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Sugar-Rich Resources Mediate Geographic Morphological Variation in a Dominant, Neotropical Savanna Ant

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ABSTRACT

Aim: Trait variation across geographic gradients can reveal how species respond to different environmental settings, which is crucial under the growing threat of climate change. Although on the basis of evolutionary theory, the patterns and drivers of intraspecific functional variation remain largely underexplored. In ants, pilosity and body size are morphological traits associated to thermoregulation and heat tolerance, which are critical concerns in the context of global warming. Here, we focused on the dominant ant *Camponotus crassus* to investigate trait variation and its potential drivers across a latitudinal gradient in the Brazilian Cerrado savanna.

Location: Brazilian Cerrado savanna.

Taxon: Camponotus crassus Mayr, 1862 (Hymenoptera: Formicidae).

Methods: We measured mesosoma pilosity and body size of *C. crassus* across multiple sites, and evaluated their relationship with *temperature*, *rainfall*, *solar radiation*, *vegetation* and *sugar-rich resource* variables. We also assessed morphological and genetic covariation to search for possible phenotypic plasticity or adaptation in *C. crassus*.

Results: Only sugar-rich resources were found to significantly influence *C. crassus* pilosity. Specifically, a negative relationship between ant pilosity and *sugar-rich resources* (i.e., proportion of plants with extrafloral nectaries and hemipteran trophobionts) was found. No covariation between pilosity and genetic dissimilarities was observed, suggesting phenotypic plasticity. None of the variables were significant to predict body size, while this trait positively covaried with genetics.

Main Conclusions: Our findings suggest resource availability as a critical factor for species thermoregulation under environmental change, a hypothesis previously reported in the literature. We emphasise the importance of examining intraspecific variation and phenotypic plasticity across large geographic scales, particularly under the scenario of rapid global change and the current threat to Cerrado savanna. Our work covers a still poorly investigated aspect of intraspecific variation of tropical eusocial

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insects, and sheds new light on trait variation associated with large geographical gradients and resource availability in a major ecosystem.

1 | Introduction

Trait-based ecology, which emphasises measurable characteristics affecting organisms' fitness (McGill et al. 2006), provides a framework for predicting the impacts of environmental changes on distinct biological levels (Schleuning et al. 2023). By making use of easily measurable morphological traits, this approach makes the assessment of organisms' phenotypic responses more feasible at large geographical scales such as latitudinal gradients, in which the diverse environmental conditions offer great opportunity to draw predictions into species' adaptability to changes (Bujan et al. 2020; Harvey et al. 2023; Schleuning et al. 2023; Gibb et al. 2024). This method is powerful given the Earth's rising temperatures—the hottest in 125,000 years (IPCC 2022)-which have amplified concerns about thermoregulation, especially for ectotherms that lack internal mechanisms of heat production (Davenport 1992). Insects, the most diverse animal lineage, are particularly vulnerable to warming (Williams et al. 2016; Wagner 2020; Halsch et al. 2021), risking essential ecosystem functions and services (Dangles and Casas 2019; Elizalde et al. 2020). Their small size and high surface area make them susceptible to temperature and rainfall variations, particularly in the tropics where many insects already live near their physiological limits (Deutsch et al. 2008; García-Robledo et al. 2016; Diamond and Chick 2018).

Many morphological traits-leg length, pilosity, cuticle roughness, body colour and size (Chown and Nicolson 2004; Nascimento et al. 2022)-correlates with thermoregulation in insects, making them suitable for studies across environmental gradients. The present study focuses on two of those traits: pilosity and body size, because the former may be a reliable predictor of insect physiology, and the latter may reduce dehydration and increase tolerance to higher temperatures (Buxton et al. 2021; Nascimento et al. 2022). Although the mechanisms connecting trait variation and environmental factors are unclear (Gibb et al. 2024), there are several hypotheses on how body size and pilosity are affected by biotic and abiotic factors. Body size is a fundamental physiological and ecological trait, often correlated with energy use, resource exploitation, species abundance and geographic distribution (Calder 1984; Peters 1983; Schimidt-Nielsen 1984). Smaller body sizes generally facilitate faster heat exchange, aligning with Bergmann's rule, which predicts the occurrence of larger individuals in colder environments (Bergmann 1847). Additionally, rainfall may positively influence body size, since larger individuals lose less water in dry conditions (Hadley 1994). In spite of these evidence, there are some contradictory examples in insects. Studies show that median ant body size decreases with higher temperatures and precipitation globally (Gibb et al. 2018), while wasps exhibit larger bodies in wetter environments (Kang et al. 2023). However, local studies with beetles found larger bodies at higher temperatures (Williamson et al. 2022). Pilosity, or body hair density (i.e., the number of hairs corrected by body size), is also associated with climatic variables, and is highlighted as a useful trait to predict responses by Hymenoptera to environmental changes (Peters

et al. 2016). Pilosity influences heat and desiccation tolerance, reduces water loss, enhances UV reflectance, increases thermal limits and is directly linked to maximal critical thermal limits (Wittlinger et al. 2007; Parr et al. 2017; Buxton et al. 2021). Pilosity is generally higher in colder environments, as observed in bees across elevational gradients (Peters et al. 2016), although an opposite pattern has been reported for beetles (Williamson et al. 2022). High pilosity also protects insects from solar radiation (Shi et al. 2015) and has also been reported in wetter environments (Oliveira et al. 2022).

Not only climatic variables are expected to impact insects' thermoregulatory morphological traits. Vegetation density, which is predicted to increase under global changes (De Frenne et al. 2019), impacts microclimates and may ultimately influence body size and pilosity. For example, large-bodied and hairier ants are often found in open environments (Gibb and Parr 2010; Arnan et al. 2014; Gibb et al. 2015). Resource availability is another concern under global changes, since warming can reduce primary productivity and food sources for many insects, drastically reducing their biomass (Hallmann et al. 2017; Huey and Kingsolver 2019). Diet is highly associated with thermal limits because carbohydrates (such as sucrose) provide quick energy and support heat-shock protein synthesis, crucial for thermal stress resistance (King and MacRae 2015). Thus, the decrease in sugar-rich carbohydrates predicted for dry forests under climate change (Magrin et al. 2014) would then directly compromise species thermoregulatory responses. Although there is no clear prediction on how resource availability impacts thermoregulatory morphological traits, larger body sizes in ant communities correlate with greater interaction with plants offering sugarrich resources (Chamberlain and Holland 2009). Although the relationship between resources and pilosity remains unclear, it warrants further investigation. Given the potential influences of climate, vegetation, and resources on body size and pilosity, simultaneous evaluation of these factors is valuable (Merilä and Hendry 2014). Latitudinal gradients, which encompass variation in multiple biotic and abiotic factors, are then particularly useful for investigating the effect of distinct environmental setups on insect's morphological variation (Gibb et al. 2024).

Ants serve as excellent models for investigating morphological trait-environment correlations. Besides their huge contribution to ecosystem functions (Lach et al. 2010; Del Toro et al. 2012; Oliveira and Koptur 2017), ants' quasi-sessile nature, nesting habits and wide morphological variation make them highly responsive to environmental changes (Wilson 1971; Hölldobler and Wilson 1990; Purcell et al. 2016; Menzel and Feldmeyer 2021). The role of pilosity and body size in ant thermoregulation was mechanistically demonstrated in a study of over 70 ant species and morphospecies. The findings showed that species with high pilosity and small body sizes tend to have higher critical thermal maxima, with high pilosity also associated with reduced water loss (Buxton et al. 2021). Although many studies have examined ant functional traits at the interspecific level (Gibb and Parr 2010; Arnan et al. 2013; Gibb et al. 2015, 2018, 2024), intraspecific variation remains underexplored (Barbosa et al. 2023; but see Oliveira et al. 2022). Yet, this variation is critical for understanding population responses to environmental changes. Indeed, high functional diversity within populations increases resilience to environmental shifts by preserving favourable genotypes (Hooper et al. 2005; Des Roches et al. 2017; Wong and Carmona 2021). Lastly, field studies combining ant morphological traits and genetics are scarce, making it challenging to disentangle phenotypic plasticity from genetic adaptation (Whitman and Ananthakrishnan 2009; Merilä and Hendry 2014). Thus, expanding research on intraspecific trait variation, particularly thermoregulatory traits, could clarify the roles of plasticity and adaptation in response to global changes (Oms et al. 2017).

The Brazilian Cerrado savanna offers a great setting for such investigations. As one of the world's biodiversity hotspots (Myers et al. 2000), the Cerrado covers nearly a quarter of Brazil's territory and extends over 2 million km² (Oliveira and Marquis 2002). It encompasses a mosaic of vegetation types, from grasslands to woodlands and harbours a rich biodiversity (Oliveira-Filho and Ratter 2002). Despite this, the Cerrado faces rapid vegetation loss, with only 8% of its original area under legal protection (Colli et al. 2020). Moreover, global change has made the Cerrado hotter and drier (Hofmann et al. 2021), likely affecting species' thermoregulatory responses. Ants occur in high abundance and

at high species diversity on the Cerrado ground and foliage (Campos et al. 2011). This biodiversity is partly explained by the prevalence of sugar-rich resources such as extrafloral nectaries (EFNs) and honeydew-producing insects (trophobionts), which are present in approximately 30% of the Cerrado plants (Oliveira and Freitas 2004).

To our knowledge, there is no study on how ant intraspecific thermoregulatory traits vary across latitudinal gradients in Cerrado, as well as their potential correlation to climate, vegetation and resource variables and covariation with genetic makeup. Here, we aimed to fill this gap by investigating the intraspecific body size and pilosity variation in the dominant ant species Camponotus crassus Mayr, 1862 (Formicinae; Figure 1A) at seven Cerrado sites across a latitudinal gradient. The choice for this species relies on the huge variation of body size and mesosoma pilosity within C. crassus, with individuals ranging from low (Figure 1B) to high (Figure 1C) levels of pilosity across different Cerrado areas (S. F. Sendoya, personal observation). Specifically, we examined whether pilosity and body size (Figure 1D) vary across the Cerrado gradient, and how these traits correlate with temperature, rainfall, solar radiation, vegetation and sugar-rich resources. Additionally, we evaluated the covariation between genetic and morphological traits to distinguish phenotypic plasticity from genetic adaptation. We hypothesize that C. crassus pilosity and body size will correlate with latitude due to climatic gradients. Specifically, we expect temperature, rainfall, solar radiation



FIGURE 1 | Morphological traits of *Camponotus crassus* used in this work, and corresponding hypotheses about this abundant ant species, commonly seen at liquid food sources in the Brazilian Cerrado savanna. (A) Worker of *C. crassus* tending a hemipteran trophobiont. (B) Specimen of *C. crassus* from Serra da Canastra (state of Minas Gerais, SE Brazil), showing low pilosity on the mesosoma and (C) worker from Itirapina, (state of São Paulo, SE Brazil), showing high pilosity on the mesosoma. (D) Schematic lateral view of an ant, 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 crossing the edge of the mesosoma profile (7 hairs on the side are not counted). (E) Hypotheses on the influence of conceptual predictors (temperature, rainfall, solar radiation, vegetation and sugar-rich resources) on ant pilosity and body size.

and *vegetation* traits to negatively affect body size, whereas *sugar-rich resources* affect it positively (Figure 1E). For pilosity, we predict negative effect from *temperature* and *vegetation* traits, and positive effects from *solar radiation* and *rainfall*. Given limited evidence on ant pilosity's response to resources, both positive and negative correlations are considered plausible (Figure 1E).

2 | Material and Methods

2.1 | 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* has an aggressive behaviour toward other insects nearby their food sources, effectively defending many plant species against herbivores in the Cerrado (Oliveira et al. 1987; Oliveira and Freitas 2004; Sendoya et al. 2009; Lange et al. 2019; Calixto et al. 2021).

2.2 | Study Sites and Data Collection

Between November 2012 and April 2013, woody plants at seven sites of Cerrado sensu stricto (dense scrub of shrubs and trees; Oliveira-Filho and Ratter 2002) were searched for ant-plant interactions (Figure 2A): (1) Estação Ecológica de Itirapina (state of São Paulo), (2) Parque Nacional Serra da Canastra and (3) Parque Nacional Serra do Cipó (both in the state of Minas Gerais), (4) Parque Nacional das Emas and (5) Parque Nacional Chapada dos Veadeiros (both in the state of Goiás), (6) Parque Nacional de Brasília (in Distrito Federal, Central Brazil) and (7) Parque Estadual Serra Azul (state of Mato Grosso). These sites are well preserved Cerrado areas, with pairwise distance between them ranging from about 173 km to 990 km (Table S1). In each locality, 5 transects of 200 m were established, at least 1 km apart from one another. Transects were divided into 20 parcels of 10 m length and data were collected in 10 alternate parcels (Figure 2B). To avoid subsampling large trees, in each parcel, we sampled all small woody plants (with Diameter at Soil Height-DSH-of 2 to 30 mm) up to 1 m from the central line of the transect (totalizing an area of 100 m² for small tree sampling) and all large trees (with DSH > 30 mm) up to 2 m from the central line of the transect (totalling an area of $200 \,\mathrm{m}^2$ for large tree sampling) (Figure 2B). For each plant, we recorded the DSH, height, the occurrence of EFNs and/or insect trophobionts and the foraging ants on foliage. Plants and ants were collected, morphotyped and identified at the species level whenever possible. Ants were preserved in 100% ethanol.

Each transect was characterised by its climatic, vegetational and resource variables, previously hypothesized to influence ant pilosity and body size (Figure 1E). We choose different explanatory operational variables to characterise the transect's



FIGURE 2 | Sampling sites and design. (A) Brazil (in grey) with the distribution of Cerrado savanna (in orange). Sampling sites are indicated by black dots. (B) Sampling design in each transect.

temperature, rainfall, solar radiation, vegetation and *sugar-rich resources* availability (Table 1). Mean annual temperature (Bio1), temperature seasonality (Bio4), annual precipitation (Bio12) and precipitation seasonality (Bio15) were obtained from WorldClim 2.0 database (Fick and Hijmans 2017), with resolution of 30s. *Solar radiation* was characterised by the annual direct normal irradiation (i.e., part of the solar irradiance directly reaching a surface; DNIy) and diffuse horizontal irradiation (i.e., solar irradiance scattered by the atmosphere, DIFy) from the Global Solar Atlas 2.0 (2023). *Vegetation* was characterised by (i) mean density of small and large plants (by dividing the number of small and large plants by the sampling area of 100 m² and 200 m², respectively); (ii) mean plant DSH; (iii) mean plant height. Finally, *sugar-rich resources* were described as the proportion of plants

TABLE 1Operational explanatory variables used for each conceptual predictor of Camponotus crassus morphological variation in Cerradosavanna.

Conceptual predictor	Operational variable	Abbreviation	
Temperature	Annual mean temperature	Bio1	
	Temperature seasonality		
Rainfall	Annual precipitation	Bio12	
	Precipitation seasonality	Bio15	
Solar radiation	Annual direct normal irradiation	DNIy	
	Annual diffuse horizontal irradiation	DIFy	
Vegetation	Mean vegetation density	Density	
	Mean plant height	Height	
	Mean plant diameter at soil height	DSH	
Sugar-rich resources	Proportion of plants with extrafloral nectaries	% EFN	
	Proportion of plants with trophobionts	% Trophobionts	

with EFNs (% EFN) and proportion of plants with trophobionts (% Trophobionts).

2.3 | Morphological and Genetic Data of *Camponotus crassus*

For morphological characterisation and Single Nucleotide Polymorphisms (SNPs) genotyping, we selected C. crassus specimens from samples collected 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 characterisation and SNP genotyping in subsequent molecular analyses, we did not pin the ants prior to morphological measurements. Accordingly, we used aquarium blue sand, previously sterilised in autoclave. This sand was chosen because it does not release residuals, enables easy positioning of the ants and creates a good contrast to observe the hairs on the mesosoma of the ants (Figure 1B,C). After ethanol evaporation, the workers were positioned in lateral view and pilosity was estimated following the Global Ants Database (GLAD) guidelines, based on which we counted the total number of hairs crossing the mesosoma profile (Gibb et al. 2015; Parr et al. 2017) (Figure 1D). We also estimated the Weber's length (a reliable estimate of body size in ants, see Kaspari and Weiser 1999), which consists of the distance between the anterodorsal margin of the pronotum and the posteroventral margin of the propodeum (Figure 1B; Weber 1938). All morphological analyses were performed under a M205C stereomicroscope (Leica) with the same zoom for all individuals. After morphological characterisation, ants were stored in 100% ethanol until DNA extraction. A total of 173 workers of C. crassus were morphologically described (2 to 9 individuals per transect) (Table S2).

Given that *C. crassus* pilosity can vary with body size, we estimated hair density by dividing the mesosoma pilosity by the Weber's length. We performed Pearson's correlation among the three morphological variables using the function *corPlot* implemented in the R package *psych* (Ravelle 2022). Pilosity was positively correlated with hair density and Weber's length (Figure S1). We decided to maintain the three morphological estimates, separately, as response variables in the subsequent analyses.

After morphological characterisation, we obtained the total genomic DNA of *C. crassus* workers. To preserve the specimens, genomic DNA was obtained through non-destructive DNA extraction, with DNeasy Blood & Tissue Kit (Qiagen) (see details in the Supporting Information S1). Due to degradation and low yields of DNA, we used a PCR based approach known as "multiplexed inter-simple-sequence repeats (ISSR) genotyping by sequencing" (MIG-seq) (Suyama and Matsuki 2015; Suyama et al. 2021). MIG-seq is an effective methodology for population genetic studies on aged and museum specimens, validated for ants (Eguchi et al. 2020). After amplification, SNP calling, filtering and testing for loci putatively under selection (see details in the Supporting Information S1), we obtained a total of 186 SNPs for 143 individuals of *C. crassus* (Table S2).

2.4 | Statistical Analyses

2.4.1 | Morphological Variation Across Sites

We used the transects as sample units for the statistical analyses (N=34). We estimated the mean pilosity, Weber's length, and hair density in ants for each transect. To investigate the morphological differences among sites (i.e., the seven study sites as the explanatory variables), 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* (van Lampe and Schellenberg 2023) and compared them among sites using the same procedure implemented for the means. We did not detect differences in the coefficients of variation among sites for all the

three morphological estimates (Figure S3), which indicates that variation is homogeneous in our dataset, and supports the use of means as response variables.

Additionally, we evaluated the morphological variation of *C. crassus* in response to *latitude* and *longitude* using linear mixed models (LMM) implemented in the function *lmer* in the R package *lme4* (Bates et al. 2015). Sampling sites (i.e., the seven Cerrado localities) were included as random effects. 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 to validate model assumptions using the function *simulateResiduals* implemented in the R package *DHARMa* (Hartig 2022).

2.4.2 | Morphological Variation in Response to Temperature, Rainfall, Vegetation and Sugar-Rich Resources

To evaluate the effects of temperature, rainfall, vegetation and sugar-rich resources on the morphological variation of C. crassus, we used LMM. We constructed a full model for each morphological trait including all explanatory operational variables as predictors (i.e., Bio1, Bio4, Bio12, Bio15, DNIy, DIFy, Density, DSH, Height, % EFN, % Trofobionts; Table 1). All variables were scaled prior modelling using the function scale in R. Study sites (i.e., the seven Cerrado localities) were included as random effects. To account for the differences in the number of characterised 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). We adopted a conservative approach, removing variables with VIF > 3 from the full models (Table S3). Model residuals were inspected (Figure S4). We used an automated model selection approach implemented in the function dredge in the R package MuMIn, based on Akaike Information Criterion corrected for small samples (AICc) to select the models that best explain C. crassus morphology. Models with $\Delta AICc < 2$ were considered the most plausible among candidates (Burnham and Anderson 2002). Because more than one model was plausible, we performed a model averaging (full average) using the function model.avg in the MuMIn package. Standardised effect size of each predictor of the averaged model was estimated using the function effectsize implemented in the R package effectsize (Ben-Shachar et al. 2020).

2.4.3 | Hierarchical Partitioning of the Marginal Coefficient of Determination (R²m)

To evaluate the contribution of each predictor to the power of explanation of the best model, we also performed a hierarchical partitioning of the R²m among explanatory operational variables using the function *glmm.hp* implemented in the R package *glmm.hp* (Lai et al. 2022). Given that more than one model was

selected for pilosity and hair density (see Results), hierarchical partitioning of R^2m was estimated for each model separately, and then the mean and standard deviation was taken for each predictor. Explanatory operational variables were grouped into conceptual predictors, following Table 1.

2.4.4 | Correlations Between Morphological, Genetic and Geographic Variation

To assess if morphological variation is correlated to neutral genetic variation between transects in C. crassus, we performed a Mantel analysis implemented with the function mantel in the R package vegan (Oksanen et al. 2022). For each morphological variable, we created a matrix of pairwise Euclidian distance between the means of the transects 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), and transformed them into a dissimilarity index by calculating 1-Dps and using it 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, we used the function mantel. partial implemented in the package vegan. Mantel and partial Mantel tests were conducted based on 999 permutations. Given that we were unable to obtain genomic data for all individuals, for these analyses we maintained only the individuals with both SNPs and morphological characterisation (Table S2).

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

3 | Results

3.1 | Morphological Variation Across Sites

We found differences in pilosity and hair density among sites, but not for Weber's length (Figure S5). No morphological trait presented a significant relationship when modelled in response to latitude or longitude (Figure S6).

3.2 | Morphological Variation in Response to *Temperature, Rainfall, Vegetation* and *Sugar-Rich Resources*

We found four and eight best models for ant pilosity and hair density, respectively, with Bio1 (annual mean temperature), Bio15 (precipitation seasonality), density (vegetation density), annual direct normal irradiation (DNIy), and the proportion of plants with EFN (% EFN) and trophobionts (% Trophobionts) being included in the final full averaged model (Figure 3A,B, Table S4). Diffuse horizontal irradiation (DIFy) was included



FIGURE 3 | Standardised effect sizes and 95% confidence intervals of predictors (explanatory operational variables) from the averaged model for (A) pilosity and (B) hair density. Significant effect size, whose confidence interval does not overlap zero (dashed line), is highlighted by the asterisk. Hierarchical partitioning of marginal r-squared (R^2m) for the best linear mixed effect models constructed for evaluating the (C) pilosity and (D) hair density of the mesosoma of *Camponotus crassus*. Hierarchical partitioning of R^2m was estimated for each model selected after automated model selection, then the mean (points) and standard deviation (line) was estimated for each predictor. Explanatory operational variables were grouped into conceptual predictors, following Table 1. Each plot shows the mean R^2m among models and the mean individual effect of each conceptual predictor (i.e., the contribution of each predictor to the power of explanation of the models). For Weber's length results are not shown because the null model was the best one after model selection. For additional information on averaged models, see Table S4. Bio1 = annual mean temperature, Bio15 = precipitation seasonality, DNIy = annual direct normal irradiation, DIFy = annual diffuse horizontal irradiation, Density = mean vegetation density, % Trophobionts = proportion of plants with trophobionts, % EFN = proportion of plants with extrafloral nectaries.

only in the pilosity full averaged model (Figure 3A). Despite the inclusion of *temperature*, *rainfall* and *vegetation variables* in the final averaged models, only *sugar-rich resource* variables were found to have a significant effect size on *C. crassus* pilosity (both % EFN and % trophobionts) and hair density (only % EFN) (Figure 3A,B; confidence intervals crossing zero indicate no significative variables). Importantly, these effect sizes were negative (Figure 3A,B), showing that *sugar-rich resources* had a negative effect on pilosity and hair density (i.e., ants had fewer hairs where resources were more abundant). For body size, the best selected model was the null one (Table S4), indicating that all the explanatory operational variables for *temperature*, *rainfall*, *vegetation* and *sugar-rich resources* evaluated in this work do not play an important role in shaping the size of *C. crassus* workers. For detailed information of full averaged best models, please see Table S4.

Do not play an important role in shaping the size of *C. crassus* workers. For detailed information of full averaged best models, please see Table S4.

3.3 | Hierarchical Partitioning of the Marginal Coefficient of Determination (R²m)

In addition to the negative and significant effect *of sugar-rich resources* on *C. crassus* pilosity and hair density, our hierarchical partitioning analyses revealed that such variables had the highest contribution to the explanatory power of the models (i.e., R²m; Figure 3C,D). The proportion of plants with trophobionts (% Trophobionts) presented a slightly higher individual effect on ant pilosity compared with the proportion of plants with EFN (% EFN). For detailed information on hierarchical partitioning for each selected model for *C. crassus* pilosity and hair density, please see Table S5.

3.4 | Correlations Among Morphological, Genetic and Geographic Variation

In Mantel and partial Mantel analyses, we did not detect significant correlations between *C. crassus* morphological variation (both pilosity and hair density) and genetic distance between transects, even when controlling for geographic distance (Table 2). Correlation between pilosity and geographic distance was not significant as well. Similar patterns were found for hair density (Table 2). Contrastingly, we found a positive correlation for Weber's length and genetic distance, even when controlling for geographic distance, suggesting that the more different ants are in size, the more they differ genetically (Table 2). Finally, there was a positive correlation between genetic and geographic distances, suggesting genetic distance in ants increases with geographic distance (Mantel; r=0.178, p=0.008).

4 | Discussion

In this field-based and geographically large-scale study, we evaluated potential drivers of variation in functional traits associated with thermoregulation in *Camponotus crassus*, an abundant ant of the Cerrado savanna. Our models suggested that pilosity and hair density on the mesosoma of *C. crassus* are negatively associated with *sugar-rich resources* (i.e., proportion of plants with EFNs and trophobionts). That is, the higher the availability of liquid resources on leaves, the lower the quantity of hairs on the mesosoma of *C. crassus*. Indeed, *sugar-rich resources* were the variables that most contributed to the explanatory power of the models for pilosity. Such relationship, however, was not detected for ant body size. The variation in ant pilosity and hair density did not covary with genetic dissimilarities between ants, but genetic distance did covary with body size, even when accounting for geographic distance. Genetic distance also covaried with geographic distances, an isolation by distance pattern commonly found in genetic structure of natural populations.

4.1 | Morphological Variation Across Sites

Camponotus crassus pilosity and hair density presented significant differences among studied sites, but they were not predicted by latitude. Body size, in turn, was statistically similar among sites and did not vary in response to latitude. These results contradict previous findings on interspecific ant body size across latitudinal gradients in the British Isles, where body size increased with latitude (Cushman et al. 1993), suggesting that variation at the intraspecific level can diverge from expectations at community scale. Although variances in all morphological traits did not differ statistically among sites, they varied greatly among transects within the same site. It has been shown that most trait variation across ant species (i.e., at the interspecific level) 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). Moreover, such patterns are attributed to the fact that local habitats support a large diversity of niches, then promoting a higher trait diversification between species (Chown and Gaston 2010). Our results corroborate with this previous literature, suggesting that the local variation can also be higher when considering the intraspecific levels of functional trait variation.

4.2 | Morphological Variation in Response to *Temperature, Rainfall, Vegetation* and *Sugar-Rich Resources*

Contrary to our hypothesis, results showed that none of the evaluated factors significantly mediated *C. crassus* body size. In addition to body size association with organism physiology (Peters 1983) and thermoregulation (Oms et al. 2017), body size in ants can be positively correlated with competitive ability and aggression (large-bodied ants can be more aggressive toward competitors; Nowbahari et al. 1999), migration ability (large-bodied ants may migrate faster than small-bodied ones), starvation resistance (large-bodied ants potentially resist to longer periods of starvation; Cushman et al. 1993), resource exploitation (ants

TABLE 2 | Mantel and partial Mantel tests between morphological (pilosity, Weber's length and hair density), genetic and geographic distances.

		Ma	Partial mantel			
	Genetic distance		Geographic distance		Genetic distance	
Morphological trait	r	р	r	р	r	р
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

Note: 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.

with large bodies present greater resource removal; Kaspari 1996; Nooten et al. 2022), and even soil granulometry (small ant species are more frequently found on fine-grains soils; da Costa-Milanez et al. 2017). Although most of these factors were evaluated at the interspecific level, it is possible that multiple factors in the Cerrado landscape can also mediate body size in *C. crassus*. We acknowledge that our work did not cover these variables, and further investigation is needed to sort out potential drivers and mechanisms shaping this trait in Cerrado ants.

Although the models that best explained pilosity and hair density in *C. crassus* included *temperature*, *rainfall*, *solar radiation* and *vegetation* predictors, they were not significant (confidence intervals of effect sizes crossed zero; Figure 3A,B), which contradicted our initial expectations. Only *sugar-rich resources* were found to have a significant and negative effect on pilosity and hair density, mostly contributing to the power of explanation of the models. Such finding is new, opening new avenues of research on ant morphology and thermoregulation. When phenotype-environment correlations are found across multiple independent sites, the importance of environmental drivers of phenotypic variation increases (Merilä and Hendry 2014). Thus, our models pointed out that *sugar-rich resources* are important throughout our large-scale study, suggesting they likely play a role mediating *C. crassus* pilosity and hair density.

Availability of liquid resources had a large and negative effect on pilosity and hair density in C. crassus. In ants, the foraging area commonly encompasses the immediate vicinity of 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 (Parr 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 12m from the nest entrance (Lange et al. 2019), suggesting that the ants can adjust their foraging terrain in response to resource availability. Since the distribution of resources determines 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 area and spend 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, metabolised 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; Freires et al. 2023; Zuanon et al. 2023). 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. We found that C. crassus pilosity and hair density variation is negatively associated with the proportion of plants with EFNs and (only for pilosity) honeydew producing insects. Yet, for pilosity, plants with trophobionts showed a slightly higher effect than EFN. Indeed, hemipteran honeydew was already reported to be nutritionally different from EFNs, with ants preferring the former liquid source (Del-Claro and Oliveira 1993; Blüthgen et al. 2004; Sendoya et al. 2016). It is known that heattolerant 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, we hypothesize that in places with fewer plants bearing EFNs and trophobionts, it is possible that other strategies for thermoregulation take place, including an increase in hair pilosity. In other words, it is possible that a trade-off between higher physiological thermal tolerance and greater thermoregulatory capacity due to morphological characteristics (in this case, pilosity) exists for ants. However, further investigation is needed on this topic.

Our findings sum to the scarce literature about ant intraspecific variation across environments in the Neotropics. A recent work in Brazilian dry forest (Caatinga) across a gradient of disturbance and aridity investigated intraspecific trait variation of four ant species and found that species may respond differently to climate and disturbance predictors (Oliveira et al. 2022). Moreover, a positive correlation was found between pilosity and rainfall for some species in Caatinga (Oliveira et al. 2022), which was not observed in our results in Cerrado. Thus, it is likely that functional trait responses vary with species and biome, which requires more research effort to unveil the context-dependent drivers of ant intraspecific variation and susceptibility to global changes.

We acknowledge that the mechanisms behind the negative influence of *sugar-rich resources* on *C. crassus* pilosity were not addressed here, since this is beyond the scope of this study. However, the increasing evidence that high amounts of *sugarrich resources* increase thermal limits in ants (Freires et al. 2023; Zuanon et al. 2023), make our findings reliable and open a new avenue of investigation on the interplay of ants' physiological and morphological responses to global changes.

4.3 | Correlations Among Morphological, Genetic and Geographic Variation

Few studies have attempted to unveil the genetic basis of thermal tolerance in ants and how it varies across populations (Roeder et al. 2021). Under selective pressure, trait variation can reveal longstanding processes such as adaptation, character divergence and even speciation processes (Richardson et al. 2014). Conversely, when derived from phenotypic plasticity, traits can rapidly change within few generations and differ remarkably among different habitats (West-Eberhard 1989). Although we used neutral markers, our results revealed a possible genetic adaptation in *C. crassus* morphological 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 biotic (e.g., competition) or abiotic (e.g., climate) selective pressure or nonadaptative processes, such as genetic drift, gene flow and inbreeding (Merilä and Hendry 2014). Thus, further investigation is needed to elucidate the nature of inter-habitat 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. However, we recognise that further genetic analyses including genes associated with ant pilosity would be required to draw more concrete conclusions. 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 (Whitman and Ananthakrishnan 2009). Despite the limitations to clarify such mechanisms, phenotypic plasticity conceivably plays a central role in species acclimation and adaptation to changing climates, as it can facilitate adaptative phenotypes in ecological timescales (Ghalambor et al. 2007) and allows more time for species to respond genetically over evolutionary timescales. Therefore, plastic organisms should be favoured in constantly changing environments (Whitman and Ananthakrishnan 2009) and may be a crucial mechanism for species persistence 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, intraspecific variation is crucial to predict the effects of biodiversity loss and environmental changes (Des Roches et al. 2017). For instance, trait variation can increase niche complementarity, which reduces competition between different species (Bolnick et al. 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 to 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).

4.4 | Conclusions

Morphological differences between individuals can also lead to a diversity in demographic parameters, defence and competitive abilities, parasite resistance, tolerance to abiotic changes, resource exploitation (Bolnick et al. 2011). Moreover, intraspecific variation may alter community structure and dynamics (Bolnick et al. 2011). Specifically, regarding ants, heat tolerance is key for community dynamics (Menzel and Feldmeyer 2021). Ants have thermal niches, with aggressive and dominant ants occupying the high-quality thermal niches, whereas subordinate ones are commonly reported to forage close to their maximum thermal limit, presenting higher thermal tolerance (Fitzpatrick et al. 2014; Nascimento et al. 2022). Under global warming, timid and subordinate ants (with better heat resistance) can predominate, decreasing the effectiveness of ant-induced benefits to plants (see Christianini and Oliveira 2013; Leal et al. 2017; 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 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 the availability of sugar-rich resources for ant geographical ecology, as well as for ant ecophysiology, morphology and behaviour. Importantly, to our best knowledge, this is the first work that shows the role of sugar-rich resources in shaping intraspecific variation in pilosity across a latitudinal gradient in Cerrado, filling a gap of knowledge for the Neotropics. 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; Oliveira 1997; Sendoya et al. 2009; Calixto et al. 2021). This study reveals 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 Cerrado (Colli et al. 2020). Moreover, the findings here support previous works suggesting that the impact of global changes on resource availability will be a key to ant persistence, with ants in environments with high availability of sugar-rich resources being more likely to endure the negative effects of warming (Freires et al. 2023). Our work covers a still poorly investigated aspect of intraspecific variation of tropical eusocial insects and sheds new light on the trait variation associated with latitudinal gradients and resource availability in a major ecosystem.

Author Contributions

M.A.-S., S.F.S. and P.S.O. conceived the ideas and designed methodology; S.F.S. and A.N. designed sampling scheme and collected the field data; S.F.S. and A.N. identified ant and plant species, respectively. M.A.-S. collected morphological and genetic data; M.A.-S., P.A.S.L., Y.S., S.K.H., G.M.M. and A.P.S. sequenced genetic data and performed bioinformatic analyses; M.A.-S., M.C.C. and S.F.S. analysed the data; M.A.-S. and P.S.O. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data is available as a Supporting Information and in Dryad repository (https://doi.org/10.5061/dryad.4xgxd25mp). All raw reads of MIG-seq data were submitted to the NCBI Sequence Read Archive (BioProject ID: PRJNA1098407) and can be accessed through the reviewer link (https://dataview.ncbi.nlm.nih.gov/object/PRJNA1098407?reviewer=6bftk695cuc70jik7nkt92ae2m).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.