

Temperature drives caste-specific size and shape clines in North American ants

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Abstract

What regulates the distribution of plants and animals on Earth remains a long-standing question in ecology and biogeography. Since an organism's morphology relates to virtually all aspects of its life history, understanding what drives size and shape variation is fundamental to elucidate the factors that limit species distribution. Eusocial insects, especially ants, epitomize morphological variation with their unique castes; workers are typically wingless sterile females whereas queens and males are fertile and winged. Thus, the environment may select for different morphologies in each caste. As such, ants offer a unique opportunity to assess how selection operates on different components of insect societies. Here, I use global climate data and over 40,000 morphometric measurements on multiple species of closely related *Formica* ants to assess how abiotic factors affect morphology at the continental scale. Specifically, I examine caste-specific variation in morphology along broad-scale climatic gradients. My results show that (1) body size is strongly and positively related to geographic variation in temperature, especially in queens and males (2) temperature drives geographic variation in shape in the worker caste, but not in queens or males. Specifically, workers had relatively thinner and smaller heads in warmer environments, whereas their legs, antennae and thorax were longer. This study highlights the importance of temperature in regulating species distributions and shows the value of simultaneously examining all castes in ant ecology.

Keywords: Biogeography, Bergmann's rule, Size-grain hypothesis, Morphospace, Formicidae

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Introduction

Species, even closely related ones, vary tremendously in the extent of their geographic distributions (Thuiller *et al.* 2004; Guénard & Weiser 2018). Understanding what drives these differences remains a challenge. A promising approach is to examine how functional traits vary along broad-scale environmental gradients (Swenson & Weiser 2010; Pollock *et al.* 2012; Swenson *et al.* 2012). Of these traits, some may play determinant roles in limiting species distributions. For instance, small changes in size and shape can drastically enhance performance (i.e. quantitative measure of the ability of an organism to conduct an ecologically relevant task (Irschick & Le Galliard 2008)) (Koehl 1996). Morphological variations affect species abundance (Blackburn & Gaston 1999), species range size (James H. Brown *et al.* 1996) and population growth (Savage *et al.* 2004). Variation in morphology could thus potentially make the difference between populations of a species persisting in an environment or not. Therefore, assessing how the size and shape of species vary along gradients, and how such differences affect performance, could better our understanding of what regulates their distribution.

Size along gradients

Body size is a key ecological trait that relates to the physiology and life history of a species. Variation in body size may thereby translate into patterns of population size and distributions (Brown *et al.* 1993; Brown 1995; Gaston & Blackburn 1996; Blackburn & Gaston 1999, 2001; Hildrew *et al.* 2007; Olson *et al.* 2009; Chown & Gaston 2010). As such, it can be particularly insightful to understand how species' traits, such as body size, relate to variation in the abiotic environment underlying their distributions (Grinnell 1904; Antoine *et al.* 1998; Guisan &

Thuiller 2005; Lenoir *et al.* 2008; Elith & Leathwick 2009). An organism's size relates to virtually all aspects of its life history. For example, variation in body size relates to variation in energy use, in abundance, and in the extent of the geographic distribution of a species (Brown *et al.* 2004; White *et al.* 2007). Specifically, if body size relates to individual fitness (e.g. by allowing organisms to produce a larger quantity or better quality of offspring), then it could influence the ability of individuals to reproduce and of populations to grow (Hildrew *et al.* 2007; Horne *et al.* 2015). Therefore, assessing how and why body size varies within and among species can help to understand which factors regulate species distributions.

General patterns of body size variation along the latitudinal gradient differ between endotherms and ectotherms. For endothermic vertebrates, body size is often positively related to latitude. The greater surface to volume ratio of large species would explain why we find them in colder environments (i.e. Bergmann's rule). An organism's heat production positively relates to its volume, while heat loss negatively relates to its surface area (Bergmann 1847). Consequently, larger animals produce more heat and lose relatively less; an advantage in colder climates (Meiri & Dayan 2003). Although this mechanism is applicable to endotherms, it cannot account for geographic patterns of size variation in ectotherms, which rely on their external environment to reach their optimal temperature. While a majority of endotherms (i.e. birds and mammals) follow Bergmann's rule (Meiri & Dayan 2003), patterns of body size are inconsistent in ectotherms (Shelomi 2012). In insects, a roughly equal number of studies found that their size increases (Arnett & Gotelli 1999b; Heinze *et al.* 2003; Bernadou *et al.* 2016), decreases (Masaki 1967; Mousseau 1997; Schutze & Clarke 2008; Beasley *et al.* 2018) or does not vary (Geraghty *et al.* 2007; Adams & Church 2008) across latitudinal and altitudinal gradients (reviewed by Shelomi 2012). These opposite trends may result from two non-mutually exclusive evolutionary processes

(Blanckenhorn & Demont 2004). A temperature effect would drive the positive relationship (i.e. Bergmann's rule). Specifically, lower temperatures slow ectotherms' growth rate linearly but increase their development time exponentially, thereby leading to maturation at a larger size (Porter 1988; Atkinson & Sibly 1997; Chown *et al.* 1999; Angilletta *et al.* 2004; Blanckenhorn & Demont 2004; Chown & Gaston 2010). In contrast, a seasonality effect can potentially drive the negative relationship. Shorter growing seasons restrict time for development and would cause a decrease in size at maturation (Masaki 1967; Blanckenhorn & Fairbairn 1995; Chown & Klok 2003). Hence, I expect that temperature and seasonality will regulate size along broad-scale gradients.

Patterns of body size variation along latitudinal gradients are highly inconsistent in ectotherms, perhaps because they can overcome environmental restrictions in several ways, which might obscure size clines. For instance, a species can evolve a faster growth rate and shorter development time to maintain an optimal size despite brief growing seasons (Blanckenhorn & Demont 2004). Along gradients, individuals of these species would thus differ in their growth rate and development time, but not in body size (Blanckenhorn 2018). Another potential strategy for species to compensate for short seasons is to change the number of generations completed per year. Multivoltine species can, contrary to univoltine species, diminish their number of generations per year to maintain an optimal body size where seasons are shorter (Kivelä *et al.* 2011). Conversely, some species can spread their developmental period over more than a year to reach their adult size – semivoltine organisms take two years to develop while partivoltine take three or more. A shift in voltinism sharply modifies the time available for development. Such shifts may produce a saw-tooth body size cline (Roff 1980; Mousseau 1997; Shelomi 2012), whereby for each additional generation the time available for maturation

decreases, thus resulting in smaller adult sizes. Therefore, an ectotherm's size at maturity is not solely regulated by temperature and seasonality, but also by its voltinism, developmental time and growth rate.

In truly social organisms such as ants, it is unclear whether it is the size of an individual or the size of the colony that is analogous to the body size of non-social animals. In ants, the performance of all individuals trickles down to explain the colony's fitness, thereby it encompasses the performance of both its somatic (i.e. workers) and reproductive units (i.e. males and queens) (Oster & Wilson 1978). Body size can thus be described as the size of individuals within the colony or as the summed sizes of its component individuals (i.e. the size of the 'superorganism') (Kaspari & Vargo 1995).

Individual ants within a colony are separated between multiple castes, namely workers, males and queens. These castes typically differ substantially in size (Hölldobler & Wilson 1990). Usually, members of the reproductive castes (i.e. males and queens) are larger than the workers (Hölldobler & Wilson 1990; Tribble & Kronauer 2017) – queens being the largest, with developed thoraxes harboring their flight muscles (Keller *et al.* 2014) and enlarged gasters housing their developed ovaries (Tribble & Kronauer 2017). These size differences relate to the unique life history of each caste; males and queens are typically winged individuals with reproduction as their main purpose whereas workers are wingless females that are sterile (Abouheif & Wray 2002). Accordingly, in ants, size can relate positively with fecundity in both males (Wiernasz *et al.* 2001) and females (Vargo & Fletcher 1989; Smith *et al.* 2007). As for the functionally sterile worker caste, they can display a variety of morphology even within a colony (Wilson 1953; Hölldobler & Wilson 1990). This phenomenon, termed worker polymorphism, occurs in 29% of ant species (Larichelière, in preparation). The evolution of worker polymorphism is thought to be

driven by resource exploitation (Wilson 1978a, 1980; Waller 1989; Powell & Franks 2006) and competitive interactions (Wilson & Hölldobler 1985; Hölldobler & Wilson 1990; Powell 2008); worker subcastes can have an optimized morphology to accomplish specific tasks. However, since genetic and environmental factors interact to determine worker polymorphism (Schwander *et al.* 2010), abiotic variation along broad-scale gradients could affect the size of workers. Furthermore, because positive selection can differ between castes (Harpur *et al.* 2014; Privman *et al.* 2018), selective pressures could be caste-specific and produce diverging size clines.

Patterns of size clines in ants are inconsistent across studies. The superorganism's size (i.e. colony size) either increases with latitude (Kaspari & Vargo 1995; Yang 2006) or shows no clear pattern (Heinze *et al.* 2003; Geraghty *et al.* 2007; Bernadou *et al.* 2016). In individual workers, size either increases with latitude (Cushman *et al.* 1993) and temperature (Kaspari 2005), or shows no consistent trend (Geraghty *et al.* 2007) among species. Within species, individual ants mostly follow Bergmann's rule along latitudinal (Heinze *et al.* 2003) and altitudinal gradients (Bernadou *et al.* 2016; Purcell *et al.* 2016). Therefore, there is still no consensus as to what mechanisms would drive size clines in ants. An important limitation of the current body of research is that no study assesses size clines in ants both intra- and interspecifically. An additional shortcoming of previous work is that most studies assess morphological variations exclusively in the worker caste. Indeed, to my knowledge, only one study examined both the size of workers and queens of a species along large-scale gradients (Heinze *et al.* 2003), and none examined all castes simultaneously. This is problematic because natural selection acts at the colony-level; colony growth and defense depend on the workers, whereas its reproductive output depends on males and queens (Oster & Wilson 1978). Therefore, a colony's fitness relies on the cumulative performance of the members of each caste.

At the level of individuals within a colony, research on ant body size has focused largely on workers (Wilson 1983; Kaspari 1996; Cerdá & Retana 1997; Burd 2000; Schöning *et al.* 2005; Powell & Franks 2006; Huang 2010); very few studies examined size variation in queens (Vargo & Fletcher 1989; Ruppell & Heinze 1999; Wiernasz & Cole 2003; Helms & Godfrey 2016) and males (Davidson 1982; Abell *et al.* 1999). Moreover, no study to date has examined more particularly how the size of the reproductive caste varies along abiotic gradients. Depending on the caste, an increase in size can lead to various benefits, including higher fecundity (Vargo & Fletcher 1989; Wiernasz *et al.* 2001; Smith *et al.* 2007) and success in colony founding (Wiernasz & Cole 2003). It also increases resistance to various stresses, such as starvation (Kondoh 1977; Kaspari & Vargo 1995; Heinze *et al.* 2003; Modlmeier *et al.* 2013), heat (Cerdá & Retana 1997; Wendt & Verble-Pearson 2016) and desiccation (Hood & Tschinkel 1990; Kaspari 1993). However, larger sizes might decrease resistance to cold temperatures by elevating the supercooling points of freeze avoidant species (i.e. species that do not survive freezing of internal body fluids (Sinclair *et al.* 2015)), which is the case in ants (Hahn *et al.* 2008). Additionally, size variation in the worker caste can relate to diet breadth. For instance, in a granivorous desert ant, a higher range of worker size allows colonies to exploit a higher range of seed sizes (Davidson 1978). Despite the ecological importance of ants (Lach *et al.* 2010), there are still relatively few studies that assess the causes and consequences of variation in their body size along abiotic gradients. Moreover, these studies obtained inconsistent results.

A thorough examination of caste-specific size clines could help to reveal the underlying mechanisms driving patterns observed in previous studies and identify factors limiting species distribution. For instance, castes differ in their development time (Porter 1988). Therefore, if season length restricts size in ants, we should expect that the castes with longer development

times would be more affected. If, on the other hand, size variations in ants result from temperature variations, we should expect that each caste would be affected more uniformly. Furthermore, we should expect that selection will drive different size clines across castes. Sexual selection should drive males and queens to larger sizes (Blanckenhorn 2000), whereas for the workers, which are not subjected to sexual selection, natural selection should drive a higher size variability within colonies to optimize colony efficiency (Davidson 1978; Wilson 1978a, 1980; Waller 1989), or larger worker sizes to widen the spectrum of conditions they can forage at (Kaspari 1993; Cerdá & Retana 1997). Evaluating caste-specific size clines will further allow to identify if environmental restrictions on one caste could limit an ant species distributions. For example, constraints acting on queens, who take care of the reproduction within the colony, could limit a species distribution. Indeed, queenless colonies are bound to disappear since workers cannot produce diploid females (i.e. workers and queens); workers with functional ovarioles can only produce males by arrhenotokous parthenogenesis (Bourke 1988). In eusocial insects, we should expect that the colony's requirements, which encompass the respective restrictions acting on each caste, will reflect its species distribution. Nonetheless, to my knowledge, no study simultaneously examined size variation in all castes (i.e. workers, queens and males) at a broad geographic scale.

Shape along gradients

Most work asking how morphology varies geographically centers on body size, often neglecting to examine variation in shape. The shape of an organism relates to life history traits such as movement (Losos 1990a, b; Wootton 1992), diet (Bernays *et al.* 1991; Nogueira *et al.* 2009) and habitat preference (Schluter 1993; Herrell *et al.* 2001). In ants, certain shapes are adaptations to

competition (Powell 2008, 2009; Huang 2010), task partitioning (Powell & Franks 2006), sexual selection (Abell *et al.* 1999), locomotion ability (Kaspari & Weiser 1999; Espadaler & Gómez 2001; Farji-Brener *et al.* 2004), groundcover (Wiescher *et al.* 2012) and diet (Brandão *et al.* 1991; Weiser & Kaspari 2006; Cerdá & Dejean 2011; Ohkawara *et al.* 2017). However, it is still unclear if the shape of ants should vary systematically along abiotic gradients.

Shape of ants could relate to broad-scale variation in the environment in several ways. Assuming that biotic interactions intensify at lower altitudes and latitudes (as suggested by Jeanne (1979), Roslin *et al.* (2017) and Schemske *et al.* (2009)), then we might expect a shift in shape relating to defense ability or competitive performance that co-varies with latitude. Simultaneously, productivity (Gillman *et al.* 2015) and the diversity of prey species (Price *et al.* 1995; Brown 2014) increases towards the tropics, which might produce a shift in shape relating to resource acquisition. Therefore, we might expect that traits linked to head shape in the worker caste, which relate to defense ability (Powell 2008; Powell & Dornhaus 2013) and diet (Weiser & Kaspari 2006; Cerdá & Dejean 2011; Ohkawara *et al.* 2017), will vary along broad-scale gradients. For instance, we might expect a higher shape variation in workers within a colony. Concurrently, there is a higher prevalence of polymorphism towards the tropics (Wilson 1978b). Nevertheless, notwithstanding the recognized importance of shape, it is still unclear if abiotic factors drive shape variation in ants and, if so, if responses are consistent among castes.

Hypotheses

In this study, I use morphometric measurements taken on more than 3000 georeferenced specimens belonging to 34 closely related species in the genus *Formica* (*fusca* group) distributed across the Nearctic region. My dataset includes an extensive number of specimens within

species, thereby it allows to account for both intraspecific and interspecific variation. Thus, I mitigate phylogenetic non-independency while extending the range of conditions experienced by specimens within the study. I use this comprehensive dataset to examine how morphology varies along abiotic gradients in the aim of furthering our knowledge on what regulates species distribution. I test the hypothesis that abiotic factors (i.e. temperature, precipitation and seasonality) drive size and shape variation in ants. Specifically, I tested whether body size decreases with increasing temperature within and among species of *Formica*, which is expected if they follow Bergmann's rule. I predicted that temperature variation would affect species similarly, and that size differences among species would reflect their respective distributions. For example, if low temperatures increase the size of *Formica*, a decrease in temperature would increase the size of both northern and southern species at the same rate, but northern species would be larger than southern species since they live in colder environments.

I then measured functional traits that relate to foraging efficiency, defense ability, prey type and locomotion speed. Furthermore, I examined how each trait varies relative to general size. Specifically, a proportional variation of a trait relative to size would indicate isometry. In contrast, a relative increase or decrease would indicate a positive and negative allometry, respectively (Fig. S3). By evaluating if these functional traits follow a positive or negative allometry along gradients, I hoped to gain insights into their relative importance. My predictions were that head width would follow a positive allometry along a temperature gradient in the worker caste. Specifically, this means that workers' head width relative to body size should increase with increasing temperatures. In contrast, since changes in allocation of resources to one trait could produce compensatory changes in the relative size of other traits (Nijhout & Emlen 1998), I expected that the other morphological traits (e.g. leg length, scape length and mesosoma

length) would follow a negative allometry. As for the sexuals, I anticipated that their shape would remain stable. This is because I expected that selective pressures acting on dispersal, colony founding, and fecundity would be more consistent along a temperature gradient. Thus, I predicted that, compared to workers, males and queens would vary little in shape.

Materials & Methods

Morphological data

To estimate body size and shape of ants, I used morphological data previously compiled for a taxonomic revision of the Nearctic *Formica* species (Francoeur 1973) within the *fuscus* group (Creighton 1950). It consists of morphological measurements taken on 3280 mounted specimens, which came from several museum collections (listed in Francoeur 1973). Of these specimens, ($n = 411$) are queens, ($n = 329$) are males and ($n = 2540$) are workers. Morphometric measurements were taken using a three-dimensional stage and a stereoscope Zeiss model III equipped with an ocular micrometer ($0.05 \text{ mm} \pm 0.005 \text{ mm}$). Amongst the specimens, a total of 34 different species were morphologically characterized. I had measurements on workers for all 34 species, on males for 24 species and on queens for 28 species. In this study, I used the 12 measurements taken on the greatest number of specimens: head width, minimal head width, head length, mesosoma length, pronotum width, petiole width, femur width, femur length, distance between the eyes, eye diameter, distance between carenas and scape length (Fig. 1, Table 1). The traits are appropriate for this study because they are associated with ecological functions (summarized in Table 1).

Geographic Location

To extract information about the environmental conditions of a specimen's locality, I obtained their coordinates. Since the specimens within my dataset were solely labeled with a locality name (e.g. Montréal, Qc), I obtained latitude and longitude coordinates for the centroid of the provided locality using GeoHack (<https://tools.wmflabs.org/geohack/>). For instance, a landmark (e.g. "Ithaca") was typed in google. I then clicked on the coordinates icon listed in its Wikipedia page, which redirected me to the GeoHack page with the coordinates of the landmark provided by GoogleMaps. To diminish the effect of geographic inaccuracy I removed specimens for which I had only a province or state name; counties were the lowest resolution used for a locality name. For the localities not present in GeoHack, such as street names, I used GoogleMaps to extract their coordinates. For such precise localities, I typed the locality's name (e.g. "rue Saint-Jean, Qc") in Google's search engine and directly took the coordinates provided by GoogleMaps. Using these coordinates, I mapped the specimens' locations using QGIS (QGIS Development Team 2015) (Fig. 2).

Environmental Data

Temperature has been shown to affect the morphology of ants (Molet *et al.* 2017; Oms *et al.* 2017). Moreover, previous studies demonstrated that temperature (VanVoorhies 1996; Chown & Gaston 2010), seasonality (Blanckenhorn & Demont 2004), and humidity (Stillwell *et al.* 2007) relate to insect morphology (VanVoorhies 1996; Blanckenhorn & Demont 2004; Stillwell *et al.* 2007; Chown & Gaston 2010). Therefore, to test if ant morphology varies predictably along environmental gradients, I modeled morphological traits against the following variables; mean annual temperature (MAT), mean annual precipitation (MAP) and temperature seasonality (TS)

(Hijmans *et al.* 2005). I corrected for elevation, because ants have been shown to respond to altitudinal gradients (Bernadou *et al.* 2016; Purcell *et al.* 2016), and altitude correlates with temperature (Janzen 1967). As such, I added altitude estimates (DEM) (Farr *et al.* 2007) as a covariate in my models to correct for the effect of elevation, which might influence the abiotic variables. I included DEM as a covariate in all models because I was not interested in testing for its effect as much as controlling for it, given that the data was skewed towards low elevation sites

Statistical analyses

I performed all analyses using R (R Core Team 2017). Prior to running my models, I used a square root transformation on altitude estimates to attenuate their skewness and respect the normality assumption of regression analyses. I used the Aitchison transformation (Aitchison 1986) on the morphometric traits, because it extracts the size and shape components embedded in morphometric measurements (Peres-Neto & Magnan 2004; Bastien-Henri *et al.* 2010). It also scales the data, which is essential prior to running principal component analyses (PCAs). Since I used 12 measurements, the Aitchison transformation produced 12 size-free shape variables and 1 shape-free size variable. I used the shape-free size variable (hereafter referred as size) as a size proxy. The 12 size-free shape variables represent the scaling of each morphometric measurement in relation to overall size. I used these 12 size-free shape variables to run a principal component analysis (PCA), a dimension-reducing technique (Jolliffe 2011). I then extracted the first principal component (PC1) to create a univariate shape variable which could then be used in linear models (Gotelli & Ellison 2004).

Morphological characterization of Formica castes

To characterize each caste morphologically, I started by establishing if body size variation differs among them. To do so, I compared the coefficient of variation for body size between castes using an asymptotic test (Feltz & Miller 1996) embedded within the package *cvequality* (Marwick & Krishnamoorthy 2018), a method that tests for the equality of coefficients of variation from k populations (i.e. here castes). Then, I assessed how species and castes segregate within the morphospace using a principal component analysis (PCA) on the 12 morphometric measurements (Fig. 1, Table 1). Principal component analyses were plotted to visualise how castes and species segregate along the first two principal component axes (Fig. S1). In doing so, I assessed if species and caste form independent clusters in the morphospace.

Relationship between abiotic factors and morphology

To assess the relationship between morphology (i.e. size and shape) and environmental variables (MAT, MAP, TS), I used *lme4* (Bates *et al.* 2014) to build a suite of linear mixed effect models. To identify the best predictors for size and shape I compared models with the Akaike Information Criterion (AIC) using the *AICcmodavg* package (Marc J. Mazerolle 2015). Models with the lowest AIC scores were selected. When multiple models were within 2 AIC units of the lowest score, the most parsimonious model was selected (Arnold 2010).

To test my models for the normality and homoscedasticity assumptions, I verified my models' residual plots visually (Chatterjee & Hadi 2006). Marginal R^2 (i.e. proportion of variance explained by fixed factors) and conditional R^2 (i.e. proportion of variance explained by both the fixed and random factors) values for mixed models were obtained using the package *MuMIn* (Bartoń 2016).

For the multivariate analyses, I only used specimens for which all 12 traits were measured (queens, $n = 337$; males, $n = 200$; workers, $n = 1648$). This way, I could run principal component analyses and incorporate the size and shape variation embedded within all 12 traits for each of our specimen. Although it reduced the number of specimens I could incorporate in our subsequent analyses, it allowed me to produce size and shape proxies incorporating variation embedded within more traits.

Abiotic factors drive size variation in ants

I compared linear mixed models to identify the abiotic factors driving size variation across *Formica* species. As the response variable, I used size, a variable I produced by extracting the size component of the 12 morphometric traits of study. As fixed effects, I entered environmental variables (i.e. MAT, MAP, TS and DEM) and, as random effects, I entered species. Accounting for phylogenetic non-independence is important, because similarities among taxa may arise as a result of their common ancestry instead of as a response to similar forces of selection (Bell 1989). However, there are still no species-level phylogeny available for the Nearctic species of *Formica* in the *fuscus* group. Therefore, I entered the taxonomic rank of species as a random effect to account for phylogenetic non-independence. To account for the effect of altitude, I included DEM in all my models. To determine if the relationship between temperature and size differed among caste, I built a linear model testing for an interaction between caste and temperature. I used size as the response variable and, as fixed effects, I entered caste and temperature. Finally, I examined the relationship between size and temperature within species. To do so, I built linear models testing for an interaction between species and temperature for

each caste. I used size as the response variable and, as fixed effects, I entered species and temperature.

Abiotic factors drive shape variation in ants

I compared linear mixed models to identify if abiotic factors affect shape in *Formica*. As the response variable, I used shape. As fixed effects, I entered environmental variables (MAT, MAP and TS) and, as random effects, I entered species. To account for the effect of altitude, I included DEM in all my models.

Results

Morphological characterization of *Formica* castes

A visual representation of the range of size for each species showed a clear demarcation between castes (Fig. 3). Specifically, workers had a greater range of sizes than the alates (i.e. males and queens). Further supporting this, castes differed in their coefficient of variation for size ($df = 2, p = 0$).

Morphology varied more among castes than among species (Fig. S1). Specifically, size differed more among castes than among species (caste: $df = 2, F_{2, 2203} = 1612, p < 2.2^{-16}$; species: $df = 32, F_{32, 2173} = 8.106, p < 2.2^{-16}$). Similarly, shape differed more among castes than among species (caste: $df = 2, F_{2, 2203} = 18183, p < 2.2^{-16}$; species: $df = 32, F_{32, 2173} = 3.46, p = 2.75^{-10}$). Moreover, within the morphospace, species overlapped considerably across the first two principal component axes. This was apparent even within a caste, especially for the workers. In

contrast, each caste formed a distinct cluster within the morphospace. This indicates that caste, but not species, form discrete morphological groups.

Abiotic factors drive size variation in ants

Mean annual temperature alone was the best predictor for size variation in all castes (Table 2).

Temperature drove size variation among Nearctic *Formica* species and, contrary to my prediction, drove the converse to Bergmann's rule (Table 3). This pattern was consistent among all castes; queens ($t = 3.605$, $df = 322.4$, $p = 3.61 \cdot 10^{-4}$, $R^2_{(m)} = 0.04379$, $R^2_{(c)} = 0.52034$), males ($t = 3.132$, $df = 196.4$, $p = 0.002$, $R^2_{(m)} = 0.04039$, $R^2_{(c)} = 0.66691$) and workers ($t = 3.347$, $df = 888.5$, $p = 8.52 \cdot 10^{-4}$, $R^2_{(m)} = 0.01222$, $R^2_{(c)} = 0.15778$) all increased in size with increasing temperatures (Fig. 4). The effect of temperature on size differed among castes; there was a significant interaction between caste and temperature in my linear model ($df = 2$, $F_{2, 2200} = 5.4998$, $p = 4.14 \cdot 10^{-3}$). Thus, the rate at which size varies with temperature is caste-dependent. In contrast, the effect of temperature on size did not differ among species; there was no interaction between species and temperature in any caste (queens: $df = 21$, $F_{21, 288} = 0.8271$, $p = 0.6856$; males: $df = 14$, $F_{14, 175} = 1.1935$, $p = 0.2839$; workers: $df = 28$, $F_{28, 1596} = 1.2568$, $p = 0.1672$). However, both temperature and species explained size variation (Fig. S2. queens: $p < 2.2 \cdot 10^{-16}$, $r^2 = 0.6391$, $df = 309$; males: $p < 2.2 \cdot 10^{-16}$, $r^2 = 0.6755$, $df = 189$; workers: $p < 2.2 \cdot 10^{-16}$, $r^2 = 0.1858$, $df = 1624$). Thus, although species differ in size, temperature affects their size similarly (Fig. S2).

Abiotic factors drive shape variation in ants

My model selection showed that precipitation, seasonality and temperature were significant predictors of shape and that, of these, temperature had the strongest effect. However, the drivers of shape differed between caste (Table 4). None of the abiotic factors affected the shape of queens and males (Table 5). In contrast, precipitation, seasonality and temperature affected the shape of workers (Table 5). Specifically, temperature had the strongest influence on worker's shape (Table 5). Thus, temperature affected the shape of worker's ($t = -2.595$, $df = 1644$, $p = 9.53 \cdot 10^{-3}$, $R^2_{(m)} = 0.00274$, $R^2_{(c)} = 0.72763$), but not the shape of males ($t = 0.109$, $df = 206.1$, $p = 0.913$, $R^2_{(m)} = 0.00005$, $R^2_{(c)} = 0.59344$) or queens ($t = -1.213$, $df = 331.7$, $p = 0.226$, $R^2_{(m)} = 0.00452$, $R^2_{(c)} = 0.57597$) (Fig. 5). In workers, the shape proxy I used correlated strongly with the shape of various structures. Specifically, the principal traits positively correlated with shape were inter-ocular distance ($corr = 0.817$, $p = 0$), inter-carenal distance ($corr = 0.759$, $p = 2.25 \cdot 10^{-311}$) and head width ($corr = 0.756$, $p = 1.64 \cdot 10^{-306}$), whereas the principal traits negatively correlated with shape were scape length ($corr = -0.79$, $p = 0$), femur length ($corr = -0.77$, $p = 0$) and mesosoma length ($corr = -0.67$, $p = 6.22 \cdot 10^{-213}$) (Table S1). The shape component relates to the scaling of each morphometric measurement relative to overall body size. A positive correlation between a trait and the shape component indicates a positive allometry relative to size. Conversely, a negative correlation between a trait and the shape component indicate a negative allometry relative to size. I found that the worker's shape component increased with decreasing temperatures. Thus, positively correlated traits show a positive allometry with decreasing temperatures (i.e. inter-carenal distance, inter-ocular distance and head width), while negatively correlated traits show a negative allometry with decreasing temperatures (i.e. scape length, femur length and mesosoma length). In colder environments, *Formica* workers have relatively shorter limbs, antennae and

mesosoma, but larger heads. Conversely, in warmer environments, workers have relatively thinner heads, but longer limbs, antennae and mesosoma.

Discussion

Within the field of ant ecology, few studies examine the validity of ecological rules by simultaneously using the worker, queen and male castes. Here, I investigate each caste to elucidate if abiotic factors drive size and shape variation in ants. First, I find that body size is positively related to temperature and that the relationship is particularly strong for queens and males. Moreover, this pattern is consistent within and across species. Second, I find that shape relates to geographic variation in climate in the worker caste, but not in males or queens. In sum, I show that the morphology of *Formica* ants relates to abiotic factors, which potentially plays a role in regulating their species distributions. Furthermore, I show that responses to abiotic gradients differ between castes, which suggests that selective pressures by the abiotic environment are caste-specific.

Morphological characterization of castes

I find that the range of size within the worker caste is higher than within the male and queen castes. I explain this discrepancy of size variations between the workers and sexuals in two ways: (1) selective pressures acting on queens and males stabilize their size-range, and (2) selective pressures promote a higher variation in worker-sizes. For the alates, I propose that selection for fecundity (Honek 1993; Blanckenhorn 2000) and dispersal (Keller *et al.* 2014) counterbalanced by environmental restrictions stabilize size variation. Conversely, for the worker caste, a wide-

range of sizes might be adaptive because it could optimize task partitioning within the colony (Davidson 1978; Wilson 1980). In multiple ant genera, such as the *Atta* leaf-cutting ants, size classes determine the workers' role (e.g. gardeners, harvesters and soldiers) (Hölldobler & Wilson 1990, 1994). Improved task partitioning could then partially explain the higher size variation of workers I observe.

The morphological characterization of *Formica* ants yields three main observations. The first is that, within the morphospace, castes form discrete clusters. The second is that species cluster poorly within the morphospace. This is apparent even within each caste. The third, and perhaps most important, is that queens and males occupy a smaller morpho-space than workers, which suggest that their shapes are less plastic. I conclude that within the *Formica* genus, morphological variations are higher among castes than among species. This illustrates the drastic morphological differences between each caste and, I argue, demonstrates that caste-specific comparisons could yield valuable insights into understanding what drives size and shape variations in ants.

Abiotic factors drive size variation in ants

In this study, I show that environmental factors affect the body size of *Formica* ants. Principally, the effect of temperature on size is apparent in all castes; queens, males, and workers increase in size with increasing temperatures. Furthermore, this pattern holds both at the inter- and intraspecific level. Within a caste, temperature affects body size similarly across species. However, the strength of the effect of temperature differs between castes. Thus, *Formica* ants get increasingly smaller in colder environments; they follow the converse to Bergmann's rule. This is contrary to my initial prediction and the pattern found in previous ant studies (Cushman *et al.*

1993; Heinze *et al.* 2003; Bernadou *et al.* 2016; Purcell *et al.* 2016). My results contradict the rationale behind Bergmann's rule in ectotherms, where higher temperatures would shorten development time and lead to smaller adult sizes (as previously observed in ants (Molet *et al.* 2017; Oms *et al.* 2017)). This is potentially because *Formica* is a Nearctic genus for which low temperatures are probably most limiting. Following this rationale, if extreme temperatures restrict the size ants can reach, then I could expect that high temperatures would be more limiting for southern species whereas low temperatures would be more limiting for northern species. Supporting this, a lab experiment found that colonies of *Myrmecina nipponica* subjected to cold temperatures produced smaller queen morphs (Kikuchi *et al.* 1999). However, these results come from experimental manipulations which may not represent conditions experienced by colonies in nature, where ants use various means to thermoregulate their nests (Kadochová & Frouz 2013). Nevertheless, a field study found that colder nests of *Formica rufa* failed to produce alates (Rosengren *et al.* 1987), which underlines the importance of nest temperature for their development.

Cold temperatures are generally associated with an increase in body size (VanVoorhies 1996; Blanckenhorn & Demont 2004; Chown & Gaston 2010). Nonetheless, in this study I found the opposite pattern. This could be a result of seasonal restrictions. Indeed, if I consider mean annual temperature as a proxy for the number of degree days in a locality (i.e. growing season length), my results concur with the mechanism thought to drive the converse to Bergmann's rule – shorter growing seasons in cold environments constrain the size ectotherms can reach. This indicates that there is a trade-off between size and season length in *Formica* ants, which are univoltine (Kipyatkov 1993). Thus, they cannot palliate seasonal restrictions by having fewer generations per growing season (Kivelä *et al.* 2011; Horne *et al.* 2015). Therefore, unless they

can fasten their growth rate and shorten their development time, season length will restrict the size they can reach in colder environments. I find that *Formica* ants decrease in size with decreasing temperatures, which suggests that they cannot compensate for seasonal restrictions by increasing their growth rates. Similarly, a study on *Myrmica rubra* found that larvae developing at low temperatures pupate at smaller sizes in order to mature before the end of the growing season (Kipyatkov & Lopatina 2005). Development in ants is temperature dependent, whereas low temperatures slow their growth rate linearly and augment their development time non-linearly (Porter 1988). For instance, the development time of the pupal stage of *Formica subsericea* more than doubles with a decrease in temperature of 9°C (Penick *et al.* 2017). In effect, this makes short and cold summers especially restrictive, because they diminish growth rates and limit the time available to reach maturity. Since ants can reprogram the critical size for initiating metamorphosis (Tschinkel *et al.* 2003), they may lower it in order to mature in time in cold environments, albeit at a smaller size.

The different responses of the workers and alates further supports this explanation. Namely, temperature affects the size of queens and males more than the size of workers. If seasonality imposes size restrictions, I expect that temperature variation should affect the members of the reproductive castes more strongly since they are larger. Corroborating this, a study found that the development time of nanitics (i.e. the first brood of a founding queen) was 35% faster than in minor workers, whereas the development time of queens was 27% slower (Porter 1988). These increments in development time concur with size differences; nanitics are smaller than minor workers, which are in turn smaller than queens. Therefore, since queens and males are larger than the workers in this study, I expect them to require more time (i.e. degree days) to mature. This is in fact the pattern I obtain. Hence, I posit that it is the seasonal

restrictions of colder environments that limit the size *Formica* ants can reach in colder environments, especially for the males and queens. I propose that size restrictions in males and queens could have several consequences.

Size and fecundity

I find that males and queens of *Formica* are smaller in colder environments. This could modify their fitness in several ways. Smaller sizes could lower the fecundity of both males and queens (Vargo & Fletcher 1989; Honek 1993; Blanckenhorn 2000; Wiernasz *et al.* 2001; Schluns *et al.* 2003). For males, lower fecundity could impact a colony's fitness in various ways because, within the *Formica* genus, queen reproductive behavior goes from monoandrous (i.e. queens mate with one male) to polyandrous (i.e. queens mate with two or more males) (Pamilo 1982). Thus, the impact of less fecund males would depend on the mating dynamics of the species. Ant queens obtain their lifetime supply of sperm during one nuptial flight (Baer *et al.* 2006). Thus, monoandrous queens could have a lower reproductive output if they mate with smaller and less fecund males. Accordingly, polyandrous queens might need to mate with more males to reach a similar reproductive output as queens of populations harboring larger and more fecund males. Although an alternative behavior (i.e. increased number of matings) could hypothetically palliate for less fecund males in polyandrous species, it could also increase queen vulnerability to predation (Fjerdingstad & Boomsma 1998) and horizontally transmitted infections (e.g. bacteria in the genus *Asaia* are found in ants (Kautz *et al.* 2013) and are sexually transmittable in insects (Gonella *et al.* 2012)). Also, previous authors found that larger males have higher mating success (Davidson 1982; Abell *et al.* 1999), suggesting that smaller males are not as potent at gaining and keeping access to females.

As for queens, lower fecundity due to a smaller size could limit the number of offspring they produce. Queens typically have only one mating event in their lifetime (Hölldobler & Wilson 1990; Baer *et al.* 2006), and they subsequently deplete their sperm reserves as they age (Tschinkel 1987). For monogynous colonies, smaller less fecund queens could lower the colony's ultimate reproductive output since they would potentially produce less alates (i.e. males and future queens) throughout their lives (Fjerdingstad & Boomsma 1998). Moreover, smaller colonies could have a lower fitness since fewer workers would be available to cater to the colony's needs (e.g. resource acquisition, brood care and nest defense). The colony could, however, replace the resident queen with depleted sperm reserves with a more fecund queen (Tschinkel & Howard 1978). Additionally, size also influences longevity in ants, with larger workers living longer (Calabi & Porter 1989). Therefore, smaller queens could have a lower longevity, which could further limit a monogynous colony's overall production of alates. As for polygynous species, colonies would need a higher number of small queens to achieve a similar reproductive output as in populations harboring large queens. Therefore, I expect that the environmental restrictions on male and queen size I observe have cascading effects on the reproductive biology of *Formica* species. Specifically, size reductions could lower the reproductive ability of both males and queens, which could restrict species ranges to warmer environments.

Size and dispersal ability

Constraints on size could influence the dispersal ability of both queens and males during their mating flight. For instance, if more energy allocation is spent towards the accumulation of lipid stores in queens, this would lower their dispersal ability by reducing their flight muscle ratio

(Helms & Godfrey 2016). Queens harboring relatively more lipid stores (Helms & Godfrey 2016) and smaller males might have lower flight abilities (Fjerdingstad & Boomsma 1997), which would worsen their capacity to disperse. However, in multiple *Myrmica* species, it is usually the large macrogynes that disperse while the small microgynes come back to their natal nest after mating (Brian & Brian 1955; Elmes 1991). Thus, contrary to the expectation that smaller queens would have a higher flight ability, in these species the macrogynes would be the most potent dispersers. Similarly, in *Formica truncorum*, macrogynes are more likely to disperse (Sundström 1995). Concurrently, where growing seasons are short, queens that do not disperse at all could take advantage in lowering their construction and provisioning costs (Heinze & Tsuji 1995). As for males, larger individuals are more likely to disperse (Sundström 1995), and are thought to be better flyers (Fjerdingstad & Boomsma (1997), but see Davidson (1982)). Larger sizes could allow males to disperse farther. This could decrease spatial variation in allele frequencies, especially in populations where queens found colonies dependently. Here, I find that males and queens grow smaller in colder environments. If their flight muscle ratio stays the same as they grow smaller, alates would be able to disperse farther (Helms & Godfrey 2016). However, if the reduction in size I observe is due to a trade-off where individuals invest more in energy reserves than in flight muscles, they would have a lower dispersal ability. Most of the evidence found in previous studies suggest that smaller sizes would lower the dispersal ability of males and queens. Therefore, I propose that climatic restrictions on size negatively affects the dispersal ability of *Formica* ants and might contribute to limiting their species distributions.

Size and colony founding

A crucial stage in a colony's existence happens directly after the nuptial flight, when newly mated queens must run the gauntlet of colony founding. After their mating flight, claustral queens (i.e. queens that found their colony independently and do not forage outside the nest) seal themselves in a new nest and rear their first brood by histolizing their wing muscles (Heinze & Tsuji 1995; Peeters 2012). This unforgiving period would be even harsher if shorter and colder summers during queen development restrict the size they can reach, which is what I find support for. Indeed, since larger queens are more likely to survive the initial stages of colony founding (Wiernasz & Cole 2003), I expect that smaller queens of monogynous species will struggle to establish colonies. This could be a result of the relationship between size and resistance to starvation. Indeed, larger workers resist better to starvation (Heinze *et al.* 2003; Modlmeier *et al.* 2013). Concurrently, smaller queens have relatively less fat reserves (Wagner & Gordon 1999). This suggests that the smaller *Formica* queens of colder environments are more at risk to starve during colony founding. Once a queen produces a number of workers though, I expect that her risk of starvation would drop, because queens surrounded by workers survive to starvation longer (Kaspari & Vargo 1995). Similarly, groups of ants resist better to starvation than single workers (Modlmeier *et al.* 2013). Thus, during colony ontogeny, I expect that the risk of starvation of founding queens will be highest during their first overwintering period, and that it will further diminish in subsequent seasons as the number of resident workers in the colony grows.

An additional limitation for small founding queens might be a lower tolerance to desiccation and heat. Indeed, desiccation resistance is positively related to body size (Hood & Tschinkel 1990). Concurrently, in a study of Neotropical species, smaller ants preferred to forage

in moist conditions whereas larger ants could forage in both moist and dry conditions (Kaspari 1993). Thus, small queens might sustain an elevated risk of desiccation while seeking a nest site and establishing a colony. Furthermore, smaller queens might be less resistant to heat (Cerdá & Retana 1997; Wendt & Verble-Pearson 2016), which might also put smaller queens at a higher risk while seeking a new nest. However, smaller ants have lower supercooling points (Hahn *et al.* 2008). Accordingly, across multiple species within the genus *Solenopsis*, the workers, which are smaller than males and queens, have a slightly lower supercooling point than the reproductives (Francke *et al.* 1986). Thus, since evidence shows that ants are not freeze-tolerant (Hahn *et al.* 2008), smaller queens with lower supercooling points might be more adapted to avoid freezing during the cold spells of winter.

The pattern I obtain here, namely that queens get smaller with decreasing temperatures, suggest that it is riskier for monogynous queens to establish colonies independently in colder environments. If seasonal restrictions are too stringent, they might have to shift to polygyny and dependent colony founding to keep subsisting. Accordingly, across ant taxa, independent foundresses have a relatively higher fat content than dependent foundresses (Keller & Passera 1989). This is also apparent in species where both types of colony founding exist. For instance, in the species *Formica truncorum*, larger females harboring more fat and glycogen preferentially disperse, whereas smaller females with less fat and glycogen reserves preferentially dealate without accomplishing a mating flight (Sundström 1995). Thus, environmental restrictions on size might promote polygyny, which would help explain why facultatively polygynous species are more prevalent in boreal habitats (Heinze 1993) and at higher altitudes (Reymond *et al.* 2013; Heinze & Rueppell 2014). Further supporting this idea, when intraspecific polymorphism of queens is present (i.e. microgynes and macrogynes), macrogynes establish monogynous colonies

independently, whereas microgynes establish polygynous colonies with the aid of workers (Meunier & Chapuisat 2009).

Similarly, short summers and long cold winters pose a high cost to independent colony founding strategies. This may promote behaviors such as social parasitism (Heinze & Tsuji 1995), where a queen invades a nest of its host species to replace the resident queen (Buschinger 2009). This strategy could be especially adaptive for the smaller *Formica* queens of colder environments, because it could augment their odds of founding a colony successfully. Supporting this idea, multiple forms of social parasitism exist in *Formica*, such as temporary parasitism, facultative slavery, and dulosis (Buschinger 2009). Moreover, social parasitism is especially prevalent in boreal and alpine habitats, where as much as 30% of ant species are social parasites (compared to the estimated 3% of all ant species that would exhibit some form of social parasitism) (Heinze & Tsuji 1995). Social parasites are also smaller than their host species (Aron *et al.* 1999). Therefore, I propose that environmental constraints on size might drive a shift from independent to dependent colony founding, associated with a shift from monogyny to polygyny. Moreover, I propose that size restrictions may promote social parasitism in colder environments.

Size and foraging efficiency

My results show that workers decrease in size in colder environments. Since body size positively relates to heat resistance (Cerdá & Retana 1997; Wendt & Verble-Pearson 2016) and desiccation resistance (Hood & Tschinkel 1990), larger workers might be able to forage under a higher range of environmental conditions. Therefore, smaller workers might not be able to forage as much as larger workers, which could decrease the amount of energy brought back to the colony and limit its growth. Environmental restrictions on worker size could thus act as an additional limit to

Formica species distributions. However, smaller workers have lower supercooling points (Hahn *et al.* 2008), which may be associated with an increase in their survival in environments where winters experience severe cold spells. Smaller sizes in workers could thus illustrate a trade-off between cold resistance and foraging efficiency; in warmer environments, workers reach a larger size and are more efficient foragers, whereas in cold environments, they reach smaller sizes and are more resistant to cold temperatures.

Abiotic factors drive shape variation in ants

According to my predictions, no abiotic factors drove shape variation consistently among castes in *Formica*. I find no evidence for environmental drivers of shape within the reproductive castes. In contrast, I find that precipitation, seasonality and temperature drive shape variation in workers. Of these abiotic factors, temperature had the strongest effect on worker's shape. I speculate that this clear dichotomy between the worker and the reproductive castes could originate from two different underlying mechanisms. In the case of workers, selective pressures may vary along gradients, thereby not stabilizing their morphology. On the contrary, strong selective pressures on queens and males might stabilize their shape, since queens and males have specific roles within the colony for which there might exist an ideal shape. Accordingly, slight deviations from this optimal morphology could have a high cost. Specifically, selective pressures on the reproductive success (Fjerdingstad & Boomsma 1997) and the flight ability (Keller *et al.* 2014) of the sexuals might drive their phenotype. Indeed, sexuals need large gasters to contain their reproductive organs (Trible & Kronauer 2017), but also large thoraxes to accommodate their wing muscles (Keller *et al.* 2014) and metabolic reserves (Peeters 2012). In *Formica*, an increase in gaster size might be counterbalanced by an increase in thorax size to conserve a

similar flight muscle to weight ratio (Helms & Godfrey 2016), thus heightening fecundity while minimizing drawbacks on dispersal ability. These selective pressures might be consistent along environmental gradients, which would explain the static shape of the alates.

Species within my dataset are found across North America (Fig. 2). Thus, although *Formica* is primarily a monomorphic genus (Wilson 1953), it is not surprising that I find some degree of shape variation in the worker caste along broad-scale gradients. I find that abiotic factors, especially temperature, affect workers' shape. However, contrary to my prediction that head size would show a positive allometry along a temperature gradient, my results show that eye distance, inter-carenal distance, and head width follow a negative allometry. Thus, as ants increase in size at higher temperatures, they have relatively thinner and smaller heads. Simultaneously, scape length, femur length and mesosoma length show a positive allometry. Thus, the workers' scape, femur and mesosoma become relatively longer in warmer environments.

In this study, I show that ants increase in size in warmer environments. This environmental release on size could allow workers to grant a relatively higher investment to certain structures as they become larger. These differences could reflect the importance of each trait for this genus. The relative decrease in head width could show that there is only a very weak diet shift, if any at all, associated with temperature variations. Indeed, head size relates to prey size, with larger heads allowing the capture of larger preys (Kaspari 1993; Sarty *et al.* 2006; Schofield *et al.* 2016). If there are no larger potential preys in southern habitats, there may be no advantage in using additional energy to produce larger head sizes. *Formica* ants rely heavily on carbohydrates (Way 1963; Carroll & Janzen 1973) (Fig. S4). Moreover, to obtain protein, multiple species of *Formica* scavenge for dead insects (McIver & Loomis 1993; Mikheyev &

Tschinkel 2004). Both strategies for food collection do not require large mandibles. Thus, if size restrictions are weaker in warmer environments, it might be more adaptive to invest in other structures than head size for *Formica* workers. Relatively longer legs could allow workers to move faster and make the environment more planar, as predicted by the size-grain hypothesis (Kaspari & Weiser 1999). Having relatively longer scapes could facilitate following pheromone trails (Weiser & Kaspari 2006). Furthermore, ants with relatively longer scapes might enable foragers to avoid collisions and might increase the speed at which they follow pheromone trails (Couzin & Franks 2002; Schöning *et al.* 2005). Body length correlates positively with uptake rate and load size of liquid foods in Formicines (Davidson *et al.* 2004). Increased mesosoma length may allow workers to carry a larger load in their crop. They could thus transport a higher amount of carbohydrates (i.e. honeydew), which is a major component in the diet of *Formica* (Way 1963). A larger mesosoma in workers could also allow for more T1-associated muscles, which could enhance their capacity for load carriage (Keller *et al.* 2014). My results show that, as *Formica* workers increase in size along a temperature gradient, they invest relatively more energy in morphological traits relating to foraging efficiency. This suggests that in colder environments, *Formica* workers may be less efficient foragers, which could slow colony growth. Therefore, environmental constraints on worker shape might negatively impact colony fitness, and act as a factor limiting *Formica* species distributions towards the poles.

Caveat

I hypothesize that the strength of the relationship between size and temperature is stronger in the reproductive castes than in the workers because of size differences among castes. This is because larger ants take more time to develop (Porter 1988) and, in the *Formica fusca* group, queens and

males are larger than workers (Fig. 3). Therefore, I expect them to be more limited by restrictions in growing seasons. However, I acknowledge that the worker model might be weaker because of the high size variation of this caste. For instance, during colony ontogeny there is an increase in mean worker size (Brian 1957; Wilson 1983; Tschinkel 1988, 1993). I could not correct for colony size in my analyses, which may partially explain why the relationship between temperature and the size of workers was weaker. I expect that if I had corrected for colony size, the relationship between worker size and temperature would be stronger. Next, the relatively small slopes of the relationship between size and temperature may be a result of the noise embedded in my dataset. Specifically, specimens were collected over a wide range of years and their labels did not contain coordinates. Furthermore, temperature differences at the local scale may also be important. For instance, if the nest is exposed to sunlight as opposed to being in a shaded area, the brood would experience different temperatures as they mature. Similarly, nest material could also have an influence because of the thermal properties of the substrate (e.g. under a rock versus within a log). As a result, the environmental data I assigned for each specimen are a gross estimate of the conditions these ants experienced as they matured. However, I expect that higher geographic resolution, microclimatic data and information on nest substrate would only strengthen the relationship I observe in this study.

Additionally, behavioural thermoregulation could attenuate the relationship along the temperature gradient. Ants of colder environments could palliate for the shortened temperature window by relocating brood to suitably warm chambers, by using metabolic heat to maintain a heat core (Kadochová & Frouz 2013), and by nesting under appropriately thick rocks to elevate temperatures and accelerate larval development (McCaffrey & Galen 2011). Nonetheless, despite

these limitations, my results support the hypothesis that short growing seasons in cold environments restrict the size that univoltine insect species can reach.

Lastly, I did not account for the influence of nutrition, which is a relevant component since resource availability affects the size ectotherms can reach (Moczek 1998; Arnett & Gotelli 1999a; Chown & Klok 2003; Stillwell *et al.* 2007). Specifically, higher food quality and quantity can shorten developmental time and lead to larger individuals (Trichilo & Leigh 1988; Danner & Joern 2004). Furthermore, diet influences caste determination in social insects (Schwander *et al.* 2010; Smith & Suarez 2010) and the regulation of juvenile hormone in ants (Leboeuf *et al.* 2016), which plays a crucial part in the development of arthropods. Therefore, I expect that accounting for the effect of nutrition would render the relationship between temperature and body size more evident. Thus, future studies aiming to disentangle the drivers of size clines in ants should also take diet into consideration.

Summary

Throughout this study, I identify caste-specific phenomena. I find that environmental factors affect both the size and shape of *Formica* ants, but that the magnitude of clinal variation differs between castes. All castes, but especially the males and queens, decreased in size with decreasing temperatures, consistent with the converse to Bergmann's rule. This pattern held both at the interspecific and intraspecific levels. My results highlight the potentially negative effects that colder temperatures can have on alates, which could limit the extent of ant species distributions by restricting the size they are able to reach. As for shape, temperature, seasonality, and precipitation each affected worker shape, but not the shape of queens or males. I posit that

this divergence stems from different selective forces driving the shape of sexuals versus the shape of workers. Specifically, I hypothesize that an environmental release in warmer environments allows workers to have a more efficient shape for foraging. Because of their morphological distinctness and the numerous caste-specific patterns I identify, I propose that each caste acts as a discrete selection unit. Consequently, for a species to persist, I argue that each caste must meet its unique ecological necessities. This highlights the validity and importance of examining all castes to establish ant species distribution.

The eminent Robert MacArthur said that “To do science is to search for repeated patterns, not simply to accumulate facts”. I argue that my study shows that comparing caste-specific patterns is a powerful tool to identify the drivers of species distributions in ants. I hope that, despite the difficulty to collect data on ant males and queens, this research will further motivate myrmecologists to employ a more holistic approach and to heighten their focus on alates in future studies.

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Legend of Tables & Figures

Table 1 Selected morphological traits from the Nearctic Formica dataset (Francoeur 1973).

Table 2 Results of the linear mixed models using environmental variables as predictors of size (i.e. response variable) for each caste. As random effects, I had intercept for species to account for phylogenetic relationships. To control for the effect of altitude I included DEM as a covariate in all models. The abbreviations used in the table are MAT (mean annual temperature), TS (temperature seasonality), DEM (Global elevation data). The size variable was obtained by extracting the size component of 12 morphological measurements with the Aitchison transformation.

Table 3 Summary of selected models for size. Models with the lowest scores were chosen. When multiple models were within 2 AIC units, the most parsimonious model was used.

Table 4 Results of the linear mixed models using environmental variables as predictors of shape (i.e. response variable) for each caste. As random effects, I had intercept for species to account for phylogenetic relationships. To control for the effect of altitude I included DEM as a covariate in all models. The abbreviations used in the table are MAT (mean annual temperature), TS (temperature seasonality), DEM (Global elevation data). The shape variable is the PC1 of a PCA on the 12 size-free shape variables extracted by doing the Aitchison transformation on 12 morphological measurements.

Table 5 Summary of selected models for shape. Models with the lowest scores were chosen. When multiple models were within 2 AIC units, the most parsimonious model was used.

Figure 1 Selected morphological traits from the Nearctic Formica dataset (Francoeur 1973).

Figure 2 Distribution map of the Formica specimens of the dataset I used (Francoeur 1973).

Figure 3 Size variation in (a) queens, (b) males and (c) workers. Variation in head width (mm) for (d) queens, (e) males and (f) workers. Species are ordered by the mean latitude of their distribution. The line within each boxplot represents the median value of size. The box edges represent the 25th and 75th percentiles, the extended vertical lines represent values within the certain distance (1.5 times the inter-quartile range) and the points represent extreme values.

Figure 4 Relationship between size and geographic variation in temperature (°C) in (a) queens, (b) males and (c) workers. Relationship between head width (mm) and geographic variation in temperature (°C) in (d) queens, (e) males and (f) workers. Significant P values for linear mixed models marked with asterisks (*** for $p < 0.001$, ** for $p < 0.01$ and * for $p < 0.05$).

Figure 5 Relationship between shape and variation in mean annual temperature (°C) in (a) queens, (b) males and (c) workers. Significant P values for linear mixed models marked with asterisks (*** for $p < 0.001$, ** for $p < 0.01$ and * for $p < 0.05$).

Tables & Figures

Table 1 Selected morphological traits from the Nearctic Formica dataset (Francoeur 1973).

Measurement Characteristic	Trait measured	Measurement method	Functional significance
Head size	Maximum Head width	In full face view, maximum head width according to a line crossing the center of the eyes	Head size vary allometrically with body size, head size relates to predatory strategies, with wider heads allowing for larger mandibles and therefore larger prey (Kaspari 1993; Sarty et al. 2006; Schofield et al. 2016). Head width is also a proxy for body size (Bernadou et al. 2016; Purcell et al. 2016). Head length is the best estimator of mass in Formicinae (Kaspari & Weiser 1999).
	Minimum Head width	In full face view, minimum head width measured at the base of the genae, at the level of the articular condyles of the mandibles	
	Head length	In full face view, maximum head length measured from the extreme median point of the clypeus to the central point of the occipital border	
Scape size	Scape length	Maximum scape length excluding the condyle, while the scape is seen perpendicularly to the articulation plan of the funiculus.	Longer scape facilitates following pheromone trails (Weiser & Kaspari 2006). It may also enable foragers to avoid collisions and follow pheromone trails at higher speeds (Couzin & Franks 2002; Schöning et al. 2005).
Eye size	Eye width	Measure of the largest diameter of the composed eye	Wider eyes may be found in more predatory species or may depend on foraging period, with low light level foragers having bigger eyes (Weiser & Kaspari 2006; Narendra et al. 2013; Schofield et al. 2016).
Eye position	Inter-ocular distance	In full face view, shortest distance between the composed eyes	More dorsally positioned eyes (larger inter-ocular distance) are characteristic of visual predators (Schofield et al. 2016).
Carenal Width	Inter-carenal distance	Inter-carenal width – in full face view, distance between the end of each carena.	Unknown
Mesosoma	Mesosoma length (i.e. Weber's length)	Mesosoma length – maximum length of the mesosoma measured from the anterodorsal side of the pronotum to the crest posteroventral of the propodeum (i.e. epinotum, the first segment of the abdomen which is fusionned to the thorax to form the alitrunk in the adult).	Indicative of overall body size, which correlates with metabolic characteristics (Gibb et al. 2015). It may also relate to resource use (Kaspari & Weiser 1999; Schofield et al. 2016). Pronotum width is the best single predictor of total mass for myrmicine ants (Kaspari & Weiser 1999). Enlarged T1 and T1-associated muscles in workers provide superior strength and mobility to the neck controlling head movements, whereas enlarged T2 in queens relate to developed wing muscles (Keller et al. 2014).
	Pronotum width	Pronotum width – maximum width in dorsal view. Measured in front of the tegulae for the queens and males.	
Petiole size	Petiole width	maximum width of the petiole seen dorsally or posteriorly	The pedicel refers to the connection between the gaster and the mesosoma and, depending on the subfamily, consist of 1 segment (petiole) or 2 segments (petiole and postpetiole). Its size might affect an ant ability to maneuver its gaster.
Leg size	Femur length	Femur length – length of the mesofemur, measured from the base of the coxa to the insertion of the tibia.	In simple habitats, longer leg length increases locomotion speed and in more complex habitat, shorter leg length allows more manoeuvrability and the possibility to explore tighter spaces (Sarty et al. 2006; Gibb & Parr 2010, 2013; Schofield et al. 2016). Foreleg length relates to swimming speed (Gripshover et al. 2018).
	Femur width	Femur width – maximum width of the mesofemur in dorsal view	

Table 2 Results of the linear mixed models using environmental variables as predictors of size (i.e. response variable) for each caste. As random effects, I had intercept for species to account for phylogenetic relationships since a species-level phylogeny for this group is not available. To control for the effect of altitude I included DEM as a covariate in all models. The abbreviations used in the table are MAT (mean annual temperature), TS (temperature seasonality), MAP (mean annual precipitation) and DEM (Global elevation data). The size variable was obtained by extracting the size component of 12 morphological measurements with the Aitchison transformation.

Caste	Model	AIC
Worker	DEM	4408.18
Worker	DEM + MAT	4399.30
Worker	DEM + MAP	4406.90
Worker	DEM + TS	4402.68
Worker	DEM + MAT + MAP	4399.46
Worker	DEM + MAT + TS	4399.89
Worker	DEM + MAT + MAP + TS	4401.11
Queen	DEM	665.68
Queen	DEM + MAT	660.02
Queen	DEM + MAP	667.36
Queen	DEM + TS	663.45
Queen	DEM + MAT + MAP	660.67
Queen	DEM + MAT + TS	661.98
Queen	DEM + MAT + MAP + TS	662.50
Male	DEM	425.30
Male	DEM + MAT	419.88
Male	DEM + MAP	422.34
Male	DEM + TS	422.00
Male	DEM + MAT + MAP	417.46
Male	DEM + MAT + TS	421.19
Male	DEM + MAT + MAP + TS	421.90

Table 3 Summary of selected models for size. Models with the lowest scores were chosen. When multiple models were within 2 AIC units, the most parsimonious model was selected. If not, the most parsimonious model was used. The abbreviations used in the table are MAT (mean annual temperature), TS (temperature seasonality), MAP (mean annual precipitation) and DEM (Global elevation data).

Caste	Fixed effects	Estimate	Standard Error	df	t value	Pr (> t)
Worker	DEM	-2.555 ⁻⁰⁴	2.099 ⁻⁰⁴	1523	- 1.217	0.223
	MAT	2.754 ⁻⁰³	8.330 ⁻⁰⁴	864.9	3.306	9.85 ⁻⁰⁴ ***
Queen	DEM	-1.097 ⁻⁰³	2.713 ⁻⁰⁴	333	- 4.044	6.54 ⁻⁰⁵ ***
	MAT	2.638 ⁻⁰³	9.345 ⁻⁰⁴	319.4	2.823	5.06 ⁻⁰³ **
Male	DEM	-3.732 ⁻⁰⁴	2.682 ⁻⁰⁴	196.3	- 1.391	0.16569
	MAT	2.312 ⁻⁰³	8.376 ⁻⁰⁴	194.2	2.760	6.34 ⁻⁰³ **

Table 4 Results of the linear mixed models using environmental variables as predictors of shape (i.e. response variable) for each caste. As random effects, I had intercept for species to account for phylogenetic relationships since a species-level phylogeny for this group is not available. To control for the effect of altitude I included DEM as a covariate in all models. The abbreviations used in the table are MAT (mean annual temperature), TS (temperature seasonality), MAP (mean annual precipitation) and DEM (Global elevation data). The shape variable is the PC1 of a PCA on the 12 size-free shape variables extracted by doing the Aitchison transformation on 12 morphological measurements.

Caste	Model	AIC
Worker	DEM	2938.98
Worker	DEM + MAT	2934.25
Worker	DEM + MAP	2938.01
Worker	DEM + TS	2938.14
Worker	DEM + MAT + MAP	2934.55
Worker	DEM + MAT + TS	2924.86
Worker	DEM + MAT + MAP + TS	2921.13
Queen	DEM	865.63
Queen	DEM + MAT	866.72
Queen	DEM + MAP	866.17
Queen	DEM + TS	865.43
Queen	DEM + MAT + MAP	867.66
Queen	DEM + MAT + TS	867.50
Queen	DEM + MAT + MAP + TS	868.95
Male	DEM	446.06
Male	DEM + MAT	448.13
Male	DEM + MAP	447.97
Male	DEM + TS	446.97
Male	DEM + MAT + MAP	450.06
Male	DEM + MAT + TS	448.05
Male	DEM + MAT + MAP + TS	450.20

Table 5 Summary of selected models for shape. Models with the lowest scores were chosen. When multiple models were within 2 AIC units, the most parsimonious model was selected. The abbreviations used in the table are MAT (mean annual temperature), TS (temperature seasonality), MAP (mean annual precipitation) and DEM (Global elevation data).

Caste	Fixed effects	Estimate	Standard Error	df	t value	Pr (> t)
Worker	DEM	-1.019 ⁻⁰⁴	1.088 ⁻⁰⁴	1626	-0.937	0.3491
	MAT	-1.978 ⁻⁰³	5.076 ⁻⁰⁶	1636	-3.896	1.02 ⁻⁰⁴ ***
	MAP	-1.120 ⁻⁰⁵	4.673 ⁻⁰⁶	1630	-2.397	1.66 ⁻⁰² *
	TS	-3.899 ⁻⁰⁵	9.907 ⁻⁰⁶	1638	-3.936	8.65 ⁻⁰⁵ ***
Queen	DEM	4.005 ⁻⁰⁵	3.543 ⁻⁰⁴	331.5	1.13	0.2592
	TS	3.423 ⁻⁰⁵	2.291 ⁻⁰⁵	327.0	1.494	0.1361
Male	DEM	8.251 ⁻⁰⁵	4.228 ⁻⁰⁴	197.8	0.195	0.845

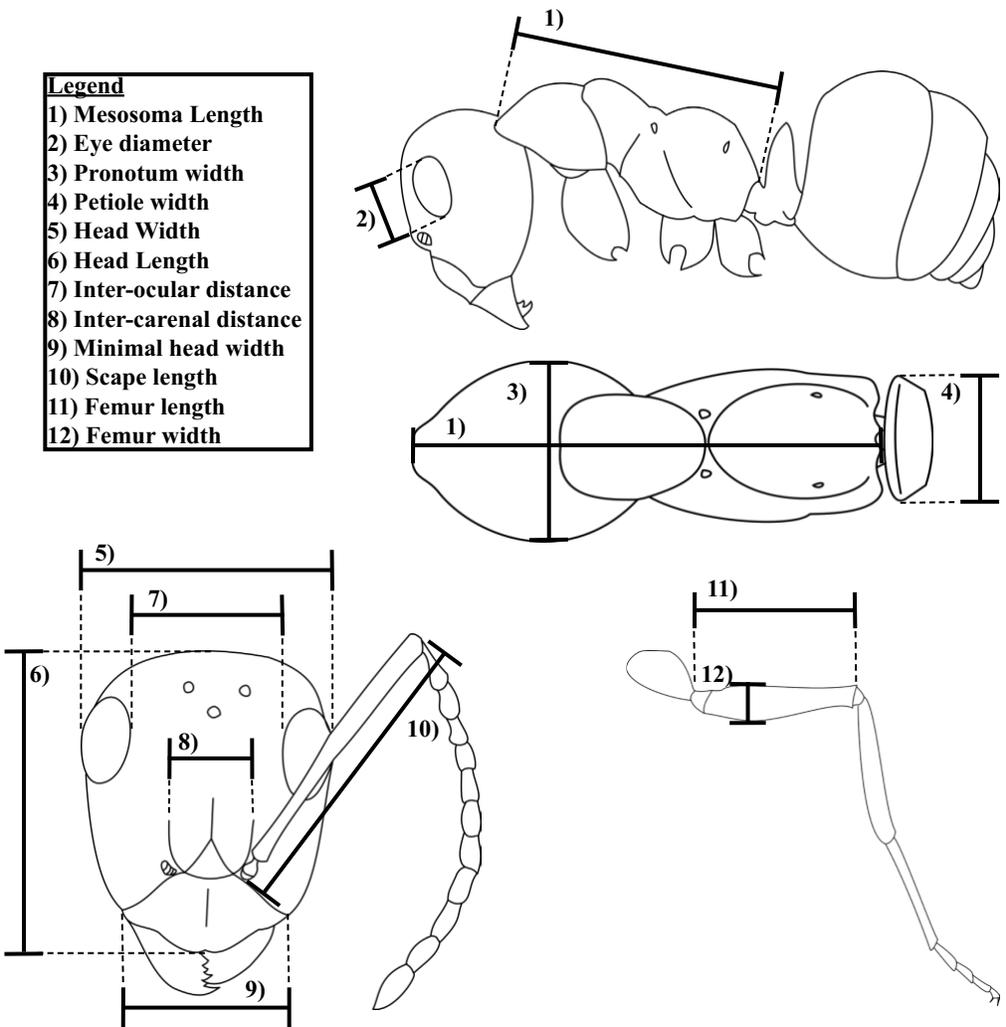


Figure 1 Selected morphological traits from the Nearctic *Formica* dataset (Francoeur 1973).

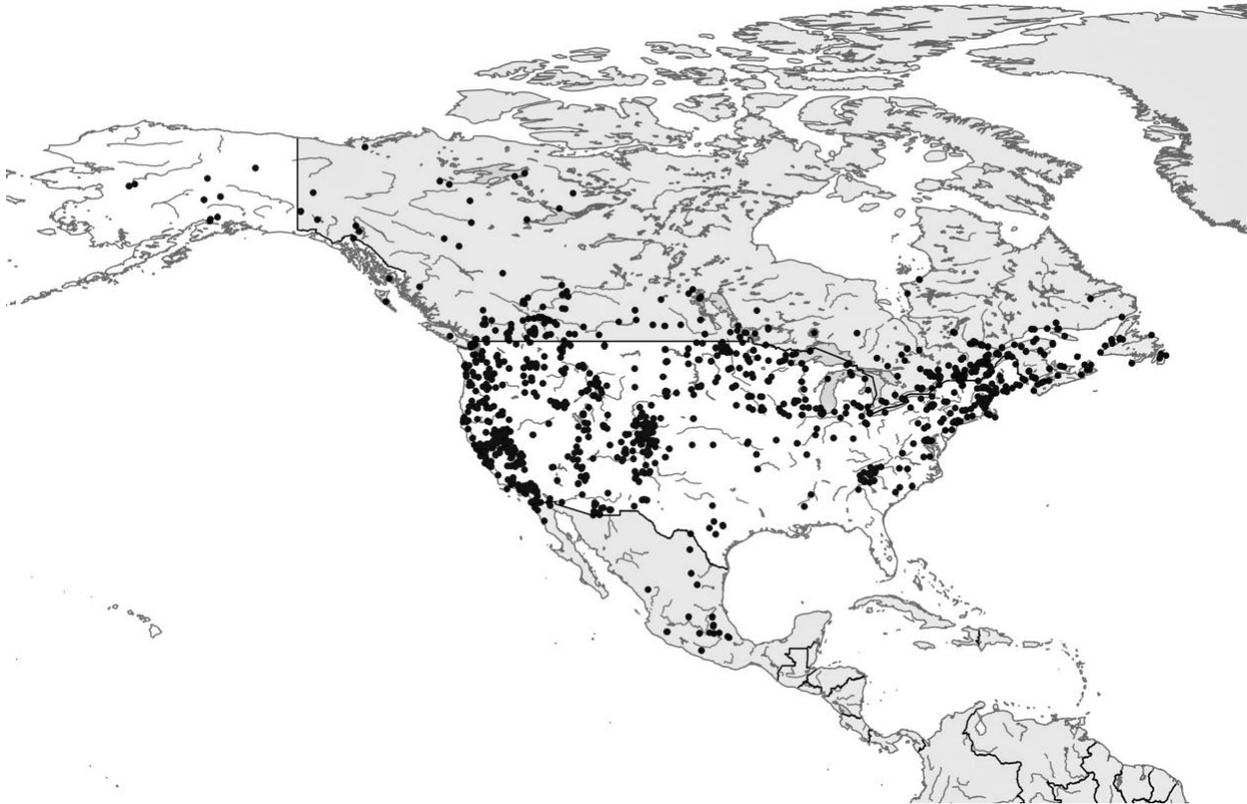


Figure 2 Distribution map of the *Formica* specimens used (Francoeur 1973).

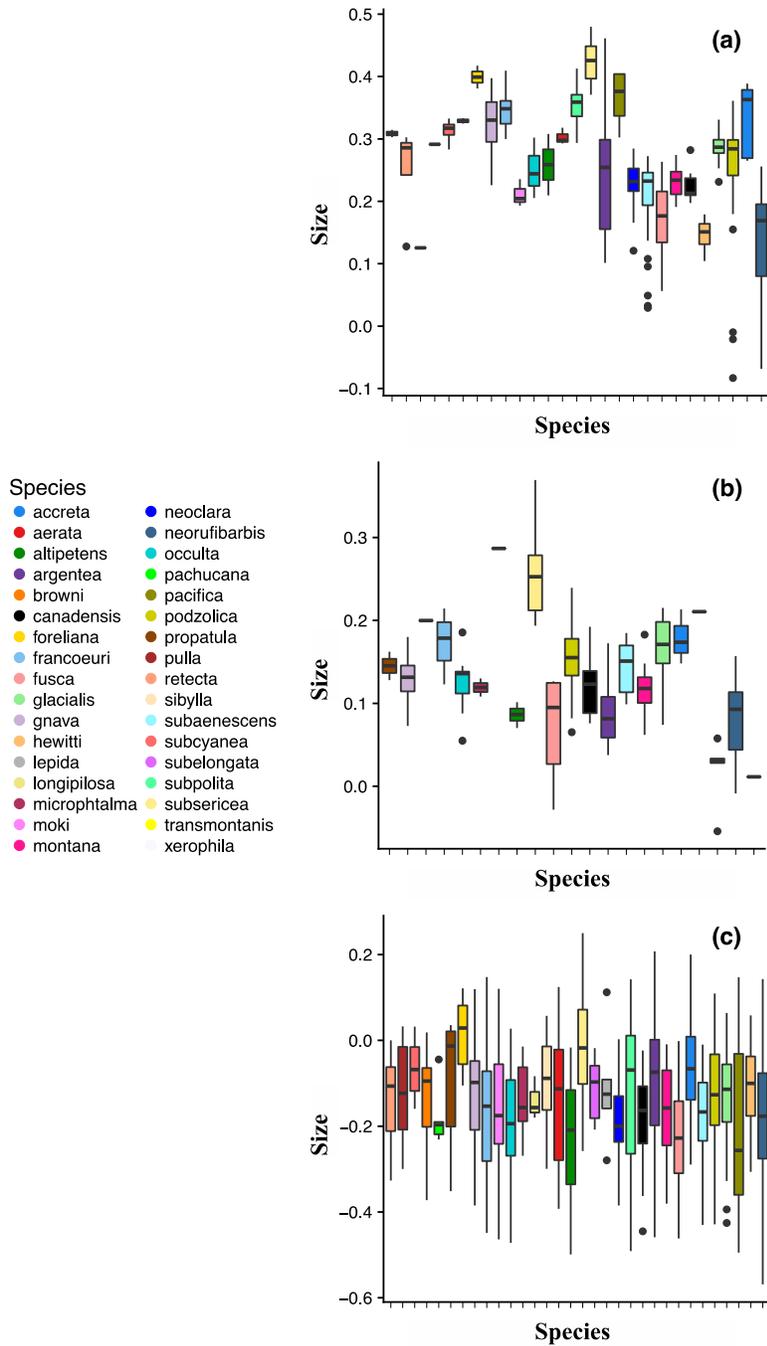


Figure 3 Size variation in (a) queens, (b) males and (c) workers. Here, size represents a shape-free variable produced by using the Aitchison transformation on 12 morphological traits. Species are ordered by the mean latitude of their distribution. The line within each boxplot represents the median value of size. The box edges represent the 25th and 75th percentiles, the extended vertical lines represent values within the certain distance (1.5 times the inter-quartile range) and the points represent extreme values.

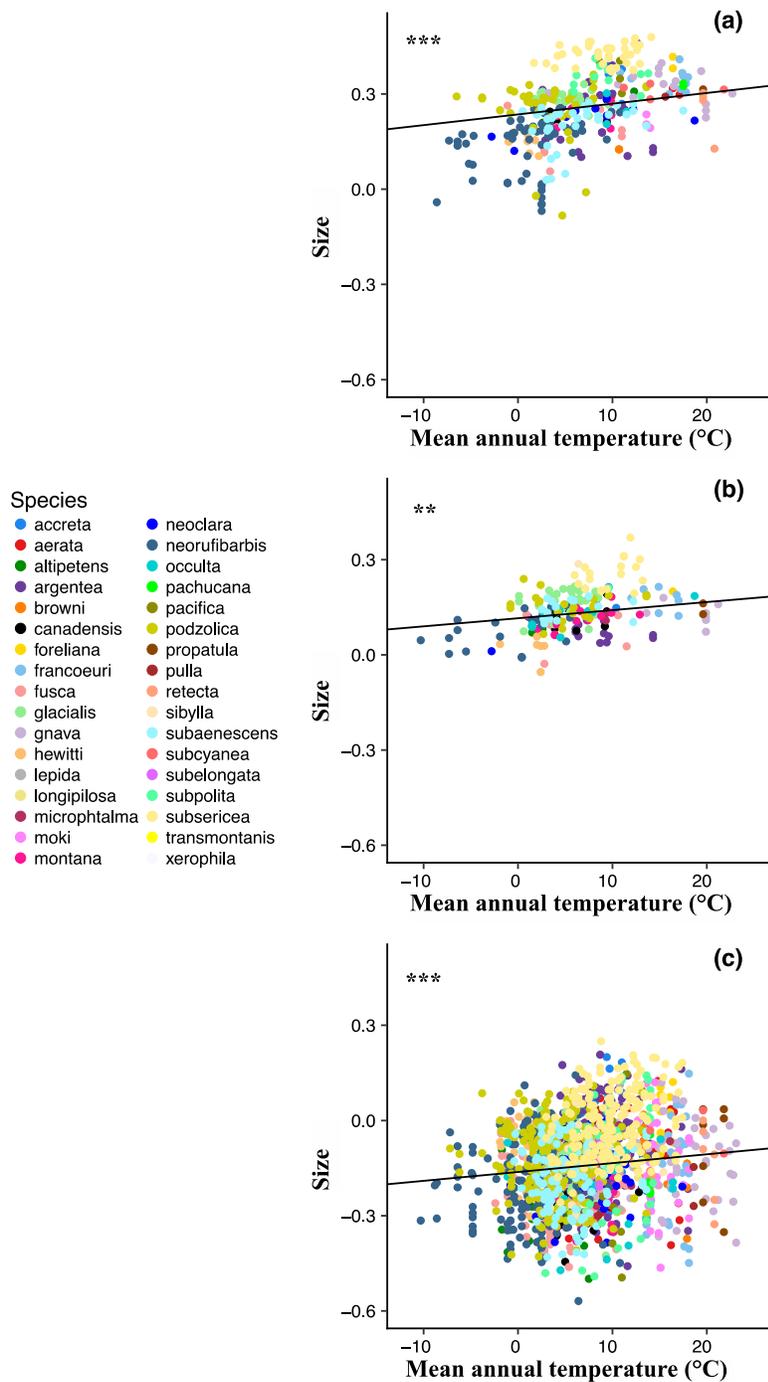


Figure 4 Relationship between size and geographic variation in temperature (°C) in (a) queens, (b) males and (c) workers. Here, size represents a shape-free variable produced by using the Aitchison transformation on 12 morphological traits. Significant P values for linear mixed models marked with asterisks (***) for $p < 0.001$, ** for $p < 0.01$ and * for $p < 0.05$).

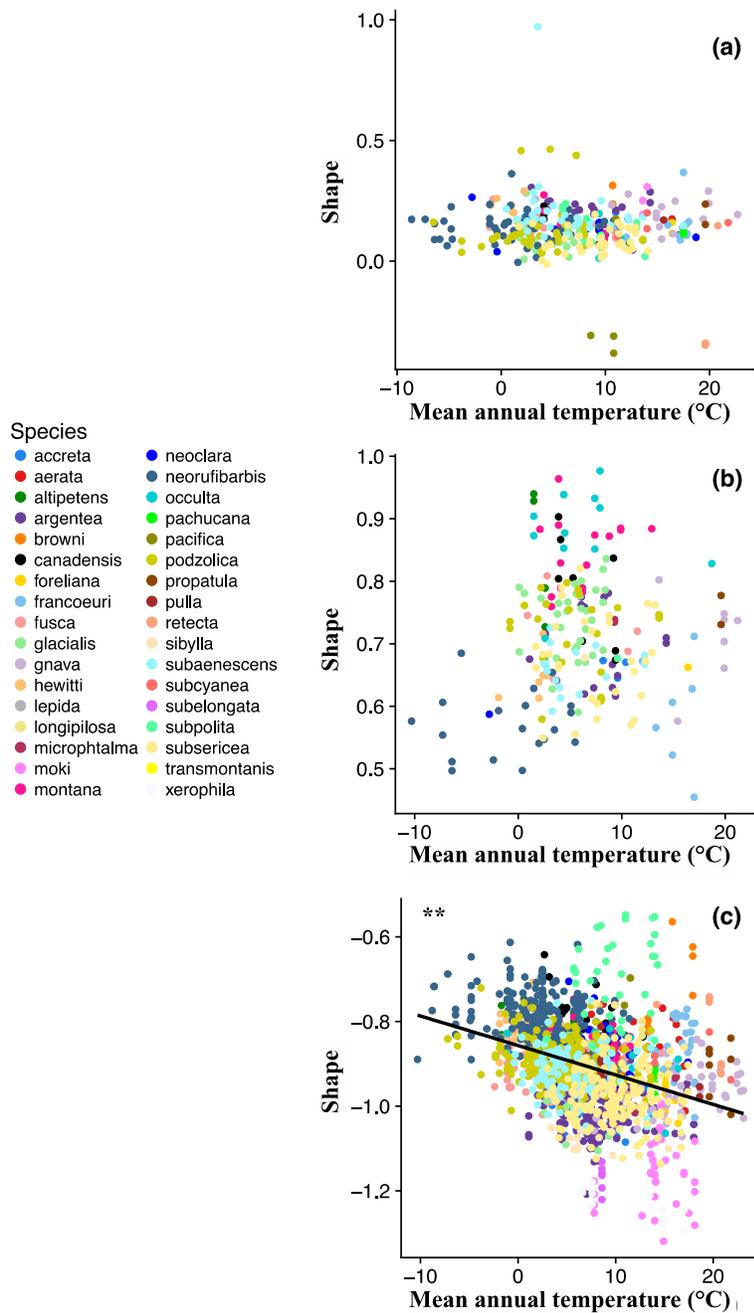


Figure 5 Relationship between shape and variation in mean annual temperature (°C) in (a) queens, (b) males and (c) workers. Here, shape is the PC1 of the 12 size-free shape variables produced by using the Aitchison transformation on 12 morphological traits. Significant P values for linear mixed models marked with asterisks (***) for $p < 0.001$, ** for $p < 0.01$ and * for $p < 0.05$).

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Supplementary Material

Table S1 Correlation of traits with shape proxy for workers.

Figure S1 Principal component analyses (PCAs) using 12 log-transformed morphometric measurements to visualise how (a) queens, (b) workers, (c) males and (d) all castes of multiple *Formica* species segregate in the morphospace.

Figure S2 Relationship between size and geographic variation in temperature (°C) in (a) queens, (b) males and (c) workers. Each regression line represents a species that had a significantly different intercept for size. Significant P values for linear mixed models marked with asterisks (*** for $p < 0.001$, ** for $p < 0.01$ and * for $p < 0.05$).

Figure S3 Schematic representation of scaling. Traits varying proportionately with size show isometry ($b = 1$). Traits increasing relative to size show a positive allometry ($b > 1$). Traits decreasing relative to size show a negative allometry ($b < 1$).

Figure S4 Worker of *Formica* sp. (*fusca* group) tending aphids for honeydew.

Table S1 Correlation of traits with shape proxy for workers ($n = 1658$). Shown in bold are correlations above 0.6.

	Correlation	p. value
Inter-ocular diameter	0.818	0
Inter-carenal distance	0.759	2.25^{-311}
Head width	0.756	1.64^{-306}
Minimal head width	0.342	1.04^{-46}
Petiole width	0.225	1.72^{-20}
Pronotum width	0.133	4.83^{-08}
Femur width	-0.301	5.60^{-36}
Eye diameter	-0.342	8.26^{-47}
Mesosoma Length	-0.666	6.22^{-213}
Femur Length	-0.770	0
Scape length	-0.794	0

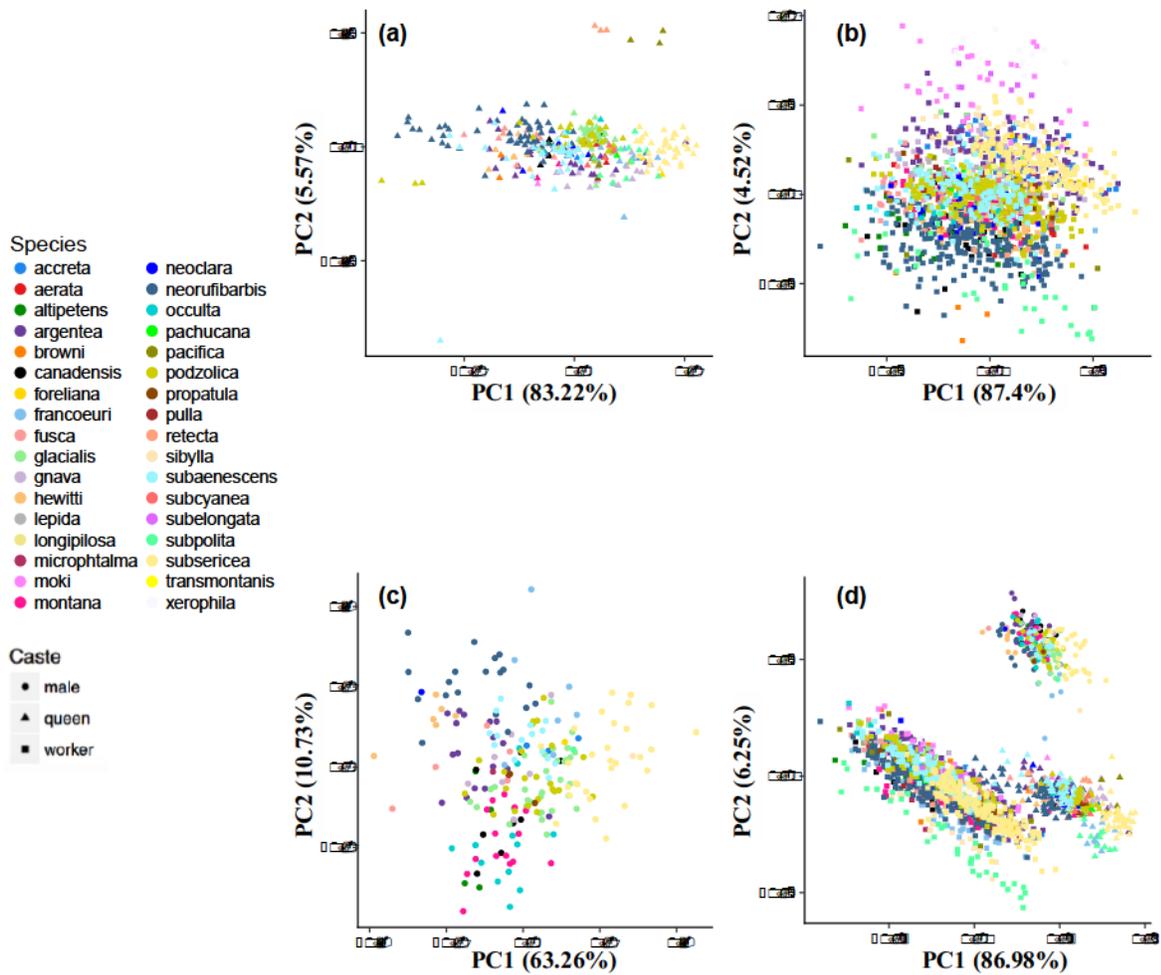


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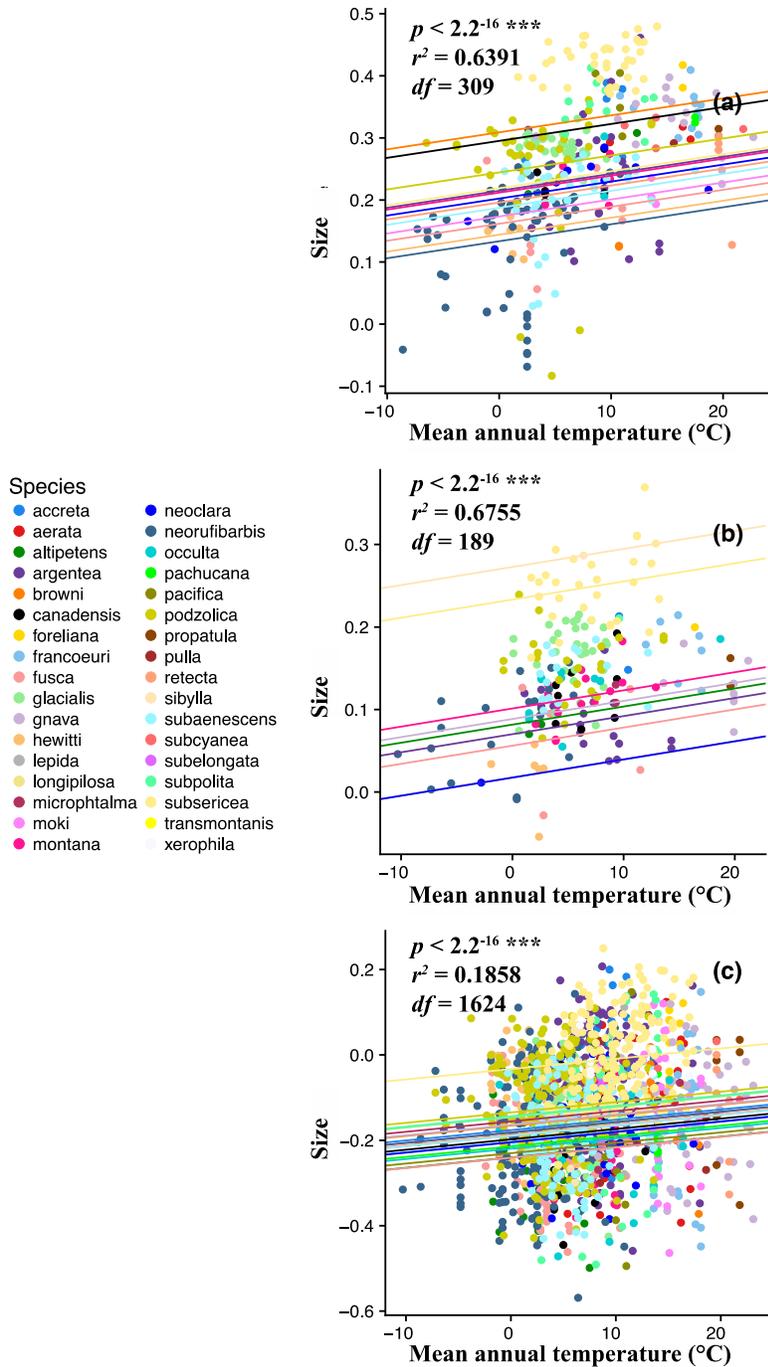


Figure S2 Relationship between size and geographic variation in temperature (°C) in (a) queens, (b) males and (c) workers. Here, size represents a shape-free variable produced by using the Aitchison transformation on 12 morphological traits. Each regression line represents a species that had a significantly different intercept for size. Significant P values for linear mixed models marked with asterisks (***) for $p < 0.001$, ** for $p < 0.01$ and * for $p < 0.05$).

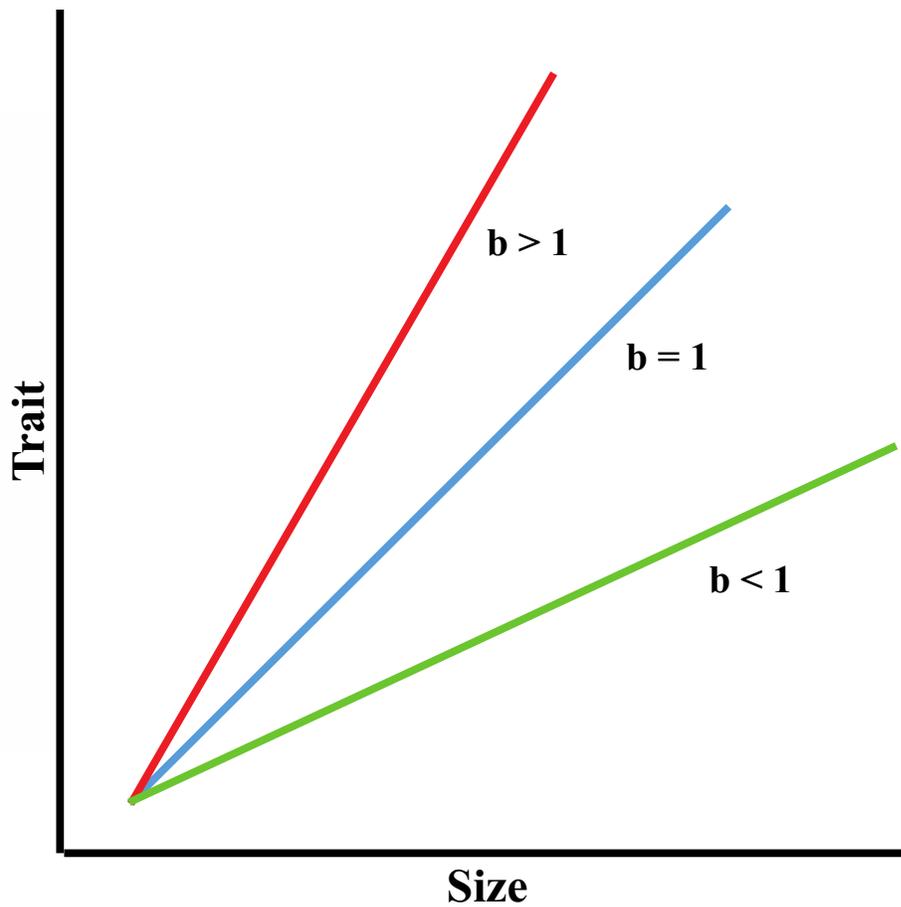


Figure S3 Schematic representation of scaling. Traits varying proportionately with size show isometry ($b = 1$). Traits increasing relative to size show a positive allometry ($b > 1$). Traits decreasing relative to size show a negative allometry ($b < 1$).



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