 plots of 10 x 2 m; data were collected in each alternate plot in the transect, totaling 10 plots per transect as illustrated in Figure 1E.

We focused our study on the arboreal ant community and all data collected during fieldwork were based on observation of ant activity on plants. We considered all small wood plants (with diameter at breast height, DAP, of 2 to 30 mm) up to 1 m from the central line of the transect, covering an area of 100 m^2 per transect (Figure 1E). We sampled all large trees (with DAP > 30 mm) up to 2 m from the central line of the transect, covering a total of 200 m^2 per transect (Figure 1E). DAP measures were taken at 30 cm from the ground. Plants were identified at the species level or, when identification was not possible, classified in morphotypes. Although we observed the presence of EFNs during fieldwork, we made an extensive search in the Cerrado literature to guarantee presence or absence of EFNs in the collected plants (Oliveira and Leitão-Filho 1987; Oliveira and Oliveira-Filho 1991; Machado et al. 2008; Schoereder et al. 2010; Boudouris and Queenborough 2013; Muehleisen 2013; Keeler et al. “World List of Plants with Extrafloral Nectaries”).

Each plant was observed during two intervals of 1 minute, during which we noted: the number of ants foraging on the plant, the presence of trophobiont insects, and the presence of ant nests. Ants were collected and identified at the species level or, when identification was not possible, classified in morphospecies.

Overall, a total of 3,345 plants were recorded and observed for ant activity, comprising 198 plant and 97 ant species (Table S1). One transect located in the Chapada dos Veadeiros was lost due to a fire during sampling procedure, resulting in a total of 34 transects analyzed in this work. All analyses were performed using R software v4.2.2 (R Core Team 2022).

Ant-plant interactions in Cerrado

The proportion of plants with ants (**n.interaction**) was used as an estimate of ant-plant interactions along the Cerrado gradient. In each transect at a given locality, we divided the number of plants with ants by the total number of plants sampled. All estimated variables within major biotic and abiotic parameters associated with ant-plant interactions are presented in Table 1.

Major parameters

1. *Environmental heterogeneity*

Environmental heterogeneity is commonly associated with the structure of the physical environmental promoted by the vegetation (Tews et al. 2004). In each transect we estimated the density of small (**D.small**) and large (**D.large**) plants by dividing the number of small and large plants by the sampling area of 100 m^2 and 200 m^2, respectively. Given DAP is considered a good proxy of plant biomass in Cerrado (Roitman et al. 2018), we also estimated the mean DAP per transect (**DAP**). From each transect, we made a composite soil sampling and 300 g, which was analyzed in the Instituto Agronômico de Campinas to determine the amount of organic matter (**M**) in the soil. In addition to the data collected during fieldwork, we also obtained the mean annual temperature (**T**) and annual precipitation (**P**) (bio 1.C and bio 12, respectively) from the WorldClim database (Fick and Hijmans 2017), with resolution of 30 seconds. Net primary productivity (**NPP**) for the period of November 2012 to April 2013 was obtained from the mean of Moderate Resolution Imaging Spectroradiometer (MODIS) on NASA’s Terra and Aqua satellites (Running and Zhao 2019), with resolution of 500 m.

2. *Resources on Cerrado foliage*

To characterize the resources potentially mediating ant-plant interactions in each transect, we focused on the three main resources used by ants in Cerrado vegetation: extrafloral nectaries (EFNs), trophobionts and nesting sites (Oliveira and Freitas 2004). We then estimated the proportion of plants bearing EFNs (**n.efn**) by dividing the number of plants bearing EFNs by the total number of plants sampled in the transect. Similarly, we estimated the proportion of plants with trophobionts (**n.tropho**) and proportion of plants with ant nests (**n.nests**).

3. *Plant and ant communities*

Resources to ants in Cerrado vegetation are greatly influenced by plant species richness, being also affected by plant taxonomic families (Morais 1980, Oliveira and Leitão-Filho 1987; Oliveira and Oliveira-Filho 1991; Lopes 1995; Table 2). Ant identity and dominance relationships seem also important in determining ant presence on vegetation (Leston 1973; Ribas and Schoereder 2004; Blüthgen and Stork 2007; Schoereder et al. 2010). Therefore, in each transect, plant and ant communities were characterized by three estimates: rarified species richness, phylogenetic diversity, and co-occurrence index.

Given that there are distinct number of plants per transect, species richness (**S.plants**) was rarefied to the minimum number of plants across all transects based on Hurlbert’s (1971) formulation, implemented in the function *rarefy* in the R package *vegan* (Oksanen et al. 2022). The same procedure was used to estimate ant rarefied species richness (**S.ants**).

To estimate phylogenetic diversity of plants, we used a megaphylogeny for seed plants (Smith and Brown 2018). Species names were standardized based on *World Flora Online (WFO) Plant List* (https://wfoplantlist.org/plant-list), using the R package *Taxonstand* (Cayuela et al. 2012). To link species or genera names to those in the megaphylogeny, we ran the R function *S.PhyloMaker* (scenario 3; see Qian and Jin 2016). This approach adds missing species or genus to the phylogeny within the taxa with known branch lengths. Complete phylogeny was then pruned to include only the species in our dataset. This final phylogeny was used as reference tree from which phylogenetic diversity could be estimated for each transect. Phylogenetic diversity of plants (**PD.plants**) was calculated using the *ses.pd* function in the R package *picante* (Kembel et al. 2010). Similar approach was used to estimate ant phylogenetic diversity (**PD.ants**). However, given that we lack a good ant phylogeny at the species level, we used a phylogeny at the genus level (Moreau et al. 2006), which is also appropriate for studies on phylogenetic structure of communities (Qian and Jin 2021). Generic names were standardized manually to converge to the ones listed in the phylogeny, which was then pruned to include only the genus present in our dataset. Phylogenetic diversity of ants was also calculated using the *ses.pd* function in *picante* (Kembel et al. 2010).

To evaluate the co-occurrence of species in each transect, we estimated the checkboard score (*C-score*; Stone and Roberts 1990) implemented in the function *C.score* in the R package *bipartite* (Dormann et al. 2008). *C-score* estimates the randomness between two or more species distributions: the higher the *C-score*, the lower the randomness in species distribution within communities (i.e. the higher the probability of the distribution of one species to be affected by another species; Stone and Roberts 1990). For each transect, plots and plants were used as sampling units to estimate *C-score* in plant (**C.plants**) and ant (**C.ants**) community, respectively.

For ant communities, we also estimated de mean number of ants per plant (**n.ants**) as a proxy of ant abundance on plants.

Table 1. Summary of the variables measured in the sampling transects, with their respective code, type (mainly for path analysis), probability distribution, mean and standard error. Mean and stardard error (SE) are reported for each variable considering all transects (N = 34) across the seven sampled localities (Estação Ecológica de Itirapina, Parque Nacional Serra da Canastra, Parque Nacional Serra do Cipó, Parque Nacional das Emas, Parque Nacional Chapada dos Veadeiros, Parque Nacional de Brasília, and Parque Estadual Serra Azul).

Conceptual variable	**Operational variables (unit)**	**Code**	**Type**	**Distribution**	**Mean (± SE)**
Latitude	Latitude	Lat	Explanatory	-	-
Environmental heterogeneity	Annual mean temperature (°C)^1	T	Response/Explanatory	Gaussian	21.08 ± 0.29
	Annual precipitation (mm)^2	P	Response/Explanatory	Gaussian	1578.03 ± 21.4
	Mean net primary productivity (g C/m^2/yr)^3	NPP	Response/Explanatory	Gaussian	10395.12 ± 371.58
	Soil organic matter (g/dm^3)	M	Response/Explanatory	Gaussian	3.93 ± 0.23
	Mean trunk diameter at soil height (mm)	DAP	Explanatory	-	35.67 ± 1.68
	Density of small plants (number of plants/m^2)	D.small	Explanatory	-	0.58 ± 0.05
	Density of large plants (number of plants/m^2)	D.large	Explanatory	-	0.19 ± 0.01
Plant community	Rarefied species richness	S.plants	Response/Explanatory	Gaussian	16.64 ± 0.68
	Co-occurrence (c score)	C.plants	Response/Explanatory	Gaussian	2.39 ± 0.08
	Phylogenetic diversity	PD.plants	Explanatory	-	0.14 ± 0.19
Resources	Proportion of the total number of plants bearing extrafloral nectaries	n.efn	Response/Explanatory	Binomial	0.23 ± 0.02
	Proportion of the total number of plants with trophobionts	n.tropho	Response/Explanatory	Binomial	0.04 ± 0.01
	Proportion of the total number of plants with ant nests	n.nests	Response/Explanatory	Binomial	0.14 ± 0.01
Ant community	Rarefied species richness	S.ants	Response/Explanatory	Gaussian	8.17 ± 0.27
	Co-occurrence (c score)	C.ants	Response/Explanatory	Gaussian	13.43 ± 1.41
	Phylogenetic diversity	PD.ants	Explanatory	-	0.25 ± 0.13
	Mean number of ants per plant	n.ants	Response/Explanatory	Gaussian	1.73 ± 0.18
Ant-plant interaction	Proportion of the total number of plants with ants	n.interaction	Response	Binomial	0.45 ± 0.03

[1] Data obtained from WordClim (bio 1.C); [2] data obtained from WordClim (bio 12); [3] data obtained from NASA Earth Observation System (MOD17). All other variables were obtained during fieldwork.

Data analyses: Evaluating context-dependence of ant-plant interactions in Cerrado

1. *Generalized modelling*

To investigate the effects of *latitude*, *environmental heterogeneity*, *resources*, and *plant and ant communities* on the proportion of plants with ants in Cerrado, we used generalized linear mixed models (GLMM) implemented in the function *glmer* in the R package *lme4* (Bates et al. 2015). A binomial distribution was used to deal with the nature of the response variable (proportion). Sampling sites were included as random effects. We ran the models separately for each major parameter, enabling us to explore which operational variables are influencing the proportion of plants with ants in Cerrado (Table S2). Except for the model with latitude as explanatory variable, all operational variables were scaled prior to modelling by subtracting their means and dividing by their standard deviation. We checked collinearities for all models by calculating the variance inflation factor (VIF) for each prediction using the function *check_collinearity* implemented in the R package *performance* (Lüdecke et al 2021). Exclusion of variables follow two methods: (i) variables with VIF pointed as moderate or high collinear and/or (ii) variables with VIF > 3 (Zuur et al. 2009) (Table S3). Model residuals were inspected for model assumptions using the function *simulateResiduals* implemented in the R package *DHARMa* (Hartig 2022) (Figure S1). We used an automated model selection approach implemented in the function *dredge* in the R package *MuMIn* (Barton 2022), based on Akaike Information Criterion corrected for small samples (AICc) to select the best models within each conceptual predictor. Models with ΔAICc < 2 were considered the most plausible among candidates (Zuur et al. 2009). When more than one model was pointed as plausible, we performed a model averaging (full average) using the function *model.avg* in the *MuMIn* package. Marginal (fixed effects only; R^2m) and conditional (all effects; R^2c) coefficient of determination were estimated for each model using the function *r.squaredGLMM* implemented in the *MuMIn* package (for averaged models, the range of R^2m and R^2c was reported). Finally, final model residuals were tested for spatial autocorrelation, using Moran I test implemented in the function *Moran.I* in the R package *ape* (Paradis and Schliep 2019).

2. *Structural equation modelling*

Having identified the main variables explaining the proportion of plants with ants in Cerrado, we synthesized our analyses by investigating the importance of explanatory variables and their interrelations in a logical causal path. To do so, we applied a piecewise structural equation modelling (SEM) framework (Lefcheck 2016). SEM is a modelling approach that enables testing hypothetical pathways of cause-effect relationships (Grace et al. 2015). In this sense, models that represent causal hypothesis are built based on prior knowledge, and observational data can be used to test such hypothesis under the light of SEM (Grace et al. 2015). Both direct and indirect effects can be tested, being possible to access the strength and direction of the relationship between variables (Grace et al. 2015). We built a full model for the hypothesis of direct and indirect impacts of latitude, environmental heterogeneity, resources and plant and ant communities on the proportion of plants with ants in Cerrado (Figure 2A). All the hypothesized pathways were based on literature survey (Table 2). The path model consisted of a set of Linear Mixed Models (LMM) and GLMM for response variables with gaussian and binomial distributions, respectively (Table 1). Sampling sites were included as random effects in all internal models. LMM and GLMM were performed using, respectively, the *lmer* and *glmer* functions implemented in *lme4* package. As in the previous section, internal models were checked for collinearity, with exclusion of variables with VIF > 3 (Table S4). Model assumptions were also checked (Figure S2). Path model was fitted using the *psem* function implemented in R package piecewiseSEM (Lefcheck 2016). piecewiseSEM uses Shipleys's (2000) direct-separation (d-separation) test to identify significative non-hypothesized, independent paths that would improve internal models. Missing links were incorporated to internal models if ecologically justifiable (Table S5). Non-significant paths were removed iteratively and model AICc was estimated (see in Table S6 the step-by-step process of addition and removal of links up to the final, best model). Internal models of the final SEM were again checked for collinearity (Table S7) and model assumptions (Figure S3), and no issue was detected. Residuals of the final SEM models were tested using *Moran.I* in *ape* package, and no spatial autocorrelation was detected (Table S8). The goodness of fit of the final model were assessed using Fisher's C statistic, whose P > 0.05 indicates a good fit (Shipley 2009). R^2m and R^2c were estimated for all models incorporated in the SEM. Standardized path coefficients were estimated. Indirect effects were calculated by multiplying the partial path coefficients, and the overall effect was calculated by summing all direct and indirect effects.

Table 2. Internal models of full structure equation modelling (SEM), with literature support to hypothesized pathways of how the proportion of plants with ants could be explained by direct and indirect effects of latitude, environmental heterogeneity, plant community, resources, and ant community.

Response variable	Literature support	Explanatory variables	Expected effect
T	• Annual mean temperature presents an inverse relationship with latitude, increasing toward the equator (Sanderson 1999).	**Lat**	negative
P	• Although there is not a clear pattern, annual precipitation has been shown to vary with latitude (Linacre and Geerts 1997).	**Lat**	undefined
NPP	• Net primary productivity is higher near the equator (Gillman e al. 2015), where precipitation is high, and temperature is uniform along the year. • Considering all tropical forests, annual mean temperature is the best predictor of above ground net primary productivity (Cleveland et al. 2011). • Organic matter influences physical, chemical, and biological properties of the soil. Negatively-charged particles of organic matter interact with positively-charged particles and can mediate phosphorus absorption by the plants (see Fink et al. 2016). In turn, phosphorus in the soil explains a significant proportion of above ground net primary productivity in tropical forests (Cleveland et al 2011). • Net primary productivity is positively associated with the total biomass of the plant community, which is dependent on individual biomass and number of individuals in the community (Kerkhoff and Enquist 2006). Diameter at soil height is a reliable predictor of plant biomass (Roitman et al. 2018).	**Lat** **T** **P** **M** **DAP** **D.small*** **D.large**	positive positive positive undefined positive positive positive
M	• The distribution of organic carbon in the soil is greatly influenced by vegetation density and physiognomy. Moreover, the total amount of organic carbon in the soil increases with precipitation and decreases with environmental temperature (Jobbági and Jackson 2000). • Given that ants carry food to the interior of their nests, they tend to promote the accumulation of organic matter in the soil (Frouz and Jilková 2008).	**T** **P** **D.small** **D.large** **S.ants****	negative positive positive positive positive
S.plants	• Species richness of vascular plants is positively associated to net primary productivity (Gillman e al. 2015).	**NPP**	positive

C.plants	• *C-score* is correlated with species richness (Ulrich et al. 2018). • Species co-occurrence may be phylogenetic clustered if species traits are evolutionarily conserved, and environmental filtering is strong (Emerson and Gillespie 2008). However, the coexistence of closely related species may be less frequent due to competition, given their higher probability to present similar ecological traits (Hardin 1960).	**S.plants**	positive
		PD.plants	undefined
n.efn	• Plants bearing EFNs may represent more than 30% of arboreal species in Cerrado, being mainly found in Mimosaceae, Bignoniaceae and Vochysiaceae plant families (see Oliveira and Leitão-Filho 1987; Oliveira and Oliveira-Filho 1991).	**S.plants**	positive
		PD.plants	undefined
		C.plants	undefined
		D.small	positive
		D.large	positive
n.tropho	• Similar to EFNs, trophobionts may occur in approximately 30% of the plant species in Cerrado communities (Lopes 1995);	**S.plants**	positive
		PD.plants	undefined
		C.plants	undefined
		D.small	positive
		D.large	positive
n.nests	• There are many plant species inside which ant colonies can be found in Cerrado (e.g. Morais 1980); • Ants may change nest location to nearby food resources (e.g. Wagner and Nicklen 2010; Burns et al. 2021)	**S.plants**	positive
		PD.plants	undefined
		C.plants	undefined
		D.small	positive
		D.large	positive
		n.efn	positive
		n.tropho	positive
S.ants	• There is a positive association between ant species richness, precipitation and net primary productivity in Cerrado (Vasconcelos et al. 2018). • Ant species richness increases with plant richness in Cerrado and other grasslands (Ribas et al. 2003; Aguiar et al. 2022).	**P**	positive
		NPP	positive
		S.plants	positive
C.ants	• *C-score* is correlated with species richness (Ulrich et al. 2017); • Species co-occurrence may be phylogenetic clustered if species traits are evolutionarily conserved, and environmental filtering is strong (Emerson and Gillesoie 2008). However, the coexistence of closely related species may be less	**S.ants**	positive
		PD.ants	undefined

	frequent due to competition, given their higher probability to present similar ecological traits (Hardin 1960).		
n.ants	• Across distinct Brazilian biomes, ant foraging behavior tends to be higher in sites with increased humidity, net primary productivity, and less variation in temperature (Lasmar et al. 2021); • Association between ants and plants is generally mediated by the offer of structures for nesting and liquid resources from EFNs and trophobionts (Oliveira and Brandão 1991; Oliveira and Freitas 2004; Rico-Gray and Oliveira 2007; Sendoya 2016) • Mean number of ants on plants should be dependent of their co-occurrence (*C-score*).	**P** **NPP** **T** **n.nests** **n.efn** **n.tropho** **C.ants**	positive positive undefined positive positive positive undefined
n.interactions	• According to Figure 2, in our study system there is a negative relationship between latitude and the proportion of plants with ants in Cerrado. • Ant-plant interaction is mediated by the ant community and resources presented on plants (Oliveira and Freitas 2004; Rico-Gray and Oliveira 2007)	**Lat** **S.ants** **C.ants** **PD.ants** **n.ants*** **n.efn** **n.tropho** **n.nests**	negative undefined undefined undefined undefined positive positive positive

* Explanatory variable removed from models due to high or moderate collinearity (see Material and Methods); ** variable removed because it creates a looping in SEM structure, which is not allowed for analysis in piecewiseSEM. **T**: Annual mean temperature; **P**: Annual Precipitation; **NPP**: Mean net primary productivity; **M**: Soil organic matter; **DAP**: Mean diameter at soil height; **D.small**: Density of small plants; **D.large**: Density of large plants; **S.plants**: Rarefied plant species richness; **C.plants**: Plants co-occurrence index; **PD.plants**: Plants phylogenetic diversity; **n.efn**: Proportion of the total number of plants bearing extrafloral nectaries; **n.tropho**: Proportion of the total number of plants with trophobionts; **n.nests**: Proportion of the total number of plants with ant nests; **S.ants**: Rarefied ant species richness; **C.ants**: Ants co-occurrence index; **PD.ants**: Ants phylogenetic diversity; **n.ants**: Mean number of ants per plant; **n.interaction**: Proportion of the total number of plants with ants.

RESULTS

1. *Generalized modelling*

We found a significant negative relationship between the proportion of plants with ants in Cerrado vegetation (**n.interaction**) and latitude (Estimate = -0.128; P = 0.003; R^{2}m = 0.495; Figure 2). In all individual conceptual models, **n.interaction** varied depending on some operational variables (Table 3). Among the *environmental heterogeneity* operational variables, **n.interaction** increased with the mean plant diameter at soil height (**DAP**; Estimate = 0.139; P = 0.005) and decreased with the organic matter in the soil (**M**; Estimate = -0.46; P = 0) (GLMM; R^{2}m = 0.7 - 0.867; Table 3; Table S2). For *plant community*, all variables were found to influence **n.interaction**, which increased with. the *C-score* of plants (**C.plants**; Estimate = 0.118; P = 0.019) and decreased with the plant species richness (**S.plants**; Estimate = -0.186; P = 0.002) and phylogenetic diversity (**PD.plants**; Estimate = -0.104; P = 0.02) (GLMM; R^{2}m = 0.214; Table 3; Table S2). Regarding *resources*, the proportion of plants with EFNs (**n.efn**; Estimate = 0.232; P = 0) and trophobionts (**n.tropho**; Estimate = 0.292; P = 0) were found to positively impact **n.interaction** (GLMM; R^{2}m = 0.507 - 0.526; Table 3; Table S2). Finally, among *ant community* operational variables, the *C-score* (**C.ants**; Estimate = 0.241; P = 0) and the mean number of ants per plant (**n.ants** Estimate = 0.329; P = 0) were found to increase **n. interaction** (GLMM; R^{2}m = 0.749 - 0.758; Table 3; Table S2). In all models, we did not detect spatial autocorrelation (Figure 2; Table S2).

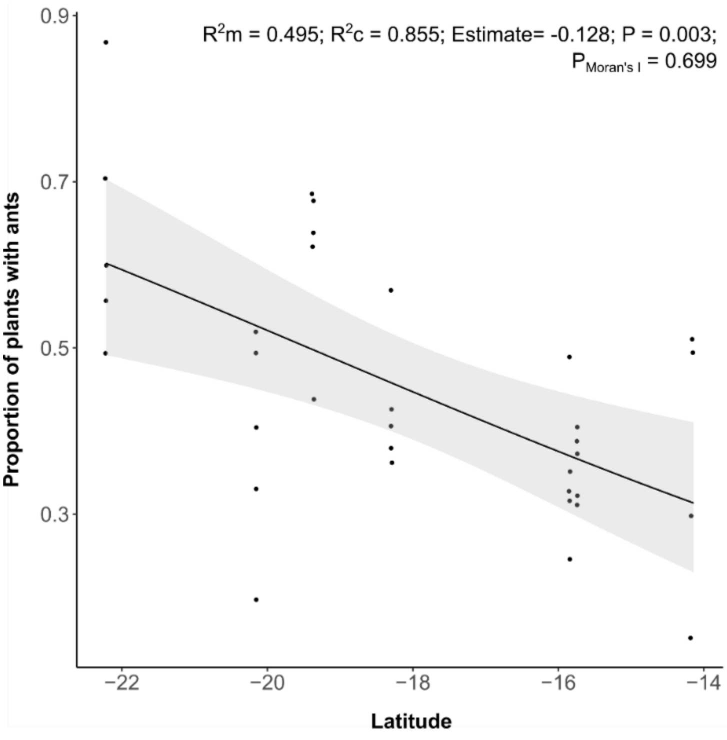


Figure 2. Generalized linear mixed model with binomial error for the proportion of plants with ants in response to latitude in Brazilian Cerrado savanna. Study sites were included as random factor. Each point represents a transect. R^2m, R^2c, Estimate, P-value, and P-value for Moran I spatial correlation test are shown.

Table 3. Significant (GLMM; $P < 0.05$) drivers (operational variables) of the proportion of plants with ants in Cerrado vegetation for each conceptual predictor. Arrows represent positive (↑) or negative (↓) effects. For detailed GLMMs, see Table S2.

Major parameters	**Operational variables**	**Directionality of the effect**
Environmental heterogeneity	**M**	↓
	DAP	↑
Plant community	**S.plants**	↓
	C.plants	↑
	PD.plants	↓
Resources	**n.efn**	↑
	n.tropho	↑
Ant community	**C.ants**	↑
	n.ants	↑

M: Soil organic matter; **DAP**: Mean diameter at soil height; **S.plants**: Rarefied plant species richness; **C.plants**: Plants co-occurrence index; **PD.plants**: Plants phylogenetic diversity; **n.efn**: Proportion of the total number of plants bearing extrafloral nectaries; **n.tropho**: Proportion of the total number of plants with trophobionts; **C.ants**: Ants co-occurrence index; **n.ants**: Mean number of ants per plant.

2. *Structural equation modelling*

Piecewise SEM selection resulted in a final path directly or indirectly relating all major parameters to the proportion of plants with ants in Cerrado vegetation (**n.interaction**): *latitude*, *environmental heterogeneity*, *plant community*, *resources* and *ant community*. The final model presented a good fit to our data (Fisher's C = 181.23, d.f. = 166, P = 0.198; Figure 3; Table S6). Only two environmental heterogeneity variables and two ant community variables presented a direct effect on **n.interaction**: net primary productivity (**NPP**; direct effect = 0.06), organic matter in the soil (**M**; direct effect = -0.11), mean number of ants per plant (**n.ants**;direct effect = 0.14), and *C-score* of ants (**C.ants;** direct effect = 0.12) (Table S8). With respect to overall effects, *latitude* was found to indirectly and negatively affect *n.interaction*, (overall effect = -0.04; Figure 3; Table 4; Table S8). *Environmental* *heterogeneity* (overall effect = -0.132) and *plant community* (overall effect = -0.063) were also found to negatively impact **n.interaction** (Figure 3; Table 4; Table S8). Finally, *resources* (overall effect = 0.198) and *ant community* (overall effect = -0.178) presented the highest and positive overall effects on **n.interaction** (Figure 3; Table 4; Table S8). We did not detect any spatial autocorrelation in the final piecewise SEM model (Table S9).

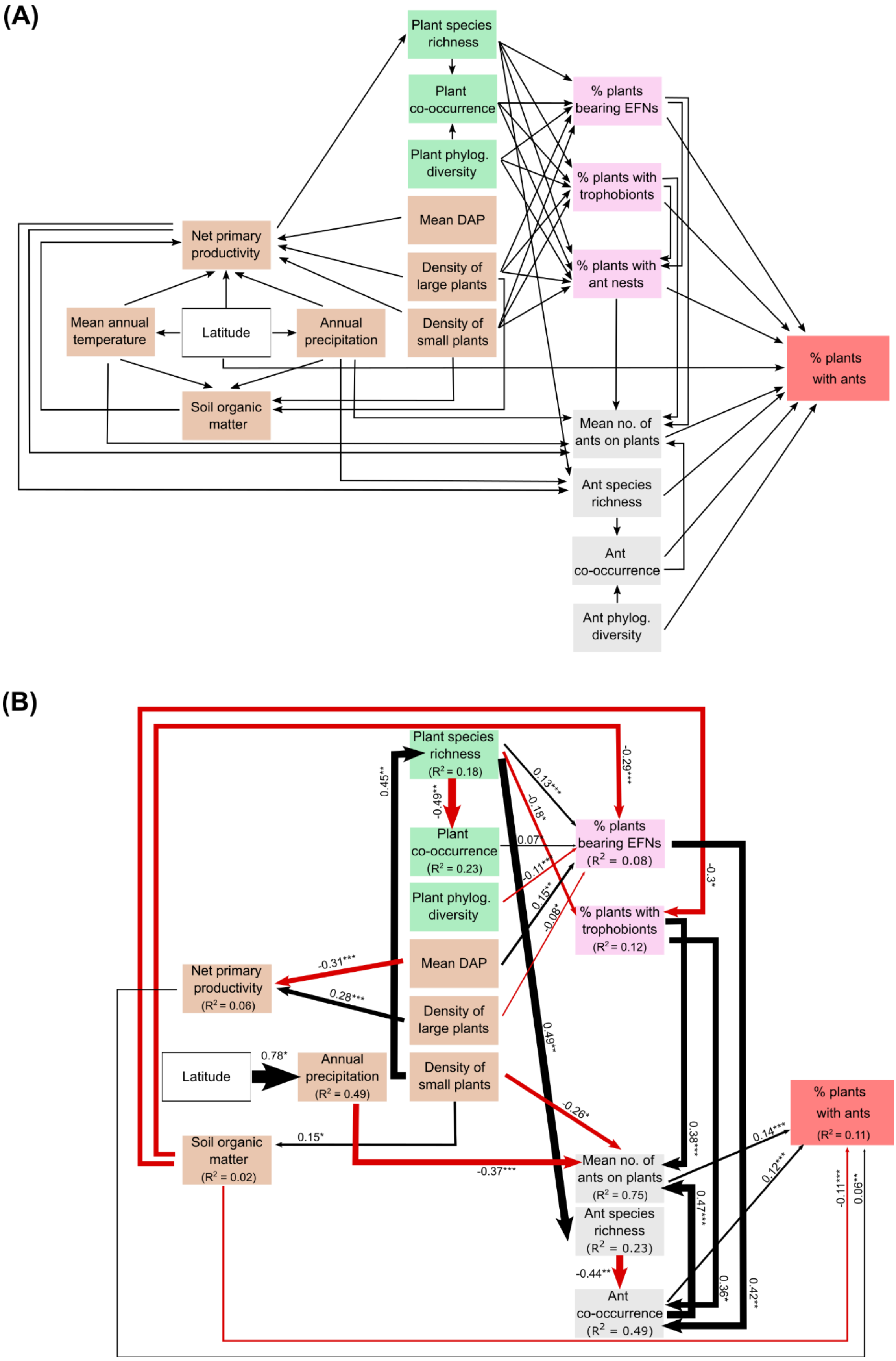


Figure 3. Structural equation model. (A) Full model with hypothesized direct and indirect effects of *latitude*, *environmental heterogeneity* (brown), *plant community* (green), *resources*

(pink), and *ant community* (grey) on the proportion of plants interacting with ants in Brazilian Cerrado savanna. (B) Final model with missing paths incorporated based on d-separation tests, and non-significant pathways removed. Standardized coefficients are shown for each path, with significance levels denoted as ***p < 0.001, **p < 0.01, *p < 0.05. R^2m is shown for each component response variable. Black arrows represent positive links and red arrows, negative links. The thickness of arrows is proportional to the strength of the effect.

Table 4. Standardized effects of paths of the conceptual predictors retained in the best fitting structural equation model for the proportion of plants with ants in Cerrado savanna. Indirect effects are calculated by multiplying the partial path coefficients, and the overall effect is calculated by summing all direct and indirect effects. For detailed path effects, see Table S8.

Major parameter	**Direct effects**	**Indirect effects**	**Overall effects**
Latitude	-	-0.04	-0.04
Environmental heterogeneity	-0.05	-0.082	-0.132
Plant Community	-	-0.063	-0.063
Resources	-	0.198	0.198
Ant community	0.26	-0.082	0.178

DISCUSSION

In this large scale, field-based work, we highlighted the distinct effects of *latitude*, *environmental heterogeneity*, *plant* and *ant communities* and *resource availability*, as drivers of ant presence on foliage of Cerrado. In our modelling approach, we found an inverse latitudinal gradient for ant-plant interactions, whose frequency increased with the distance from equator. Moreover, plant diameter at soil height (a proxy to biomass) and soil organic matter were found as the main environmental traits influencing the proportion of plants with ants in Cerrado, followed by plant species richness, co-occurrence, and phylogenetic diversity. Among resources and ant community, we also found that ant-plant interactions were mediated by the abundance and co-occurrence of ants on vegetation, and the proportion of plants bearing EFNs and trophobionts. Our structural equation modelling highlighted these same variables as drivers of ant-plant interactions, but in a causal network that reveals how they directly and indirectly impact the presence of ants on vegetation. Ant-plant interactions were indirectly and negatively associated with *latitude*. *Environmental heterogeneity* and *plant community* presented a negative overall effect on interactions, with the latter showing the lowest effect. On the other hand, *resources* and *ant communities* presented positive and the highest effects on the proportion of plants with ants on Cerrado vegetation. *Ant community* characteristics were the drivers with the highest direct effect on ant-plant interactions.

Arboreal ant species richness in Cerrado has already been reported as negatively correlated with latitude, with precipitation as the main variable mediating the association (Vasconcelos et al. 2018). Our results are consistent with these findings, with the proportion of plants interacting with ants also presenting an inverse latitudinal gradient in Cerrado. We found that ant-plant interactions are indirectly associated with latitude via the negative impact of annual precipitation on ant abundance on vegetation. Indeed, water-related variables are thought to be the main drivers of animal diversity gradients in the tropics (Hawkins et al. 2003). Water availability also mediates soil nutrient absorption, which may limit the production of extrafloral nectar (Yamawo 2017). Contrary to this expectation, however, we found soil organic matter to negatively impact ant-plant interactions by decreasing the proportion of plants bearing EFN and trophobionts. Cerrado soils are well drained, predominantly acid, poor in available mineral nutrients, and present toxic levels of aluminum and accelerated decomposition processes, which lead to low concentration of organic matter (Coutinho 1982; Haridasan et al. 2008; Carvalho et al. 2009). Indeed, Cerrado plants present physiological and morphological adaptations to deal with these soil characteristics, and plant communities may change with variation in soil traits (Lira-Martins et al. 2022). Therefore, it is possible that interhabitat variation in soil organic matter affects the floristic composition throughout the Cerrado domain, impacting the local number of plants bearing EFNs and trophobionts across habitats.

Among *environmental heterogeneity* variables, net primary productivity was found to have a direct and positive impact on ant-plant interactions in Cerrado, corroborating a positive association between biotic defense in plants and primary productivity (Yamawo 2017). Indeed, Dátillo and Vasconcelos (2019) have also reported primary productivity as the main driver of ant-plant networks in Cerrado, increasing network size and diversity. Such association would presumably result from weak interspecific competition in more productive localities, favoring ant species coexistence and, therefore, ant-plant interactions (Sebastián-González et al. 2015; Trojelsgaard and Olesen 2013). Additionally, we found that DAP and plant densities affect ant-plant interactions via indirect pathways, as shown in SEM analysis. Given that an increase in environmental heterogeneity would lead to an increment in habitat types, resources, and spatial complexity (Stein et al. 2014), the availability of niche space would also tend to increase (Tews et al. 2004), affecting how frequent ants interact with plants in Cerrado. Moreover, changes in the physical environmental heterogeneity can also promote changes in microclimate variables, which in turn can affect ant foraging behavior and, consequently, the ant-plant interactions (Ribas et al. 2003).

The *plant community* was found to present little negative impact on ant-plant interactions, which derived mainly from negative impacts of phylogenetic diversity and plant species richness on plant species co-occurrence. Indeed, liquid resources from EFNs in Cerrado are mainly found in some plant families including Mimosaceae, Bignoniaceae and Vochysiaceae (Oliveira and Leitão-Filho 1987; Oliveira and Oliveira-Filho 1991). A similar pattern accounts for trophobiont occurrence on foliage (Lopes 1995). As a result, it is possible that an increase in plant species richness and phylogenetic diversity would reduce the number of plant species with available liquid resources, modulating the presence of ants on vegetation. We have shown that the proportion of plants with liquid *resources* was the most important factor influencing the presence of ants on vegetation, corroborating our initial hypothesis and literature that resource availability is a major driver explaining ant-plant interaction occurrence (Hoek et al. 2016; Pringle 2016). Our results reinforce food availability as crucial factor mediating ant-plant interactions (Chamberlain and Holland 2009; Díaz-Castelazo et al. 2017). Although both EFNs and trophobionts indirectly account for ant-plant interactions in Cerrado (Oliveira and Freitas 2004), the proportion of plants with trophobionts was the main factor mediating ant abundance on vegetation. Indeed, liquid resources from EFNs and trophobionts may differ in their nutritional quality (Blüthgen et al. 2004) and affect ant foraging and behavior (Styrsky and Eubanks 2007). In tropical habitats, ants may behave aggressively, monopolizing plants with trophobionts (Del-Claro and Oliveira 1993; Blüthgen et al. 2004). Specifically, in Cerrado, Sendoya et al. (2016) reported that honeydew produced by trophobionts have the strongest effect on ant visitation rates to foliage. Although ant visitation is also commonly affected by the distribution of arboreal nests (Powell et al. 2011), the presence of ant nests had no significant effect on ant-plant interactions in our study. In Cerrado, most arboreal ant nests are constructed opportunistically in hollow stems left by boring beetles, cavities galls, or even using leaves (e.g. Araujo et al. 1995). Additionally, nesting can be primarily conditioned by the presence of food resources (e.g. Wagner and Nicklen 2010; Burns et al. 2020). Therefore, the generalist and opportunistic character of ants in Cerrado vegetation, may turn nests as less determinant of ant presence on plants. In ecosystems where specialized myrmecophytes (plants with adaptations to house ant colonies) are more abundant – such as the Amazon rainforest (Benson 1985) – nest space are presumably important promoters of ant-plant interactions.

Ant community traits (abundance and co-occurrence) had the higher direct effects on ant-plant interactions in Cerrado. Ant abundance was greatly influenced by the proportion of plants with trophobionts, while ant co-occurrence (here evaluated with *C-score*) was mediated by both sources of liquid food – trophobionts and EFNs. We found that the proportion of plants with ants is associated with the ant *C-score*. In other words, interspecific ant-ant interactions on foliage increase with the number of plants interacting with ants in Cerrado. Although co-occurrence does not imply ecological interactions (Blanchet et al. 2020), there is evidence that exudate-feeding ant species affect one another on Cerrado foliage (Oliveira and Brandão 1991; Del-Claro and Oliveira 2000; Schoereder et al. 2010). Ant interspecific competition and territoriality commonly create ant mosaics in tropical ecosystems (Leston 1973; Ribas and Schoereder 2004), which can be detectable even at small scales (Sendoya et al. 2016). This suggests that ant species are affecting one another in our study system. For instance, it is known that dominant ants tend to forage on fast growing hosts, while subordinate ants are displaced to forage on plants with slow resource supply (Davidson and Mckey 1993; Díaz-Castelazo et al. 2004). Investigating ant-plant networks in Cerrado, Sendoya et. al (2016) reported that fast and opportunistic ants (e.g. *Pseudomyrmex*) are more frequently found on foliage free from dominant ants (*Camponotus*). While dominant ants forage on high quality resources, numerous smaller and timid ants may be the main visitors of plants with fewer or less productive EFNs (Sendoya et al. 2016). Therefore, ant-ant interspecific interactions can be relevant as mediators of the distribution and frequency of ant-plant interactions on Cerrado foliage.

Our study revealed different sources and pathways of context-dependence in ant-plant interactions on Cerrado foliage. However, given that ant-plant-herbivore interactions are conditioned by third-party (benefits to plants will generally occur only in the presence of herbivores; Bronstein and Barbosa 2002), it is imperative to evaluate the effects of herbivores as drivers of ant presence on Cerrado foliage, and vice-versa. Moreover, as pointed above, species ant identity and behavior are crucial determinants shaping ant-plant interactions (Beattie and Hughes 2002; Rico-Gray and Oliveira 2007). For instance, it is known that foliage-dwelling *Camponotus* ants act as efficient bodyguards in Cerrado (Del-Claro and Oliveira 2000; Oliveira and Freitas 2004; Sendoya et al. 2009), whereas other genera may not protect plants efficiently (e.g. Byk and Del-Claro 2010). Finally, the presence and distribution of alternate food sources (other than EFNs and trophobionts) may also influence ant-plant interactions, since they can lead less specialized ants (e.g. *Pheidole*, *Ectatomma*) to forage on plants (Sendoya et al. 2016). Clearly, further investigation is needed on alternative, context-dependent variables mediating ant-plant interactions in Cerrado.

This study produced a reliable causality network to understand the relationships and effects of *latitude*, *environmental heterogeneity*, *ant* and *plant communities*, and *resource* variables, on ant-plant interactions along a gradient in the Cerrado domain. Although traditionally addressed through meta-analytical approaches (Hoeksema and Bruna 2015), we were able to reinforce structural equation modelling as a trustworthy strategy to unveil context-dependence in interspecific interactions. Our results using SEM highlighted the causality pathways between variables. Field data corroborated our initial hypothesis – *resources* (indirectly) and *ant abundance* and *co-occurrence* (directly) are major drivers of ant-plant interactions on Cerrado foliage. As a step forward in the understanding of ant-plant interactions in a tropical savanna, this work also opens new avenues of investigation. Additional large-scale and field-based studies should help clarify the complexity of ant interspecific interactions in different ecosystems at variable ecological settings. Moreover, given that we did not measure the outcomes of ant-plant interactions, we encourage studies on this topic and how the evaluated major parameter mediates the benefit of ant presence on plants. Our work helps to synthesize the current knowledge towards the ant-plant interaction in Cerrado and encourage similar approaches in other ecosystems.

SUPPLEMENTARY INFORMATION

Table S1. List of plant and ant species identified in 34 transect along a latitudinal gradient of Brazilian Cerrado Savanna.

Plant species
Anadenanthera sp, Acosmium dasycarpum, Aegiphila verticillata, Agonandra brasiliensis, Amphilophium mansoanum, Anacardium occidentale, Andira cujabensis, Andira humilis, Anemopaegma arvense, Annona coriacea, Annona crassiflora, Annona tomentosa, Aspidosperma macrocarpon, Aspidosperma tomentosum, Baccharis sp1, *Banisteriopsis anisandra, Banisteriopsis hirsuta, Banisteriopsis malifolia, Banisteriopsis nummifera, Banisteriopsis stellaris, Banisteriopsis variabilis, Barbacenia* sp1, *Bauhinia brevipes, Bauhinia burchellii, Bauhinia rufa, Blepharocalyx salicifolius, Brosimum gaudichaudii, Byrsonima coccolobifolia, Byrsonima gardneriana, Byrsonima intermedia, Byrsonima pachyphylla, Byrsonima vacciniifolia, Byrsonima verbascifolia, Calliandra* sp1, *Callisthene major, Campomanesia adamantium, Campomanesia pubescens, Campomanesia* sp1, *Caryocar brasiliense, Casearia sylvestris, Chamaecrista orbiculata, Chromolaena pungens, Chromolaena* sp1, *Cinnamomum hatschbachii, Coccoloba* sp1, *Connarus suberosus, Cordiera sessilis, Couepia grandiflora, Cupania platycarpa, Curatella americana, Cuspidaria sceptrum, Dalbergia miscolobium, Dalbergia* sp2, *Dasyphyllum sprengelianum, Davilla elliptica, Diospyros inconstans, Diospyros lasiocalyx, Duguetia furfuracea, Duguetia* sp1, *Duguetia* sp2, *Enterolobium gummiferum, Eremanthus glomerulatus, Eremanthus goyazensis, Eremanthus mattogrossensis, Eremanthus* sp1, *Eriotheca gracilipes, Eriotheca pubescens, Erythroxylum daphnites, Erythroxylum deciduum, Erythroxylum pruinosum, Erythroxylum* sp1, *Erythroxylum* sp3, *Erythroxylum suberosum, Erythroxylum tortuosum, Erythroxylum umbu, Erythroxylum vaccinifolium, Eugenia aurata, Eugenia bimarginata, Eugenia punicifolia, Eugenia pyriformis, Fridericia platyphylla, Guapira campestris, Guapira noxia, Guapira opposita, Guarea canjerana, Hancornia speciosa, Handroanthus impetiginosus, Handroanthus ochraceus, Heteropterys byrsonimifolia, Heteropterys eglandulosa, Heteropterys escalloniifolia, Heteropterys umbellata, Himatanthus obovatus, Hymenaea stigonocarpa, Hypenia brachystachys, Hyptidendron asperrimum, Hyptis villosa, Ilex velutina, Jacaranda caroba, Kielmeyera coriacea, Kielmeyera rubriflora, Lafoensia pacari, Leandra lacunosa, Licania humilis, Machaerium opacum, Mangifera indica, Manihot tripartita, Maprounea guianensis, Miconia albicans, Miconia alborufescens, Miconia fallax, Miconia irwinii, Miconia ligustroides, Miconia pepericarpa, Miconia rubiginosa, Miconia sellowiana, Miconia stenostachya, Microstachys corniculata, Mimosa claussenii, Mimosa setosa, Monteverdia* sp1, *Moquiniastrum densicephalum, Moquiniastrum pulchrum, Myrcia bella, Myrcia guianensis, Myrcia lanuginosa, Myrcia pulchra, Myrcia* sp1, *Myrcia* sp2, *Myrcia* sp3, *Myrcia* sp4, *Myrcia splendens, Myrcia vestita, Myrsine monticola, Myrsine* sp, *Myrsine* sp1, *Myrsine umbellata, Nectandra* sp1, *Neea theifera, Ocotea pulchella, Ouratea hexasperma, Ouratea semiserrata, Ouratea spectabilis, Palicourea rigida, Peixotoa goiana, Peixotoa hatschbachii, Peixotoa reticulata, Peritassa campestris, Piptocarpha rotundifolia, Plathymenia reticulata, Plenckia populnea, Pouteria ramiflora, Pouteria torta, Protium ovatum, Psidium grandifolium, Psidium laruotteanum, Psidium myrsinites, Pterodon emarginatus, Qualea cordata, Qualea grandiflora, Qualea multiflora,*

Qualea parviflora, Roupala montana, Rourea induta, Sabicea brasiliensis, Salacia crassifolia, Salacia elliptica, Salacia macrantha, Schefflera macrocarpa, Schefflera malmei, Sclerolobium aureum, Senna rugosa, Senna sp1, *Serjania obtusidentata, Siparuna guianensis, Siphoneugena densiflora, Stryphnodendron adstringens, Stryphnodendron obovatum, Styrax camporum, Symplocos nitens, Symplocos oblongifolia, Tabebuia aurea, Tachigali subvelutina, Tapirira guianensis, Tetrapterys microphylla, Tetrapterys ramiflora, Tocoyena formosa, Vatairea macrocarpa, Vellozia* sp1, *Vellozia* sp2, *Vochysia elliptica, Vochysia herbacea, Vochysia rufa, Vochysia thyrsoidea, Vochysia tucanorum, Xylopia aromatica, Xylopia frutescens, Zeyheria montana*

Ant species

Apterostigma sp1, *Atta* sp2, *Azteca* sp1, *Azteca* sp2, *Azteca* sp3, *Azteca* sp4, *Azteca* sp5, *Brachymyrmex australis, Brachymyrmex patagonicus, Brachymyrmex* sp3, *Brachymyrmex* sp4, *Brachymyrmex* sp5, *Camponotus blandus, Camponotus crassus, Camponotus leydigi, Camponotus mus, Camponotus novogranadensis, Camponotus pallecens, Camponotus personatus, Camponotus renggeri, Camponotus rufipes, Camponotus* sp1, *Camponotus* sp10, *Camponotus* sp13 , *Camponotus* sp16, *Camponotus* sp2, *Camponotus* sp4, *Camponotus* sp6, *Cephalotes atratus, Cephalotes pavonii, Cephalotes persimilis, Cephalotes pusillus, Cephalotes* sp2 , *Cephalotes* sp6, *Crematogaster obscurata, Crematogaster* sp1, *Crematogaster* sp12, *Crematogaster* sp13, *Crematogaster* sp14, *Crematogaster* sp15, *Crematogaster* sp16, *Crematogaster* sp2, *Crematogaster* sp6, *Crematogaster* sp9, *Crematogaster torosa, Dolichoderus germaini, Dolichoderus* sp3, *Dorimyrmex brunneus, Dorimyrmex* sp1, *Dorimyrmex* sp2, *Dorimyrmex* sp3, *Ectatomma permagnum, Ectatomma* sp1, *Ectatomma* sp4, *Ectatomma tuberculatum, Gnamptogenys* sp1, *Leptothorax* sp1, *Leptothorax* sp2, *Linepithema gallardoi, Linepthema neotropicum, Myrmelachista* sp2, *Myrmelachista* sp4, *Neoponera villosa, Nesomyrmex* sp1, *Nesomyrmex* sp4, *Nesomyrmex* sp6 , *Nylanderia* sp1, *Ochetomyrmex semipolitus, Pachycondyla* sp3, *Pachycondyla* sp4, *Pheidole capillata, Pheidole fracticeps, Pheidole oxyops, Pheidole radoszkowskii, Pheidole* sp1, *Pheidole* sp13, *Pheidole* sp17, *Pheidole* sp20, *Pheidole* sp22, *Pheidole* sp24, *Pheidole* sp29, *Pheidole suzannae, Pheidole triconstricta, Pseudomyermex gracilis, Pseudomymex* sp13, *Pseudomyrmex flavidulus, Pseudomyrmex* sp10, *Pseudomyrmex* sp11, *Pseudomyrmex* sp12, *Pseudomyrmex* sp3, *Pseudomyrmex* sp6, *Pseudomyrmex* sp7, *Solenopsis* sp1, *Solenopsis* sp5, *Solenopsis* sp6, *Solenopsis* sp7, *Wasmania* sp1

Table S2. Generalized mixed effect models with binomial error constructed for evaluating the proportion of plants with ants in Cerrado vegetation (**n.interaction**) in response to *environmental heterogeneity*, *plant community*, *resources*, and *ant community*. Locality was included as random effect. Explanatory variables were scaled prior to modelling. Full model and the averaged best models (after automated model selection) are shown. Models with ΔAICc < 2 were considered the most plausible among candidates (Zuur et al. 2009). For each model, it is shown: Akaike Information Criteria corrected for small samples (AICc); degrees of freedom (df); explanatory variables included in the model (Parameters; see Table 1); Estimate, standard error (SE), Z-value, and P-value (* when significant) for each parameter; marginal (R^2m) and conditional (R^2c) r-squared (for averaged models, the range of R^2m and R^2c is reported); and significance value of Moran I spatial autocorrelation test (Moran's I; $p < 0.05$ indicates significant spatial autocorrelation in the model). [1] Explanatory variable removed from models due to high or moderate collinearity (see Table S2). [2] full model was the unique best model, so averaged model is not shown. Plots of models diagnostics is shown in Figure S2.

Major Parameters	**Model**	**AICc**	**df**	**Variables**	**Estimate**	**SE**	**Z-value**	**P-value**	**R^2m**	**R^2c**	**Moran's I**
Environmental heterogeneity	full	316.6	8	Intercept	-0.186	0.037	-4.993	0.000*	0.875	0.875	0.379
				T	-0.017	0.050	-0.345	0.730			
				P	-0.089	0.063	-1.430	0.153			
				NPP	0.185	0.050	3.698	0.000*			
				M	-0.365	0.050	-7.293	0.000*			
				DAP	0.201	0.054	3.734	0.000*			
				D.large	-0.066	0.042	-1.544	0.123			
				D.small[1]							
	average	-	-	Intercept	-0.198	0.083	2.296	0.022*	0.7-0.867	0.872-0.9	-
				DAP	0.139	0.048	2.778	0.005*			
				M	-0.460	0.081	5.449	0.000*			
				T	-0.113	0.123	0.902	0.367			
				NPP	0.050	0.094	0.530	0.596			
Plant community	full[2]	292.5	7	Intercept	-0.211	0.222	-0.948	0.343	0.214	0.928	0.168

				S.plants	-0.186	0.059	-3.133	0.002*			
				C.plants	0.118	0.050	2.347	0.019*			
				PD.plants	-0.104	0.044	-2.328	0.020*			
Resources	full	286.0	5	Intercept	-0.228	0.138	-1.654	0.098	0.526	0.899	0.991
				n.efn	0.232	0.048	4.805	0.000*			
				n.tropho	0.292	0.054	5.383	0.000*			
				n.nests	-0.091	0.049	-1.875	0.061			
	average	-	-	Intercept	-0.226	0.139	1.565	0.118	0.507-0.526	0.897-0.899	-
				n.efn	0.229	0.048	4.555	0.000*			
				n.nests	-0.054	0.058	0.912	0.362			
				n.tropho	0.276	0.056	4.750	0.000*			
Ant community	full	232.0	6	Intercept	-0.214	0.099	-2.160	0.031	0.748	0.909	0.994
				S.ants	-0.006	0.053	-0.111	0.911			
				C.ants	0.239	0.067	3.558	0.000*			
				PD.ants	0.073	0.050	1.458	0.145			
				n.ants	0.334	0.067	4.979	0.000*			
	average	-	-	Intercept	-0.215	0.097	2.125	0.034	0.749-0.758	0.908-0.909	-
				C.ants	0.241	0.060	3.867	0.000*			
				n.ants	0.329	0.066	4.786	0.000*			
				PD.ants	0.033	0.047	0.677	0.498			

T: Annual mean temperature; **P**: Annual precipitation; **NPP**: Mean net primary productivity; **M**: Soil organic matter; **DAP**: Mean diameter at soil height; **D.small**: Density of small plants; **D.large**: Density of large plants; **S.plants**: Rarefied plant species richness; **C.plants**: Plants co-occurrence index; **PD.plants**: Plants phylogenetic diversity; **n.efn**: Proportion of the total number of plants bearing extrafloral nectaries; **n.tropho**: Proportion of the total number of plants with trophobionts; **n.nests**: Proportion of the total number of plants with ant nests; **S.ants**: Rarefied ant species richness; **C.ants**: Ants co-occurrence index; **PD.ants**: Ants phylogenetic diversity; **n.ants**: Mean number of ants per plant; **n.interaction**: Proportion of the total number of plants with ants.

Table S3. Variance inflation factor (VIF) and 95% confidence interval (CI) to check for collinearity among explanatory variables in generalized mixed effect models, with binomial error constructed for evaluating the proportion of plants with ants in Cerrado vegetation in response to environmental heterogeneity, plant community, resources, and ant community. Locality was included as random effect. Exclusion of variables follow two methods: (i) variables pointed as moderate or high collinear according to James et al. 2013 (indicated by *check_collinearity* function implemented in *performance* R package) and (ii) VIF > 3 (Zuur et al. 2009). See models in Table 2.

Model	**Predictor**	**VIF**	**CI**
Environmental heterogeneity	Low correlation		
	T	1.73	[1.33, 2.62]
	P	3.79	[2.60, 5.88]
	NPP	1.82	[1.38, 2.75]
	M	2.98	[2.09, 4.58]
	D.large	2.64	[1.88, 4.04]
	DAP	5.04	[3.37, 7.88]
	Moderate correlation		
	D.small	4.85	[3.26, 7.58]
Environmental heterogeneity (after **D.small** removal)	Low correlation		
	T	1.7	[1.30, 2.63]
	P	2.86	[2.00, 4.48]
	NPP	1.81	[1.37, 2.80]
	M	1.75	[1.33, 2.70]
	DAP	2.04	[1.50, 3.16]
	D.large	1.43	[1.15, 2.24]
Plant community	Low correlation		
	S.plants	1.16	[1.02, 2.52]
	C.plants	1.16	[1.02, 2.58]
	PD.plants	1.01	[1.00, 5.01e+07]
Resources	Low correlation		
	n.efn	1.04	[1.00, 36.37]
	n.tropho	1.22	[1.04, 2.33]
	n.nests	1.2	[1.03, 2.38]
Ant community	Low correlation		
	S.ants	1.54	[1.19, 2.50]
	C.ants	2.16	[1.55, 3.49]
	PD.ants	1.21	[1.04, 2.26]
	n.ants	1.77	[1.32, 2.85]

T: Annual mean temperature; **P**: Annual precipitation; **NPP**: Mean net primary productivity; **M**: Soil organic matter; **DAP**: Mean diameter at soil height; **D.small**: Density of small plants; **D.large**: Density of large plants; **S.plants**: Rarefied plant species richness; **C.plants**: Plants co-occurrence index; **PD.plants**: Plants phylogenetic diversity; **n.efn**: Proportion of the total number of plants bearing extrafloral nectaries; **n.tropho**: Proportion of the total number of plants with trophobionts; **n.nests**: Proportion of the total number of plants with ant nests; **S.ants**: Rarefied ant species richness; **C.ants**: Ants co-occurrence index; **PD.ants**: Ants phylogenetic diversity; **n.ants**: Mean number of ants per plant; **n.interaction**: Proportion of the total number of plants with ants.

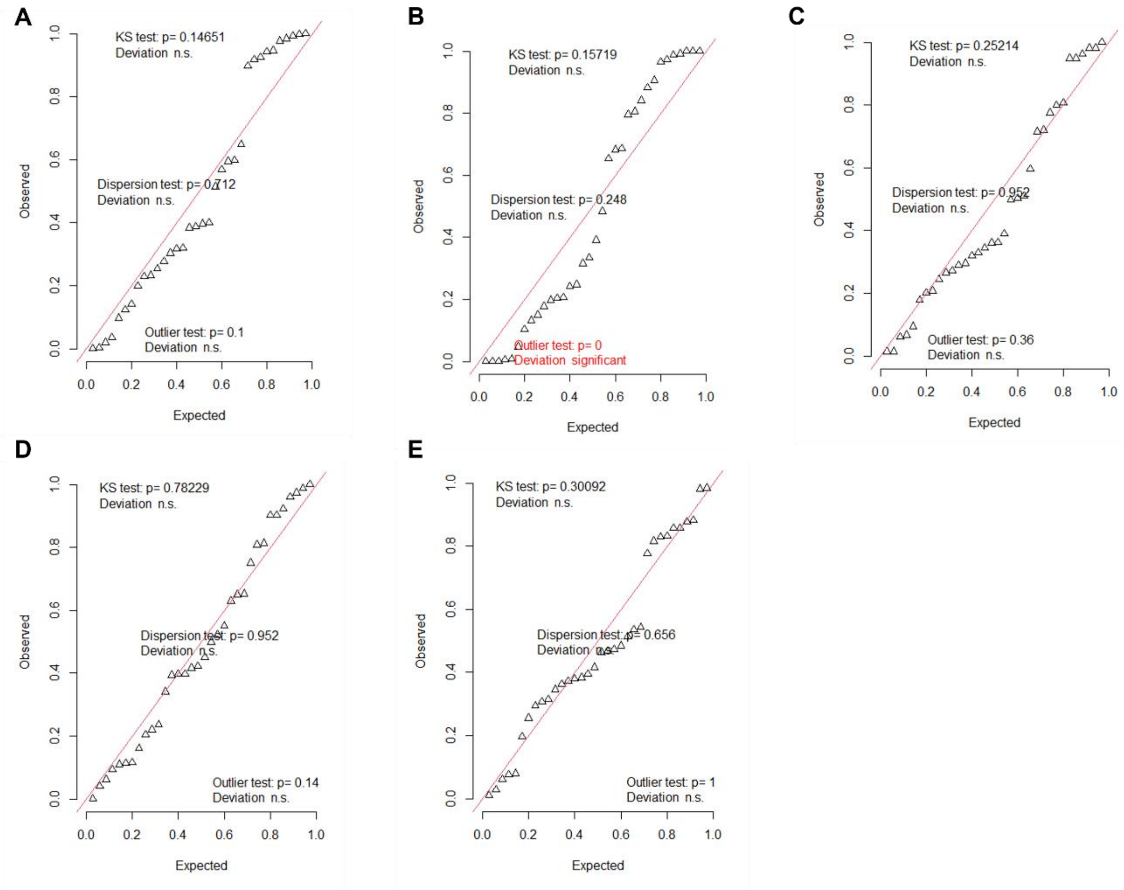


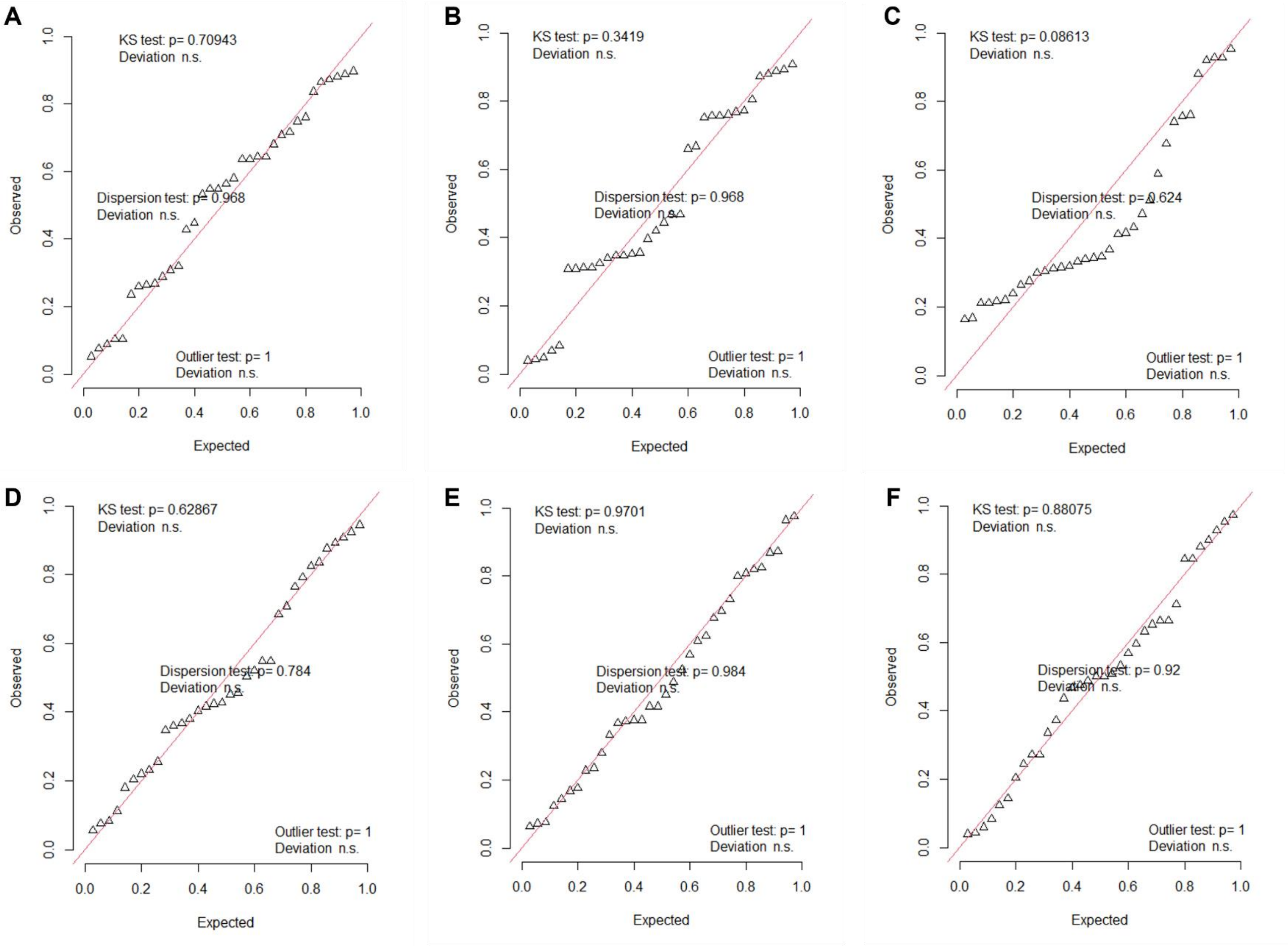
Figure S1. Diagnostic plots for the generalized mixed effect models with binomial error constructed for evaluating the proportion of plants with ants on Cerrado vegetation in response to (A) *latitude*, (B) *environmental heterogeneity*, (C) *plant community*, (D) *resources*, and (E) *ant community*. Local was included as random effect (Table S1). In each plot is shown a qq-plot to detect overall deviations from the expected distribution, including tests for correct distribution (KS test), dispersion and outliers. n.s.= non-significant.

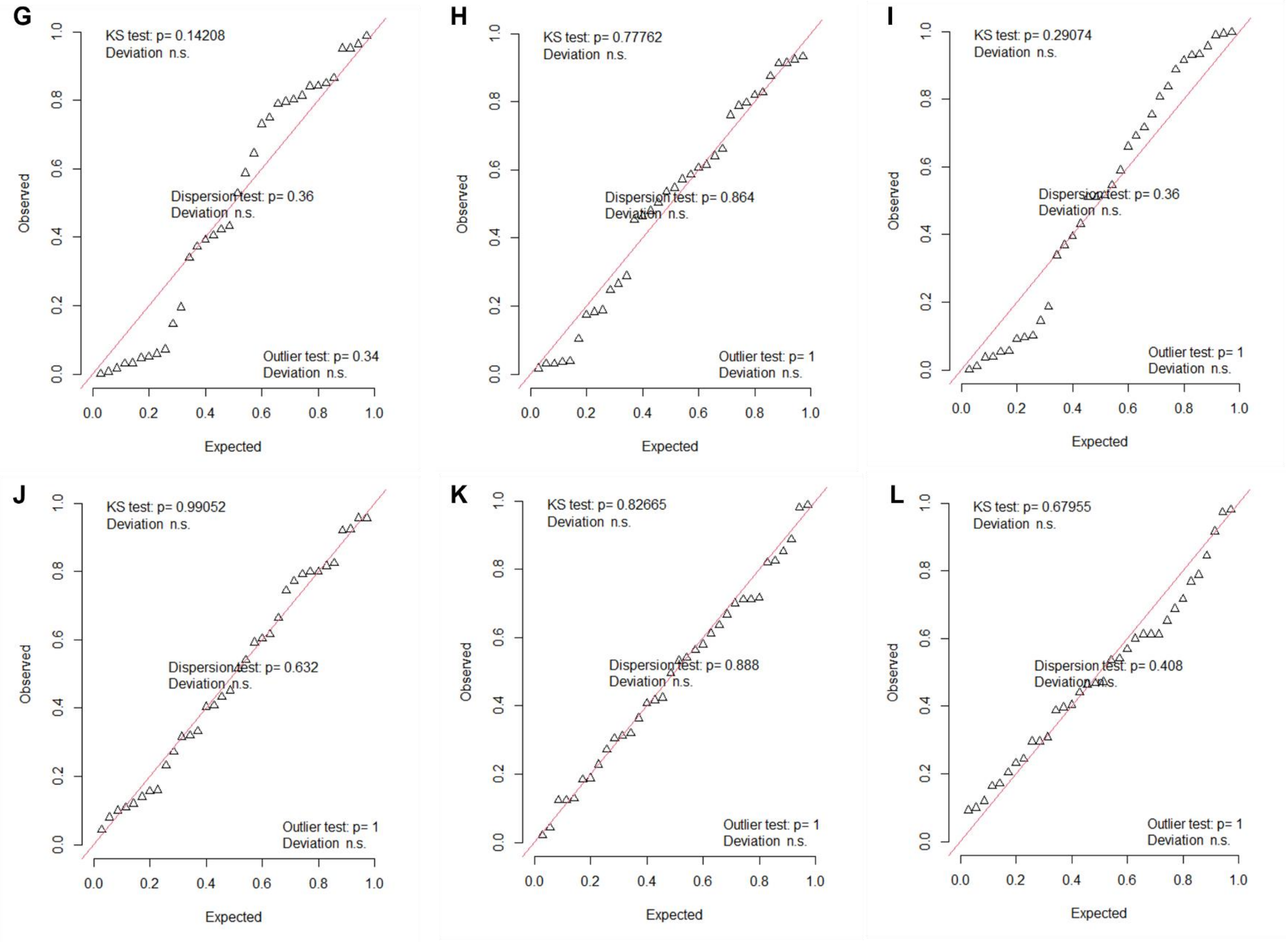
Table S4. Variance inflation factor (VIF) and 95% confidence interval (CI) to check for collinearity among explanatory variables in internal models initialed included in the full structural equation model. Exclusion of variables follow two methods: (i) variables pointed as moderate or high collinear according to James et al. 2013 (indicated by *check_collinearity* function implemented in *performance* R package) and (ii) VIF > 3 (Zuur et al. 2009). See models in Table 2.

Response variable	**Predictor**	**VIF**	**CI**
NPP	Low correlation		
	Lat	2.86	[2.02, 4.39]
	T	2.05	[1.52, 3.11]
	P	2.23	[1.63, 3.39]
	M	1.37	[1.12, 2.13]
	D.large	3.48	[2.40, 5.38]
	DAP	5.09	[3.40, 7.96]
	Moderate correlation		
	D.small	2.87	[2.03, 4.41]
NPP	Low correlation		
(after **D.small**	**Lat**	2.9	[2.02, 4.54]
removal)	**T**	2.03	[1.50, 3.15]
	P	2.12	[1.55, 3.29]
	M	1.36	[1.11, 2.16]
	DAP	2.53	[1.79, 3.94]
	D.large	2.07	[1.52, 3.20]
M	Low correlation		
	T	1.14	[1.01, 2.58]
	P	1.2	[1.03, 2.27]
	D.small	1.14	[1.01, 2.57]
	D.large	1.1	[1.00, 3.48]
C.plants	Low correlation		
	S.plants	1.07	[1.00, 8.46]
	PD.plants	1.07	[1.00, 8.46]
n.efn	Low correlation		
	S.plants	1.51	[1.18, 2.40]
	C.plants	1.27	[1.06, 2.16]
	PD.plants	1.08	[1.00, 4.15]
	D.small	1.47	[1.16, 2.35]
	D.large	1.18	[1.03, 2.25]
n.tropho	Low correlation		
	S.plants	1.56	[1.21, 2.48]
	C.plants	1.32	[1.09, 2.19]
	PD.plants	1.16	[1.02, 2.33]
	D.small	1.68	[1.28, 2.66]
	D.large	1.15	[1.02, 2.36]
n.nests	Low correlation		
	S.plants	2.18	[1.60, 3.31]
	C.plants	1.75	[1.34, 2.66]
	PD.plants	1.05	[1.00, 10.20]

	D.small	2.52	[1.81, 3.85]
	D.large	1.34	[1.11, 2.10]
	n.efn	1.22	[1.05, 2.04]
	n.tropho	1.43	[1.16, 2.20]
S.ants	Low correlation		
	P	1.31	[1.07, 2.30]
	NPP	1.45	[1.14, 2.44]
	S.plants	1.14	[1.01, 2.70]
C.ants	Low correlation		
	S.ants	1.07	[1.00, 9.42]
	PD.ants	1.07	[1.00, 9.42]
n.ants	Low correlation		
	P	1.39	[1.13, 2.15]
	NPP	1.49	[1.19, 2.28]
	T	1.54	[1.22, 2.35]
	n.efn	1.37	[1.12, 2.13]
	n.tropho	1.5	[1.19, 2.29]
	n.nests	1.45	[1.16, 2.22]
	C.ants	1.5	[1.20, 2.29]
n.interaction	Low correlation		
	Lat	1.28	[1.08, 1.99]
	S.ants	1.86	[1.42, 2.77]
	C.ants	2.6	[1.88, 3.90]
	PD.ants	1.48	[1.19, 2.21]
	n.efn	1.47	[1.18, 2.20]
	n.tropho	2.47	[1.80, 3.70]
	n.nests	1.3	[1.09, 2.01]
	n.ants	3.2	[2.26, 4.84]
n.interaction	Low correlation		
(after **n.ants** removal)	**Lat**	1.05	[1.00, 10.58]
	S.ants	1.78	[1.36, 2.70]
	C.ants	2.15	[1.58, 3.28]
	PD.ants	1.34	[1.11, 2.10]
	n.efn	1.42	[1.15, 2.18]
	n.tropho	1.64	[1.27, 2.48]
	n.nests	1.27	[1.07, 2.05]

T: Annual mean temperature; **P**: Annual precipitation; **NPP**: Mean net primary productivity; **M**: Soil organic matter; **DAP**: Mean diameter at soil height; **D.small**: Density of small plants; **D.large**: Density of large plants; **S.plants**: Rarefied plant species richness; **C.plants**: Plants co-occurrence index; **PD.plants**: Plants phylogenetic diversity; **n.efn**: Proportion of the total number of plants bearing extrafloral nectaries; **n.tropho**: Proportion of the total number of plants with trophobionts; **n.nests**: Proportion of the total number of plants with ant nests; **S.ants**: Rarefied ant species richness; **C.ants**: Ants co-occurrence index; **PD.ants**: Ants phylogenetic diversity; **n.ants**: Mean number of ants per plant; **n.interaction**: Proportion of the total number of plants with ants.


A
KS test: p= 0.70943
Deviation n.s.
Dispersion test: p= 0.968
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
B
KS test: p= 0.3419
Deviation n.s.
Dispersion test: p= 0.968
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
C
KS test: p= 0.08613
Deviation n.s.
Dispersion test: p= 0.624
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
D
KS test: p= 0.62867
Deviation n.s.
Dispersion test: p= 0.784
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
E
KS test: p= 0.9701
Deviation n.s.
Dispersion test: p= 0.984
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
F
KS test: p= 0.88075
Deviation n.s.
Dispersion test: p= 0.92
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
Observed
Expected
0.0
0.2
0.4
0.6
0.8
1.0


G
KS test: p= 0.14208
Deviation n.s.
Dispersion test: p= 0.36
Deviation n.s.
Outlier test: p= 0.34
Deviation n.s.
Observed
Expected
H
KS test: p= 0.77762
Deviation n.s.
Dispersion test: p= 0.864
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
Observed
Expected
I
KS test: p= 0.29074
Deviation n.s.
Dispersion test: p= 0.36
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
Observed
Expected
J
KS test: p= 0.99052
Deviation n.s.
Dispersion test: p= 0.632
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
Observed
Expected
K
KS test: p= 0.82665
Deviation n.s.
Dispersion test: p= 0.888
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
Observed
Expected
L
KS test: p= 0.67955
Deviation n.s.
Dispersion test: p= 0.408
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
Observed
Expected
0.0
0.2
0.4
0.6
0.8
1.0

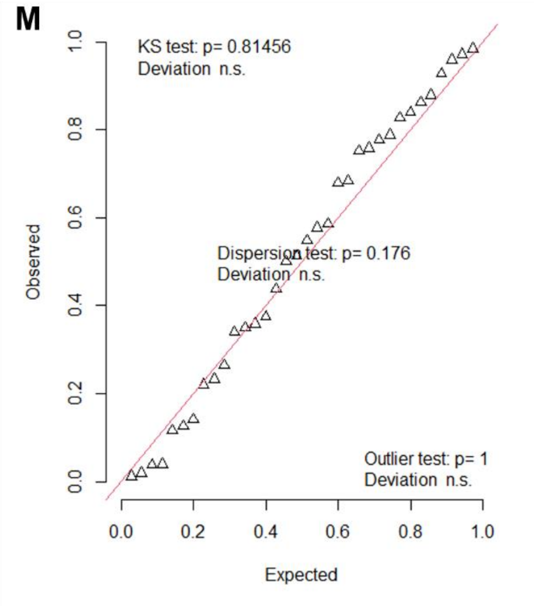


Figure S2. Diagnostic plots for the internal models included in the full structural equation model, whose response variables are (A) **T**; (B) **P**; (C) **NPP**; (D) **M**; (E) **S.plants**; (F) **C.plantas**; (G) **n.efn**; (H) **n.tropho**; (I) **n.nests**; (J) **S.ants**; (K) **C.ants**; (L) **n.ants**; (M) **n.interaction**. Locality was included as random effect. In each plot is shown a qq-plot to detect overall deviations from the expected distribution, including tests for correct distribution (KS test), dispersion and outliers. n.s.= non-significant. See models in Table 2.

Table S5. Significant missing paths obtained through d-separation tests to full structural equation model. Variables were added as covariate or model term depending on justificative.

Response variable	Explanatory variable	P-value	Action	Justificative
T	**DAP**	0.0013	added as covariate	Vegetation height and density may alter climate variables, such as air temperature (Song et al. 2013). On the other hand, precipitation and temperature regulate species diversity and biomass (Yao et al. 2022). Due to this mutual influence, we included these variables as covariates in the final SEM model
T	**D.small**	0.0333	added as covariate	
P	**DAP**	0.0198	added as covariate	
P	**D.small**	0.0371	added as covariate	
P	**T**	0	added as covariate	
S.plants	**D.small**	0.0396	added	Given that small plants are the most abundant in our study, it is expected that the number of small plants increases with plant species richness. Thus, we included this variable in the respective model.
n.efn	**DAP**	0	added	There is evidence that climate variables may affect the amount of plant species bearing extrafloral nectaries in Cerrado communities (e.g. Boudouris and Queenborough 2013). Then, we hypothesize that other environmental variables should also affect the number of plants with EFNs. We included these variables in the respective model.
n.efn	**M**	0	added	
n.tropho	**NPP**	0.0001	added	Insect abundance in Cerrado (including honeydew-producing hemipterans) are affected by climate variables (da Silva et al. 2011). Then, we hypothesize that other environmental variables should also affect the number of plants with trophobionts in the Cerrado community. We included these variables in the respective models.
n.tropho	**M**	0.0104	added	
n.tropho	**n.efn**	0.0432	added as covariate	Plants invest in different direct and indirect resources to attract ants (Beattie and Hughes 2002). We then hypothesize that these sugary liquid resources should covary, but not necessarily directly affect one another. We then included this relationship as a covariate.

n.nests	**T**	0.0017	added	Given that ants use plant branches as nest sites, and plant biomass is affected by environmental variables, we decided to include these variables in the respective model.
n.nests	**NPP**	0.0095	added	
n.nests	**M**	0.0286	added	
C.ants	**n.efn**	0.0011	added	Co-occurrence of ants on plants should be influenced by the number of plants with liquid resources in communities (Oliveira and Freitas 2004). We included these variables in the respective model.
C.ants	**n.tropho**	0.001	added	
n.ants	**D.small**	0.0154	added	Ants are dominant organisms on Cerrado foliage (Oliveira and Freitas 2004; Schoereder et al. 2010). We then hypothesize that the number of plants should affect the number of ants on vegetation. We included this variable in the respective model.
n.interaction	**DAP**	0.0169	added	Our exploratory analyses revealed that the proportion of plants interacting with ants in Cerrado is affected by environmental heterogeneity, resources and ant community factors (see Table 3). We thus included these variables in the respective model.
n.interaction	**D.small**	0.0029	added	
n.interaction	**NPP**	0	added	
n.interaction	**M**	0.0115	added	
n.interaction	**n.ants**	0	added	

T: Annual mean temperature; **P**: Annual precipitation; **NPP**: Mean net primary productivity; **M**: Soil organic matter; **DAP**: Mean diameter at soil height; **D.small**: Density of small plants; **D.large**: Density of large plants; **S.plants**: Rarefied plant species richness; **C.plants**: Plants co-occurrence index; **PD.plants**: Plants phylogenetic diversity; **n.efn**: Proportion of the total number of plants bearing extrafloral nectaries; **n.tropho**: Proportion of the total number of plants with trophobionts; **n.nests**: Proportion of the total number of plants with ant nests; **S.ants**: Rarefied ant species richness; **C.ants**: Ants co-occurrence index; **PD.ants**: Ants phylogenetic diversity; **n.ants**: Mean number of ants per plant; **n.interaction**: Proportion of the total number of plants with ants.

Table S6. Model fitting procedure undertaken in structural equation modelling. Significant and ecologically justifiable missing paths were included in the full model based on d-separation tests (step 2; see Table S3). Non-significant pathways were removed iteratively (step 3 to 44). Model adequacy was assessed with Fisher's C test. For each step of modelling, it is reported: Akaike Information Criteria corrected for small samples (AICc), degrees of freedom for information criteria (K), Fisher's C statistic (Fisher's C), with respective degrees of freedom (df) and P-value (P). Fisher's C statistic with P > 0.05 indicates good adequacy of the model. In all models, sampling site were included as random effect.

Step	**Full model variables/ changings in full model**	**AICc**	**K**	**Fisher's C**	**df**	**P**
1 (full model)	**T ~ Lat** **P ~ Lat** **NPP ~ Lat+T+P+M+DAP+D.large** **M ~ T+P+D.small+D.large** **S.plants ~ NPP** **C.plants ~ S.plants+PD.plants** **n.efn~S.plants+C.plants+PD.plants+D.small+D.large** **n.tropho~S.plants+C.plants+PD.plants+D.small+D.large** **n.nests~S.plants+C.plants+PD.plants+D.small+D.large+n.efn+n.tropho** **S.ants ~ P+NPP+S.plants** **C.ants ~ S.ants+PD.ants** **n.ants ~ P+NPP+T+n.efn+n.tropho+n.nests+C.ants** **n.interaction~Lat+S.ants+C.ants+PD.ants+n.efn+n.tropho+n.nests**	2670.56	86	443.067	210	0
2 (full model after inclusion of variables based on d-	**T ~ Lat** **P ~ Lat** **NPP ~ Lat+T+P+M+DAP+D.large** **M ~ T+P+D.small+D.large** **S.plants ~ NPP+D.small** **C.plants ~ S.plants+PD.plants** **n.efn~S.plants+C.plants+PD.plants+D.small+D.large+M+DAP** **n.tropho~S.plants+C.plants+PD.plants+D.small+D.large+NPP+M** **n.nests~S.plants+C.plants+PD.plants+D.small+D.large+n.efn+n.tropho+T+NPP+M** **S.ants ~ P+NPP+S.plants**	2607.436	102	194.224	166	0.066

sepatarion tests)	**C.ants ~ S.ants+PD.ants+n.efn+n.tropho** **n.ants ~ P+NPP+T+n.efn+n.tropho+n.nests+C.ants+D.small** **n.interaction~Lat+S.ants+C.ants+PD.ants+n.efn+n.tropho+n.nests+DAP+D.small+NPP+M+n.ants** **P%~~%T** **T%~~%DAP** **T%~~%D.small** **P%~~%DAP** **P%~~%D.small** **n.tropho**%~~%**n.efn**					
3	Covariation ‘**T**%~~%**D.small**’ removed	2607.436	102	201.027	168	0.042
4	Covariation ‘**P**%~~%**D.small**’ removed	2607.436	102	207.615	170	0.026
5	Covariation ‘**n.tropho**%~~%**n.efn**’ removed	2607.436	102	210.973	172	0.023
6	Model ‘**T ~ Lat**’ removed	2583.723	98	195.604	164	0.046
7	Explanatory variable ‘**Lat**’ removed from ‘**NPP**’ model	2597.242	97	199.328	166	0.04
8	Explanatory variable ‘**T**’ removed from ‘**NPP**’ model	2608.823	96	206.056	168	0.024
9	Explanatory variable ‘**P**’ removed from ‘**NPP**’ model	2611.322	95	213.047	170	0.014
10	Explanatory variable ‘**M**’ removed from ‘**NPP**’ model	2622.711	94	213.6	172	0.017
11	Explanatory variable ‘**T**’ removed from ‘**M**’ model	2618.397	93	213.874	174	0.021
12	Explanatory variable ‘**P**’ removed from ‘**M**’ model	2609.338	92	220.926	176	0.012
13	Explanatory variable ‘**D.large**’ removed from ‘**M**’ model	2608.887	91	219.651	178	0.018

14	Explanatory variable ‘**NPP**’ removed from ‘**S.plants**’ model	2593.909	90	217.917	180	0.028
15	Explanatory variable ‘**PD.plants**’ removed from ‘**C.plants**’ model	2588.417	89	219.944	182	0.029
16	Explanatory variable ‘**D.small**’ removed from ‘**n.efn**’ model	2587.915	88	229.972	184	0.012
17	Explanatory variable ‘**C.plants**’ removed from ‘**n.tropho**’ model	2584.598	87	231.88	186	0.013
18	Explanatory variable ‘**PD.plants**’ removed from ‘**n.tropho**’ model	2581.431	86	234.597	188	0.012
19	Explanatory variable ‘**D.small**’ removed from ‘**n.tropho**’ model	2579.01	85	234.155	190	0.016
20	Explanatory variable ‘**D.large**’ removed from ‘**n.tropho**’ model	2576.05	84	226.62	192	0.044
21	Explanatory variable ‘**NPP**’ removed from ‘**n.tropho**’ model	2573.293	83	219.028	194	0.105
22	Explanatory variable ‘**PD.plants**’ removed from ‘**n.nests**’ model	2570.467	82	224.567	196	0.079
23	Explanatory variable ‘**D.small**’ removed from ‘**n.nests**’ model	2566.035	81	223.564	198	0.103
24	Explanatory variable ‘**D.large**’ removed from ‘**n.nests**’ model	2562.587	80	223.721	200	0.12
25	Explanatory variable ‘**n.efn**’ removed from ‘**n.nests**’ model	2561.147	79	229.347	202	0.091
26	Explanatory variable ‘**S.plants**’ removed from ‘**n.nests**’ model	2560.063	78	233.846	204	0.074
27	Explanatory variable ‘**M**’ removed from ‘**n.nests**’ model	2558.059	77	233.968	206	0.088
28	Explanatory variable ‘**P**’ removed from ‘**S.ants**’ model	2545.419	76	235.004	208	0.096
29	Explanatory variable ‘**NPP**’ removed from ‘**S.ants**’ model	2526.868	75	235.147	210	0.112

30	Explanatory variable ‘**PD.ants**’ removed from ‘**C.ants**’ model	2526.962	74	234.231	212	0.141
31	Explanatory variable ‘**NPP**’ removed from ‘**n.ants**’ model	2505.72	73	235.148	214	0.153
32	Explanatory variable ‘**T**’ removed from ‘**n.ants**’ model	2498.277	72	234.117	216	0.189
33	Explanatory variable ‘**n.efn**’ removed from ‘**n.ants**’ model	2496.213	71	235.771	218	0.195
34	Explanatory variable ‘**n.nests**’ removed from ‘**n.ants**’ model	2495.409	70	235.799	220	0.221
35	Explanatory variable ‘**Lat**’ removed from ‘**n.interaction**’ model	2490.052	69	238.979	222	0.207
36	Explanatory variable ‘**S.ants**’ removed from ‘**n.interaction**’ model	2485.772	68	244.011	224	0.171
37	Explanatory variable ‘**PD.ants**’ removed from ‘**n.interaction**’ model	2484.452	67	222.552	202	0.153
38	Explanatory variable ‘**n.efn**’ removed from ‘**n.interaction**’ model	2481.44	66	229.223	204	0.109
39	Explanatory variable ‘**n.tropho**’ removed from ‘**n.interaction**’ model	2477.53	65	233.827	206	0.089
40	Explanatory variable ‘**n.nests**’ removed from ‘**n.interaction**’ model	2477.08	64	241.483	208	0.056
41	Explanatory variable ‘**DAP**’ removed from ‘**n.interaction**’ model	2473.717	63	227.31	210	0.196
42	Explanatory variable ‘**D.small**’ removed from ‘**n.interaction**’ model	2473.901	62	225.395	212	0.252
43	Covariation ‘**P%~~%T**’ removed	2473.901	62	248.382	214	0.053
44	‘**n.nests**’ model removed	2247.375	56	181.23	166	0.198
45	Covariation ‘**T%~~%DAP**’ removed **P ~ Lat**	2247.375	56	181.23	166	0.198

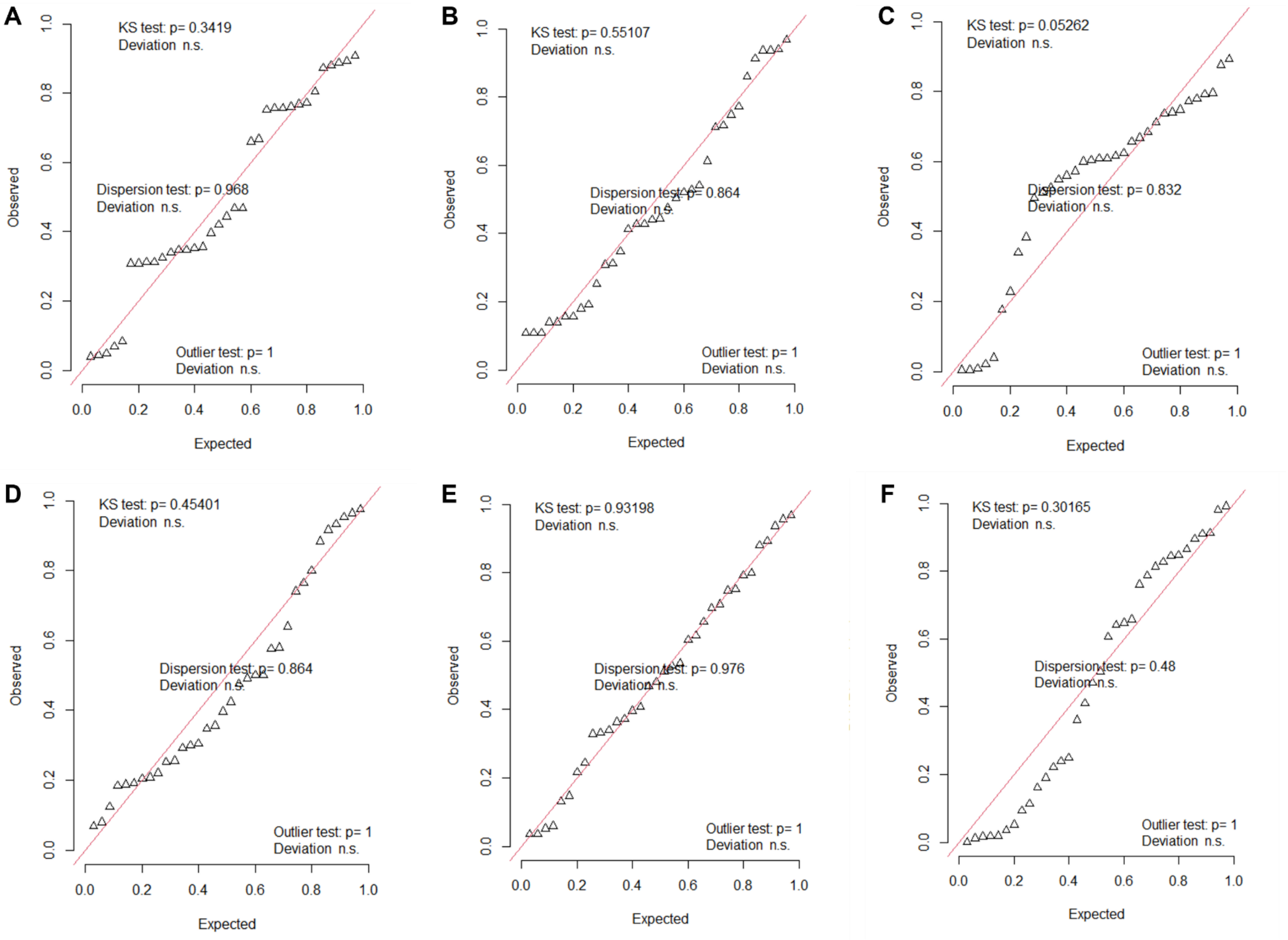
(final model)	**NPP ~ DAP+D.large** **M ~ D.small** **S.plants ~ D.small** **C.plants ~ S.plants** **n.efn~S.plants+C.plants+PD.plants+D.large+M+DAP** **n.tropho~S.plants+M** **S.ants ~ S.plants** **C.ants ~ S.ants+n.efn+n.tropho** **n.ants ~ P+n.tropho+C.ants+D.small** **n.interaction~C.ants+NPP+M+n.ants** **P%~~%DAP**

T: Annual mean temperature; **P**: Annual precipitation; **NPP**: Mean net primary productivity; **M**: Soil organic matter; **DAP**: Mean diameter at soil height; **D.small**: Density of small plants; **D.large**: Density of large plants; **S.plants**: Rarefied plant species richness; **C.plants**: Plants co-occurrence index; **PD.plants**: Plants phylogenetic diversity; **n.efn**: Proportion of the total number of plants bearing extrafloral nectaries; **n.tropho**: Proportion of the total number of plants with trophobionts; **n.nests**: Proportion of the total number of plants with ant nests; **S.ants**: Rarefied ant species richness; **C.ants**: Ants co-occurrence index; **PD.ants**: Ants phylogenetic diversity; **n.ants**: Mean number of ants per plant; **n.interaction**: Proportion of the total number of plants with ants.

Table S7. Variance inflation factor (VIF) and 95% confidence interval (CI) to check for collinearity among explanatory variables in internal models included in the final structural equation model. See original models and final models in Table 2 and Table S4, respectively.

Response variable	Predictors	VIF	CI
NPP	Low Correlation		
	DAP	1.71	[1.27, 2.87]
	D.large	1.71	[1.27, 2.87]
n.efn	Low Correlation		
	S.plants	1.32	[1.09, 2.13]
	C.plants	1.27	[1.06, 2.10]
	PD.plants	1.11	[1.01, 2.65]
	D.large	2.28	[1.64, 3.53]
	M	1.16	[1.02, 2.23]
	DAP	2.12	[1.55, 3.29]
n.tropho	Low Correlation		
	S.plants	1	[1.00, Inf]
	M	1	[1.00, Inf]
C.ants	Low Correlation		
	S.ants	1.04	[1.00, 59.69]
	n.efn	1.25	[1.05, 2.30]
	n.tropho	1.26	[1.05, 2.30]
n.ants	Low Correlation		
	P	1.21	[1.04, 2.26]
	n.tropho	1.28	[1.06, 2.23]
	C.ants	1.31	[1.08, 2.24]
	D.small	1.16	[1.02, 2.41]
n.interaction	Low Correlation		
	C.ants	1.81	[1.34, 2.91]
	NPP	1.06	[1.00, 8.29]
	M	1.56	[1.21, 2.53]
	n.ants	2.49	[1.73, 4.02]

P: Annual precipitation; **NPP**: Mean net primary productivity; **M**: Soil organic matter; **DAP**: Mean diameter at soil height; **D.small**: Density of small plants; **D.large**: Density of large plants; **S.plants**: Rarefied plant species richness; **C.plants**: Plants co-occurrence index; **PD.plants**: Plants phylogenetic diversity; **n.efn**: Proportion of the total number of plants bearing extrafloral nectaries; **n.tropho**: Proportion of the total number of plants with trophobionts; **S.ants**: Rarefied ant species richness; **C.ants**: Ants co-occurrence index; **n.ants**: Mean number of ants per plant; **n.interaction**: Proportion of the total number of plants with ants.


A
KS test: p= 0.3419
Deviation n.s.
Dispersion test: p= 0.968
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
Observed
Expected
B
KS test: p= 0.55107
Deviation n.s.
Dispersion test: p= 0.864
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
Observed
Expected
C
KS test: p= 0.05262
Deviation n.s.
Dispersion test: p= 0.832
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
Observed
Expected
D
KS test: p= 0.45401
Deviation n.s.
Dispersion test: p= 0.864
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
Observed
Expected
E
KS test: p= 0.93198
Deviation n.s.
Dispersion test: p= 0.976
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
Observed
Expected
F
KS test: p= 0.30165
Deviation n.s.
Dispersion test: p= 0.48
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
Observed
Expected
0.0
0.2
0.4
0.6
0.8
1.0

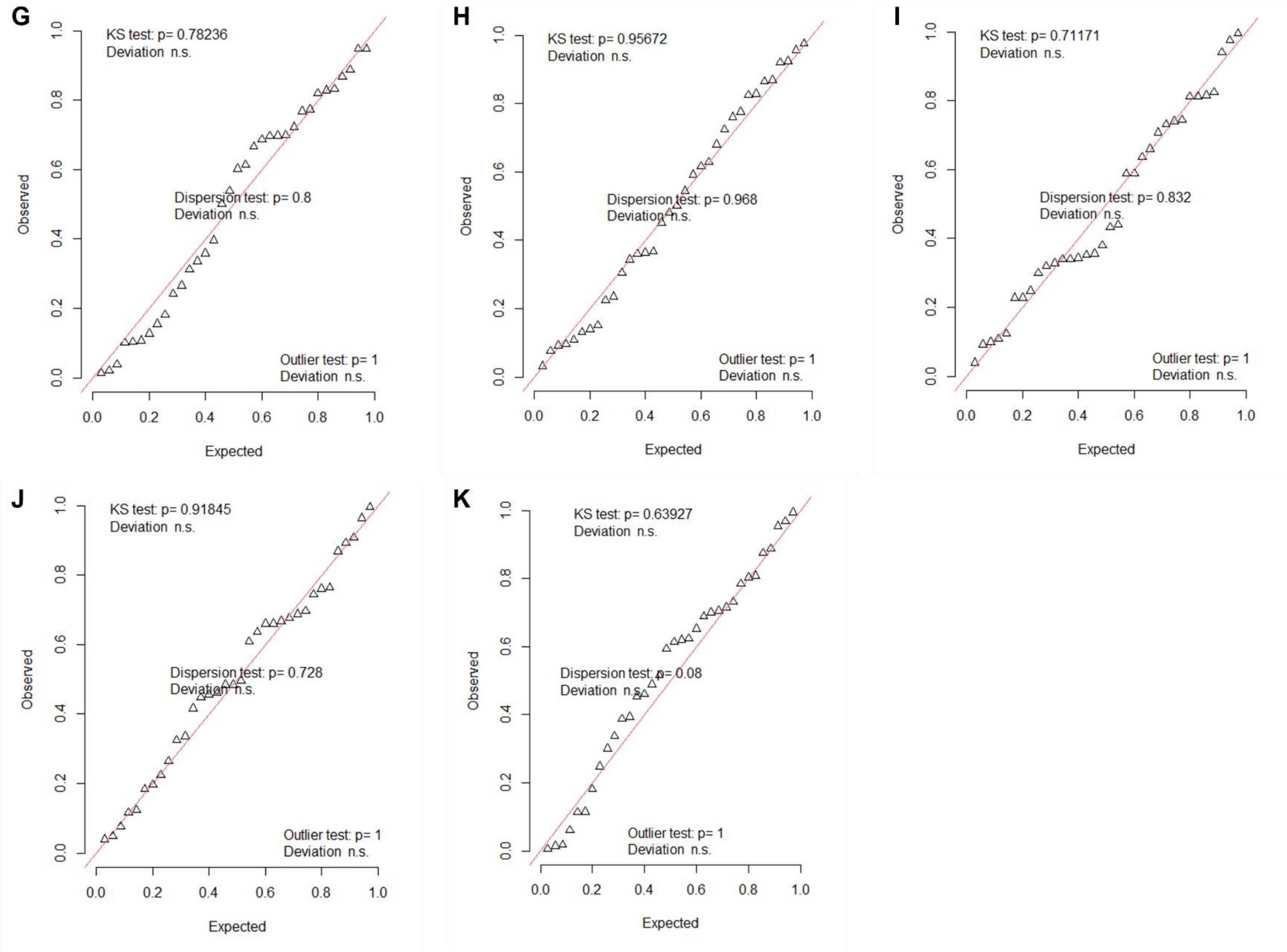

G
KS test: p= 0.78236
Deviation n.s.
Dispersion test: p= 0.8
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
Observed
Expected
0.0
0.2
0.4
0.6
0.8
1.0
H
KS test: p= 0.95672
Deviation n.s.
Dispersion test: p= 0.968
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
Observed
Expected
I
KS test: p= 0.71171
Deviation n.s.
Dispersion test: p= 0.832
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
Observed
Expected
J
KS test: p= 0.91845
Deviation n.s.
Dispersion test: p= 0.728
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
Observed
Expected
K
KS test: p= 0.63927
Deviation n.s.
Dispersion test: p= 0.08
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
Observed
Expected

Figure S3. Diagnostic plots for the internal models included in the final structural equation model, whose response variables are (A) **P**; (B) **NPP**; (C) **M**; (D) **S.plants**; (E) **C.plantas**; (F) **n.efn**; (G) **n.tropho**; (H) **S.ants**; (I) **C.ants**; (J) **n.ants**; (K) **n.interaction**. Locality was included as random effect. In each plot is shown a qq-plot to detect overall deviations from the expected distribution, including tests for correct distribution (KS test), dispersion and outliers. n.s.= non-significant. See initial and final models in Table 2 and Table S6, respectively.

Table S8. Detailed standardized effects of the paths retained in the best fitting structural equation model for the proportion of plants with ants in Cerrado savanna. Indirect effects are calculated by multiplying the partial path coefficients and the overall effect is calculated by summing all direct and indirect effects.

Major parameter	**Pathways**	**Direct effects**	**Indirect effects**	**Overall effects**
Latitude	**Lat → P → n.ants → n.interaction**	-	-0.040	-0.040
Environmental heterogeneity	**NPP → n.interaction**	0.06	-	-0.132
	M → n.interaction	-0.11	-	
	P → n.ants → n.interaction	-	-0.053	
	D.large → NPP → n.interaction	-	0.017	
	D.large → n.efn → C.ants → n.ants → n.interaction	-	-0.002	
	D.large → n.efn → C.ants → n.interaction	-	-0.004	
	D.small → M → n.interaction	-	-0.017	
	D.small → M → n.efn → C.ants → n.ants → n.interaction	-	-0.001	
	D.small → M → n.efn → C.ants → n.interaction	-	-0.002	
	D.small → M → n.tropho → C.ants → n.ants → n.interaction	-	-0.001	
	D.small → M → n.tropho → C.ants → n.interaction	-	-0.002	
	D.small → M → n.tropho → n.ants → n.interaction	-	-0.002	
	D.small → S.plants → n.efn → C.ants → n.ants → n.interaction	-	0.002	
	D.small → S.plants → n.efn → C.ants → n.interaction	-	0.003	
	D.small → S.plants → C.plants → n.efn → C.ants → n.ants → n.interaction	-	0.000	
	D.small → S.plants → C.ants → n.efn → C.ants → n.interaction	-	-0.001	
	D.small → S.plants → n.tropho → C.ants → n.ants → n.interaction	-	-0.002	
	D.small → S.plants → n.tropho → C.ants → n.interaction	-	-0.003	
	D.small → S.plants → n.tropho → n.ants → n.interaction	-	-0.004	
	D.small → S.plants → S.ants → C.ants → n.ants → n.interaction	-	-0.006	

	D.small → S.plants → S.ants → C.ants → n.interaction	-	-0.012	
	D.small → n.ants → n.interaction	-	-0.036	
	DAP → NPP → n.interaction	-	-0.019	
	DAP → n.efn → C.ants → n.ants → n.interaction	-	0.004	
	DAP → n.efn → C.ants → n.interaction	-	0.008	
Plant community	**S.plants → n.efn → C.ants → n.ants → n.interaction**	-	0.004	-0.063
	S.plants → n.efn → C.ants → n.interaction	-	0.007	
	S.plants → C.plants → n.efn → C.ants → n.ants → n.interaction	-	-0.001	
	S.plants → C.ants → n.efn → C.ants → n.interaction	-	-0.002	
	S.plants → n.tropho → C.ants → n.ants → n.interaction	-	-0.004	
	S.plants → n.tropho → C.ants → n.interaction	-	-0.008	
	S.plants → n.tropho → n.ants → n.interaction	-	-0.010	
	S.plants → S.ants → C.ants → n.ants → n.interaction	-	-0.014	
	S.plants → S.ants → C.ants → n.interaction	-	-0.026	
	PD.plants → n.efn → C.ants → n.ants → n.interaction	-	-0.003	
	PD.plants → n.efn → C.ants → n.interaction	-	-0.006	
Resources	**n.efn → C.ants → n.ants → n.interaction**	-	0.028	0.198
	n.efn → C.ants → n.interaction	-	0.050	
	n.tropho → C.ants → n.ants → n.interaction	-	0.024	
	n.tropho → C.ants → n.interaction	-	0.043	
	n.tropho → n.ants → n.interaction	-	0.053	
Ant community	**n.ants → n.interaction**	0.140	-	0.178
	C.ants → n.interaction	0.120	-	
	S.ants → C.ants → n.ants → n.interaction	-	-0.029	
	S.ants → C.ants → n.interaction	-	-0.053	

P: Annual precipitation; **NPP**: Mean net primary productivity; **M**: Soil organic matter; **DAP**: Mean diameter at soil height; **D.small**: Density of small plants; **D.large**: Density of large plants; **S.plants**: Rarefied plant species richness; **C.plants**: Plants co-occurrence index; **PD.plants**: Plants phylogenetic diversity; **n.efn**: Proportion of the total number of plants bearing extrafloral nectaries; **n.tropho**: Proportion of the total number of plants with trophobionts; **S.ants**: Rarefied ant species richness; **C.ants**: Ants co-occurrence index; **n.ants**: Mean number of ants per plant; **n.interaction**: Proportion of the total number of plants with ants.

Table S9. Significance value of Moran I spatial autocorrelation test for the residuals of internal models of the final structural equation model (P < 0.05 indicates significant spatial autocorrelation in the model).

Response variable	**P**
P	0.180
NPP	0.205
M	0.124
S.plantas	0.642
C.plants	0.903
n.efn	0.352
n.tropho	0.266
S.ants	0.510
C.ants	0.830
n.ants	0.296
n.interaction	0.293

P: Annual precipitation; **NPP**: Mean net primary productivity; **M**: Soil organic matter; **DAP**: Mean diameter at soil height; **D.small**: Density of small plants; **D.large**: Density of large plants; **S.plants**: Rarefied plant species richness; **C.plants**: Plants co-occurrence index; **PD.plants**: Plants phylogenetic diversity; **n.efn**: Proportion of the total number of plants bearing extrafloral nectaries; **n.tropho**: Proportion of the total number of plants with trophobionts; **S.ants**: Rarefied ant species richness; **C.ants**: Ants co-occurrence index; **n.ants**: Mean number of ants per plant; **n.interaction**: Proportion of the total number of plants with ants.

CAPÍTULO 2

RESOURCE AVAILABILITY DRIVES FUNCTIONAL TRAIT VARIATION IN A DOMINANT ANT OF TROPICAL CERRADO SAVANNA

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ABSTRACT

Climate change has been highlighted as one of the major threats to biodiversity, affecting mostly ectotherms, such as ants. Thermoregulation and heat tolerance are crucial for ants and two morphological traits are related with these functions: pilosity and body size. Although highly ignored, describing the patterns and unveiling the drivers of intraspecific functional variation is fundamental in the current scenario of global climate change. Here, we use a dominant ant of the Brazilian Cerrado savanna – *Camponotus crassus* – to investigate trait variation along a latitudinal gradient of the Brazilian Cerrado savanna. We measured mesosoma pilosity and body size of *C. crassus* across multiple independent localities, and evaluated their relationship with physical *environmental heterogeneity*, *plant* and *ant communities*, and *resource availability*. We also assessed morphological and genetic covariation to differentiate phenotypic plasticity and adaptation in *C. crassus*. Our results suggest that all evaluated drivers play a role in shaping *C. crassus* pilosity, but not body size. *Resource availability* had the highest effect mediating *C. crassus* pilosity. When disentangling resource effect on trait variation, we found an inverse relationship between the number of plants with sugary liquid resources (extrafloral nectaries and hemipteran trophobionts) and ant pilosity. Variation in ant pilosity did not covariate with genetic dissimilarities, suggesting phenotypic plasticity. This study enhances the importance of looking for intraspecific variation and phenotypic plasticity, especially under the scenario of rapid global change, and the current threat to the *cerrados*. Our work covers a still poorly investigated aspect of intraspecific variation of tropical eusocial insects and sheds new light on the study of trait variation associated with latitudinal gradient and resource availability in a major ecosystem.

INTRODUCTION

In the last decade, the Earth has experienced the hottest temperatures over the past 125 thousand years (IPCC 2022). In addition to risky consequences for humans, climate change has been highlighted as a major threat to biodiversity, directly impacting species physiology, behavior, distribution, interactions, and population persistence (Bellard et al. 2012; Habibullah et al 2022). Global temperature is believed to affect mostly ectotherm organisms (Williams et al. 2016) due to their lack of internal sources and mechanisms of heat production and retention (Davenport 1992). Insects – the most diverse lineage of animals – will be particularly affected by warming (Wagner 2020; Halsch et al. 2021; Harvey et al. 2023), risking their important contribution to ecosystem functioning and as providers of ecosystem services (Dangles and Casas 2019; Elizalde et al. 2020). Despite their ability to scape to protected microhabitats (Willmer 1982), the generally small size and relatively large surface area of insects make them more susceptible to variation in temperature and pluviosity (see Harvey et al. 2023). Such a threat may be even worse in the tropics, where insects live close to their physiological temperature limit (Deutsch et al. 2008; García-Robledo et al. 2016; Diamond et al. 2018). Thus, it is imperative to investigate species responses to temperature variation across geographic gradients of major biomes, associating thermal ecology with variation of functional traits in populations living in different environmental settings (Bujan et al. 2020; Harvey et al. 2023).

The ants

Among insects, ants are ideal study systems for evaluating species’ functional traits across large spatial scales (i.e., measurable characteristics of individual organisms that impact their fitness and performance; McGill et al. 2006). As the other social insects (see Wilson 1971), ants are considered quasi-sessile organisms due to nesting habits, being highly dependent on environmental conditions (Hölldobler and Wilson 1990; Menzel and Feldmeyer 2021). Additionally, ants present great morphological variation (Hölldobler and Wilson 1990), with high degree of phenotypic plasticity and acclimation potential (Purcell et al. 2016; Nascimento et al. 2022). Because ants present wide geographic distribution, occurring in almost all places around the world (Hölldobler and Wilson 1990), they are model organisms to evaluate the impact of different in environmental conditions on intraspecific variation using spatial variance as reference (Fitzpatrick et al. 2014). Finally, since ants are abundant and numerically dominant – summing about 20 quadrillion individuals on Earth with a total biomass of nearly 12 megatons (Schultheiss et al 2022) – they are crucial elements for ecosystem functioning and provide important services, including biological control, pollination, seed dispersal, plant protection, and nutrient cycling (see Del-Toro et al. 2012). Thus, geographic variation of abiotic (e.g., climate) and biotic (e.g., food resources) parameters should affect ant functional traits and potentially produce cascading effects on other biological levels.

Similar to other ectotherms, thermoregulation and tolerance is crucial for ants (Sinclair et al. 2016) and is commonly assessed by determining critical thermal limits (see Nascimento et al. 2022). This technique is a laboratory-based approach determining the minimum and maximum temperatures that induce individual muscle spasms or death (Sinclair et al. 2016). However, there are limitations in this approach derived from experimental procedures such as initial temperature, temperature increment and exposure time (Nascimento et al. 2022). Alternatively, informative morphological traits can be used to investigate species response to abiotic and biotic factors, mainly if they are easily measured and present functional importance (Gibb et al. 2015; Parr et al. 2017; Buxton et al. 2021). Unveiling the interactions between these traits and environmental conditions is important to evaluate of how distinct processes shape natural systems (McGill et al. 2006).

In ants, pilosity of the mesosoma is a main morphological trait related to thermoregulation and can be easily measured as the number of erect setae (hair) present on the mesosoma profile (Figure 1B; Parr et al. 2017). In addition to a possible defensive role against predation (Gibb et al. 2015; Gnatzy and Maschwitz 2006), pilosity is mainly associated to maximal critical thermal limit in ants (Buxton et al. 2021). Mesosoma pilosity is important to reduce water loss in ants, retaining the absorbed external heat and increasing UV reflectance, with a crucial role on heat and desiccation tolerance (Gibb et al. 2015; Buxton et al. 2021). Body size is also described as a functional trait related to thermoregulation in insects: the smaller the body size, the faster the heat gain and heat loss (Crown and Nicolson 2004).

Analyses of trait variation can be performed on single or multiple species (Hortal et al. 2015; Wong et al. 2019), and the importance of intraspecific trait variation in functional ecology has been stressed for several animal taxa (Bolnick et al. 2011; Des Roches et al. 2017; Wong and Carmona 2021), including insects (Gouws et al. 2011; Gentile et al. 2021). Intraspecific variation is the base of evolutionary theory (Darwin 1859). In changing environments, populations with higher functional diversity would present higher chances of including genotypes that persist under distinct environmental conditions (Hooper et al. 2005).

Thus, describing the patterns and revealing the drivers of intraspecific variation in widely distributed species is fundamental to understand how selective pressures vary across a large geographic scale (Oms et al. 2017).

Different factors can mediate morphological variation in ants. Intrinsically, evolutionary constraints, genetic makeup, developmental processes, and social organization are recognized to promote ant polymorphism (Wills et al. 2018). On the other hand, external conditions may play an important role. For instance, although morphology may vary greatly in response to elevational and latitudinal gradients (e.g. Chow and Gaston 1999; Oyen et al. 2016; Bishop et al. 2017; Shik et al. 2019), much of ant phenotypic variation occurs in response to difference in microhabitats (Kaspari et al. 2015). In addition to the physical environment, resource availability and competition are also highlighted as drivers of polymorphism in ants (Wills et al. 2018). Determining the factors influencing phenotypic variation can be a challenge. Thus, a simultaneous evaluation of multiple factors across different habitats can be helpful to unveil potential drivers of morphological variation in ants (Merila and Hendry 2014). Moreover, distinguishing between phenotypic plasticity (i.e. ability of a single genotype to be translated in a range of phenotypes under distinct environmental conditions; Whitman and Ananthakrishnan 2009) and genetic adaptation (via natural selection) is also challenging, especially in wild populations (Whitman and Ananthakrishnan 2009; Merila and Hendry 2014).

Here, we investigate intraspecific functional trait variation in a dominant ant species, widely distributed in Brazilian Cerrado savanna – *Camponotus crassus* Mayr, 1862 (Formicinae; Figure 1A). Cerrado is one of world’s biodiversity hotspots (Myers et al. 2000), covering nearly a quarter of the Brazilian territory (Oliveira and Marquis 2002; Vieira et al. 2022), and consisting of a mosaic of vegetation physiognomies (Oliveira-Filho and Ratter 2002). Despite its rich biodiversity, the so-called *cerrados* are facing a rapid vegetation loss and land use change, with only 8% of its original territory under legal protection (Colli et a. 2020). The Cerrado presents a high ant species diversity, and many species forage on plants (Campos et al. 2011).

The study species: Camponotus crassus (Formicinae)

Camponotus crassus is one of the most frequent ants on Cerrado foliage (Oliveira and Freitas 2004; Calixto et al. 2021). This ant is commonly found feeding on extrafloral nectaries (EFNs) and trophobiont insects (Oliveira and Brandão 1991; Del-Claro and Oliveira 2000), with sugar-rich liquid resources accounting for up to 80% of its diet (Lange et al. 2019).

Moreover, *C. crassus* presents an aggressive behavior toward other insects nearby their food sources, making the ants effective bodyguards for many plant species from Cerrado (Oliveira et al. 1987; Oliveira and Freitas 2004; Sendoya et al. 2009; Lange et al. 2019; Calixto et al. 2021). Finally, *C. crassus* presents a huge variation in the mesosoma pilosity, with individuals raging from low (Figure 1C) to high levels of pilosity (Figure 1D; S. F. Sendoya, personal observation). Pilosity is a functional ant trait associated with thermoregulation, and that can vary in response to the physical environment, resource availability and competition (Wills et al. 2018).

In this study, we investigated *C. crassus* morphological variation across multiple localities distributed along a latitudinal gradient of Cerrado. Specifically, we describe *C. crassus* pilosity and body size in seven sites and evaluate the relationship between these traits and multiple potential drivers: *physical environmental heterogeneity*, *plant and ant communities*, and *resource availability*. We also assessed morphological and genetic covariation to differentiate phenotypic plasticity and adaptation in *C. crassus*. Since sugary-rich liquid resources account for nearly 80% of *C. crassus* diet (Lange et al. 2019), and resources have been suggested to possible mediate ant thermoregulation (Nascimento et al. 2022), we hypothesized that *resource availability* would play a central role in *C. crassus* thermoregulation capacity, and thus, would have an effect on *C. crassus* pilosity and body size.

In this large-scale study, by comparing localities with distinct biotic and abiotic characteristics, we revealed important drivers of intraspecific variation in functional traits of an abundant ant species of the *cerrados*. Our work sheds new light on the study of trait variation associated with latitudinal gradient and resource availability in a major ecosystem.

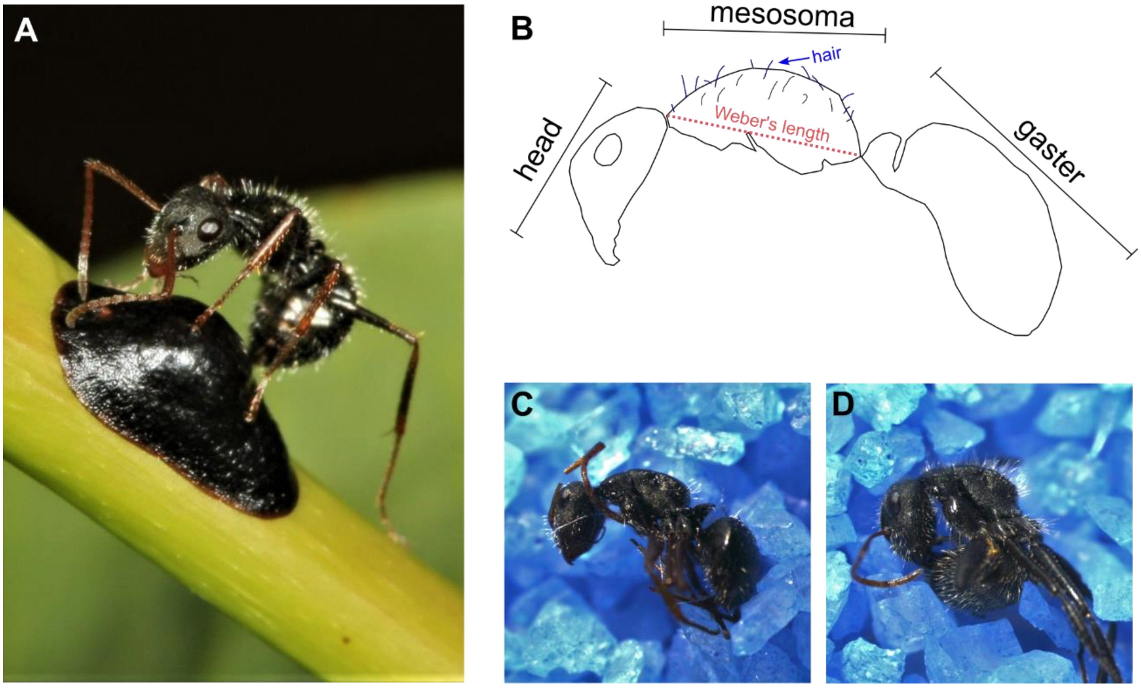

Figure 1. *Camponotus crassus*, an abundant ant species of the Brazilian *cerrados*. (A) Worker of *C. crassus* tending a honeydew-producing trophobiont. (B) Schematic lateral view of *C. crassus*, showing the hairs on the mesosoma and Weber's length - used as a measure of body size. Total pilosity was estimated following the guidelines of *The Global Ants Database*, by counting the number of hairs crossing the mesosoma profile. In the schematic example, there are 12 hairs (highlighted in blue) crossing the edge of the mesosoma profile (the 7 interior hairs are not counted). (C) Specimen of *C. crassus* from Serra da Canastra (state of Minas Gerais, SE Brazil), showing low pilosity on the mesosoma, and (D) worker from Itirapina, (state of São Paulo, SE Brazil, showing high pilosity on the mesosoma.

MATERIAL AND METHODS

Study sites and data collection

The data on physical environmental, community and resource availability used in this study resulted from our work on context-dependence of ant-plant interactions in Brazilian Cerrado (Azevedo-Silva et al. 2023 – Capítulo 1). Between November 2012 and April 2013, seven localities of Cerrado *sensu stricto* (distributed along a latitudinal gradient) were searched for ant-plant interactions: Estação Ecológica de Itirapina (state of São Paulo), Parque Nacional Serra da Canastra, Parque Nacional Serra do Cipó (both in the state of Minas Gerais), Parque Nacional das Emas, Parque Nacional Chapada dos Veadeiros (both in the state of Goiás), Parque Nacional de Brasília (in Distrito Federal), and Parque Estadual Serra Azul (state of Mato Grosso) (Figure 2A). This Cerrado physiognomy is characterized by the prevalence of herbaceous vegetation, with trees and shrubs often 3-8m tall, totalizing more than 30% crown cover (Oliveira-Filho and Ratter 2002). In each locality, 5 transects of 200 m were established, at least 1km apart from one another. Transects were divided into 20 plots of 10 m height and data were collected in 10 plots alternately (Figure 2B). In each parcel, we sampled all small wood plants (with diameter at breast height, DAP, of 2 to 30 mm) up to 1 m from the central line of the transect and all large trees (with DAP > 30 mm) up to 2 m from the central line of the transect (Figure 2B). Each plant was characterized by DAP, height, the presence of EFN, trophobionts insects, ant nests, and number of foraging ants. Plants and ants were collected, morphotyped and identified at species level whenever possible. Ants were stored in 100% ethanol. In addition to the data collected during fieldwork, mean annual temperature (T) and annual precipitation (P) (bio 1.C and bio 12, respectively) were obtained from WorldClim database (Fick and Hijmans 2017), with resolution of 30 seconds. Given ant pilosity is commonly associated to thermoregulation (Gibbs et al. 2015; Buxton et al. 2021), for this study, we also obtained the annual direct normal irradiation (i.e. part of the solar irradiance that directly reaches a surface) and diffuse horizontal irradiation (i.e. of the solar irradiance scattered by the atmosphere) from the Global Solar Atlas 2.0.

Each transect was characterized for physical *environmental heterogeneity*, *plant* and *ant communities*, and *resource availability* (Azevedo-Silva et al. 2023 – Capítulo 1). *Environmental heterogeneity* variables included: (i) density of small and large plants (by dividing the number of small and large plants by the sampling area of 100 m^2 and 200 m^2, respectively); (ii) mean DAP; (iii) mean height; (iv) mean annual temperature; (v) annual precipitation; (vi) annual direct normal irradiation, and (vii) annual diffuse horizontal irradiation). *Plant* and *ant communities* were described by: (i) rarified species richness, based on Hurlbert's (1971) formulation (implemented in the function *rarefy* in the R package *vegan* - Oksanen et al. 2022); (ii) phylogenetic diversity, using the megaphylogeny for seed plants (Smith and Brown 2018) and a phylogeny at genus level for ants (Moreau et al. 2006) (both estimates were made using the *ses.pd* function in the R package *picante* - Kembel et al. 2010); (iii) co-occurrence index (c-score implemented in the function *C.score* in the R package *bipartite* - Dormann et al. 2008), and (iv) mean number of ants per plant (only for ant community). Finally, *resource availability* was described as the proportion of plants with (i) EFN; (ii) trophobionts, and (iii) ant nests. Further details of sampling sites, design, and predictor variables are given in Azevedo-Silva et al. (2023 – Capítulo 1).

To reduce the dimensionality of each set of explanatory variables, we transformed them into one that contains most of the information, using Principal Component Analysis (PCA) implemented in the function *prcomp* in the software R (R Core Team 2022) (Figure S1). Given the first component (PC1) retained most of the variation in all PCAs (Table S1), we used the PC1 of each PCA as explanatory variables.

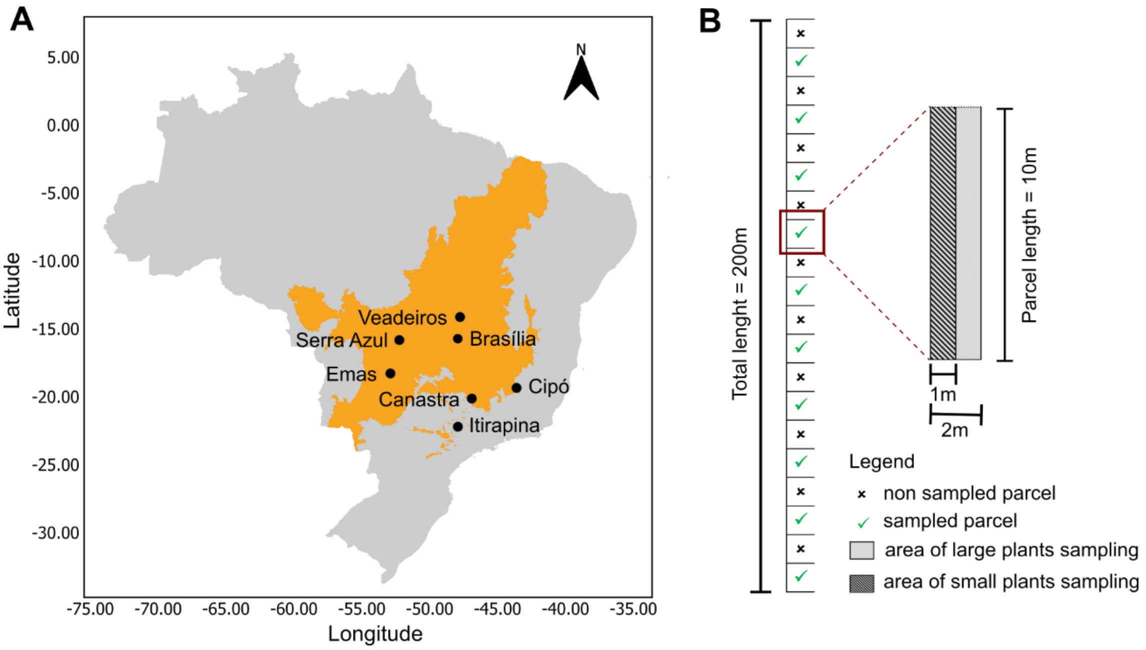

Figure 2. Sampling site and design. (A) Brazil (in grey) with the distribution of Cerrado savanna (in orange). Localities of sampling sites are indicated by black points. (B) Sampling design in each transect. Veja o que escrevi no cap 1 sobre esta fig...

Morphological data of *Camponotus crassus*

For morphological characterization and Single Nucleotide Polymorphisms (SNPs) genotyping, we collected *C. crassus* specimens during fieldwork. To reduce the chances of sampling related individuals, in each transect we chose only one *C. crassus* worker per plant (at least 20 m apart from one another). Because the same individuals were used for morphological characterization and SNP genotyping, we did not pin the ants prior to morphological measurements. Accordingly, we used aquarium blue sand, previously sterilized in autoclave. This sand was chosen because it does not release residuals, enable ants to be easily positioned, and creates a good contrast to observe the hairs on the mesosoma of ants (Figure 1C, D). After ethanol evaporation, the workers were positioned in lateral view and pilosity was estimated following the Global Ants Database (GLAD) guidelines: counting the total number of hairs crossing the mesosoma profile (Gibb et al. 2015; Parr et al. 2017) (Figure 1B). We also estimated Weber's length (a good estimate of body size in ants, see Kaspari 1996, Kaspari & Weiser 1999), which consists of the distance between the antedorsal margin of the pronotum and the posteroventral margin of the propodeum (Figure 1B; Weber 1938). For all morphological characterization, we used Estereomicroscopio Leica M205C with the same zoom for all individuals. After morphological characterization, ants were stored in 100% ethanol up to DNA extraction. A total of 173 workers of *C. crassus* were morphologically characterized.

Given that *C. crassus* pilosity can vary with Weber's length, we estimated hair density by dividing the mesosoma pilosity by Weber's length. We performed Pearson's correlation among the three morphological variables using the function *corPlot* implemented in the R package *psych* (Revelle 2022). Pilosity and hair density are positively correlated (Figure S2). Pilosity was also positively correlated with Weber's length (Figure S2). We decided to maintain the three morphological estimates, separately, as response variables in the subsequent analyses.

Genetic data of *Camponotus crassus*

After morphological characterization, we obtained the total genomic DNA of *C. crassus* workers. To preserve the ants, genomic DNA was obtained through non-destructive DNA extraction, with DNeasy Blood & Tissue Kit (Qiagen). Using an entomological needle, we did a very small hole in the mesosoma of workers and let them incubate in lyse buffer and proteinase K over 18 hours at 56°C. The subsequent procedures followed the manufacturer protocol for insects, with elution in a final volume of 50µL.

DNA extraction was performed in November 2020. Given the time between ant sampling and DNA extraction, the aged specimens resulted in degraded and low yields of DNA. These characteristics make difficult to implement classical methodologies for SNP detection (such as RADseq and GBS), which lead us to implement a PCR based approach known as "multiplexed ISSR genotyping by sequencing" (MIG-seq) (Suyama and Matsuki 2015). MIG-seq is an effective methodology for population genetic studies on aged (and even museum) specimens, being also tested and validated for ants (Eguchi et al. 2020). In this method, genome complexity is reduced by amplifying hundreds to thousands inter-simple-sequence repeats (ISSR) loci using eight pairs of 12 base di- and trinucleotide microsatellites. The amplified fragments are then purified and size selected in the range of > 250 bp. A second amplification step is conducted to add sequences to each sample that coat Illumina flow cell (P5 and P7). Products from the second amplification were pooled in equimolar concentration, purified and size-selected (350 - 800 bp). Following the manufacturer's protocol, pooled samples were sequenced in an Illumina MiSeq Sequencer (Illumina) with the MiSeq Reagent Kit v3 (150 cycles, Illumina). For detailed information on MIG-seq library preparation, see Suyama and Matsuki (2015).

SNP calling followed Suyama and Matsuki (2015). Removal of adapter sequences and low-quality reads (Phred Q score = 30 for at least 40% of the read bases) were performed using FASTX Toolkit (Hannon 2010). The filtered reads were used for *de novo* assembly in Stacks v.2.55 (Catchen et al. 2011), using the following parameters: maximum distance allowed between stacks (M) = 2; minimum depth of coverage required to create a stack (m) = 3; number of mismatches allowed between sample loci when build the catalog (n) = 2. Other parameters were set as default. For SNP filtering, all samples were considered as a single population and followed three criteria: (i) SNPs that were retained by 10% or more samples were included in the SNP dataset; (ii) any SNP site where one of two alleles had less than three counts was filtered out; (iii) the loci containing SNPs with high heterozygosity ($Ho \geq 0.6$) were removed. We also retained only biallelic SNPs. Finally, using the VCFtools v.0.1.12b (Danecek et al. 2011), we removed individuals with more than 40% of missing data.

We assessed loci putatively under selection based on two different approaches: FDIST (Fagundes et al. 2007), implemented in the LOSITAN software (Antao et al. 2008), and a Bayesian method of population differentiation implemented in the software BAYESCAN 2.1 software (Foll and Gaggiotti 2008). In both analyses, we considered the whole dataset and locality as populations. Due to high rates of false positives commonly reported for F_{ST} outlier methods (Bierne et al. 2013; Francois et al. 2016), we adopted a conservative strategy and loci were considered as putatively under selection only if indicated by both methods. Although there was evidence of outlier loci in FDIST analysis (Figure S3A), no outliers were detected by BAYESCAN (Figure S3B). We thus maintained all SNPs in the upcoming analyses, totalizing 186 SNPs for 143 individuals of *C. crassus*.

Statistical analyses

Morphological variation across localities

We used the transects as sample units for statistical analyses. We estimated the mean pilosity, Weber's length, and hair density in ants for each transect. To investigate the morphological differences among localities, we compared the means using an analysis of variance implemented in the function *aov*, followed by a pairwise post-hoc Tukey HSD implemented in the function *glht* in the R package *multcomp* (Hothorn et al. 2008). We also estimated the coefficient of variation in each transect using the function *cv* in the R package *goeveg* (von Lampe and Schellenberg 2023), and compared them among localities using the same procedure implemented for the means. We did not detect differences in the coefficients of variation among localities for all the three morphological estimates (Figure S4), showing the variation is homogeneous in our dataset, supporting the use of means as response variables.

Additionally, we evaluated the morphological variation of *C. crassus* in response to *latitude* using linear mixed models (LMM) implemented in the function *lmer* in the R package *lme4* (Bates et al. 2015). Sampling sites were included as random effect. Marginal (fixed effects only; R^2m) and conditional (all effects; R^2c) coefficient of determination were estimated for each model using the function *r.squaredGLMM* implemented in the *MuMIn* package (Barton 2022). Model residuals were inspected for model assumptions using the function *simulateResiduals* implemented in the R package *DHARMa* (Hartig 2022) (Figure S5D, E, F).

Morphological variation in response to physical *environmental heterogeneity*, *plant* and *ant communities*, and *resource availability*

To evaluate the effects of *environmental heterogeneity*, *plant* and *ant communities*, and *resource availability* on the morphological variation of *C. crassus,* we used linear mixed models (LMM) implemented in the function *lmer* in the R package *lme4*. We used the first principal component of PCAs described above as representation of explanatory variables. Sampling sites were included as random effects. To account for the differences in the number of characterized individuals in each transect, we included the number of observations as an *offset* argument in the model. We checked collinearities between predictor variables by calculating the variance inflation factor (VIF) for each predictor using the function *check_collinearity* implemented in the R package *performance* (Lüdecke et al 2021). No collinearities were found among variables (Table S2). Model residuals were inspected for model assumptions using the function *simulateResiduals* implemented in the R package *DHARMa* (Figure S6). We used an automated model selection approach implemented in the function *dredge* in the R package *MuMIn* (Barton 2022), based on Akaike Information Criterion corrected for small samples (AICc) to select the models that best explain *C. crassus* morphology. Models with ΔAICc < 2 were considered the most plausible among candidates

(Zuur et al. 2009). As more than one model was pointed as plausible, we performed a model averaging (full average) using the function *model.avg* in the *MuMIn* package.

Morphological variation in response to resource type

Given that *resource availability* presented the highest effect on *C. crassus* pilosity and hair density (see Results), we performed a linear mixed models (LMM) implemented in the function *lmer* in the R package *lme4*, using the proportion of each resource type as explanatory variables (EFN, trophobionts and ant nests). We checked the model for collinearities (Table S3) and assumptions (Figure S7). We also performed an automated model selection based on AICc to select the models that best explain the relationship between *C. crassus* morphology and resource availability. Because the full model was the best one (see Results), we estimated the effect size of each predictor using the function *effectsize* implemented in the R package *effectsize* (Ben-Shachar et al. 2020). We also performed a hierarchical partitioning of the R^2m among explanatory variables using the function *glmm.hp* implemented in the R package *glmm.hp* (Lai et al. 2022).

Correlations between morphological, genetic, and geographic variation

To access if morphological variation is congruent to genetic variation in *C. crassus,* we performed Mantel analysis implemented in the function *mantel* in the R package *vegan* (Oksanen et al. 2022). For each morphological variable, we created a matrix of Euclidian distance using the function *dist* in R. We estimated the proportion of shared alleles (Dps) between transects using the function *pairwise.propShared* implemented in the R package *PopGenReport* (Adamack and Gruber 2014). The estimates were transformed into a dissimilarity index by making 1 – Dps and used as genetic distance. Mantel tests were also performed to evaluate pairwise morphological variation in response to geographic distances (which were estimated based on latitude and longitude coordinates of the transects and using Euclidian distance implemented in the function *dist* in R). Genetic distances were also tested against geographical distances. Finally, we performed partial Mantel tests (Smouse et al. 1986) to evaluate the covariation of morphological and genetic distances when conditioned to geographic distance as a covariate (Legendre 1993). For this purpose, we used the function *mantel.partial* implemented in the package *vegan* in R. Mantel and partial Mantel tests were conducted based on 999 permutations. Given that some individuals were lost during genomic procedures, for these analyses, we maintained only the individuals with both SNPs and morphological characterization.

All analyses in this work were performed in R software v4.2.2 (R Core Team 2022).

RESULTS

Morphological variation among localities

For pilosity and hair density, we found some differences among localities (Figure 3A and C), with ants from Parque Nacional das Emas, Parque Nacional Serra do Cipó e Parque Nacional de Brasília being more differentiated from the rest of localities. We did not detect such pattern for Weber's length, with transect means not varying between localities (Figure 3B). Despite some differences between sampling sites, no morphological trait presented a significant relationship when modelled in response to latitude (LMM; Mean pilosity: Estimate = -0.453, P = 0.76, R^2m = 0.01, R^2c = 0.601; Mean Weber's length: Estimate = -0.201, P = 0.072, R^2m = 0.095, R^2c = 0.095; Mean hair density: Estimate = -0.029, P = 0.978, R^2m = 0.000, R^2c = 0.500; Figure S5A,B, and C).

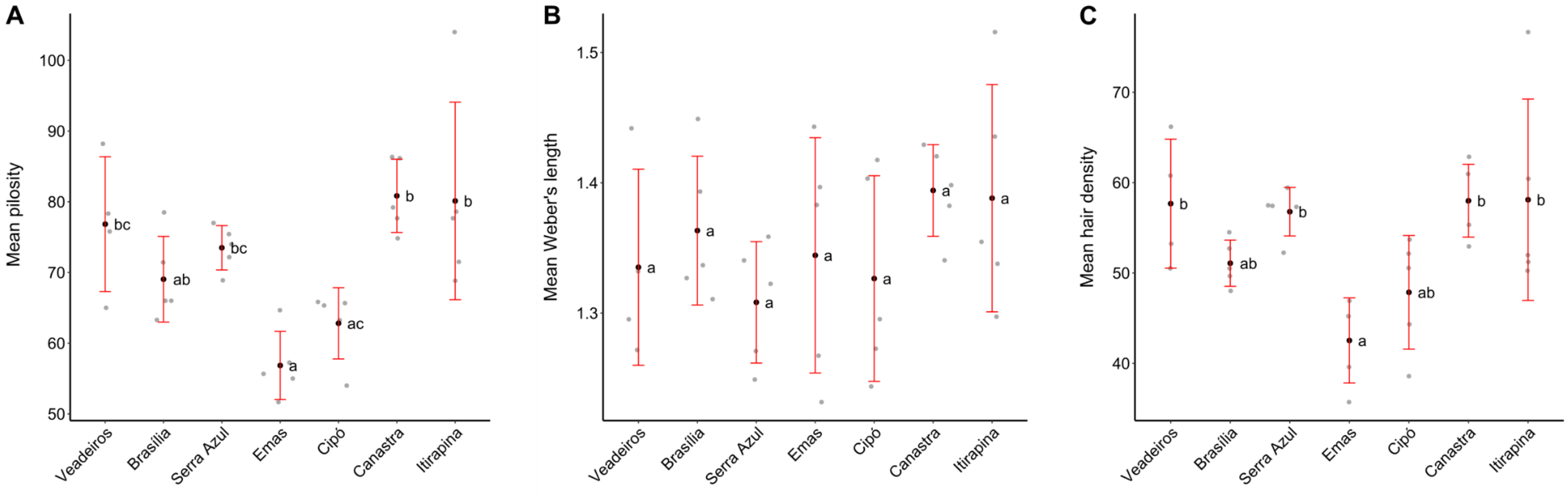

Figure 3. Variation in the mesosoma pilosity of *Camponotus crassus* by locality. (A) Mean pilosity, (B) mean Weber's length, and (C) mean hair density (= pilosity/Weber's length). Black and gray points indicated the mean hair density by locality and transect, respectively. Standard deviation is show by the red vertical line. Local means were compared using post-hoc Tukey test at 5% level of significance. Different letters indicate significant mean difference between localities.

Morphological variation in response to *environmental heterogeneity*, *plant* and *ant communities*, and *resource availability*

We found eight and seven best models for ant pilosity and hair density, respectively, and all predictors – *environmental heterogeneity*, *plant* and *ant communities*, and *resource availability* – were included in the final full averaged model (Table 1). However, *resource availability* was the only significant variable found by the Linear Mixed Models (LMM), being the only variable present in all models after model selection (Table 1). For Weber's length, the best selected model was the null one (Table 1), indicating that *environmental heterogeneity*, *plant* and *ant communities,* and *resource availability* do not play an important role in shaping the size of *C. crassus* workers.

Morphological variation in response to resource type

Given the importance of *resource availability* suggested by the above model selection, we evaluated the relationship of each component of resource availability on *C. crassus* pilosity and hair density. After the automated model selection, we found the full model – including the proportion of plants bearing extrafloral nectaries, the proportion of plants with trophobionts, and the proportion of plants with ant nests – to be the best one for both pilosity (LMM; $R^2m = 0.285$, $R^2c = 0.714$) and hair density (LMM; $R^2m = 0.268$, $R^2c = 0.61$) (Table 2). Effect size estimates revealed a negative relationship between the proportion of plants with sugary food sources (EFNs and trophobionts) and mean pilosity of *C. crassus*. The proportion of plants with trophobionts had almost twice the effect of the proportion of plants bearing EFNs (Table 2). Although the proportion of plants with ant nests had a positive effect on the mean pilosity of *C. crassus* (Table 2), the hierarchical partitioning analyses revealed that the proportion of plants bearing EFN and with trophobionts had the highest contribution to the power of explanation of the model (i.e., R^2m; Figure 4). Similar patterns were found for the modelling of mean hair density of *C. crassus* in response to resource types (Table 2; Figure 4).

Table 1. Full averaged best models (after automated model selection) obtained based on linear mixed effect models constructed for evaluating *Camponotus crassus* morphology (mean pilosity, Weber's length, and hair density) in response to environmental heterogeneity, plant and ant communities, and resources. Locality was included as random effect and the number of observations per transect as an offset argument. First principal component of each explanatory variable was used in the models. Models with ΔAICc < 2 were considered the most plausible among candidates (Zuur et al., 2009). For each model, it is shown: the number of models in which explanatory variables were included (N.models); Estimate, standard error (SE), Z-value, and P-value (* when significant) for each parameter. Plots of diagnostics and table with variance inflation factor of full models are shown in Figure S6 and Table S2. For pilosity and hair density averaged best models, the null model was not selected as best model among candidates (ΔAICc > 2).

Model	**Parameters**	**N.models**	**Estimate**	**SE**	**Z-value**	**P-value**
Mean pilosity	Intercept	-	66.346	3.348	18.977	0.000*
	Environmental heterogeneity	4	0.449	0.978	0.444	0.657
	Plant community	4	0.380	1.114	0.329	0.742
	Ant community	4	-0.008	0.882	0.009	0.993
	Resources	8	-3.469	1.353	2.454	0.014*
Mean Weber's length	NULL	NA	NA	NA	NA	NA
Mean hair density	Intercept	-	48.030	2.169	21.220	0.000*
	Environmental heterogeneity	3	0.246	0.639	0.373	0.709
	Plant community	3	0.218	0.779	0.269	0.788
	Ant community	3	-0.126	0.675	0.178	0.858
	Resources	7	-2.426	1.089	2.135	0.033*

Table 2. Effect sizes of the linear mixed effect models constructed for evaluating *Camponotus crassus* morphology (mean pilosity and hair density) in response to resource availability (proportion of plants bearing extrafloral nectaries, trophobionts, and ant nests). Locality was included as random effect and the number of observations per transect as an offset argument. For each parameter of the models, it is shown: the effect size, its respective confidence interval and P-value. Marginal (R^2m) and conditional (R^2c) r-squared of the models are also shown. Plots of diagnostics and table with variance inflation factor of full models are shown in Figure S7 and Table S3. For both models, full model was elected the unique best model after automated model selection (ΔAICc < 2).

	Dependent variable	
	Mean pilosity	**Mean hair density**
Intercept	0.01 [-0.81, 0.84] P = 0.000	0.01 [-0.75, 0.78] P = 0.000
n.efn	-0.43 [-0.91, 0.13] P = 0.034	-0.43 [-0.90, 0.16] P = 0.056
n.tropho	-0.92 [-1.44, -0.35] P = 0.005	-0.85 [-1.35, -0.28] P = 0.018
n.nests	0.48 [0.00, 0.93] P = 0.177	0.47 [-0.01, 0.91] P = 0.187
R^2m	0.285	0.268
R^2c	0.714	0.61

n.efn: proportion of plants bearing extrafloral nectaries; n.tropho: proportion of plants with trophobionts; n.nests: proportion of plants with ant nests.

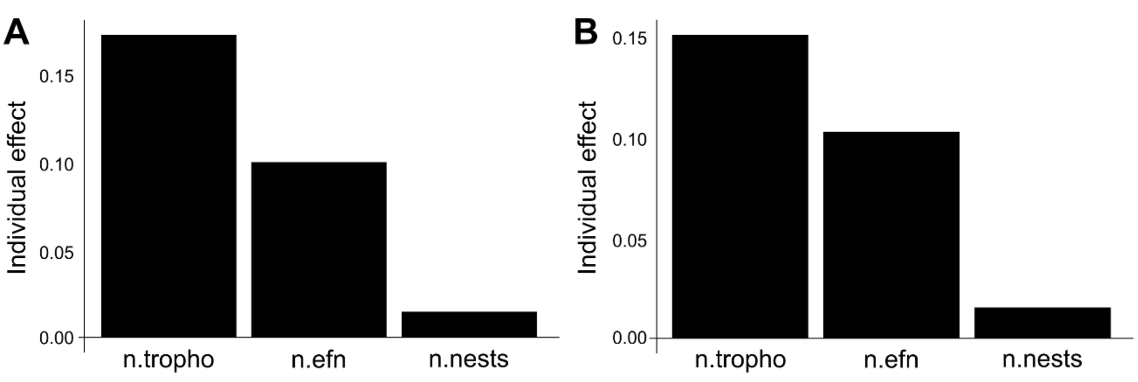

Figure 4. Hierarchical partitioning of marginal r-squared (R^2m) for the best linear mixed effect models constructed for evaluating the morphology of the mesosoma of *Camponotus crassus* in response to proportion of plants with extrafloral nectaries (n.efn), trophobionts (n.tropho), and ant nests (n.nests). In each model, response variable was mean (A) pilosity and

(B) hair density. Locality was included as random effect and the number of observations per transect as an offset argument.

Correlations among morphological, genetic, and geographic variation

In Mantel and partial Mantel analyses, we did not detect a significant correlation between *C. crassus* pilosity and genetic distance, even when controlling for geographic distance (Table 3). Correlation between *C. crassus* pilosity and geographic distance was not significant as well. Similar patterns were found for hair density (Table 3). Contrastingly, we found a positive correlation for Weber's length and genetic distance, even when controlling for geographic distance, suggesting the more different ants are in size, the more different genetically they are too (Table 3). Finally, there is a positive correlation between genetic and geographic distances, suggesting genetic distance in ants increases with geographic distance (Mantel; r = 0.178, P = 0.008).

Table 3. Mantel and partial Mantel tests between morphological (pilosity, Weber's length, and hair density), genetic, and geographic distances. In partial Mantel analyses, geographic distances were used as control. Tests were conducted based on 999 permutations. The observed Pearson's correlation (r) and P-value are shown.

	Mantel				**Partial Mantel**	
	Genetic distance		**Geographic distance**		**Genetic distance**	
Morphological trait	**r**	**P-value**	**r**	**P-value**	**r**	**P-value**
Pilosity	0.123	0.132	0.044	0.281	0.117	0.133
Weber's length	0.454	0.001	0.029	0.301	0.457	0.001
Hair density	0.171	0.1	0.024	0.366	0.17	0.088

DISCUSSION

In this field-based large-scale study, we evaluated four potential drivers of phenotypic variation in *Camponotus crassus*, an abundant ant of the Cerrado. Our models suggested that pilosity and hair density in the mesosoma of *C. crassus* are associated with the *physical environment*, *ant* and *plant communities*, and *resource availability*. Such a relationship, however, was not detected for ant body size. In agreement with our initial hypothesis, *resource availability* was found to have the higher effect in determining *C. crassus* pilosity and hair density. When disentangling resource effect on ant pilosity and hair density, we found that the number of plants with sugar-rich liquids (EFNs and trophobionts) was associated with variation in both traits, that is, the higher the availability of liquid resources on leaves, the lower the quantity of hairs on the mesosoma of *C. crassus*. The variation in ant pilosity and hair density did not covariate with genetic dissimilarities between ants, which did occur for body size, even when accounting for geographic distance. Genetic distance was also found to covariate with geographic distances, suggesting that the nearer the populations, the higher their genetic similarities.

Camponotus crassus pilosity and hair density presented significant differences among the studied localities, but it was not predicted by latitude. Body size, in turn, was statistically similar among localities and did not vary in response to latitude. Although variances in all morphological traits did not differ statistically among localities, they varied greatly among transects in the same locality. This result corroborates previous findings most trait variation in ant species can be found within local communities, which is commonly associated with microhabitat and microclimate heterogeneity (Diamond et al. 2012; Kaspari et al. 2015; Buxton et al. 2021; Nascimento et al. 2022). Our results showed that the models that best explain pilosity and hair density in *C. crassus* include *physical environmental*, *communities*, and *resource* traits at the transect scale. When phenotype-environmental correlations are found across multiple independent localities, the importance of environmental drivers of phenotypic variation increases (Merila and Hendry 2014). Thus, given that our models pointed out that the *environment, community* and *resources* are important throughout our large-scale study, likely playing a role in mediating *C. crassus* pilosity and hair density. On the other hand, none of the evaluated factors was suggested to mediate *C. crassus* body size. Body size has been directly associated with organism physiology (Peters 1983) and thermoregulation (Oms et al. 2017). In ants, body size is correlated with competitive ability (Davidson 1978), phylogenetic history, migration ability, starvation resistance (Cushman et al. 1993), resource exploitation (Okuzaki et al. 2010; Stouffer et al. 2011), and even soil granulometry (Costa-Milanez et al. 2017). It is therefore possible that multiple factors can mediate body size in *C. crassus*, and further investigation is needed to sort this out in Cerrado.

Availability of liquid resources had a high and negative effect on pilosity and hair density in *C. crassus*. In ants, the foraging area is commonly referred to as occurring nearby the nest, reducing the time to gather food and the risk of injury or death to foragers (Brown and Gordon 2000). Additionally, ant foragers are susceptible to harsh environmental conditions outside the nest (Paar and Bishop 2022). *Camponotus crassus* has a small foraging area, moving up to 8 m from the nest entrance during the rainy season in Cerrado, and visiting plants with liquid food sources 1 to 3 m from their nests (Lange et al. 2019). During the dry season, when vegetative growth and EFNs activity are lower (Silva and Oliveira 2010), *C. crassus* increases their foraging area up 12 m from the nest entrance (Lange et al. 2019), suggesting that the ants can adjust their foraging area in response to resource availability. Since the distribution of resources is determinant for ant foraging strategies (Lanan 2014, and included references), it is possible that under low resource availability in the Cerrado, workers of *C. crassus* expand their foraging terrain and expend more time outside nest. If so and if resource availability is constant during larval phase, this reduction in resource availability could lead to an increase of pilosity in response to exposure to external conditions, increasing *C. crassus* capacity of thermoregulation (Purcell et al. 2016).

King and MacRae (2015) have described a metabolic pathway associating resource type and thermoregulation in insects: ingested sucrose is stored, metabolized, and used in the synthesis of heat shock proteins, which are crucial for species dealing with heat stress. Indeed, a carbohydrate-rich diet has been shown to boost ant activity and maximum critical temperature, improving ant thermal tolerance (Blüthgen and Fiedler 2004; Bujan and Kaspari 2017; Nascimento et al. 2022). Since sugary exudates from EFNs and honeydew from trophobionts account for nearly 80% of *C. crassus* diet (Lange et al. 2019), it is possible that such carbohydrate-rich liquids play a central role in *C. crassus* thermoregulation capacity. Actually, we found that *C. crassus* pilosity and hair density variation is negatively associated with the proportion of plants with EFNs and mainly honeydew producing insects. Indeed, hemipteran honeydew was already reported to be nutritionally different from EFNs, with ants preferring to forage on the former liquid source (Del-Claro and Oliveira 1993; Blüthgen et al. 2004; Sendoya et al. 2016). It is known that heat-tolerant ants can forage close to their thermal limits when resource quality is high (Cerdá et al. 1998). As such, by consuming sugar-rich exudates, *C. crassus* workers can increase their thermal tolerance and extend their foraging activity. Therefore, in places with lower proportion of plants bearing EFNs and mainly trophobionts, it is possible that other strategies for thermoregulating take place, including an increment in hair pilosity.

Few studies have attempted to unveil the genetic basis of thermal tolerance in ants and how it varies across populations of the same species (Roeder et al. 2021). When induced by selective pressure, trait variation can reveal longstanding processes such as adaptation, character divergence, and even speciation processes (Richardson et al. 2014). On the other hand, when derived from phenotypic plasticity, traits can rapidly change within few generations and differ remarkably among different habitats (West-Eberhard 1989). Although we did not use adaptative molecular markers (instead, we focused on neutral ones), our results revealed a possible genetic adaptation in *C. crassus* morphology variation. For body size, our results showed a positive correlation with genetic distances, suggesting that different-sized ants also differ in their genetic makeup. Genetic dissimilarity can originate from selective pressure or non-adaptative processes, such as genetic drift, gene flow, and inbreeding (Merila and Hendry 2014). Thus, further investigation is needed to elucidate the nature interhabitat size variation in *C. crassus*, performing common garden or reciprocal transplant experiments (e.g., Purcell et al. 2016).

Contrary to body size, *C. crassus* pilosity and hair density did not covary with genetic distances, suggesting variation in these traits can result from phenotypic plasticity. Based on our data and analyses, we are not able to evaluate if this possible phenotypic plasticity of *C. crassus* is responsive to or anticipatory of environmental changes (Whitman and Ananthakrishnan 2009). Additionally, it is not possible to predict if such variation is active (i.e., a coordinate response of multiple regulatory genes) or passive (i.e., derived just from susceptibilities; Whitman and Ananthakrishnan 2009). Despite limitations to clarify such mechanisms, it has been argued that phenotypic plasticity plays a central role in evolutionary biology by generating phenotypic variation, a requirement for natural selection to act (Whitman and Ananthakrishnan 2009). This would facilitate adaptative evolution in ecological timescales (Ghalambor et al. 2007). Therefore, plastic organisms should be favored in constantly changing environments (Whitman and Ananthakrishnan 2009). Since phenotypic plasticity allows organisms to rapidly respond to environmental changes, it has been highlighted as a crucial mechanism under the scenario of rapid global change (Chown et al. 2007; Berg et al. 2010; Oms et al. 2017).

In addition to unveiling the drivers of intraspecific variation, it is crucial to predict the effects of biodiversity loss and environmental changes (Des Roches 2017). For instance, trait variation can increase niche complementarity, which reduces competition between different species (Bolnick 2011). Moreover, species with broader phenotypic plasticity would tolerate distinct environmental conditions, increasing their niche breadth and occurrence in a broader range of habitats (Slatyer et al.2013). Thus, species with lower levels of plasticity and tolerance tend to have more limited geographic distribution and be more vulnerable by climate change (Diamond and Chick 2018). Our results suggest that *C. crassus* has a high capacity to respond to variable environmental conditions, at a large spatial scale. Indeed, *C.*

crassus has a wide distribution in South America, occurring in Argentina, Brazil, Colombia, Paraguay, and Peru (Kempf 1972; Lange et al. 2019).

Morphological differences between individuals can also lead to a diversity in demographic parameters, defense and competitive abilities, parasite resistance, tolerance to abiotic changes, resource exploitation (Bolnick 2011). Moreover, intraspecific variation may alter community structure and dynamics (Bolnick 2011). In the specific case of ants, heat tolerance is a key aspect for community dynamics (Menzel and Feldmeyer 2021). Ants present thermal niches, with aggressive and dominant ants occupying the high-quality thermal niches, while subordinate ones are commonly reported to forage close to their maximum thermal limit (Fitzpatrick et al. 2014; Nascimento et al. 2022). Under the scenario of global warming, timid and subordinate ants can predominate, decreasing the effectiveness of herbivore deterrence on the plant surface (Halsch et al. 2021). Thus, thermotolerance adjustments (e.g., increasing pilosity) can be relevant not only for populations to survive, but also for ant-plant defense mutualisms (Nascimento et al. 2022). The consequences of trait variation in *C. crassus* reveal the need for testing the ecological impacts of ant morphological variation on interspecific interactions – a promising avenue of investigation for ant researchers.

In conclusion, by investigating multiple potential drivers of functional traits in *Camponotus crassus*, we highlighted the relevance of resource availability for ant geographical ecology, as well ant ecophysiology and behavior. The high plasticity of *C. crassus* in response to distinct environmental conditions should affect the services provided by this species in plant defensive mutualisms in Cerrado (Oliveira et al. 1987; Sendoya et al. 2009, Calixto et al. 2021a). This study enhances the importance of investigating intraspecific variation and phenotypic plasticity, especially under the scenario of rapid global change (Gouws et al. 2011; Gentile et al. 2021), and the current threat to the *cerrados* (Colli et al. 2020). Our work covers a still poorly investigated aspect of intraspecific variation of tropical eusocial insects and sheds new light on the study of trait variation associated with latitudinal gradient and resource availability in a major ecosystem.

SUPPLEMENTARY INFORMATION

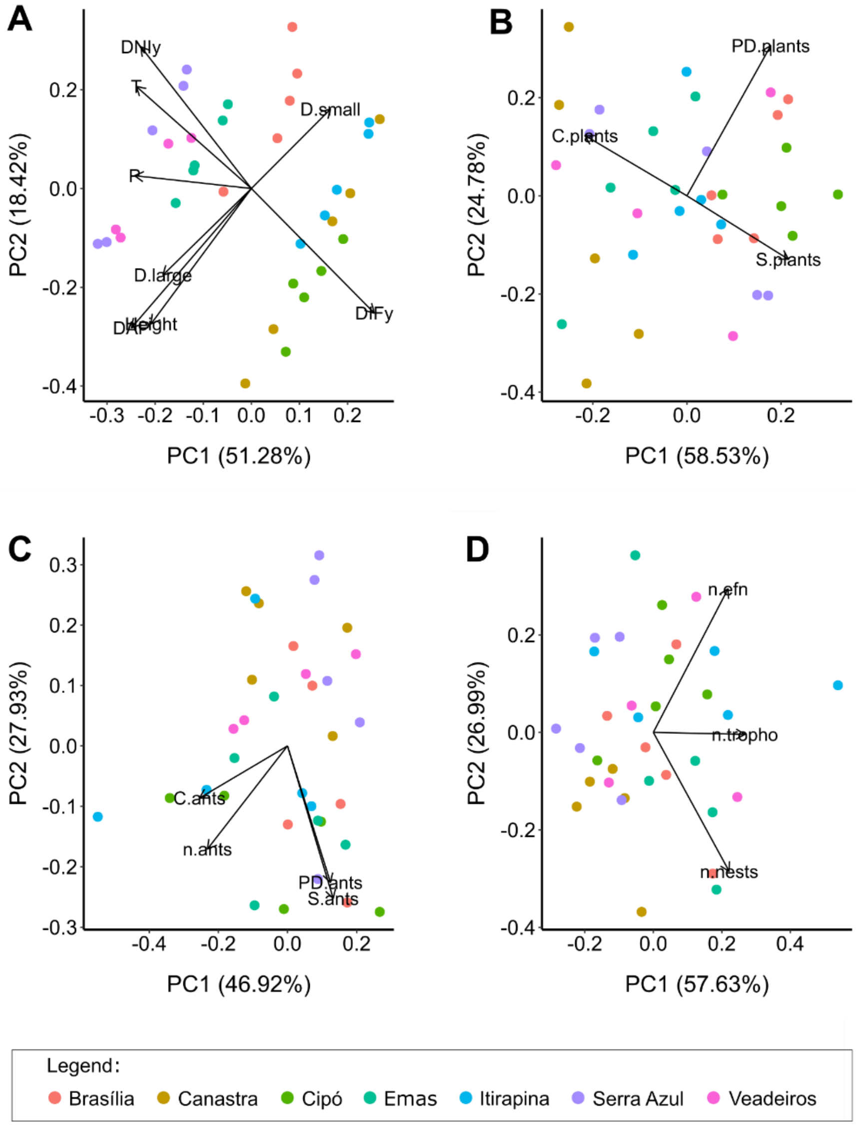

Figure S1. Principal Component Analysis (PCA) for each set of predictor variables: (A) *environmental heterogeneity*, (B) *plant community*, (C) *ant community*, and (D) *resources*. Each point represents a transect, color coded by locality according to the legend. T: annual mean temperature; P: annual precipitation; DIFy: annual diffuse solar irradiation; DNIy: annual direct solar irradiation; DAP: mean diameter at soil height; Height: mean plant height; D.small: density of small plants; D.large: density of large plants; S.plants: rarefied plant species richness; c.plants: plants co-occurrence index; PD.plants: plants phylogenetic diversity; S.ants: rarefied ant species richness; C.ants: ants co-occurrence index; PD.ants: ants phylogenetic diversity; n.ants: mean number of ants per plant; n.efn: proportion of plants bearing extrafloral nectaries; n.tropho: proportion of plants with trophobionts; n.nests: proportion of plants with ant nests.

Table S1. Principal component analyses for each set of predictor variables (PCA) used in this study. The percentage of variation explained by each principal component (PC) included in the PCA is shown, as well as the eigenvalue of each variable to the first PC (Eigenvalue – PC1-).

PCA	**Variables**	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**Eigenvalue -PC1-**
Environmental heterogeneity	mean annual temperature	0.51	0.18	0.13	0.08	0.05	0.03	0.01	0.01	-0.38
	annual precipitation									-0.38
	annual direct solar irradiation									-0.36
	annual diffuse solar irradiation									0.40
	mean diameter at soil height									-0.39
	density of small plants									0.26
	density of large plants									-0.29
	mean plant height									-0.33
Plant community	rarefied species richness	0.59	0.25	0.17	-	-	-	-	-	0.61
	co-occurrence index (c-score)									-0.61
	phylogenetic diversity									0.50
Ant community	rarefied species richness	0.47	0.28	0.20	0.06	-	-	-	-	0.34
	co-occurrence index (c-score)									-0.65
	phylogenetic diversity									0.32
	mean number of ants per plant									-0.60
Resources	proportion of plants bearing extrafloral nectaries	0.58	0.27	0.15	-	-	-	-	-	0.53
	proportion of plants with trophobionts									0.65
	proportion of plants with ant nests									0.54

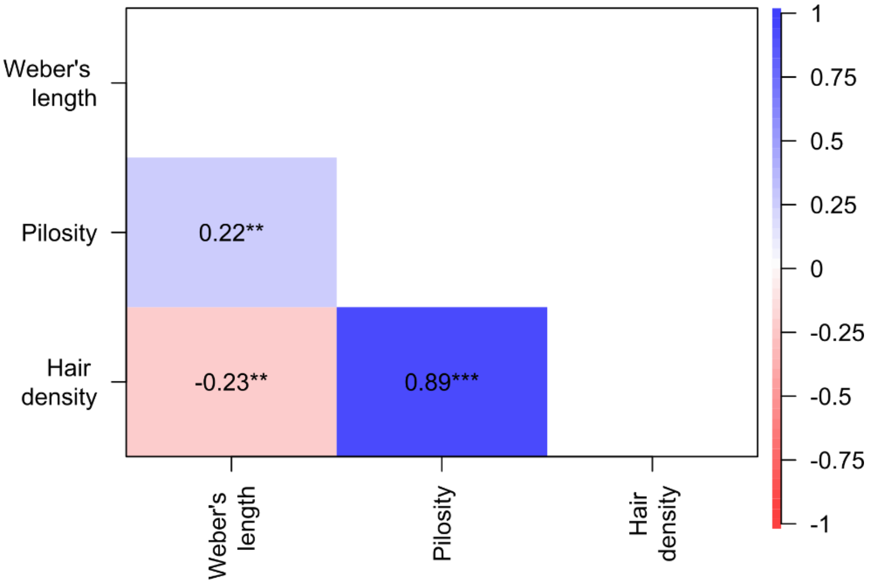

Figure S2. Correlation matrix for morphological variables measured for *Camponotus crassus*. Pearson’s correlation coefficients are shown for each comparison. Significance level is coded as *p<0.05, **p < 0.01, and ***p < 0.001.

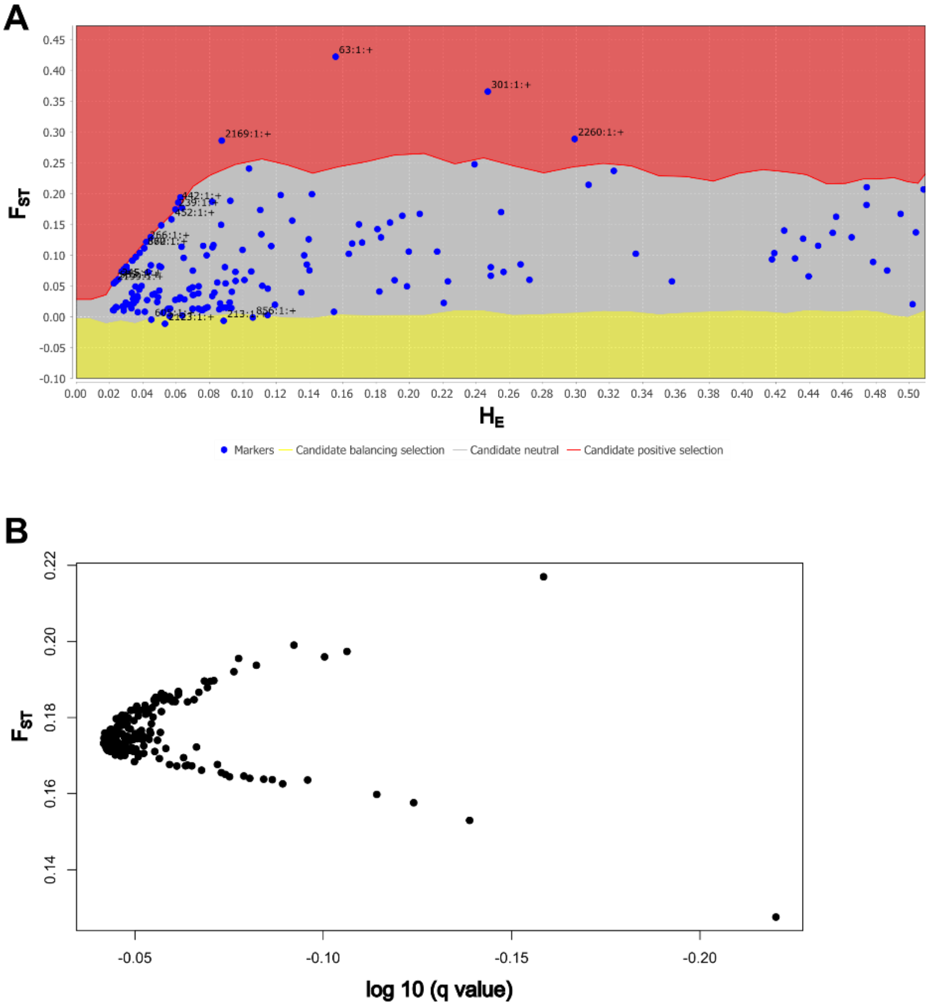

Figure S3. Methods implemented to evaluate loci putatively under selection on SNP dataset. (A) FDIST method implemented in the software LOSITAN. Each blue point represents a SNP marker. Colored areas represent the confidence intervals for (I) neutral markers (grey), (ii) under balancing selection (yellow), and (iii) under positive selection (red). Four loci are shown to be putatively under selection. (B) Bayesian method implemented in the software BAYESCAN 2.1. Each point represents a SNP marker. Log 10 (q value) is described as the False Discovery Rate (FDR), considered analog to P-value. No locus presented FDR > 0.0, suggesting no evidence of natural selection among loci.

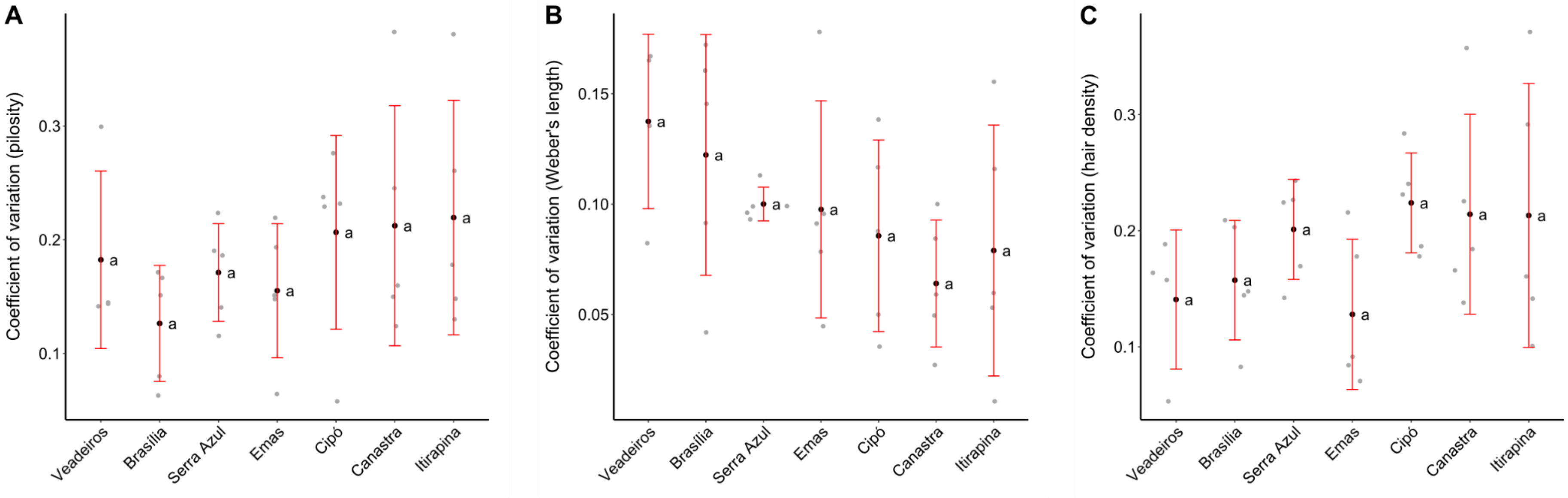

Figure S4. Coefficient of variation of morphological traits of *Camponotus crassus* mesosoma by locality. (A) Mean pilosity, (B) mean Weber's length, and (C) mean hair density (= pilosity/Weber's length). Black and gray points indicate the mean coefficient of variation of hair density by locality and transect, respectively. Standard deviation is show by the red vertical line. Local means of coefficient of variation were compared using post-hoc Tukey test at 5% level of significance. Different letters indicate significant mean difference between localities.

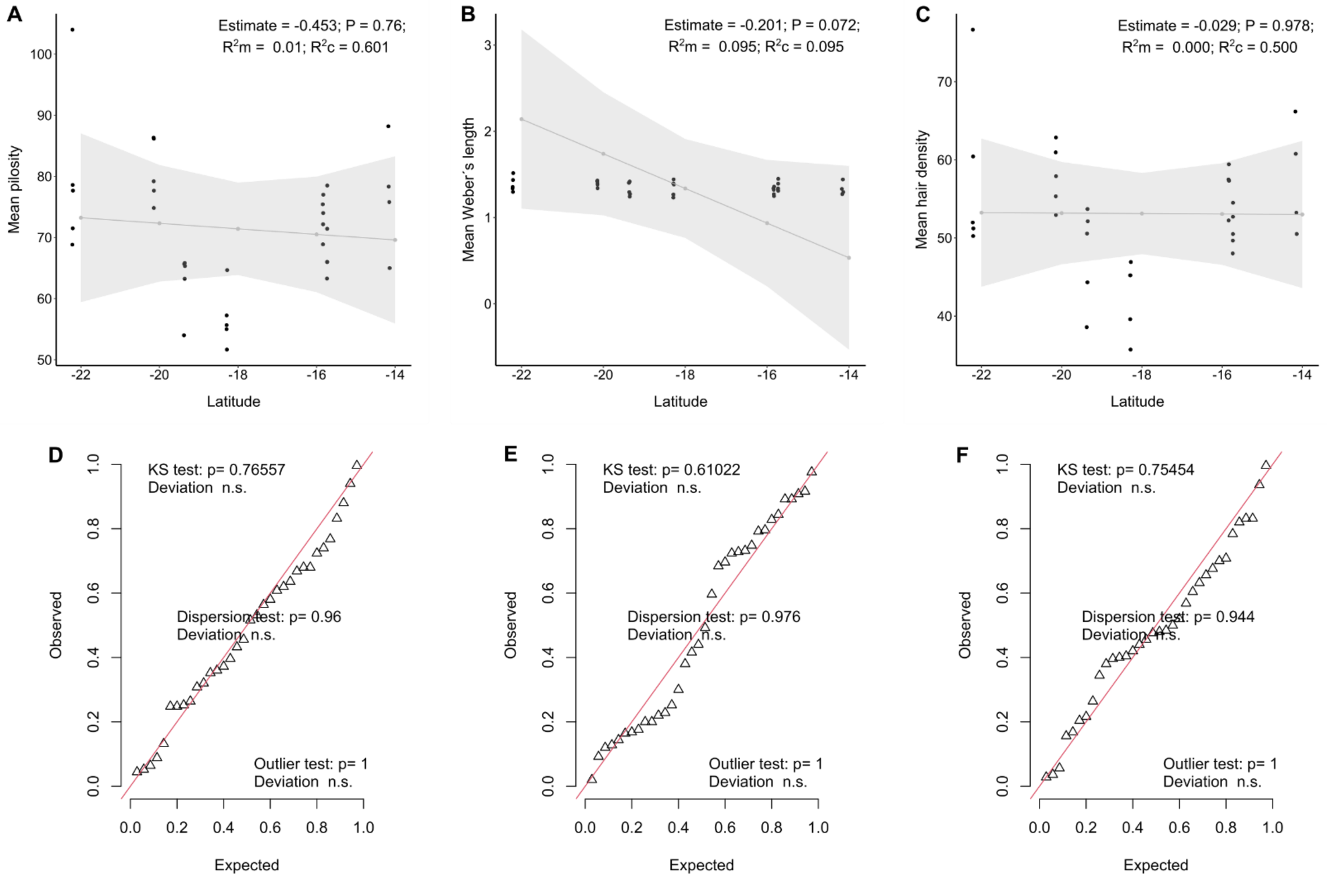

Figure S5. Linear mixed model for morphological variation in the mesosoma of *Camponotus crassus* in response to latitude. Study sites were included as random effect. (A) Mean pilosity, (B) mean Weber's length, and (C) mean hair density. For each model, it is shown the Estimate, P-value, marginal (R^2m) and conditional (R^2c) r-squared. Each point represents a transect. The solid gray line represents the model prediction and the gray area represents 95% confidence interval of each model. Diagnostic plots of models in A, B, and C are shown in D, E, and F, respectively. In each plot is shown a qq-plot to detect overall deviations from the expected distribution, including tests for correct distribution (KS test), dispersion and outliers. n.s.= non-significant.

Table S2. Variance inflation factor (VIF) and 95% confidence interval (CI) to check for collinearity among explanatory variables in the full linear mixed effect models. Models were constructed for evaluating the morphology (mean pilosity, Weber's length, and hair density) of the mesosoma of *Camponotus crassus* in response to *environmental heterogeneity*, *plant community*, *ant community*, and *resources*. Locality was included as random effect and the number of observations per transect as an offset argument. See models in Table X.

Model	**Predictors**	**VIF**	**CI**
Mean pilosity	Low correlation		
	Environmental heterogeneity	1.01	[1.00, 1.80e+10]
	Plant community	1.15	[1.02, 2.48]
	Ant community	1.57	[1.21, 2.55]
	Resources	1.4	[1.12, 2.32]
Mean Weber's length	Low correlation		
	Environmental heterogeneity	1.06	[1.00, 9.06]
	Plant community	1.16	[1.02, 2.46]
	Ant community	1.67	[1.27, 2.69]
	Resources	1.56	[1.20, 2.52]
Mean hair density	Low correlation		
	Environmental heterogeneity	1.01	[1.00, Inf]
	Plant community	1.15	[1.01, 2.52]
	Ant community	1.57	[1.21, 2.54]
	Resources	1.42	[1.13, 2.35]

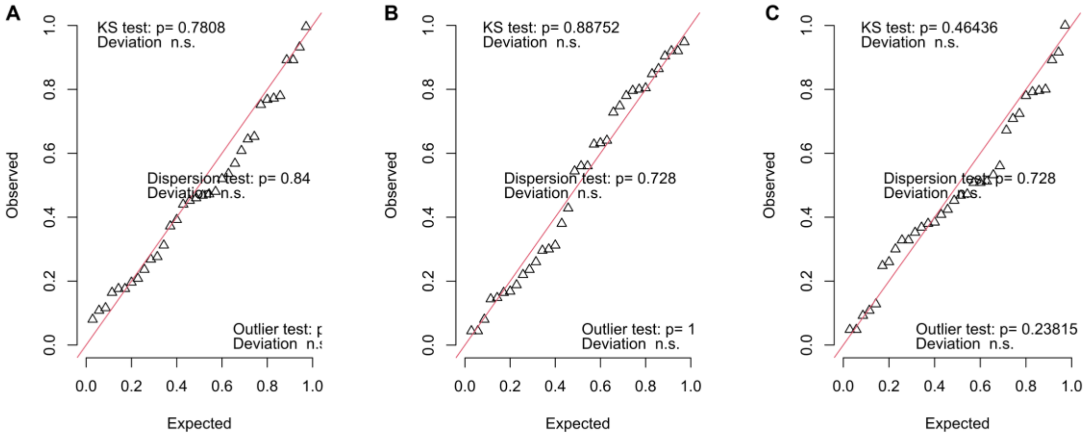

Figure S6. Diagnostic plots for the full linear mixed effect models constructed for evaluating the morphology of the mesosoma of *Camponotus crassus* in response to *environmental heterogeneity*, *plant community*, *ant community*, and *resources*. In each model, response variable was mean (A) pilosity, (B) Weber's length, and (C) hair density. Locality was included as random effect and the number of observations per transect as an offset argument. In each plot is shown a qq-plot to detect overall deviations from the expected distribution, including tests for correct distribution (KS test), dispersion and outliers. n.s.= non-significant.

Table S3. Variance inflation factor (VIF) and 95% confidence interval (CI) to check for collinearity among explanatory variables in the linear mixed effect models constructed for evaluating the morphology (mean pilosity and hair density) of the mesosoma of *Camponotus crassus* in response to proportion of plants with extrafloral nectaries, trophobionts and ant nests. Local was included as random effect and the number of observations per transect as an offset argument. See models in Table X.

Model	**Predictors**	**VIF**	**CI**
Mean pilosity	Low correlation		
	n.efn	1.08	[1.00, 4.88]
	n.tropho	1.33	[1.08, 2.32]
	n.nests	1.27	[1.06, 2.30]
Mean hair density	Low correlation		
	n.efn	1.1	[1.00, 3.95]
	n.tropho	1.34	[1.09, 2.32]
	n.nests	1.26	[1.05, 2.30]

n.efn: proportion of plants bearing extrafloral nectaries; n.tropho: proportion of plants with trophobionts; n.nests: proportion of plants with ant nests.

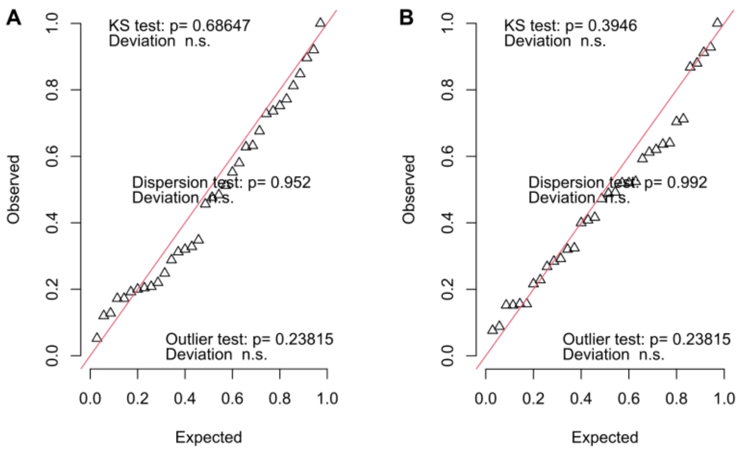

Figure S7. Diagnostic plots for the linear mixed effect models constructed for evaluating the morphology of the mesosoma of *Camponotus crassus* in response to proportion of plants with extrafloral nectaries, trophobionts and ant nests. In each model, response variable was mean (A) pilosity and (B) hair density. Locality was included as random effect and the number of observations per transect as an offset argument. In each plot is shown a qq-plot to detect overall deviations from the expected distribution, including tests for correct distribution (KS test), dispersion and outliers. n.s.= non-significant.

CAPÍTULO 3

INVESTIGATING THE DRIVERS OF ANT INTRASPECIFIC GENETIC DIVERSITY IN A LATITUDINAL GRADIENT OF NEOTROPICAL CERRADO SAVANNA

Marianne Azevedo-Silva, Marina C. Côrtes, Sebastian F. Sendoya, Pedro A. S. Longo, Anselmo Nogueira, Gustavo M. Mori, Shun K. Hirota, Yoshihisa Suyama, Anete P. Souza, Paulo S. Oliveira

ABSTRACT

One of the most consistent patterns of life distribution on Earth is the latitudinal gradient of diversity (LGD), according to which biodiversity increases toward the equator. This pattern has also been detected for intraspecific genetic diversity. Distinct factors may influence genetic variation, including species demography, community composition, food availability and environmental heterogeneity. Although distinct clades present an LGD, some groups may show an inverse pattern. For instance, in the Brazilian Cerrado savanna, ant species diversity has been reported to increase at higher latitudes. Here, we evaluated intraspecific genetic variation across a latitudinal gradient of an abundant species of Cerrado, *Camponotus crassus.* We also analyzed potential factors influencing intraspecific genetic variation, including ant community, environmental heterogeneity, resource availability, and *C. crassus* demography. All the analyzed genetic diversity estimates of *C. crassus* increase significantly with latitude. Among potential drivers of genetic variation in *C. crassus*, we found a negative correlation with precipitation and a positive correlation with resource availability. Our study casts light on the understanding of ant diversity in Cerrado by documenting a reverse latitudinal gradient for the most fundamental level of biodiversity – genetic diversity. Our large-scale geographic assessment fills a gap in the investigation of the drivers of ant intraspecific genetic diversity across major biomes. Such an approach is lacking for most ecosystems in dry and warm regions, including the Cerrado savanna. Our study casts light on the understanding of ant diversity in Cerrado by documenting a reverse latitudinal gradient for the most fundamental level of biodiversity – genetic diversity. Our large-scale geographic assessment fills a gap in the investigation of the drivers of ant intraspecific genetic diversity across major biomes. Such an approach is lacking for most ecosystems in dry and warm regions, including the Cerrado savanna.

INTRODUCTION

One of the most consistent patterns of life distribution on Earth is the latitudinal gradient of diversity (LGD), according to which biodiversity increases toward the tropics (Pianka 1966; Rosenzweig 1995; Gaston 2000; Willig et al. 2003). Although biodiversity can be defined as the variety of life at all biological scales, from genes to ecosystems (Colwell 2009), most of studies on LGD focus on species richness (Gaston 2000; Willig et al. 2003; Hillebrand 2004). Knowledge on global patterns of intraspecific genetic diversity distribution is still lacking, especially in warm regions (Pereira 2016). Similar to what has been reported for species diversity, genetic diversity also tends to increase toward the tropics (Martin and Mckay 2004; Miraldo et al. 2016; Schär et al. 2017; Gratton et al. 2017).

Indeed, species and intraspecific genetic diversity are described as potentially correlated variables (Martin and Mckay 2004; Vellend and Geber 2005; Pereira 2016), which may arise from three main causes. First, both these both levels of diversity are subjected to the same evolutionary forces: drift (that can lead to random variations in abundance of alleles or species; Vellend and Geber 2005), migration (that can introduce new alleles to populations or new species to communities; Vellend and Geber 2005), and selection (which may favor individuals from distinct species or genotypes from the same species; Vellend and Geber 2005). Secondly, genetic diversity may impact species diversity if intraspecific genotypic variation is translated into phenotypic variation with ecological effects on other species (Hughes et al. 2008). Moreover, the increasing in genotype variety enables species to differentially respond to selective pressure from competition, promoting species coexistence (Vellend 2006). Finally, environments with high species diversity are more prone to the emergence of new phenotypes, which efficiently use unexplored resources, avoiding competition (Vellend and Geber 2005; Jousset et al. 2016).

In addition to affecting and responding to species diversity, genetic variation is also associated with environmental heterogeneity both at local and global scales (Hedrick 1986; Pamilo 1988; Vellend and Geber 2005; Stein et al. 2014; Stein and Kreft 2015). Habitat complexity increases the chances of species to differentially exploit resources (Tews et al. 2004), representing a potential driver for diversifying selection (Vellend & Geber 2005). Thus, environmental heterogeneity may influence genetic variation directly through natural selection (the fitness of distinct genotypes varies under specific microhabitat conditions) or indirectly, with environmental variation affecting population demographic processes that also shapes genetic diversity (Pamilo 1988). In most terrestrial habitats, environmental heterogeneity is promoted by vegetation, since the plant community determines the physical structure of the environment, which affects animal diversity at distinct biological scales (Tews et al. 2004). Vegetation heterogeneity includes both vegetation structure (such as vegetation density) and plant diversity (Stein and Kreft 2015), which are often correlated (Qian and Kissling 2010). Moreover, environmental heterogeneity can also be determined by variations in macro and microclimate (Hillebrand 2004; Stein and Kreft 2015), both of which may play a role in determining intraspecific genetic variation.

Although distinct clades present an LGD, some groups may show an inverse pattern, such as ants in Brazilian Cerrado savanna (Vasconcelos et al. 2018). Cerrado covers nearly 26% of Brazilian territory (Oliveira-Filho and Ratter 2002; Vieira et al. 2022). Comprising a mosaic of vegetation physiognomies (Oliveira-Filho and Ratter 2002), Cerrado is one of the world’s biodiversity hotspots (Myers et al. 2000). Among tropical savannas, Cerrado presents high arboreal ant species diversity (Campos et al. 2011). The dominance of ants in Cerrado vegetation is promoted by the presence of nesting sites on plants (e.g. hollowed stems) and the high incidence of liquid food sources on leaves from extrafloral nectaries (EFNs) and trophobiont insects (Oliveira and Leitão-Filho 1987; Oliveira and Freitas 2004). Moreover, the spatial heterogeneity promoted by the physiognomic mosaic and vertical stratification of the vegetation contribute to the high ant species diversity in the *cerrados* (Ribas et al. 2003). Recently, it has been shown that ant species diversity presents an inverse latitudinal gradient, mainly driven by precipitation regime and primary productivity, with ant diversity increasing with latitude (Vasconcelos et al. 2018). An inverse latitudinal gradient has also been recorded for ant-plant interactions (Azevedo-Silva et al. 2023 – Capítulo 1). Moreover, localities at high latitudes present larger ant-based networks and increased interaction diversity and dissimilarity (Dáttillo and Vasconcelos 2019). Additionally, vertical stratification of ant assemblages was also demonstrated to be inversely correlated with latitude (Vasconcelos et al. 2023). Thus, we expect to also find such inverse latitudinal gradient for ant intraspecific genetic diversity.

Here, we evaluate intraspecific genetic variation across a latitudinal gradient of an abundant ant of Cerrado – *Camponotus crassus* Mayr, 1862 (Figure 1A). *C. crassus* is one of the most frequent ants on Cerrado foliage (Oliveira and Freitas 2004; Calixto et al. 2021), being commonly found feeding on extrafloral nectaries (EFNs) and trophobiont insects (Oliveira and Brandão 1991; Del-Claro and Oliveira 2000). These sugar-rich liquid resources accounting for up to 80% of *C. crassus* diet (Lange et al. 2019). Additionally, *C. crassus* aggressive behavior toward other insects nearby their food sources, making the ants effective bodyguards for many plant species from Cerrado (Oliveira et al. 1997; Oliveira and Freitas 2004; Sendoya et al. 2009; Lange et al. 2019; Calixto et al. 2021). We investigate *C. crassus* intraspecific genetic diversity across seven localities that comprise a latitudinal gradient within the Cerrado domain (14° S to 22° S). We analyze potential drivers of intraspecific genetic variation, including ant community (ant species richness) and environmental heterogeneity (precipitation and vegetation density). Given that (i) *C. crassus* is highly influenced by the availability of liquid food on leaves (see Azevedo-Silva et al. 2023 – Capítulo 2) and; (ii) that genetic diversity can be shaped by demographic variables (Hartl and Clark 2010), we also evaluated *C. crassus* genetic diversity in response to the proportion of plants with EFNs and trophobionts and the proportion of plants with *C. crassus*. To our knowledge, this is the first study reporting intraspecific genetic distribution in ants across a latitudinal gradient of Cerrado.

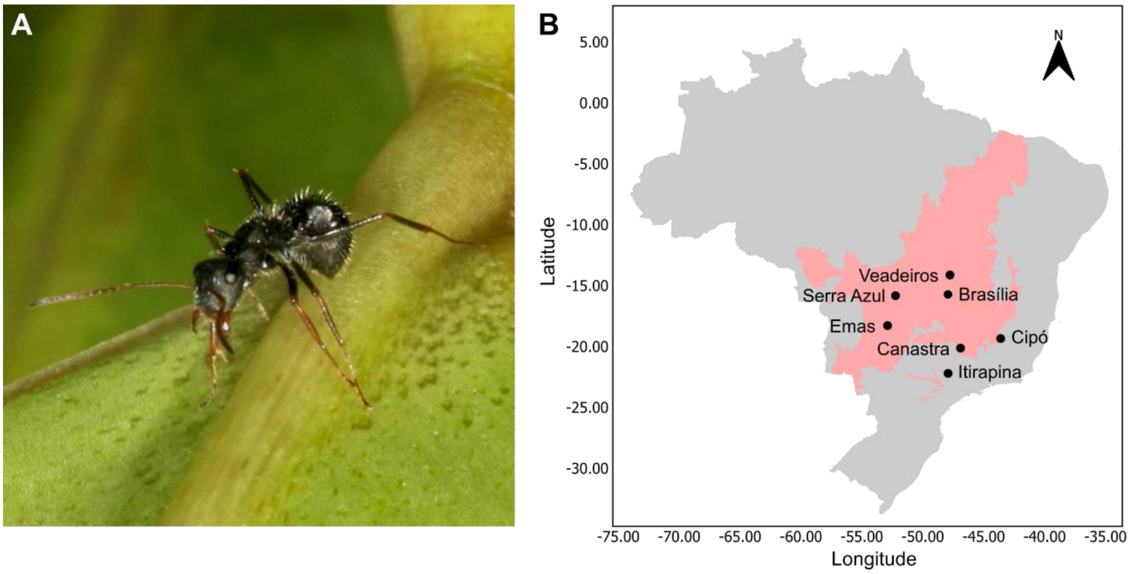

Figure 1. Focal ant species and sampling sites. (A) *Camponotus crassus* worker foraging on Cerrado foliage (photo by S. Sendoya); (B) Map of Brazil (gray), with sampling site locations (black circles) across the distribution of Cerrado savanna (pink).

MATERIAL AND METHODS

Data collection

The ecological data used in this study resulted from our work on context-dependence in ant-plant interactions in Brazilian savanna (Azevedo-Silva et al. 2023 – Capítulo 1). Briefly, seven localities distributed across a latitudinal gradient of Cerrado were chosen for surveying ant-plant interactions (Figure 1B): Estação Ecológica de Itirapina (state of São Paulo), Parque Nacional Serra da Canastra, Parque Nacional Serra do Cipó (both in the state of Minas Gerais), Parque Nacional das Emas, Parque Nacional Chapada dos Veadeiros (both in the state of Goiás), Parque Nacional de Brasília (in Distrito Federal), and Parque Estadual Serra Azul (state of Mato Grosso). Samplings in all localities were conducted in the same Cerrado physiognomy – Cerrado *sensu stricto*. This physiognomy in characterized by the prevalence of herbaceous vegetation, with trees and shrubs often 3-8m tall, totalizing more than 30% crown cover (Oliveira-Filho and Ratter 2002). In each sampling site, we established five transects of 200 m, at least 1 km apart from each other. Transects were divided into 20 plots of 10 x 2 m; data were collected in each alternate plot in the transect, totaling 10 plots per transect. In each sampled plot, we considered all small wood plants (with diameter at breast height, DAP, of 2 to 30 mm) up to 1 m from the central line of the transect. We also sampled all large trees (with DAP > 30 mm) up to 2 m from the central line of the transect. Plants were observed during two intervals of 1 minute, during which we noted: the presence of EFN, trophobiont insects, and presence of ants foraging on the plant. Plants and ants were collected and identified at the species level or, when identification was not possible, classified in morphospecies. From this work, we used the estimates of annual precipitation, mean vegetation density (here calculated as the mean of small and large plants densities), rarified ant species richness, proportion of plants with sugary resources for ants (named EFN and trophobionts), and proportion of plants with *C. crassus*. Because using total or mean abundances can be problematic for social insects (Gotelli et al. 2011), we used the proportion of plants with *C. crassus* as a proxy of its abundance. For further details of ecological data sampling, please see Azevedo-Silva et al. (2023 – Capítulo 1). Given ecological data were obtained at transect level and in this work we aimed to perform the analyses at local level, we calculated a mean for each predictor variable among transects, except for latitude and longitude. In this case, we estimated a centroid among geographic coordinates of transects from the same locality using the function *centroid* implemented in the package *geosphere* (Hijmans 2022) in R software v4.2.2 (R Core Team 2022).

Genetic data were obtained from our work on intraspecific functional trait variation in *C. crassus* (Azevedo-Silva et al. 2023 – Capítulo 2). Shortly, for each transect, *C. crassus* workers were collected during the search for ant-plant interactions. Genomic DNA were obtained from only one *C. crassus* worker per plant (at least 20 m apart from one another). Single Nucleotide Polymorphisms (SNPs) were recovered using a PCR based approach known as “multiplexed ISSR genotyping by sequencing” (MIG-seq) (Suyama and Matsuki 2015). This method reduces genome complexity by amplifying hundreds to thousands inter-simple-sequence repeats (ISSR). Sequences were treated, filtered and *de novo* assembled, and loci were inspected for being putatively under selection. We recovered 186 SNPs for 143 individuals of *C. crassus*. Per locality, a total of 23 individuals were genotyped for Estação Ecológica de Itirapina, 22 for Parque Nacional Serra da Canastra, 19 for Parque Nacional Serra do Cipó, 18 for Parque Nacional das Emas, 20 for Parque Nacional Chapada dos Veadeiros, 23 for Parque Nacional de Brasília, and 18 for Parque Estadual Serra Azul. For further details of genetic data obtention, please see Azevedo-Silva et al. (2023 – Capítulo 2). For each locality, *C. crassus* genetic diversity was characterized by observed heterozygosity (H_O) and mean gene diversity within population (H_S), both estimated using the function *basic.stats* implemented in the R package *hierfstat* (Goudet and Jombart 2022). We also estimated nucleotide diversity (π) using the function *pi.dosage* in *hierfstat*.

Statistical analyses

We evaluated the correlation among the three genetic diversity estimates using Pearson’s correlation implemented in the function *corPlot* in the R package *psych* (Revelle 2022). Although the three estimates were significantly correlated (Figure S1), we analyzed all of them as response variables in the upcoming analyses. We modeled each genetic diversity estimate in response to a single predictor variable using linear model (LM) implemented in the function *lm* in R. The predictors were the following: latitude, annual precipitation, vegetation density, rarefied ant species richness, proportion of plants with liquid resources (EFNs, trophobionts), and proportion of plants with visiting *C. crassus*. Model residuals were inspected for model assumptions using the function *simulateResiduals* implemented in the R package *DHARMa* (Hartig 2022; Figure S2 and S3). To evaluate the significance of models, we contrasted them to a null model (considering only the intercept) using likelihood-ratio tests (LRT) implemented in the package *lmtest* (Zeileis and Hothorn 2002) in R. When significantly different from the null model, we estimated the effect size of predictors using the function *effectsize* implemented in the R package *effectsize* (Ben-Shachar et al. 2020) and reported the adjusted r-squared (R^2_{adj}) obtained using the function *summary* of the LM. Finally, model residuals were tested for spatial autocorrelation, using Moran I test implemented in the function *Moran.I* in the R package *ape* (Paradis and Schliep 2019).

RESULTS

We found a significant reverse latitudinal gradient for all the three genetic diversity estimates, with H_O, H_S and π decreasing toward the equator (Figure 2).

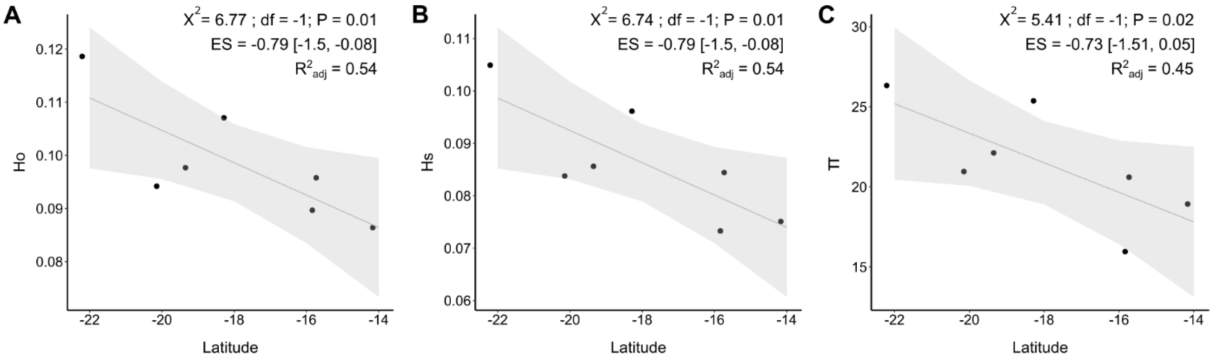

Figure 2. Linear model for *Camponotus crassus* genetic diversity estimates in response to latitude: (A) Observed heterozygosity (H_O), (B) mean gene diversity within population (H_S), and (C) nucleotide diversity (π). For each model, it is shown the likelihood ratio Chi-squared statistic (χ^2), the degrees of freedom (df) and the corresponding p-value (P) from model comparison to null model using Likelihood-ratio tests. Effect size (ES) with corresponding confidence interval and adjusted r-squared (R^2_{adj}) are also reported. In each graphic, points represent sampling sites, the solid line represents model the prediction, and the gray shadow represents 95% confidence interval of each model.

Estimates of genetic diversity in response to ecological predictors revealed that observed heterozygosity (H_O) decreases significantly as precipitation increases (LRT; $\chi^2 = 6.4$; P = 0.01), with this model explaining a good proportion of the variance ($R^2_{adj} = 0.54$; (Figure 3A). Observed heterozygosity increases with the proportion of plants with liquid resources (LRT; $\chi^2 = 4.06$; P = 0.04), also explaining a good proportion of the variance ($R^2_{adj} = 0.33$; (Figure 3D). Very similar results were obtained for estimates of mean gene diversity within population (H_S), which were negatively associated with precipitation and positively correlated with the presence of liquid resources on leaves (Figure 3F and I). Vegetation density, ant species richness and frequency of *C. crassus* on vegetation did not present significant association to H_O or H_S (Figure 3B, C, E, G, H, J). Moreover, Likelihood-ratio tests (LRT) revealed that no tested ecological variable was a good predictor for nucleotide diversity (π)

estimate (Figure 3K-O). We found no evidence of spatial autocorrelation for all evaluated models (Table S1).

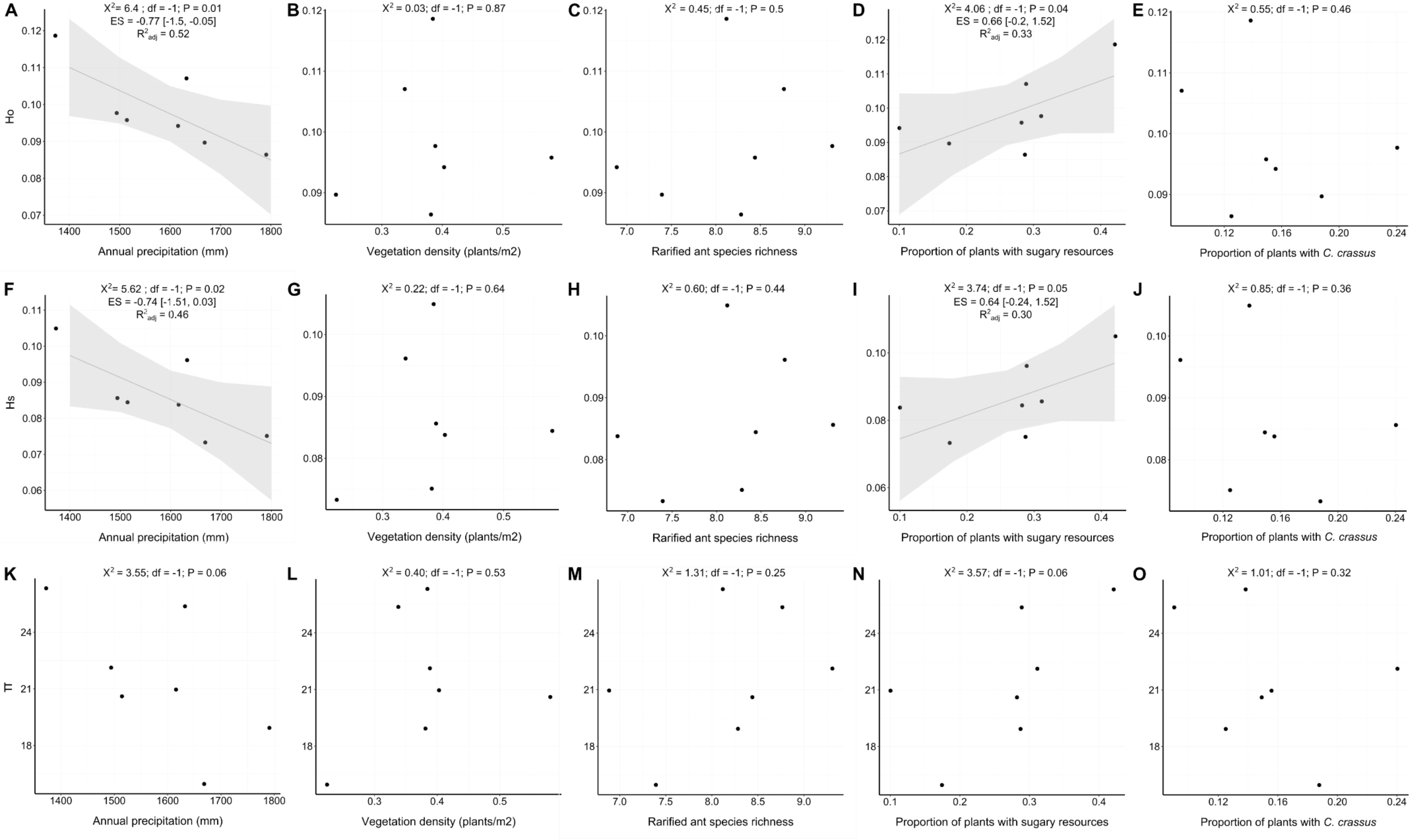
A
X2= 6.4 ; df = -1; P = 0.01
ES = -0.77 [-1.5, -0.05]
R2adj = 0.52
Ho
Annual precipitation (mm)
B
X2 = 0.03; df = -1; P = 0.87
Vegetation density (plants/m2)
C
X2 = 0.45; df = -1; P = 0.5
Rarified ant species richness
D
X2= 4.06 ; df = -1; P = 0.04
ES = 0.66 [-0.2, 1.52]
R2adj = 0.33
Proportion of plants with sugary resources
E
X2 = 0.55; df = -1; P = 0.46
Proportion of plants with C. crassus
F
X2= 5.62 ; df = -1; P = 0.02
ES = -0.74 [-1.51, 0.03]
R2adj = 0.46
Hs
Annual precipitation (mm)
G
X2 = 0.22; df = -1; P = 0.64
Vegetation density (plants/m2)
H
X2 = 0.60; df = -1; P = 0.44
Rarified ant species richness
I
X2= 3.74; df = -1; P = 0.05
ES = 0.64 [-0.24, 1.52]
R2adj = 0.30
Proportion of plants with sugary resources
J
X2 = 0.85; df = -1; P = 0.36
Proportion of plants with C. crassus
K
X2 = 3.55; df = -1; P = 0.06
TT
Annual precipitation (mm)
L
X2 = 0.40; df = -1; P = 0.53
Vegetation density (plants/m2)
M
X2 = 1.31; df = -1; P = 0.25
Rarified ant species richness
N
X2 = 3.57; df = -1; P = 0.06
Proportion of plants with sugary resources
O
X2 = 1.01; df = -1; P = 0.32
Proportion of plants with C. crassus

Figure 3. Linear model for *Camponotus crassus* genetic diversity estimates in response to biotic and abiotic factors. First, second and third lines represent models with observed heterozygosity (H_O), mean gene diversity within population (H_S), and nucleotide diversity (π) as response variables, respectively. Predictor variable is shown in each graphic. For each model, it is shown the likelihood ratio Chi-squared statistic (χ^2), the degrees of freedom (df) and corresponding p-value (P) from model comparison to null model using Likelihood-ratio tests (LRT). In each graphic, points represent sampling sites. For models with significative LRT, effect size (ES) with corresponding confidence interval and adjusted r-squared (R^2_{adj}) are also reported, the solid gray line represents model prediction and gray shadow represents 95% confidence interval of each model.

DISCUSSION

Following our initial expectations, this large-scale study revealed an inverse latitudinal gradient in *Camponotus crassus* genetic diversity, with observed heterozygosity (H_O), mean gene diversity within population (H_S), and (π) decreasing significantly toward the equator. Such findings agree with previous studies reporting a reverse latitudinal gradient for other levels of ant biodiversity. Vasconcelos et al. (2018) have shown that ant species diversity in Cerrado decreases toward the equator for arboreal and ground-dwelling ants, as well as for habitat generalists and forest specialists ants. Moreover, Azevedo-Silva et al. (2023; Capítulo 1) have shown that the proportion of plants interacting with ants decreases toward the equator. Ant-plant networks also tend to be larger away from the equator (Dáttilo and Vasconcelos 2019). Vertical stratification in Cerrado ant assemblages varies with latitude, with dissimilarity between strata decreasing at higher latitude (Vasconcelos et al. 2023). Inverse latitudinal gradient is not restricted to Cerrado. Silva and Brandão (2014) found a similar trend in ant species richness in Brazilian Atlantic rainforest, with ants at higher latitudes presenting less functional differentiation. Rainfall regimes in Cerrado and Atlantic rainforest are positively correlated, being considered the climatic variable underlying latitudinal gradients in ant biodiversity in both formations (Silva and Brandão 2014; Vasconcelos et al. 2018; Azevedo-Silva et al. 2023 – Capítulo 1).

Our analyses of *C. crassus* genetic variation revealed that observed heterozygosity (H_O) and mean gene diversity within population (H_S) are negatively associated with precipitation (i.e. genetic diversity decreases with higher annual rainfall). Indeed, water availability is thought to be the most meaningful variable in warm climate regions, such as Cerrado (Hawkins et al. 2003). In ants, precipitation is highly correlated with nuptial flights -- “the mating flight of the winged queens and males” (Hölldobler and Wilson 1990). Nuptial flights tend to be more frequent after rainy periods (Hölldobler and Wilson 1990). For some tropical species, nuptial flights are shown to be less common during the dry season, occurring in lower number and size in dry forests (Torres et al. 2001). However, for other ant species, mating flights may occur in high frequency under hotter and dryer climatic conditions (Boomsma and Leusink 1981), and rains usually occur after the flight (Depa 2006). For these species, the increment in the number of clouds, humidity, and wind velocity can even interrupt nuptial flights (Boomsma and Leusink 1981). Thus, it is possible that precipitation influences the period of nuptial flights of *C. crassus* in Cerrado, which would directly affect mating and then genetic diversity. However, we still lack information on *C. crassus* reproductive phenology and breeding system, a topic awaiting further investigation for a better understanding of genetic variation in this species. Increased genetic diversity of *C. crassus* in areas of low precipitation may facilitate this species to adapt in case the Cerrado becomes hotter and drier under climate change (Hoffmann et al. 2021). Given that genetic variation is imperative for adaptation to new environmental conditions (Hartl and Clark 2010), our results suggest that populations at higher latitudes tend to respond better to climate change.

Our results also showed that *C. crassus* genetic diversity increases with resource availability (i.e. H_O and H_S increase in areas with higher proportion of plants bearing liquid food resources). Although food availability is dependent on population densities, it can mediate demographic parameters (e.g. Tavecchia et al. 2007). In ants, colony growth has been demonstrated to increase in higher rates at high resource abundance in the environment (McGlynn et al. 2002). Thus, we speculate that abundance of EFNs and honeydew-producing hemipterans in Cerrado would reduce environmental constraints for *C. crassus*, making populations less vulnerable to demographic fluctuations, and leading to increased genetic variation. Besides potential impact on demography, resources quantity and quality have also been shown to influence both intra and interspecific competition (Tilman 1982; Groover 1997; Weider et al. 2008). Under competition, genotypes can be selected depending on resource availability, with superior competitive species tending to show higher heterozygosity (Weider et al. 2008). *Camponotus crassus* is a highly abundant and dominant on cerrado foliage (Oliveira and Brandão 1991; Oliveira and Freitas 2004; Calixto et al. 2021). Although we did not detect a significative relationship between *C. crassus* genetic variation and ant species richness, Vasconcelos et al. (2018) reported increased ant species diversity at higher latitude in Cerrado. Moreover, an inverse latitudinal gradient in ant functional diversity has also been reported in the Atlantic rainforest (Silva and Brandão 2014). Although not yet evaluated in Cerrado, lower functional differentiation between species added to increased species richness at higher latitude, should presumably lead to increased competition within and between species. Under this scenario, new genotypes are more likely to be favored, so as to efficiently exploit liquid food resources and scape competition on foliage (Vellend and Geber 2005; Agashe and Bolnick 2010; Jousset et al. 2016). We hypothesize that this can be one of the mechanisms explaining geographic genetic variation in *C. crassus.* In this case, the genetically diverse population and the high resource competition in Cerrado areas at higher latitudes should lead to faster rates of evolutionary niche expansion in resource use in *C. crassus* (Agashe and Bolnick 2010).

In this study, we investigated mainly exogenous drivers of *C. crassus* genetic diversity. However, other extrinsic and intrinsic factors can influence intraspecific genetic diversity such as fecundity, life history traits, mutation, and recombination rates (Ellegren and Galtier 2016), individual size (Romiguier et al. 2014), and even phylogenetic history (Leffler et al. 2012). For instance, it has been shown that breeding systems (polygyny, polyandry) directly impact the genetic diversity outcomes in *Camponotus* ants in Cerrado (Azevedo-Silva et al. 2020; Azevedo-Silva et al. 2023a). The genetic diversity of these ants is also mediated by the physiognomic mosaic of the *cerrados*, which influences ant dispersal (Azevedo-Silva et al. 2023b). Moreover, habitat fragmentation can also impact ant genetic variation in Cerrado, as demonstrated for *Odontomachus* ants (Ponerinae), in which heterozygosity was found to be higher in colonies at the border of fragments (Azevedo-Silva et al. 2023a). Natural history studies of *C. crassus* have been carried out in single locations (e.g. Lange et al. 2019; Calixto et al. 2021). Given that natural history traits can change under distinct environmental conditions (e.g. Pereira-Romeiro et al. 2022), it is possible that fecundity and phylogenetic traits of *C. crassus* vary with location, mediating the observed inverse latitudinal gradient in this species genetic variation. However, further investigation is still needed to evaluate such hypothesis.

Our large-scale geographic assessment fills a gap in the investigation of the drivers of ant intraspecific genetic diversity across major biomes. Such an approach is lacking for most ecosystems in dry and warm regions, including the Cerrado savanna. Our study casts light on the understanding of ant diversity in Cerrado by documenting a reverse latitudinal gradient for the most fundamental level of biodiversity – genetic diversity. Our large-scale geographic assessment fills a gap in the investigation of the drivers of ant intraspecific genetic diversity across major biomes. Such an approach is lacking for most ecosystems in dry and warm regions, including the Cerrado savanna.

SUPPLEMENTARY INFORMATION

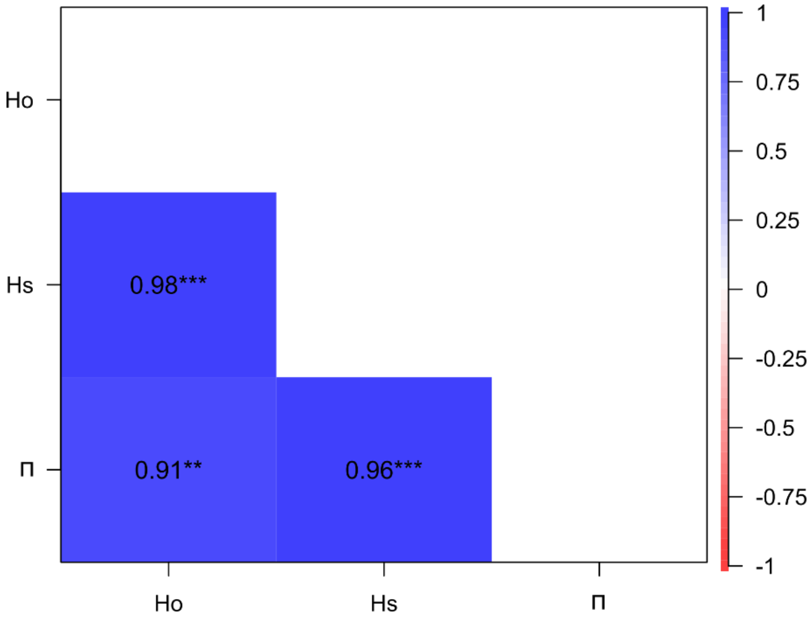

Figure S1. Correlation matrix for genetic diversity estimates for the ant *Camponotus crassus* in Cerrado vegetation. Pearson's correlation coefficients are shown for each comparison. Significance level is coded as $^{*}p<0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. H_O: observed heterozygosity, H_S: mean gene diversity within population, and π: nucleotide diversity.

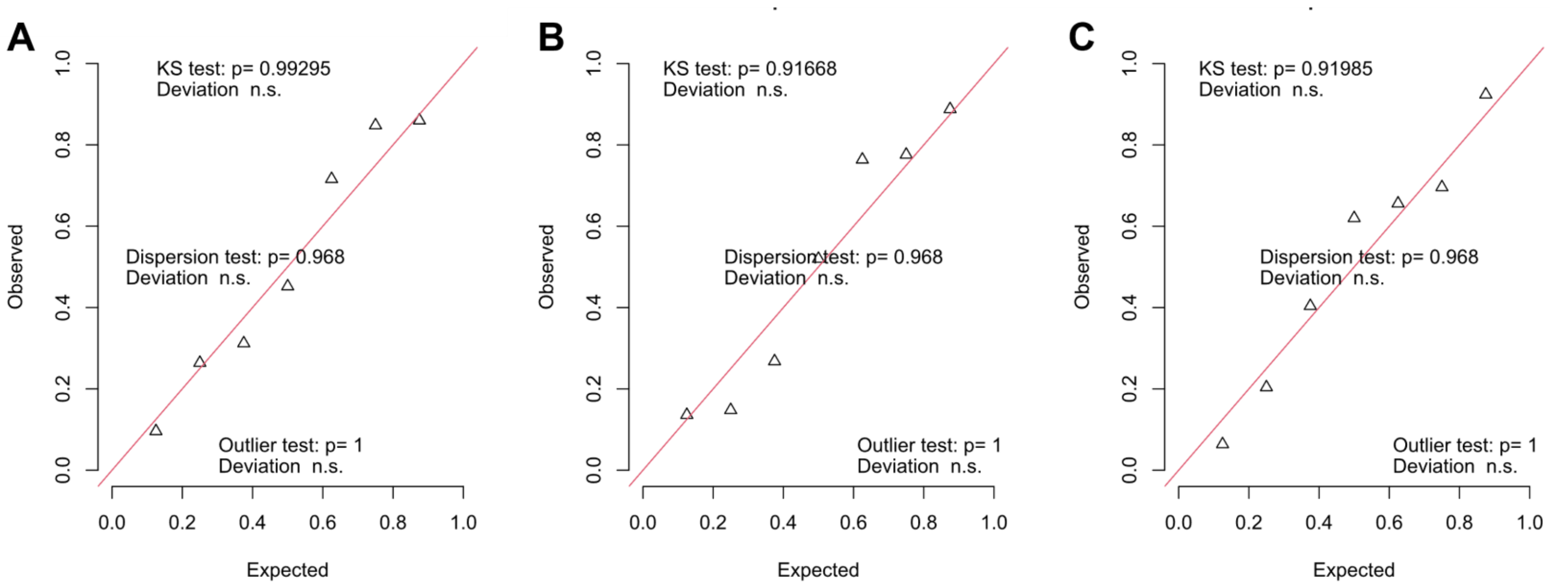

Figure S2. Diagnostic plots for the linear models constructed for evaluating *Camponotus crassus* genetic diversity estimates in response to latitude within the Cerrado domain. In each model, response variable was (A) observed heterozygosity (H_O), (B) mean gene diversity within population (H_S), and (C) nucleotide diversity (π). In each plot is shown a qq-plot to detect overall deviations from the expected distribution, including tests for correct distribution (KS test), dispersion and outliers. n.s.= non-significant.

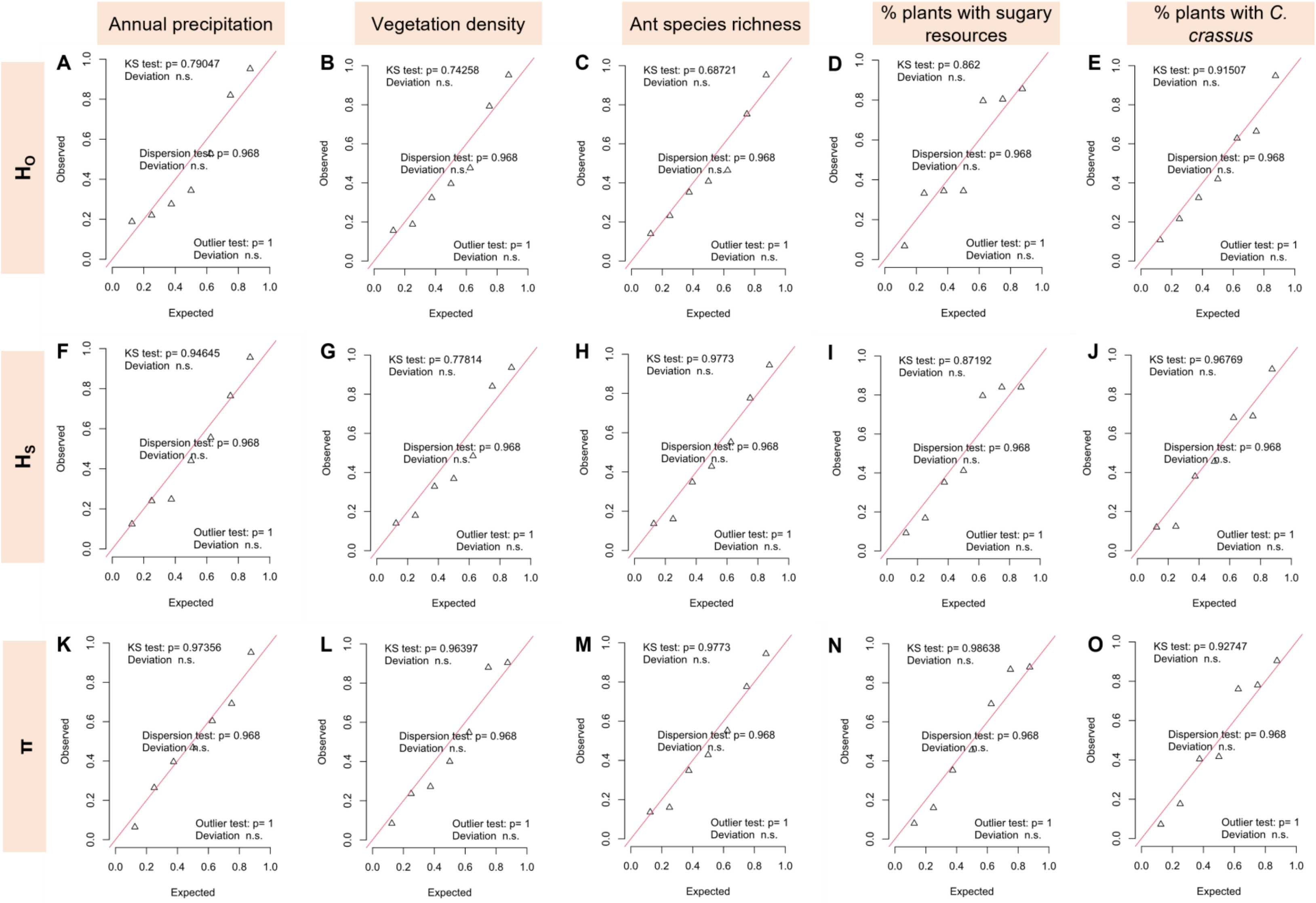
Annual precipitation
Vegetation density
Ant species richness
% plants with sugary resources
% plants with *C. crassus*
H_O
H_S
π
A
KS test: p= 0.79047
Deviation n.s.
Dispersion test: p= 0.968
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
B
KS test: p= 0.74258
Deviation n.s.
Dispersion test: p= 0.968
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
C
KS test: p= 0.68721
Deviation n.s.
Dispersion test: p= 0.968
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
D
KS test: p= 0.862
Deviation n.s.
Dispersion test: p= 0.968
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
E
KS test: p= 0.91507
Deviation n.s.
Dispersion test: p= 0.968
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
F
KS test: p= 0.94645
Deviation n.s.
Dispersion test: p= 0.968
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
G
KS test: p= 0.77814
Deviation n.s.
Dispersion test: p= 0.968
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
H
KS test: p= 0.9773
Deviation n.s.
Dispersion test: p= 0.968
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
I
KS test: p= 0.87192
Deviation n.s.
Dispersion test: p= 0.968
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
J
KS test: p= 0.96769
Deviation n.s.
Dispersion test: p= 0.968
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
K
KS test: p= 0.97356
Deviation n.s.
Dispersion test: p= 0.968
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
L
KS test: p= 0.96397
Deviation n.s.
Dispersion test: p= 0.968
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
M
KS test: p= 0.9773
Deviation n.s.
Dispersion test: p= 0.968
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
N
KS test: p= 0.98638
Deviation n.s.
Dispersion test: p= 0.968
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
O
KS test: p= 0.92747
Deviation n.s.
Dispersion test: p= 0.968
Deviation n.s.
Outlier test: p= 1
Deviation n.s.
Observed
Expected
0.0
0.2
0.4
0.6
0.8
1.0

Figure S3. Diagnostic plots for the linear models constructed for evaluating *Camponotus crassus* genetic diversity estimates (observed heterozygosity, H_O, mean gene diversity within population, H_S, and nucleotide diversity, π) in response to biotic and abiotic factors. For each model, response and predictor variables are indicated by figure row and column, respectively. In each plot is shown a qq-plot to detect overall deviations from the expected distribution, including tests for correct distribution (KS test), dispersion and outliers. n.s.= non-significant.

Table S1. Significance value of Moran I spatial autocorrelation test (Moran's I; $p < 0.05$ indicates significant spatial autocorrelation in the model). Linear models were constructed using *Camponotus crassus* genetic diversity estimates as response variables (Response), and abiotic and biotic factors as predictor variables (Predictor).

Response	**Predictor**	**Moran's I**
Ho	Latitude	0.54
	Annual precipitation	0.83
	Vegetation density	0.74
	Ant species richness	0.37
	Proportion of plants with sugary resources	0.13
	Proportion of plants with *C. crassus*	0.55
Hs	Latitude	0.45
	Annual precipitation	0.45
	Vegetation density	0.67
	Ant species richness	0.44
	Proportion of plants with sugary resources	0.31
	Proportion of plants with *C. crassus*	0.51
π	Latitude	0.27
	Annual precipitation	0.18
	Vegetation density	0.86
	Ant species richness	0.46
	Proportion of plants with sugary resources	0.64
	Proportion of plants with *C. crassus*	0.59

H_O: observed heterozygosity; H_S: mean gene diversity within population; π: and nucleotide diversity.

CONSIDERAÇÕES FINAIS E PERSPECTIVAS

Nesta tese de Doutorado, nós avaliamos diferentes níveis de diversidade biológica em um dos grupos de animais mais abundantes do planeta: as formigas. Além de descrever padrões de distribuição, também investigamos potenciais fatores que influenciam essa biodiversidade, incluindo a heterogeneidade ambiental, comunidades de plantas e formigas, e recursos líquidos disponíveis na vegetação do cerrado. É importante ainda salientar que os dados aqui utilizados foram oriundos de trabalhos de campo prévios, com coletas de forma sistemática, isto é: as sete localidades amostradas pertenciam a mesma fitofisionomia de cerrado (cerrado *sensu stricto*) e todas as amostragens seguiram o mesmo protocolo. Fazer estudos de campo em larga escala é desafiador. Neste trabalho, por exemplo, incluímos observações de interações com formigas feitas em um total de 3345 plantas. Portanto, os dados obtidos e aqui analisados são fruto de observações diretas da natureza, sendo de extrema importância para o avanço do conhecimento sobre formigas no cerrado. Além disso, as perguntas feitas nesta tese se deram posteriormente à coleta de dados, reforçando que novas perguntas são possíveis de serem feitas mesmo com dados já coletados.

No primeiro capítulo, usando modelos de equações estruturadas, nós mostramos que as interações formiga-planta apresentam um gradiente latitudinal inverso, sendo mais frequentes em maiores latitudes. Mostramos ainda como a heterogeneidade ambiental, as comunidades de formigas e plantas, e os recursos líquidos disponíveis na folhagem afetam direta e indiretamente a proporção de plantas no Cerrado que interage com formigas. Entre todos os fatores avaliados, as comunidades de formigas tiveram o maior efeito sobre as interações formiga-planta no cerrado.

No segundo capítulo, nós descrevemos a variação de características morfológicas funcionais em *Camponotus crassus*, uma formiga dominante nas plantas do Cerrado. Também avaliamos como essa morfologia é influenciada por diferentes fatores, e encontramos que a disponibilidade de recursos líquidos nas plantas tem o maior efeito sobre a pilosidade nessa espécie de formiga. Sendo a pilosidade uma característica funcional associada à termorregulação, é possível que os efeitos de alterações climáticas sobre *C. crassus* dependa, principalmente, da forma como os recursos serão afetados pelas mudanças.

No terceiro capítulo, encontramos que o gradiente latitudinal inverso de diversidade também é verdadeiro para diversidade genética de *C. crassus*, a qual é maior quanto maior a latitude. A variação genética nessa espécie foi negativa e positivamente relacionada à precipitação e disponibilidade de recursos, respectivamente.

Os resultados obtidos nesta tese representam um avanço no conhecimento da contexto-dependência das interações formiga-planta, variação funcional intraespecífica, bem como da diversidade genética de formigas no Cerrado. Nossos resultados mostram que, para todos os diferentes níveis da diversidade biológica avaliados, a disponibilidade de recursos líquidos na folhagem (néctar extrafloral e exsudados de hemípteros trofobiontes) é de extrema importância para as formigas no Cerrado. Sendo assim, essa variável deve ser medida e amplamente considerada em trabalhos futuros.

Por fim, além de responder aos objetivos propostos, essa tese contribui para abertura de novas perguntas, algumas das quais pretendemos responder em trabalhos futuros, sendo elas:

- Como a estrutura de redes de interação formiga-planta varia espacialmente e como é influenciada por características ambientais, por comunidades, e por recursos disponíveis no ambiente?

- Há variação de características funcionais (por exemplo, pilosidade) dentro das colônias de *C. crassus*? É possível observar alterações morfológicas dentro de uma mesma geração, com indivíduos expostos à diferentes condições ambientais (temperatura, umidade, disponibilidade de recursos)?

- Outras espécies de formigas também apresentam plasticidade fenotípica e funcional no gradiente latitudinal analisado? Como está distribuída a diversidade funcional entre espécies neste gradiente?

- Como está distribuída a diversidade genética (i.e estruturação genética) de *C. crassus* ao longo do gradiente latitudinal de cerrado? Dissimilaridades ambientais, de comunidades, e de recursos entre localidades são acompanhadas de dissimilaridades genéticas?

- A paisagem no entorno das áreas analisadas influencia os níveis de diversidade biológica analisados, ou as características locais (i.e. em menor escala) são mais importantes?

- Nesta tese avaliamos as formigas nas plantas do Cerrado. Os padrões aqui observados são também encontrados para as formigas de solo? Como se distribui a diversidade genética e funcional deste grupo de formigas?

Com isso, pretendemos dar continuidade aos trabalhos aqui apresentados e encorajamos futuros trabalhos a olharem para as questões levantadas, aumentando ainda mais nosso conhecimento sobre os fatores que moldam a biodiversidade nas savanas neotropicais.

REFERÊNCIAS

Introdução:

Allen, A.P., Brown, J.H. & Gillooly, J.F. (2002). Global biodiversity, biochemical kinetics, and the energetic-equivalence rule. *Science*, 297, 1545–1548.

Bronstein, E. (2015). *Mutualism*. Oxford University Press, Oxford, New York.

Brown, J.H. (2014). Why are there so many species in the tropics? *J. Biogeogr.*, 41, 8–22.

Calixto, E.S., Lange, D., Moreira, X. & Del-Claro, K. (2021). Plant species specificity of ant–plant mutualistic interactions: Differential predation of termites by *Camponotus crassus* on five species of extrafloral nectaries plants. *Biotropica*, 53, 1406–1414.

Campos, R.I., Vasconcelos, H.L., Andersen, A.N., Frizzo, T.L.M. & Spena, K.C. (2011). Multi-scale ant diversity in savanna woodlands: an intercontinental comparison. *Austral Ecology*, 36, 983–992.

Christianini, A.V., Mayhé-Nunes, A.J. & Oliveira, P.S. (2007). The role of ants in the removal of non-myrmecochorous diaspores and seed germination in a Neotropical savanna. *Journal of Tropical Ecology*, 23, 343–351.

Colwell, R.K. (2009). Biodiversity: Concepts, Patterns, and Measurement. In: *The Princeton Guide to Ecology* (eds. Levin, S.A., Carpenter, S.R., Godfray, H.C.J., Kinzig, A.P., Loreau, M., Losos, J.B., et al.). Princeton University Press, pp. 257–263.

Cowell, R.K. & Hurtt, G.C. (1994). Nonbiological gradients in species richness and a spurious Rapoport effect. *The American Naturalist*, 144, 570–595.

Currie, D.J. (1991). Energy and Large-Scale Patterns of Animal- and Plant-Species Richness. *The American Naturalist*, 137, 27–49.

Dáttilo, W. & Vasconcelos, H.L. (2019). Macroecological patterns and correlates of ant–tree interaction networks in Neotropical savannas. *Global Ecol Biogeogr*, 28, 1283–1294.

Del-Claro, K. & Oliveira, P.S. (2000). Conditional outcomes in a neotropical treehopper-ant association: temporal and species-specific variation in ant protection and homopteran fecundity. *Oecologia*, 124, 156–165.

Del-Toro, I., Ribbons, R.R. & Pelini, S.L. (2012). The little things that run the world revisited: a review of ant-mediated ecosystem services and disservices (Hymenoptera: Formicidae). *Myrmecological News*, 17, 133–146.

Díaz, S. & Malhi, Y. (2022). Biodiversity: Concepts, Patterns, Trends, and Perspectives. *Annu. Rev. Environ. Resour.*, 47, 31–63.

Gaston, K.J. (2000). Global patterns in biodiversity. *Nature*, 405, 220–227.

Grace, J.B., Scheiner, S.M. & Schoolmaster Jr, D.R. (2015). Structural equation modeling: Building and evaluating causal models. In: *Ecological Statistics: Contemporary Theory and Application* (eds. Fox, G.A., Negrete-Yankelevich, S. & Sosa, V.J.). Oxford University Press, Oxford, pp. 168–199.

Hillebrand, H. (2004). On the generality of the latitudinal diversity gradient. *Am Nat*, 163, 192–211.

Hölldobler, B. & Wilson, E.O. (1990). *The Ants:* Belknap Press, Cambridge, MA.

Leal, I.R. & Oliveira, P.S. (1998). Interactions between fungus-growing Ants (Attini), fruits and seeds in Cerrado vegetation in Southeast Brazil1. *Biotropica*, 30, 170–178.

Lopes, B.C. (1995). Treehoppers (Homoptera, Membracidae) in southeastern Brazil: use of host plants. *Ver. Bras. Zool.*, 12, 595–608.

Magalhães, V.B., Espírito Santo, N.B., Salles, L.F.P., Soares, H. & Oliveira, P.S. (2018). Secondary seed dispersal by ants in Neotropical cerrado savanna: species-specific effects on seeds and seedlings of *Siparuna guianensis* (Siparunaceae). *Ecol Entomol*, 43, 665–674.

Mittelbach, G.G., Schemske, D.W., Cornell, H.V., Allen, A.P., Brown, J.M., Bush, M.B., *et al.* (2007). Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. *Ecol Letters*, 10, 315–331.

Morais, H.C. (1980). Estrutura de uma comunidade de formigas arborícolas em vegetação de campo cerrado. Master's thesis. Unversidade Estadual de Campinas, Campinas, Brazil.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B. & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, 403, 853–858.

Oliveira, P.S. & Brandão, C.R.F. (1991). The ant community associated with extrafloral nectaries in the Brazilian Cerrados. In: *Ant-Plant Interactions* (eds. Cutler, D.F. & Huxley, C.R.). Oxford University Press, Oxford, pp. 198–212.

Oliveira, P.S. & Freitas, A.V.L. (2004). Ant-plant-herbivore interactions in the Neotropical cerrado savanna. *Naturwissenschaften*, 91, 557–570.

Oliveira, P.S. & Leitao-Filho, H.F. (1987). Extrafloral nectaries: their taxonomic distribution and abundance in the woody flora of cerrado vegetation in southeast Brazil. *Biotropica*, 19, 140–148.

Oliveira, P.S. & Oliveira-Filho, A.T. (1991). Distribution of extrafloral nectaries in the woody flora of tropical communities in Western Brazil. In: Plant-Animal Interactions: Evolutionary Ecology in Tropical and Temperate Regions (eds. Price, P.W., Lewinsohn, T.M., Fernandes, G.W. & Benson, W.W.). John Wiley & Sons, New York, pp. 163–175.

Oliveira, P.S. (1997). The ecological function of extrafloral nectaries: herbivore deterrence by visiting ants and reproductive output in *Caryocar 131rasiliense* (Caryocaraceae). *Funct Ecology*, 11, 323–330.

Oliveira, P.S. & Marquis, R.J. (2002). The Cerrados of Brazil: Ecology and Natural History of a Neotropical Savanna. Columbia University Press, New York.

Oliveira, P.S., da Silva, A.F. & Martins, A.B. (1987). Ant foraging on extrafloral nectaries of *Qualea grandiflora* (Vochysiaceae) in cerrado vegetation: ants as potential antiherbivore agents. *Oecologia*, 74, 228–230.

Oliveira-Filho, A.T. & Ratter, J.A. (2002). Vegetation physiognomies and woody flora of the Cerrado biome. In: *The Cerrados of Brazil: Ecology and Natural History of a Neotropical Savanna* (eds. Oliveira , P.S. & Marquis, R.J.). Columbia University Press, New York, pp. 91–120.

Pianka, E.R. (1966). Latitudinal Gradients in Species Diversity: A Review of Concepts. *The American Naturalist*, 100, 33–46.

Ribas, C.R., Schoereder, J.H., Pic, M. & Soares, S.M. (2003). Tree heterogeneity, resource availability, and larger scale processes regulating arboreal ant species richness. *Austral Ecology*, 28, 305–314.

Rico-Gray, V. & Oliveira, P.S. (2007). *The Ecology and Evolution of Ant-Plant Interactions*. University of Chicago Press, Chicago, IL.

Rohde, K. (1992). Latitudinal gradients in species diversity: the search for the primary cause. *Oikos*, 65, 514–527.

Rosenzweig, M.L. (1995). Species Diversity in Space and Time. Cambridge University Press, Cambridge.

Schoereder, J.H., Sobrinho, T.G., Madureira, M.S., Ribas, C.R. & Oliveira, P.S. (2010). The arboreal ant community visiting extrafloral nectaries in the Neotropical cerrado savanna. *Terr Arthropod Rev*, 3, 3–27.

Schultheiss, P., Nooten, S.S., Wang, R., Wong, M.K.L., Brassard, F. & Guénard, B. (2022). The abundance, biomass, and distribution of ants on Earth. *Proc. Natl. Acad. Sci. U.S.A.*, 119, e2201550119.

Vasconcelos, H.L., Maravalhas, J.B., Feitosa, R.M., Pacheco, R., Neves, K.C. & Andersen, A.N. (2018). Neotropical savanna ants show a reversed latitudinal gradient of species richness, with climatic drivers reflecting the forest origin of the fauna. *J Biogeogr*, 45, 248–258.

Vasconcelos, H.L., Neves, K.C. & Andersen, A.N. (2023). Vertical stratification of ant assemblages varies along a latitudinal gradient in Brazilian savanna. *Journal of Biogeography*, 50, 1331–1340.

Vieira, L.T.A., Azevedo, T.N., Castro, A.A.J.F. & Martins, F.R. (2022). Reviewing the Cerrado’s limits, flora distribution patterns, and conservation status for policy decisions. *Land Use Policy*, 115, 106038.

Willig, M.R., Kaufman, D.M. & Stevens, R.D. (2003). Latitudinal Gradients of Biodiversity: Pattern, Process, Scale, and Synthesis. *Annu. Rev. Ecol. Evol. Syst.*, 34, 273–309.

Wilson, E. (1988). *Biodiversity*. National Academies Press, Washington, D.C.

Capítulo 1:

Aguiar, J.J.M., Anjos, D.V., Carvalho, R.L., De Almeida, W., Santos, A.C.C. & Santos, J.C. (2022). Plant richness drives ant diversity in *Eucalyptus* -dominated landscape on Brazilian savanna. *Austral Ecology*, 47, 17–25.

Araújo, L.M., Lara, A.C.F. & Fernandes, G.W. (1995). Utilization of *Apion* sp. (Coleoptera Apionidae) galls by an ant community in southeastern Brazil. *Tropical Zoology*, 8, 319–324.

Barton, K. (2022). MuMIn: Multi-Model Inference.

Bates, D., Mächler, M., Bolker, B. & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Soft.*, 67.

Beattie, A. & Hughes, L. (2002). Ant-plant interactions. In: *Plant-animal interactions: an evolutionary approach* (eds. Herrera, C.M. & Pellmyr, O.). Blackwell Science Limited.

Benson, W.W. (1985). Amazon ant-plants. In: Amazonia. (eds. Prance, G.T. & Lovejoy, T.T.). Oxford: Pergamon Press, pp. 239–266.

Bentley, B.L. (1977). Extrafloral Nectaries and Protection by Pugnacious Bodyguards. *Annu. Rev. Ecol. Syst.*, 8, 407–427.

Blanchet, F.G., Cazelles, K. & Gravel, D. (2020). Co-occurrence is not evidence of ecological interactions. *Ecol Lett*, 23, 1050–1063.

Blüthgen, N., E. Stork, N. & Fiedler, K. (2004). Bottom-up control and co-occurrence in complex communities: honeydew and nectar determine a rainforest ant mosaic. *Oikos*, 106, 344–358.

Blüthgen, N. & Stork, N.E. (2007). Ant mosaics in a tropical rainforest in Australia and elsewhere: A critical review. *Austral Ecol*, 32, 93–104.

Boudouris, J. & Queenborough, S.A. (2013). Diversity and distribution of extra-floral nectaries in the cerrado savanna vegetation of Brazil. *PeerJ*, 1, e219.

Bronstein, J.L. (2015). *Mutualism*. First edition. Oxford University Press, Oxford, United Kingdom.

Bronstein, J.L. & Barbosa, P. (2002). Multitrophic/multispecies mutualistic interactions: The role of non-mutualists in shaping and mediating mutualisms. In: *Multitrophic level interactions* (eds. Tscharntke, T. & Hawkins, B.A.). Cambridge University Press, Cambridge.

Burns, D.D.R., Franks, D.W., Parr, C. & Robinson, E.J.H. (2021). Ant colony nest networks adapt to resource disruption. *J Anim Ecol*, 90, 143–152.

Byk, J. & Del-Claro, K. (2010). Nectar- and pollen-gathering *Cephalotes* ants provide no protection against herbivory: a new manipulative experiment to test ant protective capabilities. *Acta Ethol*, 13, 33–38.

Campos, R.I., Vasconcelos, H.L., Andersen, A.N., Frizzo, T.L.M. & Spena, K.C. (2011). Multi-scale ant diversity in savanna woodlands: an intercontinental comparison. *Austral Ecology*, 36, 983–992.

Carroll, C.R. & Janzen, D.H. (1973). Ecology of foraging by ants. *Annual Review of Ecology and Systematics*, 4, 231–257.

Carvalho, J.L.N., Carlos Eduardo Pelegrino, C., Feigl, B.J., Píccolo, M.D.C., Godinho, V.D.P., Herpin, U., *et al.* (2009). Conversion of cerrado into agricultural land in the south-western Amazon: carbon stocks and soil fertility. *Sci. agric. (Piracicaba, Braz.)*, 66, 233–241.

Catford, J.A., Wilson, J.R.U., Pyšek, P., Hulme, P.E. & Duncan, R.P. (2022). Addressing context dependence in ecology. *Trends in Ecology & Evolution*, 37, 158–170.

Cayuela, L., Granzow-de La Cerda, Í., Albuquerque, F.S. & Golicher, D.J. (2012). taxonstand: An r package for species names standardisation in vegetation databases. *Methods Ecol Evol*, 3, 1078–1083.

Chamberlain, S.A., Bronstein, J.L. & Rudgers, J.A. (2014). How context dependent are species interactions? *Ecol Lett*, 17, 881–890.

Chamberlain, S.A. & Holland, J.N. (2009). Quantitative synthesis of context dependency in ant–plant protection mutualisms. *Ecology*, 90, 2384–2392.

Chomicki, G., Weber, M., Antonelli, A., Bascompte, J. & Kiers, E.T. (2019). The impact of mutualisms on species richness. *Trends in Ecology & Evolution*, 34, 698–711.

Cleveland, C.C., Townsend, A.R., Taylor, P., Alvarez-Clare, S., Bustamante, M.M.C., Chuyong, G., *et al.* (2011). Relationships among net primary productivity, nutrients and climate in tropical rain forest: a pan-tropical analysis. *Ecology Letters*, 14, 939–947.

Coutinho, L.M. (1982). Ecological Effects of Fire in Brazilian Cerrado. In: *Ecology of Tropical Savannas* (eds. Huntley, B.J. & Walker, B.H.). Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 273–291.

Dáttilo, W. & Vasconcelos, H.L. (2019). Macroecological patterns and correlates of ant–tree interaction networks in Neotropical savannas. *Global Ecol Biogeogr*, 28, 1283–1294.

Davidson, D.W. & Mckey, D. (1993). The evolutionary ecology of symbiotic ant-plant relationships. *Journal of Hymenoptera Research*, 2, 13–83.

Del-Claro, K. & Oliveira, P.S. (1993). Ant-homoptera interaction: do alternative sugar sources distract tending ants? *Oikos*, 68, 202–206.

Del-Claro, K. & Oliveira, P.S. (2000). conditional outcomes in a neotropical treehopper-ant association: temporal and species-specific variation in ant protection and homopteran fecundity. *Oecologia*, 124, 156–165.

Díaz-Castelazo, C., Rico-Gray, V., Oliveira, P.S. & Cuautle, M. (2004). Extrafloral nectary-mediated ant-plant interactions in the coastal vegetation of Veracruz, Mexico. *Écoscience*, 11, 472–481.

Díaz-Castelazo, C., Chavarro-Rodríguez, N. & Rico-Gray, V. (2017). Interhabitat Variation in the Ecology of Extrafloral Nectar Production and Associated Ant Assemblages in Mexican Landscapes. In: *Ant-Plant Interactions* (eds. Oliveira, P.S. & Koptur, S.). Cambridge University Press, pp. 179–199.

Dormann, C.F., Gruber, B. & Fruend, J. (2008). Introducing the bipartite package: analysing ecological networks. *R news*, 8, 8–11.

Dunkley, K., Cable, J. & Perkins, S.E. (2020). Consistency in mutualism relies on local, rather than wider community biodiversity. *Sci Rep*, 10, 21255.

Emerson, B.C. & Gillespie, R.G. (2008). Phylogenetic analysis of community assembly and structure over space and time. *Trends in Ecology & Evolution*, 23, 619–630.

Fick, S.E. & Hijmans, R.J. (2017). WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *Int. J. Climatol*, 37, 4302–4315.

Fink, J.R., Inda, A.V., Tiecher, T. & Barrón, V. (2016). Iron oxides and organic matter on soil phosphorus availability. *Ciênc. agrotec.*, 40, 369–379.

Frouz, J. & Jilková, V. (2008). The effect of ants on soil properties and processes (Hymenoptera: Formicidae). *Myrmecological News*, 11, 191–199.

Gillman, L.N., Wright, S.D., Cusens, J., McBride, P.D., Malhi, Y. & Whittaker, R.J. (2015). Latitude, productivity and species richness. *Global Ecology and Biogeography*, 24, 107–117.

Grace, J.B., Scheiner, S.M. & Schoolmaster Jr, D.R. (2015). Structural equation modeling: building and evaluating causal models. In: *Ecological Statistics: Contemporary Theory and Application* (eds. Fox, G.A., Negrete-Yankelevich, S. & Sosa, V.J.). Oxford University Press, Oxford.

Hardin, G. (1960). The competitive exclusion principle. *Science*, 131, 1292–1297.

Haridasan, M. (2008). Nutritional adaptations of native plants of the cerrado biome in acid soils. *Braz. J. Plant Physiol.*, 20, 183–195.

Hartig, F. (2022). DHARMa: Residual diagnostics for hierarchical (Multi-Level / Mixed) regression models.

Hawkins, B.A., Field, R., Cornell, H.V., Currie, D.J., Guégan, J.-F., Kaufman, D.M., *et al.* (2003). Energy, water, and broad-scale geographic patterns of species richness. *Ecology*, 84, 3105–3117.

Hoek, T.A., Axelrod, K., Biancalani, T., Yurtsev, E.A., Liu, J. & Gore, J. (2016). Resource availability modulates the cooperative and competitive nature of a microbial cross-feeding mutualism. *PLoS Biol*, 14, e1002540.

Hoeksema, J.D. & Bruna, E.M. (2015). Context-dependent outcomes of mutualistic interactions. In: *Mutualism* (ed. Bronstein, J.L.). Oxford University Press, Oxford.

Holland, J.N., Ness, J.H., Boyle, A. & Bronstein, J.L. (2005). Mutualisms as consumer-resource interactions. In: *Ecology of predator–prey interactions* (eds. Barbosa, P. & Castellanos, I.). Oxford University Press, Oxford.

Hurlbert, S.H. (1971). The nonconcept of species diversity: a critique and alternative parameters. *Ecology*, 52, 577–586.

Jobbágy, E.G. & Jackson, R.B. (2000). The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications*, 10, 423–436.

Keeler, K.H., Porturas, L.D. & Weber, M.G. World list of plants with extrafloral nectaries. www.extrafloralnectaries.org. Accessed in November 2021.

Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D., *et al.* (2010). Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, 26, 1463–1464.

Kerkhoff, A.J. & Enquist, B.J. (2006). Ecosystem allometry: the scaling of nutrient stocks and primary productivity across plant communities. *Ecology Letters*, 9, 419–427.

Koptur, S. (1992). Extrafloral nectary-mediated interactions between insects and plants. In: *Insect-Plant Interactions* (ed. Bernays, E.A.). CRC Press.

Koptur, S. (2005). Nectar as fuel for plant protectors. In: *Plant-Provided Food for Carnivorous Insects* (eds. Wäckers, F.L., Van Rijn, P.C.J. & Bruin, J.). Cambridge University Press, pp. 75–108.

Lasmar, C.J., Bishop, T.R., Parr, C.L., Queiroz, A.C.M., Schmidt, F.A. & Ribas, C.R. (2021). Geographical variation in ant foraging activity and resource use is driven by climate and net primary productivity. *J Biogeogr*, 48, 1448–1459.

Leal, L.C., Nogueira, A. & Peixoto, P.E.C. (2023). Which traits optimize plant benefits? Meta-analysis on the effect of partner traits on the outcome of an ant–plant protective mutualism. *Journal of Ecology*, 111, 263–275.

Leal, L.C. & Peixoto, P.E.C. (2017). Decreasing water availability across the globe improves the effectiveness of protective ant-plant mutualisms: a meta-analysis: Water availability drives mutualism outcome. *Biol Rev*, 92, 1785–1794.

Lefcheck, J.S. (2016). PIECEWISESEM: Piecewise structural equation modelling in R for ecology, evolution, and systematics. *Methods Ecol Evol*, 7, 573–579.

Leston, D. (1973). The ant mosaic - tropical tree crops and the limiting of pests and diseases. *PANS Pest Articles & News Summaries*, 19, 311–341.

Linacre, E. & Geerts, B. (1997). Climates and weather explained: an introduction from a southern perspective. Routledge, London.

Lira-Martins, D., Nascimento, D.L., Abrahão, A., De Britto Costa, P., D’Angioli, A.M., Valézio, E., *et al.* (2022). Soil properties and geomorphic processes influence vegetation composition, structure, and function in the Cerrado Domain. *Plant Soil*, 476, 549–588.

Liu, O.R. & Gaines, S.D. (2022). Environmental context dependency in species interactions. *Proc. Natl. Acad. Sci. U.S.A.*, 119, e2118539119.

Lopes, B.C. (1995). Treehoppers (Homoptera: Membracidae) in southeast Brazil: use of host plants. *Revista Brasileira de Zoologia*, 12, 595–608.

Lüdecke, D., Ben-Shachar, M., Patil, I., Waggoner, P. & Makowski, D. (2021). performance: an R package for assessment, comparison and testing of statistical models. *JOSS*, 6, 3139.

Machado, S.R., Morellato, L.P.C., Sajo, M.G. & Oliveira, P.S. (2008). Morphological patterns of extrafloral nectaries in woody plant species of the Brazilian cerrado. Plant Biology, 10, 660–673.

Marquis, R.J., Morais, H.C. & Diniz, I.R. (2002). Interactions among cerrado plants and their herbivores: unique or typical? In: *The Cerrados of Brazil* (eds. Oliveira, P. & Marquis, R.). Columbia University Press, pp. 266–284.

Morais, H.C. (1980). Estrutura de uma comunidade de formigas arborícolas em vegetação de campo cerrado. Master's thesis. Universidade Estadual de Campinas, Campinas, Brasil.

Moreau, C.S., Bell, C.D., Vila, R., Archibald, S.B. & Pierce, N.E. (2006). phylogeny of the ants: diversification in the age of angiosperms. *Science*, 312, 101–104.

Muehleisen, A. (2013). incidence of extra-floral nectaries and their effect on the growth and survival of lowland tropical rain forest trees. Honors Research Thesis. The Ohio State University.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., Da Fonseca, G.A.B. & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, 403, 853–858.

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., *et al.* (2022). vegan: community ecology package .

Oliveira, P.S. & Leitao-Filho, H.F. (1987). Extrafloral nectaries: their taxonomic distribution and abundance in the woody flora of cerrado vegetation in Southeast Brazil. Biotropica, 19, 140–148.

Oliveira, P.S. & Brandão, C.R.F. (1991). The ant community associated with extrafloral nectaries in the Brazilian cerrados. In: *Ant-plant interactions* (eds. Huxley, C.R. & Cutler, D.F.). Oxford University Press, Oxford.

Oliveira, P.S. & Oliveira-Filho, A.T. (1991). Distribution of extrafloral nectaries in the woody flora of tropical communities in Western Brazil. In: *Plant-Animal Interactions: Evolutionary Ecology in Tropical and Temperate Regions* (eds. Price, P.W., Lewinsohn, T.M., Fernandes, G.W. & Benson, W.W.). John Wiley & Sons, New York, pp. 163–175.

Oliveira, P.S. & Del-Claro, K. (2005). Multitrophic interactions in a Neotropical savanna: ant–hemipteran systems, associated insect herbivores and a host plant. In: *Biotic Interactions in the Tropics* (eds. Burslem, D., Pinard, M. & Hartley, S.). Cambridge University Press, pp. 414–438.

Oliveira, P.S. & Freitas, A.V.L. (2004). Ant-plant-herbivore interactions in the Neotropical Cerrado savanna. *Naturwissenschaften*, 91, 557–570.

Oliveira, P.S. & Marquis, R.J. (2002). The Cerrados of Brazil: Ecology and Natural History of a Neotropical Savanna. Columbia University Press, New York.

Oliveira-Filho, A.T. & Ratter, J.A. (2002). Vegetation physiognomies and woody flora of the cerrado biome. In: *The cerrados of Brazil: ecology and natural history of a Neotropical savanna* (eds. Oliveira, P.S. & Marquis, R.J.). Columbia University Press, New York.

Palmer, T.M., Pringle, E.G., Stier, A. & Holt, R.D. (2015). mutualism in a community context. In: *Mutualism* (ed. Bronstein, J.L.). Oxford University Press, Oxford.

Paradis, E. & Schliep, K. (2019). ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, 35, 526–528.

Powell, S., Costa, A.N., Lopes, C.T. & Vasconcelos, H.L. (2011). Canopy connectivity and the availability of diverse nesting resources affect species coexistence in arboreal ants. *Journal of Animal Ecology*, 80, 352–360.

Pringle, E.G. (2016). Orienting the interaction compass: resource availability as a major driver of context dependence. *PLoS Biol*, 14, e2000891.

Qian, H. & Jin, Y. (2016). An updated megaphylogeny of plants, a tool for generating plant phylogenies and an analysis of phylogenetic community structure. *JPECOL*, 9, 233–239.

Qian, H. & Jin, Y. (2021). Are phylogenies resolved at the genus level appropriate for studies on phylogenetic structure of species assemblages? *Plant Diversity*, 43, 255–263.

R Core Team. (2022). *R: A language and environment for statistical computing*.

Ribas, C.R. & Schoereder, J.H. (2004). determining factors of arboreal ant mosaics in Cerrado vegetation (Hymenoptera: Formicidae). *Sociobiology*, 43, 49–68.

Ribas, C.R., Schoereder, J.H., Pic, M. & Soares, S.M. (2003). Tree heterogeneity, resource availability, and larger scale processes regulating arboreal ant species richness. *Austral Ecology*, 28, 305–314.

Rico-Gray, V. & Oliveira, P.S. (2007). The Ecology and Evolution of Ant-Plant Interactions. The University of Chicago Press.

Roitman, I., Bustamante, M.M.C., Haidar, R.F., Shimbo, J.Z., Abdala, G.C., Eiten, G., *et al.* (2018). Optimizing biomass estimates of savanna woodland at different spatial scales in the Brazilian Cerrado: Re-evaluating allometric equations and environmental influences. *PLoS ONE*, 13, e0196742.

Rosumek, F.B., Silveira, F.A.O., De S. Neves, F., De U. Barbosa, N.P., Diniz, L., Oki, Y., *et al.* (2009). Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. *Oecologia*, 160, 537–549.

Running, Steve & Zhao, Maosheng. (2019). MYD17A3HGF MODIS/Aqua Net Primary Production Gap-Filled Yearly L4 Global 500 m SIN Grid V006.

Sanderson, M. (1999). The classification of climates from Pythagoras to Koeppen. *Bulletin of the American Meteorological Society*, 80, 669–673.

Schoereder, J.H., Sobrinho, T.G., Madureira, M.S., Ribas, C.R. & Oliveira, P.S. (2010). The arboreal ant community visiting extrafloral nectaries in the Neotropical cerrado savanna. *Terr Arthropod Rev*, 3, 3–27.

Sebastián-González, E., Dalsgaard, B., Sandel, B. & Guimarães, P.R. (2015). Macroecological trends in nestedness and modularity of seed-dispersal networks: human impact matters. *Global Ecology and Biogeography*, 24, 293–303.

Sendoya, S.F., Freitas, A.V.L. & Oliveira, P.S. (2009). Egg-laying butterflies distinguish predaceous ants by sight. *The American Naturalist*, 174, 134–140.

Sendoya, S.F., Blüthgen, N., Tamashiro, J.Y., Fernandez, F. & Oliveira, P.S. (2016). Foliage-dwelling ants in a Neotropical savanna: effects of plant and insect exudates on ant communities. *Arthropod-Plant Interactions*, 10, 183–195.

Shipley, B. (2000). a new inferential test for path models based on directed acyclic graphs. *Structural Equation Modeling*, 7, 206–18.

Shipley, B. (2009). Confirmatory path analysis in a generalized multilevel context. *Ecology*, 90, 363–368.

Silva, D.P. & Oliveira, P.S. (2010). Field biology of *Edessa rufomarginata* (Hemiptera: Pentatomidae): phenology, behavior, and patterns of host plant use. *Env. Entom.*, 39, 1903–1910.

Smith, S.A. & Brown, J.W. (2018). Constructing a broadly inclusive seed plant phylogeny. *Am J Bot*, 105, 302–314.

Stein, A., Gerstner, K. & Kreft, H. (2014). Environmental heterogeneity as a universal driver of species richness across taxa, biomes and spatial scales. *Ecol Lett*, 17, 866–880.

Stone, L. (2020). The stability of mutualism. *Nat Commun*, 11, 2648.

Stone, L. & Roberts, A. (1990). The checkerboard score and species distributions. *Oecologia*, 85, 74–79.

Stone, L. & Roberts, A. (1991). Conditions for a species to gain advantage from the presence of competitors. *Ecology*, 72, 1964–1972.

Styrsky, J.D. & Eubanks, M.D. (2007). Ecological consequences of interactions between ants and honeydew-producing insects. *Proc. R. Soc. B.*, 274, 151–164.

Tews, J., Brose, U., Grimm, V., Tielbörger, K., Wichmann, M.C., Schwager, M., *et al.* (2004). Animal species diversity driven by habitat heterogeneity/diversity: the importance of keystone structures: Animal species diversity driven by habitat heterogeneity. *Journal of Biogeography*, 31, 79–92.

Thrall, P.H., Hochberg, M.E., Burdon, J.J. & Bever, J.D. (2007). Coevolution of symbiotic mutualists and parasites in a community context. *Trends in Ecology & Evolution*, 22, 120–126.

Trager, M.D., Bhotika, S., Hostetler, J.A., Andrade, G.V., Rodriguez-Cabal, M.A., McKeon, C.S., *et al.* (2010). Benefits for plants in ant-plant protective mutualisms: a meta-analysis. *PLoS ONE*, 5, e14308.

Trøjelsgaard, K. & Olesen, J.M. (2013). Macroecology of pollination networks: Macroecology of pollination networks. *Global Ecology and Biogeography*, 22, 149–162.

Ulrich, W., Kubota, Y., Kusumoto, B., Baselga, A., Tuomisto, H. & Gotelli, N.J. (2018). Species richness correlates of raw and standardized co-occurrence metrics. *Global Ecol Biogeogr*, 27, 395–399.

Vasconcelos, H.L., Maravalhas, J.B., Feitosa, R.M., Pacheco, R., Neves, K.C. & Andersen, A.N. (2018). Neotropical savanna ants show a reversed latitudinal gradient of species richness, with climatic drivers reflecting the forest origin of the fauna. *J Biogeogr*, 45, 248–258.

Vieira, L.T.A., Azevedo, T.N., Castro, A.A.J.F. & Martins, F.R. (2022). Reviewing the Cerrado’s limits, flora distribution patterns, and conservation status for policy decisions. *Land Use Policy*, 115, 106038.

Wagner, D. & Fleur Nicklen, E. (2010). Ant nest location, soil nutrients and nutrient uptake by ant-associated plants: does extrafloral nectar attract ant nests and thereby enhance plant nutrition? *Journal of Ecology*, 98, 614–624.

Yamawo, A. (2017). Plasticity and efficacy of defense strategies against herbivory in ant-visited plants growing in variable abiotic conditions. In: *Ant-Plant Interactions* (eds. Oliveira, P.S. & Koptur, S.). Cambridge University Press, Cambridge.

Zuur, A.F., Ieno, E.N., Walker, N., Saveliev, A.A. & Smith, G.M. (2009). Mixed effects models and extensions in ecology with R. Springer, New York.

Capítulo 1 – Material suplementar:

Beattie, A. & Hughes, L. (2002). Ant-plant interactions. In: *Plant-animal interactions: an evolutionary approach* (eds. Herrera, C.M. & Pellmyr, O.). Blackwell Science Limited.

Boudouris, J. & Queenborough, S.A. (2013). Diversity and distribution of extra-floral nectaries in the cerrado savanna vegetation of Brazil. *PeerJ*, 1, e219.

James, G., Witten, D., Hastie, T. & Tibshirani, R. (2013). An Introduction to Statistical Learning. Springer Texts in Statistics. Springer New York, New York, NY.

Oliveira, P.S. & Freitas, A.V.L. (2004). Ant-plant-herbivore interactions in the Neotropical cerrado savanna. *Naturwissenschaften*, 91, 557–570.

Schoereder, J.H., Sobrinho, T.G., Madureira, M.S., Ribas, C.R. & Oliveira, P.S. (2010). The arboreal ant community visiting extrafloral nectaries in the Neotropical cerrado savanna. *Terr Arthropod Rev*, 3, 3–27.

da Silva, N.A.P., Frizzas, M.R. & Oliveira, C.M.D. (2011). Seasonality in insect abundance in the "Cerrado" of Goiás State, Brazil. *Rev. Bras. entomol.*, 55, 79–87.

Song, Y., Zhou, D., Zhang, H., Li, G., Jin, Y. & Li, Q. (2013). Effects of vegetation height and density on soil temperature variations. *Chin. Sci. Bull.*, 58, 907–912.

Yao, Z., Xin, Y., Yang, L., Zhao, L. & Ali, A. (2022). Precipitation and temperature regulate species diversity, plant coverage and aboveground biomass through opposing mechanisms in large-scale grasslands. *Front. Plant Sci.*, 13, 999636.

Zuur, A.F., Ieno, E.N., Walker, N., Saveliev, A.A. & Smith, G.M. (2009). Mixed effects models and extensions in ecology with R. Springer, New York.

<u>Capítulo 2:</u>

Adamack, A.T. & Gruber, B. (2014). PopGen Report: simplifying basic population genetic analyses in R. *Methods Ecol Evol*, 5, 384–387.

Antao, T., Lopes, A., Lopes, R.J., Beja-Pereira, A. & Luikart, G. (2008). LOSITAN: A workbench to detect molecular adaptation based on a Fst-outlier method. *BMC Bioinformatics*, 9, 323.

Barton, K. (2022). MuMIn: Multi-Model Inference.

Bates, D., Mächler, M., Bolker, B. & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48.

Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W. & Courchamp, F. (2012). Impacts of climate change on the future of biodiversity: Biodiversity and climate change. *Ecology Letters*, 15, 365–377.

Ben-Shachar, M., Lüdecke, D. & Makowski, D. (2020). effectsize: estimation of effect size indices and standardized parameters. *JOSS*, 5, 2815.

Berg, M.P., Kiers, E.T., Driessen, G., Van Der Heijden, M., Kooi, B.W., Kuenen, F., *et al.* (2010). Adapt or disperse: understanding species persistence in a changing world. *Global Change Biology*, 16, 587–598.

Bierne, N., Roze, D. & Welch, J.J. (2013). Pervasive selection or is it…? Why are FST outliers sometimes so frequent? *Mol Ecol*, 22, 2061–2064.

Bishop, T.R., Robertson, M.P., Van Rensburg, B.J. & Parr, C.L. (2017). Coping with the cold: minimum temperatures and thermal tolerances dominate the ecology of mountain ants: Thermal tolerances of mountain ants. *Ecol Entomol*, 42, 105–114.

Blüthgen, N., E. Stork, N. & Fiedler, K. (2004). Bottom-up control and co-occurrence in complex communities: honeydew and nectar determine a rainforest ant mosaic. *Oikos*, 106, 344–358.

Blüthgen, N. & Fiedler, K. (2004). Preferences for sugars and amino acids and their conditionality in a diverse nectar-feeding ant community. *Journal of Animal Ecology*, 73, 155–166.

Bolnick, D.I., Amarasekare, P., Araújo, M.S., Bürger, R., Levine, J.M., Novak, M., et al. (2011). Why intraspecific trait variation matters in community ecology. *Trends in Ecology & Evolution*, 26, 183–192.

Brown, M.J.F. & Gordon, D.M. (2000). How resources and encounters affect the distribution of foraging activity in a seed-harvesting ant. *Behav Ecol Sociobiol*, 47, 195–203.

Bujan, J. & Kaspari, M. (2017). Nutrition modifies critical thermal maximum of a dominant canopy ant. *Journal of Insect Physiology*, 102, 1–6.

Bujan, J., Roeder, K.A., Yanoviak, S.P. & Kaspari, M. (2020). Seasonal plasticity of thermal tolerance in ants. *Ecology*, 101.

Buxton, J.T., Robert, K.A., Marshall, A.T., Dutka, T.L. & Gibb, H. (2021). A cross-species test of the function of cuticular traits in ants (Hymenoptera: Formicidae). *Myrmecological News*, 31–46.

Calixto, E.S., Lange, D., Moreira, X. & Del-Claro, K. (2021). Plant species specificity of ant–plant mutualistic interactions: Differential predation of termites by *Camponotus crassus* on five species of extrafloral nectaries plants. *Biotropica*, 53, 1406–1414.

Campos, R.I., Vasconcelos, H.L., Andersen, A.N., Frizzo, T.L.M. & Spena, K.C. (2011). Multi-scale ant diversity in savanna woodlands: an intercontinental comparison. *Austral Ecology*, 36, 983–992.

Catchen, J.M., Amores, A., Hohenlohe, P., Cresko, W. & Postlethwait, J.H. (2011). Stacks: building and genotyping loci de novo from short-read sequences. *G3 Genes|Genomes|Genetics*, 1, 171–182.

Cerdá, X., Retana, J. & Cros, S. (1998). Critical thermal limits in Mediterranean ant species: trade-off between mortality risk and foraging performance. *Functional Ecology*, 12, 45–55.

Chown, S. & Nicolson, S.W. (2004). Insect physiological ecology: mechanisms and patterns. Oxford University Press, Oxford ; New York.

Chown, S.L. & Gaston, K.J. (1999). Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. *Biological Reviews*, 74, 87–120.

Chown, S.L., Slabber, S., McGeoch, M.A., Janion, C. & Leinaas, H.P. (2007). Phenotypic plasticity mediates climate change responses among invasive and indigenous arthropods. *Proc. R. Soc. B.*, 274, 2531–2537.

Colli, G.R., Vieira, C.R. & Dianese, J.C. (2020). Biodiversity and conservation of the Cerrado: recent advances and old challenges. *Biodivers Conserv*, 29, 1465–1475.

Costa-Milanez, C.B. da Majer, J.D., Castro, P. de T.A. & Ribeiro, S.P. (2017). Influence of soil granulometry on average body size in soil ant assemblages: implications for bioindication. *Perspectives in Ecology and Conservation*, 15, 102–108.

Cushman, J.H., Lawton, J.H. & Manly, B.F.J. (1993). Latitudinal patterns in European ant assemblages: variation in species richness and body size. *Oecologia*, 95, 30–37.

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., *et al.* (2011). The variant call format and VCFtools. *Bioinformatics*, 27, 2156–2158.

Dangles, O. & Casas, J. (2019). Ecosystem services provided by insects for achieving sustainable development goals. *Ecosystem Services*, 35, 109–115.

Darwin, C. (1859). On the origin of species by means of natural selection. John Murray, London, UK.

Davenport, J. (1992). Basic concepts. In: *Animal Life at Low Temperature*. Springer Netherlands, Dordrecht, pp. 3–32.

Davidson, D.W. (1978). Size variability in the worker caste of a social insect (*Veromessor pergandei* Mayr) as a function of the competitive environment. *The American Naturalist*, 112, 523–532.

Del-Claro, K. & Oliveira, P.S. (1993). Ant-homoptera interaction: do alternative sugar sources distract tending ants? *Oikos*, 68, 202–206.

Del-Claro, K. & Oliveira, P.S. (2000). conditional outcomes in a neotropical treehopper-ant association: temporal and species-specific variation in ant protection and homopteran fecundity. *Oecologia*, 124, 156–165.

Del Toro, I., Ribbons, R.R. & Pelini, S.L. (2012). The little things that run the world revisited: a review of ant-mediated ecosystem services and disservices (Hymenoptera: Formicidae). *Myrmecological News*, 133–146.

Des Roches, S., Post, D.M., Turley, N.E., Bailey, J.K., Hendry, A.P., Kinnison, M.T., *et al.* (2017). The ecological importance of intraspecific variation. *Nat Ecol Evol*, 2, 57–64.

Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C., *et al.* (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl. Acad. Sci. U.S.A.*, 105, 6668–6672.

Diamond, S.E. & Chick, L.D. (2018). Thermal specialist ant species have restricted, equatorial geographic ranges: implications for climate change vulnerability and risk of extinction. *Ecography*, 41, 1507–1509.

Diamond, S.E., Sorger, D.M., Hulcr, J., Pelini, S.L., Toro, I.D., Hirsch, C., *et al.* (2012). Who likes it hot? A global analysis of the climatic, ecological, and evolutionary determinants of warming tolerance in ants. *Glob Change Biol*, 18, 448–456.

Dormann, C.F., Gruber, B. & Fruend, J. (2008). Introducing the bipartite Package: analysing ecological networks. *R news*, 8, 8–11

Eguchi, K., Oguri, E., Sasaki, T., Matsuo, A., Nguyen, D.D., Jaitrong, W., et al. (2020). Revisiting museum collections in the genomic era: Potential of MIG-seq for retrieving phylogenetic information from aged minute dry specimens of ants (Hymenoptera: Formicidae) and other small organisms. *Myrmecological News*, 30, 151–159.

Elizalde, L., Arbetman, M., Arnan, X., Eggleton, P., Leal, I.R., Lescano, M.N., et al. (2020). The ecosystem services provided by social insects: traits, management tools and knowledge gaps. *Biol Rev*, 95, 1418–1441.

Fagundes, N.J.R., Ray, N., Beaumont, M., Neuenschwander, S., Salzano, F.M., Bonatto, S.L., *et al.* (2007). Statistical evaluation of alternative models of human evolution. *Proc. Natl. Acad. Sci. U.S.A.*, 104, 17614–17619.

Fick, S.E. & Hijmans, R.J. (2017). WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *Int. J. Climatol*, 37, 4302–4315.

Fitzpatrick, G., Lanan, M.C. & Bronstein, J.L. (2014). Thermal tolerance affects mutualist attendance in an ant–plant protection mutualism. *Oecologia*, 176, 129–138.

Foll, M. & Gaggiotti, O. (2008). A Genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a bayesian perspective. *Genetics*, 180, 977–993.

François, O., Martins, H., Caye, K. & Schoville, S.D. (2016). Controlling false discoveries in genome scans for selection. *Mol Ecol*, 25, 454–469.

García-Robledo, C., Kuprewicz, E.K., Staines, C.L., Erwin, T.L. & Kress, W.J. (2016). Limited tolerance by insects to high temperatures across tropical elevational gradients and the implications of global warming for extinction. *Proc. Natl. Acad. Sci. U.S.A.*, 113, 680–685.

Gentile, G., Bonelli, S. & Riva, F. (2021). Evaluating intraspecific variation in insect trait analysis. *Ecological Entomology*, 46, 11–18.

Ghalambor, C.K., McKAY, J.K., Carroll, S.P. & Reznick, D.N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct Ecology*, 21, 394–407.

Gibb, H., Stoklosa, J., Warton, D.I., Brown, A.M., Andrew, N.R. & Cunningham, S.A. (2015). Does morphology predict trophic position and habitat use of ant species and assemblages? *Oecologia*, 177, 519–531.

Global Solar Atlas 2.0, a free, web-based application is developed and operated by the company Solargis s.r.o. on behalf of the World Bank Group, utilizing Solargis data, with funding provided by the Energy Sector Management Assistance Program (ESMAP). Available at: https://globalsolaratlas.info. Last accessed April 2023.

Gnatzy, W. & Maschwitz, U. (2006). Pedestal hairs of the ant *Echinopla melanarctos* (Hymenoptera, Formicidae): morphology and functional aspects. *Zoomorphology*, 125, 57–68.

Gouws, E.J., Gaston, K.J. & Chown, S.L. (2011). Intraspecific body size frequency distributions of insects. *PLOS ONE*, 6, e16606.

Habibullah, M.S., Din, B.H., Tan, S.-H. & Zahid, H. (2022). Impact of climate change on biodiversity loss: global evidence. *Environ Sci Pollut Res*, 29, 1073–1086.

Halsch, C.A., Shapiro, A.M., Fordyce, J.A., Nice, C.C., Thorne, J.H., Waetjen, D.P., *et al.* (2021). Insects and recent climate change. *Proc. Natl. Acad. Sci. U.S.A.*, 118, e2002543117.

Hannon, G.J. (2010). FASTX-Toolkit.

Hartig, F. (2022). DHARMa: residual diagnostics for hierarchical (Multi-Level / Mixed) Regression Models.

Harvey, J.A., Tougeron, K., Gols, R., Heinen, R., Abarca, M., Abram, P.K., *et al.* (2023). Scientists' warning on climate change and insects. *Ecological Monographs*, 93.

Hölldobler, B. & Wilson, E.O. (1990). *The Ants*. Belknap Press of Harvard University Press, Cambridge.

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., *et al.* (2005). Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecological Monographs*, 75, 3–35.

Hortal, J., De Bello, F., Diniz-Filho, J.A.F., Lewinsohn, T.M., Lobo, J.M. & Ladle, R.J. (2015). Seven shortfalls that beset large-scale knowledge of biodiversity. *Annu. Rev. Ecol. Evol. Syst.*, 46, 523–549.

Hothorn, T., Bretz, F. & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biom J*, 50, 346–363.

Hurlbert, S.H. (1971). The nonconcept of species diversity: a critique and alternative parameters. *Ecology*, 52, 577–586.

IPCC. (2022). Climate Change 2022: Mitigation of Climate Change. Contribution of Working Group III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK and New York, NY, USA.

Kaspari, M. (1996). Worker size and seed size selection by harvester ants in a neotropical forest. *Oecologia*, 105, 397–404.

Kaspari, M., Clay, N.A., Lucas, J., Yanoviak, S.P. & Kay, A. (2015). Thermal adaptation generates a diversity of thermal limits in a rainforest ant community. *Global Change Biology*, 21, 1092–1102.

Kaspari, M. & Weiser, M.D. (1999). The size–grain hypothesis and interspecific scaling in ants. *Functional Ecology*, 13, 530–538.

Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D., et al. (2010). Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, 26, 1463–1464.

Kempf, W.W. (1972). Catálogo abreviado das formigas da região Neotropical. *Studia Entomologica*, 15, 3–344.

King, A.M. & MacRae, T.H. (2015). Insect heat shock proteins during stress and diapause. *Annu Rev Entomol*, 60, 59–75.

Lai, J., Zou, Y., Zhang, S., Zhang, X. & Mao, L. (2022). glmm.hp: an R package for computing individual effect of predictors in generalized linear mixed models. *Journal of Plant Ecology*, 15, 1302–1307.

van Lampe, F. & Schellenberg, J. (2023). goeveg: functions for community data and ordinations.

Lanan, M. (2014). Spatiotemporal resource distribution and foraging strategies of ants (Hymenoptera: Formicidae). *Myrmecol News*, 20, 53–70.

Lange, D., Calixto, E.S., Rosa, B.B., Sales, T.A. & Del-Claro, K. (2019). Natural history and ecology of foraging of the *Camponotus crassus* Mayr, 1862 (Hymenoptera: Formicidae). *Journal of Natural History*, 53, 1737–1749.

Legendre, P. (1993). Spatial Autocorrelation: Trouble or New Paradigm? *Ecology*, 74, 1659–1673.

Lüdecke, D., Ben-Shachar, M.S., Patil, I., Waggoner, P. & Makowski, D. (2021). performance: an R package for assessment, comparison and testing of statistical models. *Journal of Open-Source Software*, 6, 3139.

McGill, B.J., Enquist, B.J., Weiher, E. & Westoby, M. (2006). Rebuilding community ecology from functional traits. *Trends in Ecology & Evolution*, 21, 178–185.

Menzel, F. & Feldmeyer, B. (2021). How does climate change affect social insects? *Current Opinion in Insect Science*, 46, 10–15.

Merilä, J. & Hendry, A.P. (2014). Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evol Appl*, 7, 1–14.

Moreau, C.S., Bell, C.D., Vila, R., Archibald, S.B. & Pierce, N.E. (2006). Phylogeny of the ants: diversification in the age of angiosperms. *Science*, 312, 101–104.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., Da Fonseca, G.A.B. & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, 403, 853–858.

Nascimento, G., Câmara, T. & Arnan, X. (2022). Critical thermal limits in ants and their implications under climate change. *Biological Reviews*, 97, 1287–1305.

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., *et al.* (2022). vegan: community ecology package.

Okuzaki, Y., Takami, Y. & Sota, T. (2010). Resource partitioning or reproductive isolation: the ecological role of body size differences among closely related species in sympatry. *Journal of Animal Ecology*, 79, 383–392.

Oliveira, P.S., da Silva, A.F. & Martins, A.B. (1987). Ant foraging on extrafloral nectaries of *Qualea grandiflora* (Vochysiaceae) in cerrado vegetation: ants as potential antiherbivore agents. *Oecologia*, 74, 228–230.

Oliveira, P.S. & Brandão, C.R.F. (1991). The ant community associated with extrafloral nectaries in the Brazilian cerrados. In: *Ant-plant interactions* (eds. Huxley, C.R. & Cutler, D.F.). Oxford University Press, Oxford.

Oliveira, P.S. & Marquis, R.J. (2002). The Cerrados of Brazil: Ecology and Natural History of a Neotropical Savanna. Columbia University Press, New York.

Oliveira, P.S. & Freitas, A.V.L. (2004). Ant-plant-herbivore interactions in the Neotropical cerrado savanna. *Naturwissenschaften*, 91, 557–570.

Oliveira-Filho, A.T. & Ratter, J.A. (2002). Vegetation physiognomies and woody flora of the cerrado biome. In: *The Cerrados of Brazil: Ecology and Natural History of a Neotropical Savanna* (eds. Oliveira, P.S. & Marquis, R.J.). Columbia University Press, pp. 91–120.

Oms, C.S., Cerdá, X. & Boulay, R. (2017). Is phenotypic plasticity a key mechanism for responding to thermal stress in ants? *Sci Nat*, 104, 42.

Oyen, K.J., Giri, S. & Dillon, M.E. (2016). Altitudinal variation in bumble bee (Bombus) critical thermal limits. *J Therm Biol*, 59, 52–57.

Parr, C.L. & Bishop, T.R. (2022). The response of ants to climate change. *Global Change Biology*, 28, 3188–3205.

Parr, C.L., Dunn, R.R., Sanders, N.J., Weiser, M.D., Photakis, M., Bishop, T.R., *et al.* (2017). *GlobalAnts*: a new database on the geography of ant traits (Hymenoptera: Formicidae). *Insect Conserv Divers*, 10, 5–20.

Peters, R.H. (1983). The ecological implications of body size. Cambridge University Press.

Purcell, J., Pirogan, D., Avril, A., Bouyarden, F. & Chapuisat, M. (2016). Environmental influence on the phenotype of ant workers revealed by common garden experiment. *Behav Ecol Sociobiol*, 70, 357–367.

R Core Team. (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ravelle, W. (2022). psych: procedures for personality and psychological research.

Richardson, J.L., Urban, M.C., Bolnick, D.I. & Skelly, D.K. (2014). Microgeographic adaptation and the spatial scale of evolution. *Trends in Ecology & Evolution*, 29, 165–176.

Roeder, K.A., Roeder, D.V. & Bujan, J. (2021). Ant thermal tolerance: a review of methods, hypotheses, and sources of variation. *Annals of the Entomological Society of America*, 114, 459–469.

Schultheiss, P., Nooten, S.S., Wang, R., Wong, M.K.L., Brassard, F. & Guénard, B. (2022). The abundance, biomass, and distribution of ants on Earth. *Proc. Natl. Acad. Sci. U.S.A.*, 119, e2201550119.

Sendoya, S.F., Freitas, A.V.L. & Oliveira, P.S. (2009). Egg-laying butterflies distinguish predaceous ants by sight. *Am Nat*, 174, 134–140.

Sendoya, S.F., Blüthgen, N., Tamashiro, J.Y., Fernandez, F. & Oliveira, P.S. (2016). Foliage-dwelling ants in a neotropical savanna: effects of plant and insect exudates on ant communities. *Arthropod-Plant Interactions*, 10, 183–195.

Shik, J.Z., Arnan, X., Oms, C.S., Cerdá, X. & Boulay, R. (2019). Evidence for locally adaptive metabolic rates among ant populations along an elevational gradient. *J Anim Ecol*, 88, 1240–1249.

Silva, D.P. & Oliveira, P.S. (2010). Field biology of *Edessa rufomarginata* (Hemiptera: Pentatomidae): phenology, behavior, and patterns of host plant use. *Environ Entomol*, 39, 1903–1910.

Sinclair, B.J., Marshall, K.E., Sewell, M.A., Levesque, D.L., Willett, C.S., Slotsbo, S., *et al.* (2016). Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecol Lett*, 19, 1372–1385.

Slatyer, R.A., Hirst, M. & Sexton, J.P. (2013). Niche breadth predicts geographical range size: a general ecological pattern. *Ecol Lett*, 16, 1104–1114.

Smith, S.A. & Brown, J.W. (2018). Constructing a broadly inclusive seed plant phylogeny. *Am J Bot*, 105, 302–314.

Smouse, P.E., Long, J.C. & Sokal, R.R. (1986). Multiple regression and correlation extensions of the mantel test of matrix correspondence. *Systematic Zoology*, 35, 627–632.

Stouffer, D.B., Rezende, E.L. & Amaral, L.A.N. (2011). The role of body mass in diet contiguity and food-web structure: Body mass and food-web structure. *Journal of Animal Ecology*, 80, 632–639.

Suyama, Y. & Matsuki, Y. (2015). MIG-seq: an effective PCR-based method for genome-wide single-nucleotide polymorphism genotyping using the next-generation sequencing platform. *Sci Rep*, 5, 16963.

Vieira, L.T.A., Azevedo, T.N., Castro, A.A.J.F. & Martins, F.R. (2022). Reviewing the Cerrado’s limits, flora distribution patterns, and conservation status for policy decisions. *Land Use Policy*, 115, 106038.

Wagner, D.L. (2020). Insect declines in the anthropocene. *Annu. Rev. Entomol.*, 65, 457–480.

Weber, N.A. (1938). The biology of fungus-growing ants. Part 4. Additional new forms. Part 5. The Attini of Bolivia. *Revista de Entomolgia*, 9, 154–206.

West-Eberhard, M.J. (1989). Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics*, 20, 249–278.

Whitman, D. & Ananthakrishnan, T. (Eds.). (2009). Phenotypic plasticity of insects: mechanisms and consequences. Science Publishers.

Williams, C.M., Buckley, L.B., Sheldon, K.S., Vickers, M., Pörtner, H.-O., Dowd, W.W., *et al.* (2016). Biological impacts of thermal extremes: mechanisms and costs of functional responses matter. *Integr. Comp. Biol.*, 56, 73–84.

Willmer, P.G. (1982). Microclimate and the environmental physiology of insects. In: *Advances in Insect Physiology*. Elsevier, pp. 1–57.

Wills, B.D., Powell, S., Rivera, M.D. & Suarez, A.V. (2018). correlates and consequences of worker polymorphism in ants. *Annu. Rev. Entomol.*, 63, 575–598.

Wilson, E.O. (1971). The insect societies. Harvard University Press, Cambridge.

Wong, M.K.L., Guénard, B. & Lewis, O.T. (2019). Trait-based ecology of terrestrial arthropods. *Biol Rev*, 94, 999–1022.

Wong, M.K.L. & Carmona, C.P. (2021). Including intraspecific trait variability to avoid distortion of functional diversity and ecological inference: Lessons from natural assemblages. *Methods Ecol Evol*, 12, 946–957.

Zuur, A.F., Ieno, E.N., Walker, N., Saveliev, A.A. & Smith, G.M. (2009). Mixed effects models and extensions in ecology with R. Statistics for Biology and Health. Springer, New York, NY.

Capítulo 3:

Agashe, D. & Bolnick, D.I. (2010). Intraspecific genetic variation and competition interact to influence niche expansion. *Proc. R. Soc. B.*, 277, 2915–2924.

Azevedo-Silva, M., Côrtes, M.C., Carvalho, C.S., Mori, G.M., Souza, A.P. & Oliveira, P.S. (2023a). Landscape genetics in a highly threatened environment: how relevant to ants is the physiognomic mosaic of the cerrado savanna? *Conserv Genet*.

Azevedo-Silva, M., Lemos, A.S.M., Gonçalves-Neto, S., Salles, L.F.P., Pereyra, M., Christianini, A.V., *et al.* (2023b). Are there edge effects on the genetic diversity of the trap-jaw ant *Odontomachus chelifer* (Formicidae: Ponerinae) in a Neotropical Savanna fragment? A first assessment. *Environmental Entomology*, 52, 279–285.

Azevedo-Silva, M., Mori, G.M., Carvalho, C.S., Côrtes, M.C., Souza, A.P. & Oliveira, P.S. (2020). Breeding systems and genetic diversity in tropical carpenter ant colonies: different strategies for similar outcomes in Brazilian Cerrado savanna. *Zoological Journal of the Linnean Society*, 190, 1020–1035.

Ben-Shachar, M., Lüdecke, D. & Makowski, D. (2020). effectsize: estimation of effect size indices and standardized parameters. *JOSS*, 5, 2815.

Boomsma, J.J. & Leusink, A. (1981). Weather conditions during nuptial flights of four european ant species. *Oecologia*, 50, 236–241.

Calixto, E.S., Lange, D., Moreira, X. & Del-Claro, K. (2021). Plant species specificity of ant–plant mutualistic interactions: Differential predation of termites by *Camponotus crassus* on five species of extrafloral nectaries plants. *Biotropica*, 53, 1406–1414.

Campos, R.I., Vasconcelos, H.L., Andersen, A.N., Frizzo, T.L.M. & Spena, K.C. (2011). Multi-scale ant diversity in savanna woodlands: an intercontinental comparison. *Austral Ecology*, 36, 983–992.

Colwell, R.K. (2009). Biodiversity: concepts, patterns, and measurement. In: *The Princeton Guide to Ecology* (eds. Levin, S.A., Carpenter, S.R., Godfray, H.C.J., Kinzig, A.P., Loreau, M., Losos, J.B., et al.). Princeton University Press, pp. 257–263.

Dáttilo, W. & Vasconcelos, H.L. (2019). Macroecological patterns and correlates of ant–tree interaction networks in Neotropical savannas. *Global Ecol Biogeogr*, 28, 1283–1294.

Del-Claro, K. & Oliveira, P.S. (2000). Conditional outcomes in a neotropical treehopper-ant association: temporal and species-specific variation in ant protection and homopteran fecundity. *Oecologia*, 124, 156–165.

Depa, L. (2006). Weather conditions during nuptial flight of *Manica rubida* (LATREILLE, 1802) (Hymenoptera: Formicidae) in southern Poland. *Myrmecological News*, 9, 27–32.

Ellegren, H. & Galtier, N. (2016). Determinants of genetic diversity. *Nat Rev Genet*, 17, 422–433.

Gaston, K.J. (2000). Global patterns in biodiversity. *Nature*, 405, 220–227.

Gotelli, N., Ellison, A., Dunn, R. & Sanders, N. (2011). Counting ants (Hymenoptera: Formicidae): biodiversity sampling and statistical analysis for myrmecologists. *Myrmecological News*, 15, 13–19.

Goudet, J. & Jombart, T. (2022). hierfstat: estimation and tests of hierarchical F-statistics.

Gratton, P., Marta, S., Bocksberger, G., Winter, M., Keil, P., Trucchi, E., *et al.* (2017). Which latitudinal gradients for genetic diversity? *Trends in Ecology & Evolution*, 32, 724–726.

Grover, J.P. (1997). Resource Competition. Springer US, Boston, MA.

Hartig, F. (2022). DHARMa: residual diagnostics for hierarchical (multi-level / mixed) regression models.

Hartl, D. & ClarkAG. (2010). Princípios de genética de populações. Artmed.

Hawkins, B.A., Field, R., Cornell, H.V., Currie, D.J., Guégan, J.-F., Kaufman, D.M., *et al.* (2003). Energy, water, and broad-scale geographic patterns of species richness. *Ecology*, 84, 3105–3117.

Hedrick, P.W. (1986). Genetic polymorphism in heterogeneous environments: a decade later. *Annu. Rev. Ecol. Syst.*, 17, 535–566.

Hijmans , R. (2022). geosphere: spherical trigonometry.

Hillebrand, H. (2004). On the generality of the latitudinal diversity gradient. *Am Nat*, 163, 192–211.

Hofmann, G.S., Cardoso, M.F., Alves, R.J.V., Weber, E.J., Barbosa, A.A., Toledo, P.M., *et al.* (2021). The Brazilian Cerrado is becoming hotter and drier. *Glob. Change Biol.*, 27, 4060–4073.

Hölldobler, B. & Wilson, E.O. (1990). The Ants. Belknap Press of Harvard University Press, Cambridge.

Hughes, A.R., Inouye, B.D., Johnson, M.T.J., Underwood, N. & Vellend, M. (2008). Ecological consequences of genetic diversity: Ecological effects of genetic diversity. *Ecology Letters*, 11, 609–623.

Jousset, A., Eisenhauer, N., Merker, M., Mouquet, N. & Scheu, S. (2016). High functional diversity stimulates diversification in experimental microbial communities. *Sci. Adv.*, 2, e1600124.

Lange, D., Calixto, E.S., Rosa, B.B., Sales, T.A. & Del-Claro, K. (2019). Natural history and ecology of foraging of the *Camponotus crassus* Mayr, 1862 (Hymenoptera: Formicidae). *Journal of Natural History*, 53, 1737–1749.

Leffler, E.M., Bullaughey, K., Matute, D.R., Meyer, W.K., Ségurel, L., Venkat, A., *et al.* (2012). Revisiting an old riddle: what determines genetic diversity levels within species? *PLOS Biology*, 10, e1001388.

Martin, P.R. & McKay, J.K. (2004). Latitudinal variation in genetic divergence of populations and the potential for future speciation. *Evolution*, 58, 938–945.

McGlynn, T.P., Hoover, J.R., Jasper, G.S., Kelly, M.S., Polis, A.M., Spangler, C.M., *et al.* (2002). Resource heterogeneity affects demography of the Costa Rican ant *Aphaenogaster araneoides*. *J. Trop. Ecol.*, 18, 231–244.

Miraldo, A., Li, S., Borregaard, M.K., Flórez-Rodríguez, A., Gopalakrishnan, S., Rizvanovic, M., *et al.* (2016). An Anthropocene map of genetic diversity. *Science*, 353, 1532–1535.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B. & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, 403, 853–858.

Oliveira, P.S. & Brandão, C.R.F. (1991). The ant community associated with extrafloral nectaries in the Brazilian cerrados. In: Ant-plant interactions (eds. Huxley, C.R. & Cutler, D.F.). Oxford University Press, Oxford.

Oliveira, P.S. (1997). The ecological function of extrafloral nectaries: herbivore deterrence by visiting ants and reproductive output in *Caryocar brasiliense* (Caryocaraceae). *Funct Ecology*, 11, 323–330.

Oliveira, P.S. & Freitas, A.V.L. (2004). Ant-plant-herbivore interactions in the neotropical cerrado savanna. *Naturwissenschaften*, 91, 557–570.

Oliveira, P.S. & Leitao-Filho, H.F. (1987). Extrafloral nectaries: their taxonomic distribution and abundance in the woody flora of Cerrado vegetation in Southeast Brazil. *Biotropica*, 19, 140.

Oliveira-Filho, A. & Ratter, J. (2002). Vegetation physiognomies and woody flora of the cerrado biome. In: *The Cerrados of Brazil: Ecology and Natural History of a Neotropical Savanna* (eds. Oliveira, P. & Marquis, R.). Columbia University Press, pp. 91–120.

Pamilo, P. (1988). Genetic variation in heterogeneous environments. *Annales Zoologici Fennici*, 25, 99–106.

Paradis, E. & Schliep, K. (2019). ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, 35, 526–528.

Pereira, H.M. (2016). A latitudinal gradient for genetic diversity. *Science*, 353, 1494–1495.

Pereira-Romeiro, M.P., Vanin, G.T., Azevedo-Silva, M. & Mori, G.M. (2022). Natural history of *Camponotus renggeri* and *Camponotus rufipes* (Hymenoptera: Formicidae) in an Atlantic Forest reserve, Brazil. *Insect. Soc.*, 69, 369–374.

Pianka, E.R. (1966). Latitudinal gradients in species diversity: a review of concepts. *The American Naturalist*, 100, 33–46.

Qian, H. & Kissling, W.D. (2010). Spatial scale and cross-taxon congruence of terrestrial vertebrate and vascular plant species richness in China. *Ecology*, 91, 1172–1183.

Ravelle, W. (2022). psych: procedures for personality and psychological research.

Ribas, C.R., Schoereder, J.H., Pic, M. & Soares, S.M. (2003). Tree heterogeneity, resource availability, and larger scale processes regulating arboreal ant species richness. *Austral Ecology*, 28, 305–314.

Romiguier, J., Gayral, P., Ballenghien, M., Bernard, A., Cahais, V., Chenuil, A., *et al.* (2014). Comparative population genomics in animals uncovers the determinants of genetic diversity. *Nature*, 515, 261–263.

Rosenzweig, M.L. (1995). Species Diversity in Space and Time. 1st edn. Cambridge University Press.

Schär†, S., Vila†, R., Petrović, A., Tomanović, Ž., Pierce, N.E. & Nash, D.R. (2017). Molecular substitution rate increases with latitude in butterflies: evidence for a trans-glacial latitudinal layering of populations? *Ecography*, 40, 930–935.

Sendoya, S.F., Freitas, A.V.L. & Oliveira, P.S. (2009). Egg-Laying Butterflies Distinguish Predaceous Ants by Sight. *The American Naturalist*, 174, 134–140.

Silva, R.R. & Brandão, C.R.F. (2014). Ecosystem-wide morphological structure of leaf-litter ant communities along a tropical latitudinal gradient. *PLoS ONE*, 9, e93049.

Stein, A., Gerstner, K. & Kreft, H. (2014). Environmental heterogeneity as a universal driver of species richness across taxa, biomes and spatial scales. *Ecol Lett*, 17, 866–880.

Stein, A. & Kreft, H. (2015). Terminology and quantification of environmental heterogeneity in species-richness research: Environmental heterogeneity and species richness. *Biol Rev*, 90, 815–836.

Suyama, Y. & Matsuki, Y. (2015). MIG-seq: an effective PCR-based method for genome-wide single-nucleotide polymorphism genotyping using the next-generation sequencing platform. *Sci Rep*, 5, 16963.

Tavecchia, G., Pradel, R., Genovart, M. & Oro, D. (2007). Density-dependent parameters and demographic equilibrium in open populations. *Oikos*, 116, 1481–1492.

Tews, J., Brose, U., Grimm, V., Tielbörger, K., Wichmann, M.C., Schwager, M., *et al.* (2004). Animal species diversity driven by habitat heterogeneity/diversity: the importance of keystone structures: Animal species diversity driven by habitat heterogeneity. *Journal of Biogeography*, 31, 79–92.

Tilman, D. (1982). Resource Competition and Community Structure. Princeton University Press, Princeton.

Torres, J.A., Snelling, R.R. & Canals, M. (2001). Seasonal and nocturnal periodicities in ant nuptial flights in the tropics (Hymenoptera: Formicidae). *Sociobiology. 37(3B):601-626.*, 37, 601–626.

Vasconcelos, H.L., Maravalhas, J.B., Feitosa, R.M., Pacheco, R., Neves, K.C. & Andersen, A.N. (2018). Neotropical savanna ants show a reversed latitudinal gradient of species richness, with climatic drivers reflecting the forest origin of the fauna. *J Biogeogr*, 45, 248–258.

Vasconcelos, H.L., Neves, K.C. & Andersen, A.N. (2023). Vertical stratification of ant assemblages varies along a latitudinal gradient in Brazilian savanna. *Journal of Biogeography*, 50, 1331–1340.

Vellend, M. (2006). The consequences of genetic diversity in competitive communities. *Ecology*, 87, 304–311.

Vellend, M. & Geber, M.A. (2005). Connections between species diversity and genetic diversity: Species diversity and genetic diversity. *Ecology Letters*, 8, 767–781.

Vieira, L.T.A., Azevedo, T.N., Castro, A.A.J.F. & Martins, F.R. (2022). Reviewing the Cerrado's limits, flora distribution patterns, and conservation status for policy decisions. *Land Use Policy*, 115, 106038.

Weider, L.J., Jeyasingh, P.D. & Looper, K.G. (2008). Stoichiometric differences in food quality: impacts on genetic diversity and the coexistence of aquatic herbivores in a Daphnia hybrid complex. *Oecologia*, 158, 47–55.

Willig, M.R., Kaufman, D.M. & Stevens, R.D. (2003). Latitudinal gradients of biodiversity: pattern, process, scale, and synthesis. *Annu. Rev. Ecol. Evol. Syst.*, 34, 273–309.

Zeileis, A. & Hothorn, T. (2002). Diagnostic checking in regression relationships. *R News* , 2, 7–10.

ANEXOS

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Em observância ao **§5º do Artigo 1º da Informação CCPG-UNICAMP/001/15**, referente a Bioética e Biossegurança, declaro que o conteúdo de minha Tese de Doutorado, intitulada "***ANTS, BIODIVERSITY AND LATITUDINAL GRADIENT: INVESTIGATING INTERACTIONS WITH PLANTS, FUNCTIONAL VARIATION AND GENETIC DIVERSITY IN THE CERRADO SAVANNA***", desenvolvida no Programa de Pós-Graduação em Ecologia do Instituto de Biologia da Unicamp, não versa sobre pesquisa envolvendo seres humanos, animais ou temas afetos a Biossegurança.

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