CLIMATE DRIVES GEOGRAPHIC VARIATION IN WORKER POLYMORPHISM AMONG POPULATIONS OF THE CARPENTER ANT *CAMPONOTUS HERCULEANUS*

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Abstract

CLIMATE DRIVES GEOGRAPHIC VARIATION IN WORKER POLYMORPHISM AMONG POPULATIONS OF THE CARPENTER ANT *CAMPONOTUS HERCULEANUS*

Érik Plante

In a world wherein climate is rapidly changing, understanding species' adaptations to climate is crucial. Quantifying phenotypic variation occurring across the range of a species is a useful approach to infer how populations adapt or show plastic responses to climatic regimes. In understudied groups such as eusocial ant colonies, the degree of worker polymorphism, whereby workers vary in size and scaling, is a phenotypic trait of the "superorganism". Yet, the degree to which such variation changes plastically or evolves in response to geographic variation in environmental factors remains poorly explored. Here, I first test whether the degree of variation in worker polymorphism in colonies of the carpenter ant *Camponotus herculeanus* varies predictably along a broad-scale latitudinal gradient and then examine the environmental factors driving this phenotypic variation. I found that the proportion of major workers within a colony decreased with increasing latitudes and was most related to regional climate. However, contrary to my expectation, variation in mean major worker body size did not correlate with worker polymorphism and was most related to colony size. These results suggest that change in the social phenotype of the superorganism is a plastic response to cooler and shorter seasons typical of northern latitudes, rather than resulting from the evolution of new developmental thresholds. I conclude that elucidating how genes and development interact to shape phenotypes along environmental gradients is key to improving understanding and predictions of climate adaptations in social organisms.

Keywords

Formicidae, ant castes, superorganism, environmental gradients, climate change

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Introduction

Elucidating the environmental and ecological drivers of phenotypic variation is a goal of the field of ecological evolutionary developmental biology (also known as eco-evo-devo), which aims to elucidate the complex interactions between genes, environment, and development (West-Eberhard 2005; Abouheif *et al.* 2014). Nevertheless, studies in eco-evo-devo are rarely conducted in a context enabling to link broad-scale variation in the environment to the developmental processes shaping phenotypes. Phenotypic variation occurring between different geographic populations of a same species is commonly studied to evaluate the ecological and environmental drivers of such variation, but this approach is seldom combined to concepts in evo-devo to understand the underlying mechanisms (Siefert *et al.* 2014; Valladares *et al.* 2014; Fox *et al.* 2019; Kellermann & van Heerwaarden 2019). The mechanisms promoting the microevolution or plasticity of the phenotype in different environments thus remain a key challenge, especially in understudied groups such as social organisms, where a phenotypic trait can relate to the entire colony.

Eusocial organisms are often viewed as superorganisms as they adapt their phenotype at the colony-level to variation in environmental conditions through interactions between genes, environment, and development (Kennedy *et al.* 2017). Colonies of eusocial organisms, analogous to superorganisms, are characterized by the presence of reproductive and non-reproductive castes representing the germline and soma, which maintain homeostasis in a solitary multicellular organism in relation to changes in its external environment (Lüscher 1953; Bonner 1993; Hölldobler & Wilson 2009; Boomsma & Gawne 2018). Moreover, within a single eusocial ant colony, the non-reproductive worker caste can display dramatic size variation and differences in head-to-body scaling in response to environmental and developmental factors (Oster & Wilson 1978; Wheeler 1991; Hölldobler & Wilson 2009; Lillico-Ouachour & Abouheif 2017; Wills *et al.* 2018). Superorganism phenotype can be readily quantified in ants using variation in worker morphology, also known as worker polymorphism, because it correlates to overall phenotypic variation of the colony in terms of totipotency, organization of work, communication, and functional integration (Anderson & McShea 2001; Bourke 2001).

Worker polymorphism has been shown to correlate with climate (la Richelière *et al.* 2022), habitat complexity (Schöning *et al.* 2005; Kronauer *et al.* 2007), colony size (Anderson & McShea 2001; Wills *et al.* 2018), and competition (Davidson 1978; Passera *et al.* 1996). Yang et al.'s (2004) pioneering study using *Pheidole morrisi* showed that worker polymorphism varied between three sites in the United States (Florida, North Carolina, and New York) through decrease in the proportion of major workers (workers with a disproportionately larger head than their body) in relation to the proportion of minor workers (smaller workers) and increase in body size within colonies in sites of higher latitudes. They proposed that larger proportions of major workers were due to lower interspecific competition in sites of higher latitudes, since major workers have a role for defending the colony against other ant species such as the fire ant *Solenopsis invicta* present in the native range of the species. This study raises interesting questions regarding whether the pattern is truly general or specific to these sites since environmental variables were not measured at each colony. Therefore, a broadly and well replicated study where several environmental variables are measured at each colony in one species across different geographic regions is required for further exploring the worker polymorphism variation found by Yang et al. (2004).

Whereas previous work (Yang *et al.* 2004) has focused on the proportion of majors and mean worker body size as a proxy for worker polymorphism variation, several other colony-level traits correlate differently to intraspecific worker polymorphism variation, such as worker body size variance, head size variance, and head-to-body ratio variance (Wilson 1953). Each of these traits relates to specific adaptations to ants' environment at the colony level (Table 1) and a change in these traits relates to the evolution or plasticity of ant colony phenotype for different reasons. Major worker proportion can indicate a quantitative change of the phenotype within a colony because it represents the percentage of a morphological distinct worker subcaste (Wilson 1974). The existence of major workers or soldiers reveals a developmental reprogramming event, where major worker larvae reprogram the growth of their head imaginal disk so that their head becomes disproportionately larger than their body (Wheeler 1991). Mean body size can also relate to thermal tolerance and desiccation, in a way that the largest workers of a colony are often thought to be the most resistant to temperature extremes and water loss, as it takes longer to change their internal temperature to their critical limit (Table 1). Worker body size variance, head size variance, and head-to-body ratio variance are related to scaling or allometry since the growth between head size and body size changes disproportionately among the individuals of the worker caste. These variables may be related to dietary composition, division of labor, as well as habitat complexity, as structural complexity of the microhabitat is expected to affect worker navigation, and therefore the evolutive trade-off between the growth of the workers legs, body, and head (Table 1). Geographic changes in these colony-level traits may reflect developmental plasticity or microevolution of superorganismal phenotype in ants and may be driven by specific ecological or environmental factors.

In carpenter ants (*Camponotus* spp.), worker size and scaling have been shown to be affected by environmentally sensitive factors during development (Smith 1944; Alvarado *et al.* 2015; LeBoeuf *et al.* 2016; Sinotte *et al.* 2018). For instance, the amount of food received during larval development may affect the final worker size (Smith 1942, 1944). The absence of the obligate intracellular endosymbiont *Blochmannia* has also been demonstrated to reduce worker size as well as major worker proportion in *Camponotus* colonies (Sinotte *et al.* 2018). In fact, the presence *Blochmannia* upgrades the nutritional value of its host resources by providing essential amino acids and may play a role in nitrogen recycling (Zientz *et al.* 2006; Feldhaar *et al.* 2007). Even the frequency of trophallaxis between larvae and workers can ultimately influence *Camponotus* worker size through the transfers of juvenile hormone (JH), a key developmental regulator in insects (LeBoeuf *et al.* 2016). Several epigenetic mechanisms, such as DNA methylation, can also influence *Camponotus* final worker size by mediating gene-by-environment interactions through regulation of gene transcription, where higher levels of methylation are associated with smaller worker larvae, and lower levels of methylation to larger larvae (Alvarado *et al.* 2015). All these factors are expected to affect worker size and scaling in *Camponotus*. However, how environmental drivers can mediate worker size or scaling through developmental interactions remains elusive.

Multiple environmental factors could affect the degree of polymorphism among populations of *Camponotus* spp., Moreover, different attributes of the colony phenotype could show different responses to environmental factors. First, intense competition should increase the proportion of major workers in a colony (Passera *et al.* 1996; Lillico-Ouachour & Abouheif 2017), which is expected to be higher in warm climates (Jeanne 1979), as the presence of majors can have an adaptive role for nest defense against competitive species (Table 1). Second, environmental conditions such as a colder climate should increase mean major worker body size, since a larger body is suggested to be an adaptation to temperature extremes by slowing down the influence of external thermal conditions on internal body temperature (Table 1) (Bernstein 1976; O'Donnell *et al.* 2020). Specifically, this could allow longer periods of foraging during summer or the survival of workers during the lowest temperatures of the winter. Similarly, colder climates should increase variance in worker body size and head size, as climate influences dietary composition, which also relates to size variation (Table 1) (White 2008; Robinson et al. 2017). Third, colony age, which relates to colony size, should influence average worker size and polymorphism (Tschinkel 1993; Bourke 1999). Specifically, larger colony size should also result in a larger worker body size variance and head size variance, as colony size is a proxy of division of labor, which relates to size variation because more workers will perform a wider variety of tasks that each suits their own specific size (Table 1) (Holbrook *et al.* 2011; Ferguson-Gow *et al.* 2014; Ulrich *et al.* 2018). All that said whether and how social traits are affected by colony ontogeny and environmental factors is poorly understood and largely untested.

To test these hypotheses, I sampled entire colonies of the carpenter ant *Camponotus herculeanus* along an eight-degree latitudinal gradient, from the temperate forest to the north of the boreal forest, in Eastern Canada. The northernmost colonies were located near the northern range limit of *C. herculeanus* and thus their phenotype was expected to reflect adaptations to the short summers and low annual temperatures. I therefore measured local and regional climate, colony size, competition, and nesting tree diameter at each sampled colony, and related them to five colony traits, including major worker proportion, mean major worker body size, as well as worker body size, head size and head-to-body ratio variances.

Methods

Model species

I used the carpenter ant *Camponotus herculeanus* (Linnaeus, 1758) as a study system because it is the only polymorphic eusocial insect species that is common throughout the whole study region (province of Quebec, Canada) and for which it is possible to sample entire colonies. Entire colonies of this species can often be found inside a single tree, stump or log and frequently occur in peat bogs (Ellison *et al.* 2012). In addition, since peat bogs are frequently flooded, the colonies rarely ramify into very complex tunnel systems or satellite nests in these habitats, thus allowing the complete harvest of the colony, including the queen.

C. herculeanus is a monogynous (a single egg-laying queen) species (Akre *et al.* 1994) and the most cold-tolerant ant species known (Ellison *et al.* 2012). *C. herculeanus* build their nest inside dead or live wood and exhibit marked continuous worker polymorphism. Some workers are smaller (minors) when compared to others that are larger with a disproportionally large head according to their body size (majors) (Fig. 1), which makes *C. herculeanus* useful for studying variation in superorganism phenotype (Wilson 1953). The largest colony of carpenter ants ever collected in North America was one of *C. herculeanus* and numbered over 12,000 workers (Sanders 1970). In Europe, the average number of reproductives that emerge from a single colony of *C. herculeanus* is 4,500 males and 1,800 winged queens (Hölldobler & Maschwitz 1965). Therefore, given their high number of individuals, *C. herculeanus* colonies are sufficiently large to measure worker polymorphism variation between different colonies with a high resolution.

Study system

This study took place in the province of Quebec, Canada. Sampling sites were located throughout the province, from the south, near the cities of Trois-Rivières and Québec, to the north, near the village of Radisson, which is part of the James Bay region (Fig. 2). The designated southern study areas are found in Shawinigan region (46.6°N, -72.5°W) and Sainte-Catherine-de-la-Jacques-Cartier region (46.8°N, -71.5°W). Further North, other study areas are found in Abitibi (48.6°N, -78.2°W) and Matagami region (49.7°N, -77.4°W), and the others in the Radisson region (53.8°N, -77.4°W). I selected these sampling regions according to the presence of *C. herculeanus*, the accessibility to the habitats sought by the road and their environmental differences. Specifically, the study sites cross a gradient of three different forest stand compositions structuring insect communities (Boucher *et al.* 2009; Arrowsmith *et al.* 2018). The environmental gradient ranges from stands dominated by deciduous trees to stands dominated by different conifers, which correspond to different bioclimatic domains, including (1) sugar maple-yellow birch domain, balsam fir-yellow birch, (2) balsam fir-paper birch climax zone and (3) black spruce lichen, and black spruce -moss climax zone (ESWG (Ecological Stratification Working Group) 1995).

Colony sampling

Based on our observations, *C. herculeanus* is commonly found on the edge of peat bogs. I first selected suitable sites using Google Maps. Most of the sampling sites were located at least 100 meters from roads, trails, overhead power lines or any form of human disturbance, to ensure each colony is in natural conditions. When the species was present in a selected site, I sampled at the edge of peat bogs for sites to be as comparable as possible.

I collected the colonies during summer 2021, from mid-July to mid-August, when they were active and re-established after swarming, winter mortality and spring brood hatching to not bias the colony traits (Passera 1977; Walker & Stamps 1986). Winter mortality is caused in part by predation by several species of woodpeckers and may be important enough to influence colony caste ratios, with *C. herculeanus* being a major part of their winter diet (Sanders 1964; Cramp & Simmons 1985; Mikusinski 1997). Winter mortality in workers might also be caused in part by cold temperatures, as other ant species can use workers to buffer the queen against cold temperatures (Kaspari & Vargo 1995; Shiroto *et al.* 2011).

To sample entire colonies, I selected tree trunks that were possible to open completely without danger of injury due to the top part falling and contained the desired species to harvest entire colonies. *C. herculeanus* is known to prefer softwood, especially *Abies, Picea* and *Thuja* species to build their nests (Eidmann 1929; Hölldobler 1944, 1962; Sanders 1964). I opened trunks with a saw and an axe on a tarpaulin on the ground and I directly deposited the debris containing the most individuals in ventilated plastic containers identified with the unique code of the colony. I collected other moving individuals manually. Due to the high summer temperatures during collection, ants can die quickly when they are numerous and active in a closed container. Therefore, within 30 minutes after harvesting, I placed the colony containers inside a cooler, and then in the refrigerator (4°C) when returning from the field to prevent them from becoming too active. I collected the individuals manually or with forceps from the debris in open plastic bins. Johnson's baby powder mixed with 99% ethanol was applied to the walls of the containers so that they did not escape during the transfer. Then, I isolated the ants to have only the individuals. In this way, when frozen to be measured, the workers were easier to collect and have cleaned themselves, thus making it also easier to take morphometric measurements. Species identifications were confirmed under a microscope using the criteria mentioned in Ellison *et al.* (2012).

In 2021, I harvested 27 colonies throughout the entire gradient. All colonies collected included at least 150 workers, the brood, often the queen and alates. I collected a maximum of three colonies per site, at least one kilometer from each other. Since there was almost no data on colony size in the boreal forest of eastern Canada, I also harvested small colonies of just a few hundred workers. Furthermore, several traits calculated within colonies in this study can vary depending on the age and size of ant colonies (Tschinkel 1988; Gibson 1989; Holbrook *et al.* 2011).

The sampling dates were important to ensure that the number of major workers found during sampling reflects the maximum that can be produced by the colony during the season. Therefore, I set sampling dates after the first brood development and based on differences in the onset and length of the growing season. In fact, *C. herculeanus* first brood development is independent from temperature and the second brood usually mostly enters diapause (Lopatina & Kipyatkov 1993). The main period of larval growth at latitude 46 ° and above in Eastern Canada are in June and early July and the remaining larvae often spend the winter in the nest as the life cycle can cover two years (Sanders 1964, 1972). Since freezing temperatures are still a possibility until mid-June in the northern part of the gradient (above 49° N), sampling was not conducted until mid-July to make sure that a full month has elapsed since the last frost and that worker brood was hatched.

Mating flights are also an indicator of the beginning of the species activity after diapause and therefore of brood production. In *C. herculeanus*, winged sexual castes spend the winter in the colony and mating flights can occur during spring when temperatures are above 21ºC and some snow can be remaining on the ground (Sanders 1964). Major mating flights of *C. herculeanus* take place between the second week of May and the first week of June in Eastern Canada although some can take place later (Sanders 1964, 1970, 1971; GBIF Secretariat 2021).

Environmental variables

To examine whether environmental factors affect polymorphism, I quantified both local and regional variables at each colony sampled. Local variables included the nest diameter and canopy cover. The tree diameter could affect polymorphism because the thermal insulation and rate of warming of *Camponotus* species nest in wood varies depending on the type of substrate and nest size (Chen *et al.* 2002; Vesnic *et al.* 2017), which could affect the length of development (Porter 1988). Furthermore, tree diameter is a good indicator of habitat complexity, which might affect head-to-body ratio variance (McElhinny et al. 2005, Nooten et al. 2019). I used canopy openness (portion within the canopy of the tree cover not obstructed by foliage) to estimate local climate and I determined it at the nest location with a spherical densiometer (Brand: Forest Densiometers, Model: Model-C). The canopy openness allowing the sun's rays to hit the nest, which can change the temperature of the nest, thereby altering the local conditions for larval development. In fact, temperature variables can influence larval growth rate (Porter 1988) and whether some larvae might pupate or enter diapause (Lopatina & Kipyatkov 1993), which could influence worker polymorphism in *C. herculeanus* colonies.

To characterize regional climate, I extracted several bioclimatic variables that could possibly influence worker polymorphism for each colony, using geographic coordinates obtained with a GPS (Brand: Garmin, Model: GPSMAP 64st). The bioclimatic variables included mean annual temperature, mean diurnal range (mean of monthly (maximum temperature –minimum temperature)), isothermality ((mean diurnal range/temperature annual range) ×100), temperature seasonality (standard deviation ×100), maximum temperature of warmest month, minimum temperature of coldest month, temperature annual range (maximum temperature of warmest month - minimum temperature of coldest month), and annual precipitation for the years 1970 to 2000. I obtained these variables from WorldClim version 2 (1 km^2 grid cells) using the geographic centroid coordinates of one grid per colony (Hijmans *et al.* 2005; Fick & Hijmans 2017). I performed a principal component analysis (PCA) on the eight bioclimatic variables (Table S1). I extracted the first axis, which captured 76% of the variance, and related it to the different colony traits to represent a composite of the regional climate variables. I used a PCA to summarize the bioclimatic variables because they all correlate with each other and it is difficult to isolate the effect of each of the variables on the colony-level traits along the latitudinal gradient.

Sampling potential ant competitors

I assessed the presence of potential interspecific ant competitors by active visual observation and opening wood debris within a radius of 10 meters around each colony for 15 minutes between 10:00AM and 5:00PM on days without rain. I collected workers of all species found with a mouth aspirator or manually. The presence of a single allospecific queen (i.e., of another species) without workers was not considered as a presence of a competitive species because it does not necessarily indicate the presence of a competitive nest. The presence of competitive ant species near *C. herculeanus* colonies could influence major worker production, as major workers can have a role for defense of the colony (Passera 1977). I immediately transferred the sampled species in 95% ethanol vials after their collection. Species identifications were confirmed under a microscope using the criteria mentioned in Ellison *et al.* (2012).

To estimate whether competition influences worker polymorphism, only the presence of nests of the genus *Formica* was used. North temperate ground-dwelling ant communities are often dominated by *Formica* species, which makes them important competitors of other ant species for food resources (Fellers 1987, 1989; Lessard *et al.* 2020). The ant genera I most found near *C. herculeanus* nests were *Leptothorax*, *Myrmica* and *Formica* (Table S4). The colonies of *C. herculeanus* were significantly smaller when *Formica* spp. were present (Welch two-sample t test: t = 2.70, df = 19.38, P = 0.01) but not when *Myrmica* spp. (Welch two-sample t test: t = -1.04, df = 20.53, P = 0.31) or *Leptothora*x spp. (Welch two-sample t test: t = 1.13, df = 23.45, P = 0.27) were present (Fig. S2). As such, in the analyses, I used the presence of *Formica* ants as a proxy for the presence of strong competitors.

Morphometric measurements

I measured all the workers of four colonies. I standardized the rest of the samples to subsets of 200 workers per colony, except for one colony which had 151 workers. To avoid selection bias, I created the subsets on the same day using the same method by mixing all the workers of each colony together in a plastic container with ethanol for 2 minutes and randomly collecting workers using forceps while not looking directly at the sample. In total, I measured 7 128 workers. I took photos of morphological traits using a digital microscope (Brand: Koolertron, Model: AD106S). I digitalized the dorsal and lateral views of each mounted specimen and obtained morphometric measurements using a image processing software (ImageJ version 1.53e). For each worker, I measured head width (maximum width of head including eyes) and body size (maximum diagonal length of mesosoma, i.e. Weber’s length) (Fig. 3). I used these measurements to quantify five different colony-level traits describing worker polymorphism, including major worker proportion, mean major worker body size, worker body size variance, worker head size variance and worker head-to-body ratio variance.

Major worker proportion and mean body size

I froze sampled colonies within five days of harvest, as described above to avoid influencing caste ratios. I calculated the number of major workers in each colony according to the inflection point of the allometric growth curve (third-degree polynomial formed by plotting head size against body size), which represents the point at which the second derivative of the curve is equal to zero and can be used to classify ant workers into majors (Fraser *et al.* 2000). The inflection point is useful for classifying *C. herculeanus* workers into majors because minors, medias, and majors cannot be distinguished conveniently, as the workers exhibit a continuous size variation. Major workers are those with a head width larger to the head width at the inflection point (Fig. S1). I then calculated the proportion of major workers in each colony. One colony was removed from the analyses because the inflection point was not properly detected, resulting in a total of 26 colonies analyzed. I calculated the mean size of major workers as the average of body size measurements (i.e., Weber's length) of major workers in the entire colony.

Worker body size, head size and head-to-body ratio variance

I calculated the variance of worker body size (i.e. Weber's length), head size, and the ratio between head width and worker body size for each of the sampled ant colonies. I used variance because it is the parameter that best captured the variation of each trait between the colonies (Table S2) and represents how large the variation of worker morphology is in comparison to the other workers of a same colony.

Statistical analyses

To select the variables used in the multivariate normal additive model testing the hypothesis that certain environmental factors would affect worker polymorphism, I ran separate generalized additive models (GAMs) for each trait with all predictors (Table S3) (Wood 2017). The conditional mean in a GAM is estimated as a linear combination of nonlinear basis functions, that form a smooth curve. GAMs are adequate for selecting variables because we can use p-values for testing each variable for equality to zero for deciding on which candidate variables to be removed from the model. The predictor variables I selected for the analysis after fitting a separate model for each trait were colony size and regional climate, as they were the significant predictor variables ($P < 0.05$) related to the different traits. Therefore, I did not use local climate, nest diameter and competition for further analysis because they were not significant. I then fit a single GAM to all traits, using a multivariate normal distribution family to determine whether worker polymorphism was related to specific biotic and abiotic factors, accounting for correlations between the traits themselves (Wood *et al.* 2016). The multivariate normal GAM fits a separate regression for each response variable, as a covariance matrix estimating the covariance between response residuals. The output includes a separate linear predictor for the mean for each response and the covariance matrix. Multivariate normal GAMs can be used to assess the relationship between many variables and is robust to autocorrelation. To fit this multivariate model, I used the five colony-level traits as response variables representative of ant worker polymorphism: major worker proportion, mean major worker body size, worker body size variance, head size variance and head-to-body ratio variance. I used "colony size" and "regional climate" as fixed effect predictor variables for each response. I used linear predictors instead of smoothing terms based on the model linear relationships between response and predictors residuals. Finally, I assessed the relationship between each colony trait and the predictors, and the partial effects were plotted as a visual aid for the direction of the relationships. I fitted the model using the gam function in the mgcv package (Wood & Wood 2015) in R version 4.2.0 (R Core Team 2022).

Results

In total, I collected 47 853 workers of 27 colonies in 2021. In the study area, most of the colonies nested in black spruce (*Picea mariana*) (~78%), and some of them in balsam fir (*Abies balsamea*) (~7%), eastern white cedar (*Thuja occidentalis*) (~7%), eastern white pine (*Pinus strobus*) (~4%), and tamarack (*Larix laricina*) (~4%). Colonies harvested ranged from 151 to 6 557 workers with an average of 1 793 workers. Smaller colonies of just a few hundred workers were common throughout the study region and produced majors (Fig. 5). Colony size was not related to latitude in the 27 sampled colonies (linear model, adjusted $R^2 = -0.04$, df = 25, $F = 0.03$, $P = 0.873$) (Fig. 4), but was positively related to nesting tree diameter (linear model, adjusted $R^2 = 0.30$, df = 24, $F = 11.71$, $P = 0.002$).

After I ran separate generalized additive models with all predictors to select the variables used in the analysis, I performed a multivariate normal additive model on five colony traits describing worker polymorphism and used regional climate and colony size as predictors (Table 2). I found that 33% of the deviance was explained by the model. Major worker proportion was more related to regional climate (Estimate $=-2.4 \times 10^{-2}$, SE $= 6.5\times 10^{-3}$, $P < 0.001$) than to colony size (Estimate $= 2.5 \times 10^{-5}$, SE $= 1.1 \times 10^{-5}$, $P = 0.029$). In fact, major worker proportion was lower within colonies at higher latitudes than lower latitudes of the gradient (Fig. S3). Major worker mean body size was not related to regional climate (Estimate $= 7.0 \times 10^{-3}$, SE $= 9.4 \times 10^{-3}$, $P = 0.455$) but was positively significantly related to colony size (Estimate $= 8.4 \times 10^{-5}$, SE $= 1.6 \times 10^{-5}$, $P < 0.001$). Larger colonies produced larger workers (Fig. S3). Worker body size variance was not related regional climate (Estimate $= -7.7 \times 10^{-3}$, SE $= 4.0 \times 10^{-3}$, $P = 0.056$), but was positively related to colony size (Estimate $= 1.5 \times 10^{-5}$, SE $= 7.0 \times 10^{-6}$, P = 0.028). Worker head size variance was negatively related regional climate (Estimate $= -1.5 \times 10^{-2}$, SE $= 7.0 \times 10^{-3}$, $P = 0.032$) and positively related to colony size (Estimate $= 3.0 \times 10^{-5}$, SE $= 1.2 \times 10^{-5}$, $P = 0.014$). In fact, larger colonies had a larger worker body size variance and head size variance, and higher latitudes had lower head size variance (Fig. S3). Head-to-body ratio variance was weakly related to regional climate (Estimate $= -3.6 \times 10^{-4}$, SE $= 1.5\times 10^{-4}$ P = 0.015), but not to colony size (Estimate $= 4.6 \times 10^{-7}$, SE $= 2.4 \times 10^{-7}$, $P = 0.053$) (Table 2).

Discussion

The current work provides strong evidence for the influence of environmental factors in shaping the phenotype of superorganisms at the colony-level and sets the stage for uncovering the mechanisms mediating the interactions between genes, environment, and development by combining macroecology with concepts in evo-devo. Uncovering the mechanisms through which vulnerable and understudied organisms adapt to environmental changes is urgently needed to mitigate the effects of the ongoing global climate crisis on ecosystems (Wagner 2020).

Major worker proportion is affected by regional climate

The latitudinal pattern of major worker proportion found in the present study using the carpenter ant *Camponotus herculeanus* is consistent with previous study using the ant *Pheidole morrisi* (Yang *et al.* 2004), but is not directly related to competition as the authors suggested. In the present study, major worker proportion was best explained by regional climate and weakly related to colony size. In the northern range of the latitudinal gradient, where the climate is colder and drier, the proportion of major workers among colonies was generally lower than in the south. Yang et al. (2004) observed a similar pattern of major worker proportions among colonies of *P. morrisi* in the United States in three sampling sites over a gradient of a comparable distance as the present study. They suggested that the variation in competition with other ant species between the different populations possibly causes an adaptive change in major worker proportion of *P. morrisi*. However, the authors did not measure competition or other environmental variables at the colony sampling sites to test this hypothesis. Based on the present work with *C. herculeanus*, competition is not related to major worker proportion variation between different regions, but the presence of competition reduces colony size, which is weakly related to major worker proportion. Other work with the fire ant *Solenopsis invicta* showed that colony size explained 80% of the variation in major worker proportion (Tschinkel 1988). While this may be true when different colonies are under similar climatic conditions, regional climate is a stronger driver of major worker proportion than colony size in the case of *C. herculeanus*.

Several potential ecological mechanisms may explain the observed variation in the phenotype of ant colonies along the environmental gradient. First, since the winter season is longer in the northern part of the gradient, it is possible that the high investment in the production of majors in *C. herculeanus* is worthwhile only in warmer regions where the growing season is longer and winter shorter. In *Camponotus pennsylvanicus*, it has been shown that major workers consume twice as much body nutrients than the minor workers during diapause (Cannon 1990). Therefore, a cost-benefit tradeoff could potentially mediate major worker proportion between colonies of *C. herculeanus* under different climates. Alternatively, differences in nutrition between the colonies may explain the differences in major worker proportion along the gradient. *C. herculeanus* is known to feed on both aphids' honeydew and preys (Ayre 1963; Sanders 1970). Since vitamin and protein intake of the colony can influence worker body mass, it is possible that the difference in nutrition along the gradient influence major worker production (Smith 1942, 1944; Dussutour & Simpson 2008). For instance, it is possible that preys are rare in the northern part of the gradient and more abundant in the south, which resulted in the higher proportions of major workers that were observed, since aphid honeydew is primarily composed of carbohydrates (Strong 1965; Idoine & Ferro 1988; Völkl *et al.* 1999).

In addition to ecological mechanisms, inferences about developmental mechanisms can be made to explain the major worker proportion pattern observed along the gradient. Yang et al. (2004) proposed several possibilities as to how changes in the developmental mechanism of worker caste determination in *Pheidole* are expected to affect major worker proportion and mean body size (Fig. 6). In this caste determination mechanism, any worker larvae have the potential to become either a minor, a media or a major. Whether they become one or another depends on their critical body size, which depends on nutrition (Smith 1942, 1944; Dussutour & Simpson 2008) and social interactions (Meurville & LeBoeuf 2021), and is mediated by juvenile hormone (JH) (Wheeler & Nijhout 1981; LeBoeuf *et al.* 2016). When the larvae exceed the defined threshold size, they undergo developmental reprogramming and continue growing to become majors, while larvae below the threshold size pupate to become either minors or medias (Fig. 6A). Assuming such a caste determination model is correct for *C. herculeanus*, then several predictions about the developmental mechanisms at play are possible: (1) If the larval size distribution shifts upwards, the increase in the proportion of majors will be accompanied by larger majors (Fig. 6B); (2) If the larval size distribution shifts downwards, the increase in the proportion of majors will be accompanied by smaller majors (Fig. 6C); (3) If a lower frequency of larvae exceed the threshold size to become majors, there will be a decrease in the proportion of majors but no change in the major workers body size (Fig. 6D). Yang et al. (2004) inferred that (2) was the most plausible mechanism since high proportions of majors in *P. morrisi* were accompanied by smaller mean body size and that low proportions of majors were accompanied by larger mean body size (Fig. 6C).

In the present study using *C. herculeanus*, both mechanisms in figure 6B and C, which were proposed by Yang et al. (2004) with *P. morrisi* do not correspond to the colony-level trait patterns observed along the latitudinal gradient. In fact, major worker proportion decreased with increasing latitude, but did not correlate with mean body size. Mean major worker body size was found to be related to colony size, meaning that major worker proportion and body size vary independently from each other, since colony size did not correlate with latitude. One possible explanation for this pattern could be that developmental plasticity in the colonies changes along the gradient. Specifically, it means that only the number of workers that exceed the larval threshold size might differ along the gradient (number of major workers produced), without changing the threshold size (size of major workers produced) (Fig. 6D). This would result in producing a larger number of major workers in the southern part of the gradient with a similar mean body size as in the northern part of the gradient where fewer major workers are produced.

Two different developmental mechanisms promoting *C. herculeanus* and *P. morrisi* colony-level phenotypes were inferred (Fig. 6C; Fig. 6D) and potentially evolved independently between these two ant species. *Camponotus* and *Pheidole* are not closely related phylogenetically. From the 323 ant genera in the world, *Camponotus* (Formicinae) and *Pheidole* (Myrmicinae) are the two most species-rich and are part of two different subfamilies (Economo et al. 2015). *Pheidole* is a monophyletic lineage, and crown group members of its genus have been estimated to exist since 58.4 to 61.2 million years ago (Moreau 2008). This suggests that the developmental mechanisms promoting major worker proportion patterns and variation in body size observed between different regional climates evolved independently in *Camponotus* and *Pheidole* through different developmental routes within the JH system. Further work will be needed to provide evidence of these potential mechanisms.

The first step towards testing this hypothesis is to determine whether the differences in major worker proportion are due to genetic and/or environmental differences along the gradient. Yang et al. 2004 showed that the differences of major worker proportion and mean body size observed in *P. morrisi* between three sites across the United States were consistent with microevolutionary divergence because major worker proportions persisted in a common rearing environment even after disruption. Similarly, keeping *C. herculeanus* colonies from the three different regions of the Canadian gradient under the same ambient conditions of a common rearing environment would allow to test whether the colony phenotypes are due to genetic or environmental differences. Furthermore, applying JH treatments to larvae of colonies of each region would also allow to test whether the mechanism inferred is plausible (Fig. 6D). If each region maintains a similar caste-ratio and show a similar increase in major worker proportion after a JH treatment, then it is consistent with the difference in JH levels driving major worker proportion differences along the gradient.

Mean major worker body size is affected by colony size

In the present work using *C. herculeanus*, mean major worker body size was best explained by colony size, and not by climate. Larger colonies produced larger workers, and smaller colonies produced smaller workers. This result contrasts with the previous work with *P. morrisi* by Yang et al. (2004), as the authors showed that the proportion of major workers was best explained by worker size, where higher proportions of major workers are accompanied by smaller major workers, potentially through a shift in the larval developmental threshold. This is not the case in the present work with *C. herculeanus*, and other work with *S. invicta* also showed that colony size explained mean worker body size (Tschinkel 1993).

Mean major worker body size only correlated to colony size contrasts with numerous other studies that found a relation between body size and latitudinal gradients across different insect taxa (Shelomi 2012). In the case of ants, body size has been shown to increase (Cushman *et al.* 1993; Heinze *et al.* 2003) and decrease (Diniz-Filho & Fowler 1998; Kaspari 2005; Schofield *et al.* 2016; Gibb *et al.* 2018) with latitude or show no relation (Gomez & Espadaler 2000; Geraghty *et al.* 2007; Gómez & Espadaler 2013) with latitude. One possibility that might explain these contrasting results across ants is that superorganisms differ from individualistic organisms in their way to regulate their phenotype at the colony-level according to external cues (Lüscher 1953; Bonner 1993; Hölldobler & Wilson 2009; Boomsma & Gawne 2018). In the case of *C. herculeanus*, workers are thought possess the ability to thermoregulate nest temperature through the production of metabolic heat by the workers (Sanders 1972). The production of metabolic heat by social insects, which does not occur as frequently in other non-social insect taxa, is an important additional source of heat that helps maintain the homeostasis of the nest (Kadochová & Frouz 2013). If *C. herculeanus* possesses such a thermoregulation ability, it is possible that colonies can control the temperature in which brood develops and buffer its entire larval development against temperature variation across the latitudinal gradient, thereby limiting its potential influence on body size.

One reason that larger colonies produce larger workers in *C. herculeanus* could be that they have a better access to food sources, as the colony possesses more workers to forage, thus allowing larvae to grow larger. A study with the wood ant *Formica aquilonia* showed that larger workers were produced in larger nests, and that mean worker body size and body-fat content decreased with the availability of tree-living aphids (Sorvari and Hakkarainen 2009). This could also be the case with *C. herculeanus*, as they are also largely associated with aphids for honeydew (Sanders 1970).

Other colony traits are weakly affected by climate and colony size

Worker body and head size variances were weakly affected by climate and colony size. While worker size variation has often been predicted to change adaptively depending on the environmental conditions (Davidson 1978, Bershers and Traniello 1994), it might not be directly affected by them. Major worker proportion and worker size are probably better predictors of worker size variation. The same goes for head-to-body scaling variance, which was weakly related to regional climate and not significantly related to colony size. These results support the hypothesis that changes in major worker proportion in *C. herculeanus* are developmentally plastic and not through the microevolution of a developmental mechanism linked to these other colony traits.

Conclusions

These findings attest the importance of climate and colony size in shaping phenotypic variation in superorganisms such as ants, and potential ecological and developmental mechanisms. These results also highlight that environmental factors are a major driver of the phenotype in insect populations, thereby determining a large part of their fitness. With current declines in global insect populations and ongoing environmental changes caused by anthropogenic disturbances, it is necessary to better understand how species can survive rapidly changing conditions (Wagner 2020). Fighting the climate crisis and species loss by using phenotypic variation to better understand adaptations to environmental conditions could potentially allow to protect the world's most important resource that is biodiversity.

By assessing which ant colony-level traits exhibit latitudinal variation and whether they relate environmental factors, I was able to propose potential developmental and ecological mechanisms underlying these patterns. These results suggest that geographic variation plays an important role in the development and / or evolution of phenotypic variation in ant colonies. Further work will be needed to determine how these mechanisms influence worker polymorphism and more generally phenotypic variation in superorganisms.

Works Cited

Abouheif, E., Favé, M.-J., Ibarrarán-Viniegra, A.S., Lesoway, M.P., Rafiqi, A.M. & Rajakumar, R. (2014). Eco-evo-devo: the time has come. *Adv Exp Med Biol*, 781, 107–125.

Akre, R.D., Hansen, L.D. & Myhre, E.A. (1994). Colony size and polygyny in carpenter ants (Hymenoptera: Formicidae). *J Kans Entomol Soc*, 67, 1–9.

Alvarado, S., Rajakumar, R., Abouheif, E. & Szyf, M. (2015). Epigenetic variation in the Egfr gene generates quantitative variation in a complex trait in ants. *Nat Commun*, 6, 6513.

Anderson, C. & McShea, D.W. (2001). Individual versus social complexity, with particular reference to ant colonies. *Biol Rev Camb Philos Soc*, 76, 211–237.

Araujo, M.B. & Tschinkel, W.R. (2010). Worker allometry in relation to colony size and social form in the fire ant *Solenopsis invicta*. *J Insect Sci*, 10, 1–10.

Arrowsmith, J., Shivaprakash, K.N., Larrivée, M., Turgeon, J. & Lessard, J. (2018). Environmental filtering along a broad-scale acidity gradient shapes the structure of odonate communities. *Ecosphere*, 9, e02473.

Ayre, G.L. (1963). Feeding behaviour and digestion in *Camponotus herculeanus* (L.) (Hymenoptera: Formicidae). *Entomol Exp Appl*, 6, 165–170.

Bernstein, R.A. (1976). The adaptive value of polymorphism in an alpine Ant, *Formica neorufibarbis* Gelid A. Wheeler. *PsyCh J*, 83, 180–184.

Bonner, J.T. (1993). Dividing the labour in cells and societies. *Curr Sci*, 64, 459–466.

Boomsma, J.J. & Gawne, R. (2018). Superorganismality and caste differentiation as points of no return: how the major evolutionary transitions were lost in translation. *Biol Rev*, 93, 28–54.

Boucher, Y., Arseneault, D., Sirois, L. & Blais, L. (2009). Logging pattern and landscape changes over the last century at the boreal and deciduous forest transition in Eastern Canada. *Landsc Ecol*, 24, 171–184.

Bourke. (2001). Colony size, social complexity and reproductive conflict in social insects. *J Evol Biol*, 12, 245–257.

Bourke, A.F.G. (1999). Colony size, social complexity and reproductive conflict in social insects. *J Evol Biol*, 12, 245–257.

Cannon, C.A. (1990). Demography, cold hardiness, and nutrient reserves of overwintering nests of the carpenter ant *Camponotus pennsylvanicus* (De Geer) (Hymenoptera: Formicidae). Virginia Polytechnic Institute and State University, Blacksburg.

Centorame, M., Angelino, D., Bonanni, R. & Fanfani, A. (2020). Static and evolutionary allometry in the Italian endemic ant species *Cataglyphis italica* (Emery 1906). *Ethol Ecol Evol*, 32, 16–28.

Cerdá, X. & Retana, J. (2000). Alternative strategies by thermophilic ants to cope with extreme heat: Individual versus colony level traits. *Oikos*, 89, 155–163.

Cerdá, X., Retana, J. & Cerda, X. (1997). Links between worker polymorphism and thermal biology in a thermophilic ant species. *Oikos*, 78, 467.

Chen, Y., Hansen, L.D. & Brown, J.J. (2002). Nesting sites of the carpenter ant, *Camponotus vicinus* (Mayr) (Hymenoptera: Formicidae) in northern Idaho. *Environ Entomol*, 31, 1037–1042.

Cramp, S. & Simmons, K.E. (1985). *Birds of Europe, the Middle East and North Africa*. *VV New York*.

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

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

Diniz-Filho, J.A.F. & Fowler, H.G. (1998). Honey ants (genus *Myrmecocystus*) macroecology: effect of spatial patterns on the relationship between worker body size and geographic range size. *Environ Entomol*, 27, 1094–1101.

Dussutour, A. & Simpson, S.J. (2008). Description of a simple synthetic diet for studying nutritional responses in ants. *Insectes Soc*, 55, 329–333.

Eidmann, H. (1929). Zur Kenntnis der Biologie der Roßameise (*Camponotus herculeanus* L.). *Zeitschrift für Angew Entomol*, 14, 229–253.

Ellison, A.M., Gotelli, N.J., Farnsworth, E.J. & Alpert, G.D. (2012). *A field guide to the ants of New England*. Yale University Press.

ESWG (Ecological Stratification Working Group). (1995). *A national ecological framework for Canada*. *Agriculture and Agri-Food Canada, Ottawa, Ontario/Hull, Quebec, Canada*.

Farji-Brener, A., Barrantes, G. & Ruggiero, A. (2004). Environmental rugosity, body size and access to food: A test of the size-grain hypothesis in tropical litter ants. *Oikos*, 104, 165–171.

Feldhaar, H., Straka, J., Krischke, M., Berthold, K., Stoll, S., Mueller, M.J., *et al.* (2007). Nutritional upgrading for omnivorous carpenter ants by the endosymbiont *Blochmannia*. *BMC Biol*, 5, 48.

Fellers, J.H. (1987). Interference and exploitation in a guild of woodland ants. *Ecology*, 68, 1466–1478.

Fellers, J.H. (1989). Daily and seasonal activity in woodland ants. *Oecologia*, 78, 69–76.

Ferguson-Gow, H., Sumner, S., Bourke, A.F.G. & Jones, K.E. (2014). Colony size predicts division of labour in attine ants. *Proc Royal Soc B*, 281, 20141411.

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

Fox, R.J., Donelson, J.M., Schunter, C., Ravasi, T. & Gaitán-Espitia, J.D. (2019). Beyond buying time: the role of plasticity in phenotypic adaptation to rapid environmental change. *Philos Trans R Soc B*, 374, 20180174.

Fraser, V.S., Kaufmann, B., Oldroyd, B.P. & Crozier, R.H. (2000). Genetic influence on caste in the ant *Camponotus consobrinus*. *Behav Ecol Sociobiol*, 47, 188–194.

GBIF Secretariat. (2021). *Camponotus herculeanus* (Linnaeus, 1758). *GBIF Backbone Taxonomy*.

Geraghty, M.J., Dunn, R.R. & Sanders, N.J. (2007). Body size, colony size, and range size in ants (Hymenoptera: Formicidae): are patterns along elevational and latitudinal gradients consistent with Bergmann's rule. *Myrmecol News*, 10, 51–58.

Gibb, H., Sanders, N.J., Dunn, R.R., Arnan, X., Vasconcelos, H.L., Donoso, D.A., *et al.* (2018). Habitat disturbance selects against both small and large species across varying climates. *Ecography*, 41, 1184–1193.

Gibson, R.L. (1989). Soldier production in *Camponotus novaeboracensis* during colony growth. *Insectes Soc*, 36, 28–41.

Gomez, C. & Espadaler, X. (2000). Species body-size distribution and spatial scale in Iberian ants. *Vie et milieu*, 50, 289–295.

Gómez, C. & Espadaler, X. (2013). An update of the world survey of myrmecochorous dispersal distances. *Ecography*, 36, 1193–1201.

Hasegawa, E. (1993). Nest defense and early production of the major workers in the dimorphic ant *Colobopsis nipponicus* (Wheeler) (Hymenoptera : Formicidae). *Behav Ecol Sociobiol*, 33, 73–77.

Heinze, J., Foitzik, S., Fischer, B., Wanke, T. & Kipyatkov, V.E. (2003). The significance of latitudinal variation in body size in a holarctic ant, *Leptothorax acervorum*. *Ecography*, 26, 349–355.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *Int J Climatol*, 25, 1965–1978.

Holbrook, C.T., Barden, P.M. & Fewell, J.H. (2011). Division of labor increases with colony size in the harvester ant *Pogonomyrmex californicus*. *Behav Ecol*, 22, 960–966.

Hölldobler, B. (1962). Über die forstliche Bedeutung der Roßameisen. *Waldhygiene*, 4, 228–250.

Hölldobler, B. & Maschwitz, U. (1965). Der Hochzeitsschwarm der Rossameise *Camponotus herculeanus* L. (Hym. Formicidae). *Z Vgl Physiol*, 50, 551–568.

Hölldobler, B. & Wilson, E.O. (1990). *The ants*. Harvard University Press.

Hölldobler, B. & Wilson, E.O. (2009). *The superorganism: the beauty, elegance, and strangeness of insect societies*. WW Norton & Company.

Hölldobler, K. (1944). Über die forstlich wichtigen Ameisen des nordostkarelischen Urwaldes. *Zeitschrift für Angew. Entomol*, 30, 587–622.

Hood, W.G. & Tshchinkel, W.R. (1990). Desiccation resistance in arboreal and terrestrial ants. *Physiol Entomol*, 15, 23–35.

Idoine, K. & Ferro, D.N. (1988). Aphid honeydew as a carbohydrate source for *Edovum puttleri* (Hymenoptera: Eulophidae). *Environ Entomol*, 17, 941–944.

Jeanne, R.L. (1979). A Latitudinal Gradient in Rates of Ant Predation. *Ecology*, 60, 1211–1224.

Kadochová, Š. & Frouz, J. (2013). Thermoregulation strategies in ants in comparison to other social insects, with a focus on red wood ants (*Formica rufa* group). *F1000Res*, 2.

Kaspari, M. (1993). Body size and microclimate use in Neotropical granivorous ants. *Oecologia*, 96, 500–507.

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

Kaspari, M. (2005). Global energy gradients and size in colonial organisms: Worker mass and worker number in ant colonies. *Proc Natl Acad Sci U S A*, 102, 5079–5083.

Kaspari, M. & Vargo, E.L. (1995). Colony size as a buffer against seasonality: Bergmann's rule in social insects. *Am Nat*, 145, 610–632.

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

Kellermann, V. & van Heerwaarden, B. (2019). Terrestrial insects and climate change: adaptive responses in key traits. *Physiol Entomol*, 44, 99–115.

Kennedy, P., Baron, G., Qiu, B., Freitak, D., Helanterä, H., Hunt, E.R., *et al.* (2017). Deconstructing Superorganisms and Societies to Address Big Questions in Biology. *Trends Ecol Evol*, 32, 861–872.

Kronauer, D.J.C., Schöning, C., Vilhelmsen, L.B. & Boomsma, J.J. (2007). A molecular phylogeny of *Dorylus* army ants provides evidence for multiple evolutionary transitions in foraging niche. *BMC Evol Biol*, 7, 1–11.

Lamon, B. & Topoff, H. (1981). Avoiding predation by army ants: defensive behaviours of three ant species of the genus *Camponotus*. *Anim Behav*, 29, 1070–1081.

LeBoeuf, A.C., Waridel, P., Brent, C.S., Gonçalves, A.N., Menin, L., Ortiz, D., *et al.* (2016). Oral transfer of chemical cues, growth proteins and hormones in social insects. *Elife*, 5, e20375.

Lessard, J.-P., Stuble, K.L. & Sanders, N.J. (2020). Do dominant ants affect secondary productivity, behavior and diversity in a guild of woodland ants? *Diversity (Basel)*, 12, 460.

Lillico-Ouachour, A. & Abouheif, E. (2017). Regulation, development, and evolution of caste ratios in the hyperdiverse ant genus *Pheidole*. *Curr Opin Insect Sci*, 19, 43–51.

Lopatina, E.B. & Kipyatkov, V.E. (1993). The influence of temperature on brood development in the incipient colonies of the ants *Camponotus herculeanus* (L.) and *Camponotus xerxes* Forel (Hymenoptera, Formicidae). *Proceedings of the Colloquia on Social Insects*. 61–74.

Lüscher, M. (1953). The termite and the cell. *Sci Am*, 188, 74–80.

McElhinny, C., Gibbons, P., Brack, C. & Bauhus, J. (2005). Forest and woodland stand structural complexity: its definition and measurement. *For Ecol Manage*, 218, 1–24.

Metzl, C., Wheeler, D. & Abouheif, E. (2018). Wilhelm Goetsch (1887 - 1960): Pioneering studies on the development and evolution of the soldier caste in social insects. *Myrmecol News*, 26, 81–96.

Meurville, M.-P. & LeBoeuf, A.C. (2021). Trophallaxis: the functions and evolution of social fluid exchange in ant colonies (Hymenoptera: Formicidae). *Myrmecol News*, 31.

Mikusinski, G. (1997). Winter foraging of the Black Woodpecker *Dryocopus martius* in managed forest in south-central Sweden. *Ornis Fenn*, 74, 161–166.

Molet, M., Maicher, V. & Peeters, C. (2014). Bigger helpers in the ant *Cataglyphis bombycina*: Increased worker polymorphism or novel soldier caste? *PLoS One*, 9, e84929.

Nooten, S., Schultheiss, P., Rowe, R.C., Facey, S.L. & Cook, J.M. (2019). Habitat complexity affects functional traits and diversity of ant assemblages in urban green spaces (Hymenoptera: Formicidae). *Myrmecol News*, 29.

O'Donnell, S., Bulova, S., Caponera, V., Oxman, K. & Giladi, I. (2020). Species differ in worker body size effects on critical thermal limits in seed-harvesting desert ants (*Messor ebeninus* and *M. arenarius*). *Insectes Soc*, 67, 473–479.

Oster, G.F. & Wilson, E.O. (1978). *Caste and ecology in the social insects*. Princeton University Press.

Parr, Z.J.E., Parr, C.L. & Chown, S.L. (2003). The size-grain hypothesis: a phylogenetic and field test. *Ecol Entomol*, 28, 475–481.

Passera, L. (1977). Production des soldats dans les sociétés sortant d'hibernation chez la fourmi *Pheidole pallidula* (Nyl.)(Formicidae, Myrmicinae). *Insectes Soc*, 24, 131–146.

Passera, L., Roncin, E., Kaufmann, B. & Keller, L. (1996). Increased soldier production in ant colonies exposed to intraspecific competition. *Nature*, 379, 630–631.

Porter, S.D. (1988). Impact of temperature on colony growth and developmental rates of the ant, *Solenopsis invicta*. *J Insect Physiol*, 34, 1127–1133.

Porter, S.D. & Tschinkel, W.R. (1987). Foraging in *Solenopsis invicta* (Hymenoptera: Formicidae): effects of weather and season. *Environ Entomol*, 16, 802–808.

Powell, S. & Franks, N.R. (2006). Ecology and the evolution of worker morphological diversity: a comparative analysis with *Eciton* army ants. *Funct Ecol*, 20, 1105–1114.

R Core Team. (2022). R: A Language and Environment for Statistical Computing.

Retana, J. & Cerdá, X. (1994). Worker size polymorphism conditioning size matching in two sympatric seed-harvesting ants. *Oikos*, 71, 261–266.

la Richelière, F., Muñoz, G., Guénard, B., Dunn, R.R., Economo, E.P., Powell, S., *et al.* (2022). Warm and arid regions of the world are hotspots of superorganism complexity. *Proc Royal Soc B,* 289, 20211899.

Sanders, C.J. (1964). The biology of carpenter ants in New Brunswick. *Can Entomol*, 96, 894–909.

Sanders, C.J. (1970). The distribution of carpenter ant colonies in the spruce-fir forests of northwestern Ontario. *Ecology*, 51, 865–873.

Sanders, C.J. (1971). Aggregation of alate carpenter ants in Ontario. *Proc Entomol Soc Wash*, 102, 13–16.

Sanders, C.J. (1972). Seasonal and daily activity patterns of carpenters ants (*Camponotus* spp.) in northwestern Ontario (Hymenoptera: Formicidae). *Can Entomol*, 104, 1681–1687.

Sarty, M., Abbott, K. & Lester, P. (2006). Habitat complexity facilitates coexistence in a tropical ant community. *Oecologia*, 149, 465–473.

Schofield, S.F., Bishop, T.R. & Parr, C.L. (2016). Morphological characteristics of ant assemblages (Hymenoptera: Formicidae) differ among contrasting biomes. *Myrmecol News*, 23, 129–137.

Schöning, C., Kinuthia, W., Franks, N.R. & Lindström, J. (2005). Evolution of Allometries in the Worker Caste of *Dorylus* Army Ants. *Oikos*, 110, 231–240.

Shelomi, M. (2012). Where are we now? Bergmann's rule sensu lato in insects. *Am Nat*, 180, 511–519.

Shiroto, A., Satoh, T. & Hirota, T. (2011). The importance of workers for queen hibernation survival in *Camponotus* ants. *Zoolog Sci*, 28, 327–331.

Siefert, A., Fridley, J.D. & Ritchie, M.E. (2014). Community functional responses to soil and climate at multiple spatial scales: when does intraspecific variation matter? *PLoS One*, 9, e111189.

Sinotte, V.M., Freedman, S.N., Ugelvig, L. v & Seid, M.A. (2018). *Camponotus floridanus* ants incur a trade-off between phenotypic development and pathogen susceptibility from their mutualistic endosymbiont *Blochmannia*. *Insects*, 9, 58.

Smith, F. (1942). Effect of reduced food supply upon the stature of *Camponotus* ants. *Entomol News*, 53, 133–135.

Smith, F. (1944). Nutritional requirements of *Camponotus* ants. *Ann Entomol Soc Am*, 37, 401–408.

Strong, F.E. (1965). Detection of lipids in the honeydew of an aphid. *Nature*, 205, 1242.

Tschinkel, W.R. (1988). Colony growth and the ontogeny of worker polymorphism in the fire ant, *Solenopsis invicta*. *Behav Ecol Sociobiol*, 22, 103–115.

Tschinkel, W.R. (1993). Sociometry and Sociogenesis of Colonies of the Fire Ant Solenopsis Invicta During One Annual Cycle: Ecological Archives M063-002. *Ecol Monogr*, 63, 425–457.

Ulrich, Y., Saragosti, J., Tokita, C.K., Tarnita, C.E. & Kronauer, D.J.C. (2018). Fitness benefits and emergent division of labour at the onset of group living. *Nature*, 560, 635–638.

Valladares, F., Matesanz, S., Guilhaumon, F., Araújo, M.B., Balaguer, L., Benito-Garzón, M., *et al.* (2014). The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecol Lett*, 17, 1351–1364.

Vesnic, A., Škrijelj, R., Trožić-Borovac, S. & Tomanović, Ž. (2017). Diversity and nesting preferences of *Camponotus lateralis* group species on Western Balkan Peninsula (Hymenoptera: Formicidae). *J Entomol Res Soc*, 19, 73–82.

Völkl, W., Woodring, J., Fischer, M., Lorenz, M.W. & Hoffmann, K.H. (1999). Ant-aphid mutualisms: the impact of honeydew production and honeydew sugar composition on ant preferences. *Oecologia*, 118, 483–491.

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

Walker, J. & Stamps, J. (1986). A test of optimal caste ratio theory using the ant *Camponotus* (*Colobopsis*) *impressus*. *Ecology*, 67, 1052–1062.

Wendt, C.F. & Verble-Pearson, R. (2016). Critical thermal maxima and body size positively correlate in red imported fire ants, *Solenopsis invicta*. *Southwest Nat*, 61, 79–83.

West-Eberhard, M.J. (2005). Phenotypic accommodation: adaptive innovation due to developmental plasticity. *J Exp Zool B Mol Dev Evol*, 304, 610–618.

Wheeler, D. (1991). The developmental basis of worker caste polymorphism in ants. *Am Nat*, 138, 1218–1238.

Wheeler, D.E. & Nijhout, H.F. (1981). Soldier determination in ants: New role for juvenile hormone. *Science (1979)*, 213, 361–363.

Willott, S.J., Compton, S.G. & Incoll, L.D. (2000). Foraging, food selection and worker size in the seed harvesting ant *Messor bouvieri*. *Oecologia*, 125, 35–44.

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

Wilson, E.O. (1953). The origin and evolution of polymorphism in ants. *Q Rev Biol*, 28, 136–156.

Wilson, E.O. (1974). The soldier of the ant *Camponotus* (*Colobopsis*) *fraxinicola* as a trophic caste. *PsyCh J*, 81, 182–188.

Wilson, E.O. (1976). The organization of colony defense in the ant *Pheidole dentata* Mayr (Hymenoptera: Formicidae). *Behav Ecol Sociobiol*, 1, 63–81.

Wilson, E.O. (1984). The relation between caste ratios and division of labor in the ant genus *Pheidole* (Hymenoptera: Formicidae). *Behav Ecol Sociobiol*, 16, 89–98.

Wilson, E.O. (2003). *Pheidole in the New World: a dominant, hyperdiverse ant genus*. Harvard University Press.

Wood, S. & Wood, M.S. (2015). Package 'mgcv.' *R package version*, 1, 729.

Wood, S.N. (2017). Generalized additive models: An introduction with R. *Generalized Additive Models*, 10, 9781315370279.

Wood, S.N., Pya, N. & Säfken, B. (2016). Smoothing parameter and model selection for general smooth models. *J Am Stat Assoc*, 111, 1548–1563.

Yang, A.S. (2006). Seasonality, division of labor, and dynamics of colony-level nutrient storage in the ant *Pheidole morrisi*. *Insectes Soc*, 53, 456–462.

Yang, A.S., Martin, C.H. & Nijhout, H.F. (2004). Geographic variation of caste structure among ant populations. *Curr Biol*, 14, 514–519.

Zientz, E., Beyaert, I., Gross, R. & Feldhaar, H. (2006). Relevance of the endosymbiosis of *Blochmannia floridanus* and carpenter ants at different stages of the life cycle of the host. *Appl Environ Microbiol*, 72, 6027–6033.

Figures

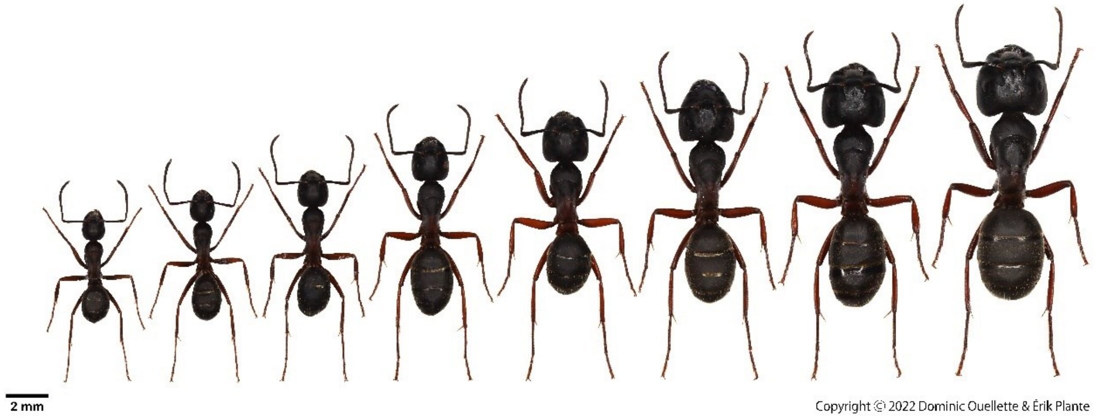

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Figure 1. *Camponotus herculeanus* worker polymorphism (left-to-right) from minors to medias and majors. All workers are from the same colony and were photographed at the same scale. They are a representation of the possible worker morphological range for a given large colony of the species in the study area in Quebec, Canada.

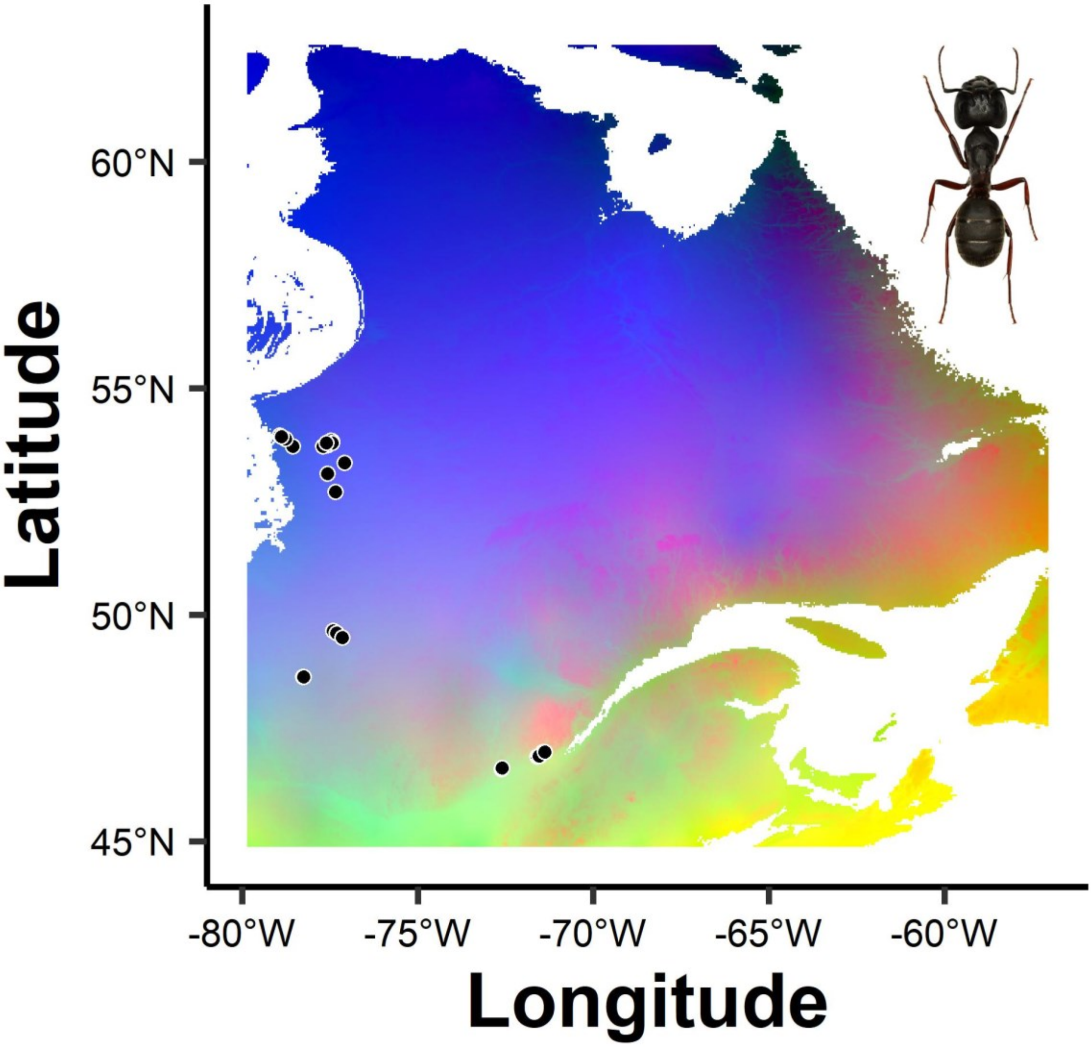


Figure 2. Sampled *Camponotus herculeanus* colonies along an environmental gradient spanning 8 degrees of latitude in the boreal forest of Eastern Canada (province of Quebec) according to three principal components of a multi-band raster displayed as a false color RGB (red, green, blue) high-resolution image of the mean annual temperature, annual precipitation, and seasonality for the years 1970 to 2000 ($n = 27$) (Fick & Hijmans 2017). Changes in colors correspond to changes in the composite formed by the three bioclimatic variables. This imagery composite helps to highlight contrasts of the bioclimatic conditions between the different sampling regions.

Figure 3. Weber's length (WL) and head width (HW) of a major worker *Camponotus herculeanus* collected in Quebec, Canada.

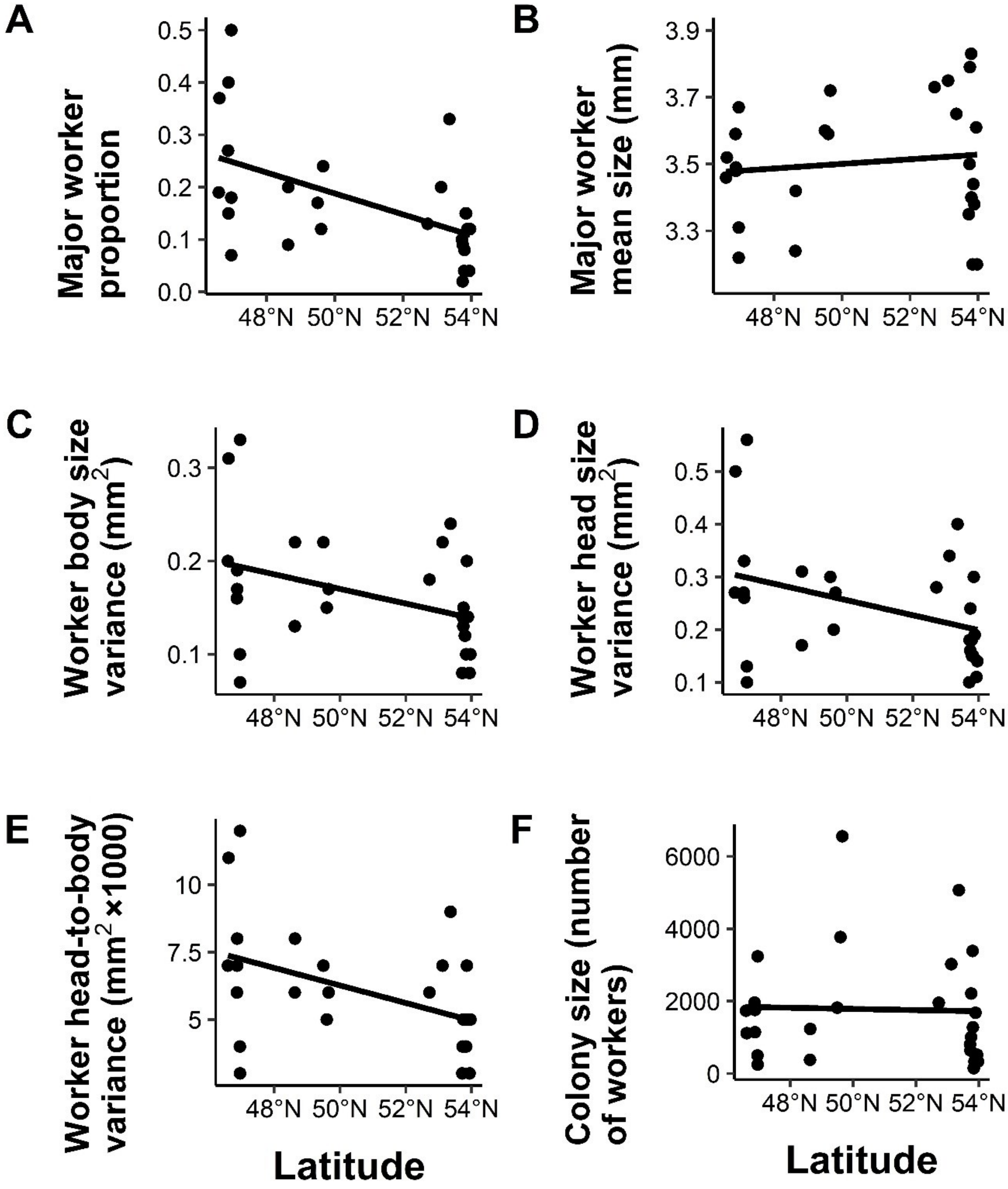


Figure 4. Colony traits of *Camponotus herculeanus* according to latitude (degrees North) ($n = 27$). (A) Proportion of major workers; (B) Major worker mean body size (mm); (C) Worker body size variance (mm^2); (D) Worker head width variance (mm^2); (E) Worker head-to-body ratio variance (mm^2 × 1000); (F) Colony size (total number of workers).

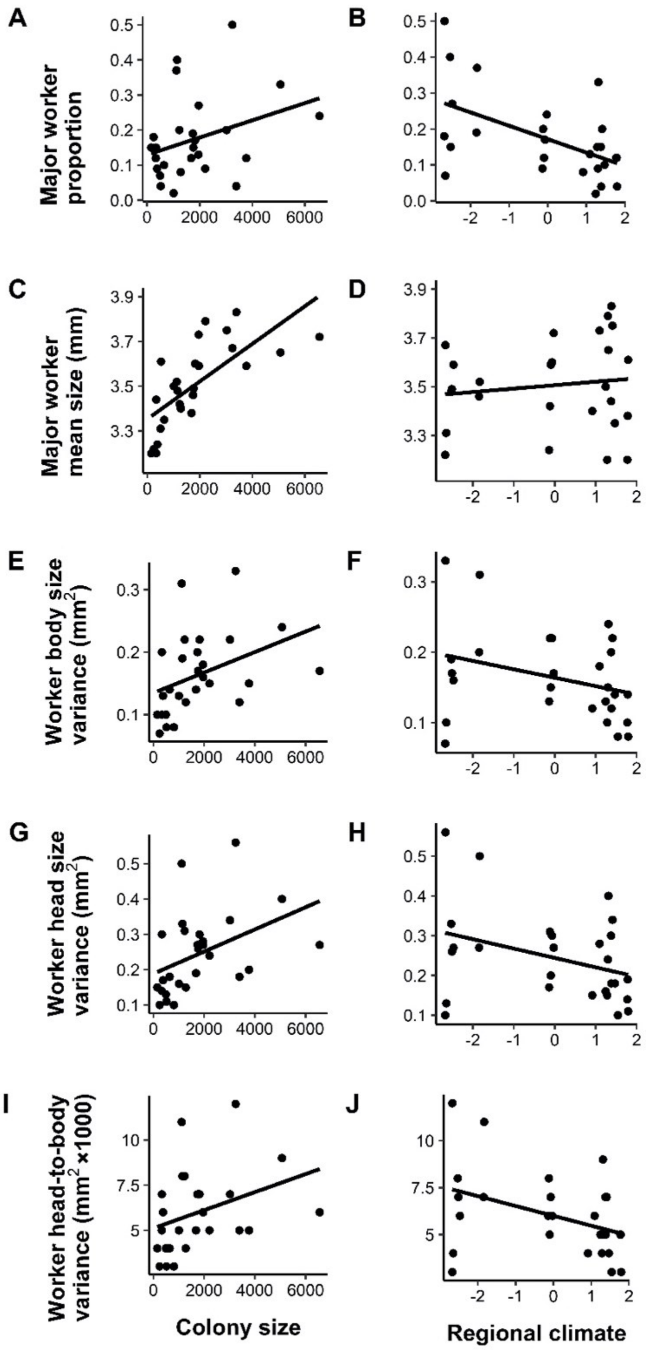
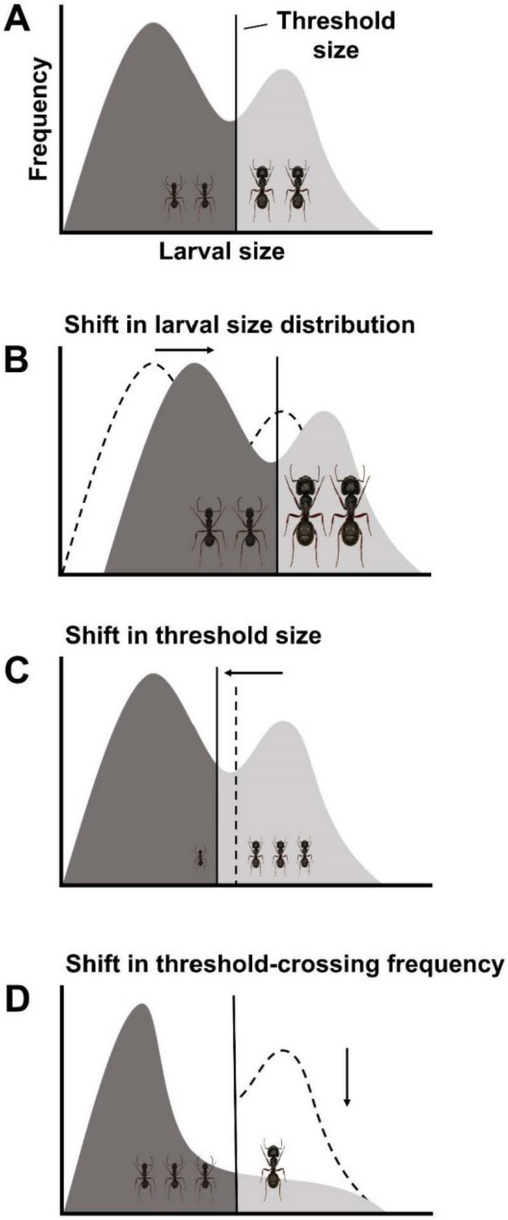


Figure 5. Colony traits *Camponotus herculeanus* collected in 2021 along a latitudinal gradient in Quebec, Canada according to colony size (total number of workers) and regional climate (PC1, positive values represent a colder and drier climate, while negative values represent a warmer and more humid climate) (n = 27). (A, B) Proportion of major workers; (C, D) Major worker mean body size (mm); (E, F) Worker body size variance (mm^2); (G, H) Worker head width variance (mm^2); (I, J) Worker head-to-body ratio variance (mm^2).



(A) In *C. herculeanus*, the worker caste size frequency distribution is bimodal. The first peak corresponds to minors while the second peak correspond to majors. The medias are the workers falling in between the two peaks. Most larvae develop into minors or medias (dark) and the larvae that reach above the threshold size develop into majors (light).

(B) When the mean size of larvae is increased relative to an initial threshold size (A), there will be a greater proportion of major workers and major workers will have a larger mean size.

(C) A larger proportion of major workers is also produced when the threshold size determination for major worker is lower than in the scenario (A). Major workers will have a smaller mean size.

(D) A lower proportion of major workers is produced when less larvae have the necessary levels of juvenile hormone to exceed the threshold size. The data of the present study is consistent with (A) representing colonies of the southern part of the gradient and (D) the northern part of the gradient.

Figure 6. How shifts in the developmental threshold of worker caste determination in *Camponotus herculeanus* are predicted to affect the proportion and mean size of majors (figure adapted from Yang et al. 2004).

Tables

Table 1. Rationale for using different worker traits of the ant colony phenotype.

Colony trait	Functions for the colony
Major worker proportion	**Defense of the colony** (Wilson 1974, 1976, 2003; Lamon & Topoff 1981; Hasegawa 1993; Yang *et al.* 2004; Molet *et al.* 2014; Metzl *et al.* 2018) **Food storage** (Wilson 1974; Yang 2006)
Major worker mean body size	**Thermal tolerance** (Bernstein 1976; Porter & Tschinkel 1987; Cerdá *et al.* 1997; Cerdá & Retana 2000; Wendt & Verble-Pearson 2016; O'Donnell *et al.* 2020) **Desiccation** (Hood & Tshchinkel 1990; Kaspari 1993)
Worker head and body size variance	**Dietary composition** (Retana & Cerdá 1994; Kaspari 1996; Willott *et al.* 2000; Powell & Franks 2006) **Division of labor** (Wilson 1953, 1984; Wheeler 1991)
Worker head-to-body ratio variance	**Habitat complexity** (Kaspari & Weiser 1999; Parr *et al.* 2003; Farji-Brener *et al.* 2004; Sarty *et al.* 2006) **Division of labor** (Hölldobler & Wilson 1990; Schöning *et al.* 2005; Araujo & Tschinkel 2010; Centorame *et al.* 2020)

Table 2. Multivariate normal additive model of major worker proportion, mean major worker body size, worker body size variance, worker head size variance and head-to-body ratio variance describing worker polymorphism according to regional climate and colony size as predictors. All significant terms are shown in bold.

Trait	Predictor	Estimate	Standard error	z-value	*P*
Major worker proportion	Intercept	1.4×10^{-1}	2.7×10^{-2}	5.0	< **0.001**
	Regional climate	-2.4×10^{-2}	6.5×10^{-3}	-3.7	< **0.001**
	Colony size	2.5×10^{-5}	1.1×10^{-5}	2.2	**0.029**
Mean major worker body size	Intercept	3.4×10^{-1}	3.9×10^{-2}	87	< **0.001**
	Regional climate	7.0×10^{-3}	9.4×10^{-3}	0.8	0.455
	Colony size	8.4×10^{-5}	1.6×10^{-5}	5.2	< **0.001**
Worker body size variance	Intercept	1.4×10^{-1}	1.7×10^{-2}	8.5	< **0.001**
	Regional climate	-7.7×10^{-3}	4.0×10^{-3}	-1.9	0.056
	Colony size	1.5×10^{-5}	7.0×10^{-6}	2.2	**0.028**
Worker head size variance	Intercept	2.0×10^{-1}	2.9×10^{-2}	7.0	< **0.001**
	Regional climate	-1.5×10^{-2}	6.9×10^{-3}	-2.1	**0.032**
	Colony size	3.0×10^{-5}	1.2×10^{-5}	2.5	**0.014**
Head-to-body ratio variance	Intercept	5.3×10^{-3}	5.6×10^{-4}	9.4	< **0.001**
	Regional climate	-3.6×10^{-4}	1.5×10^{-4}	-2.4	**0.015**
	Colony size	4.6×10^{-7}	2.4×10^{-7}	1.9	0.053
Deviance explained = 32.7%, -REML = -313.21, Scale estimate = 1, *n* = 26					

Supplementary material

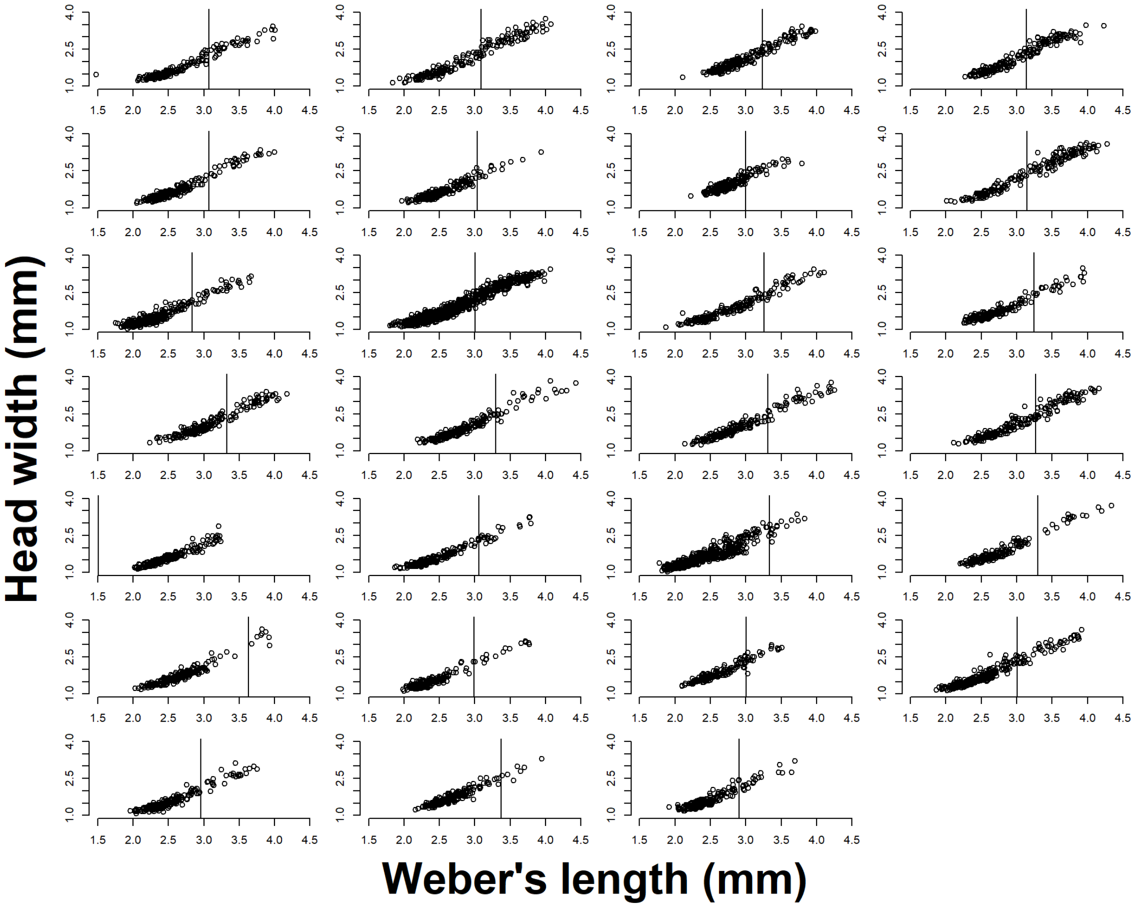


Figure S1. Inflection point of the allometric growth curve of 27 colonies of *Camponotus herculeanus* collected in 2021 along a latitudinal gradient in Quebec, Canada (one colony where the inflection point was not detected due to the near absence of large major workers was removed from the analyses).

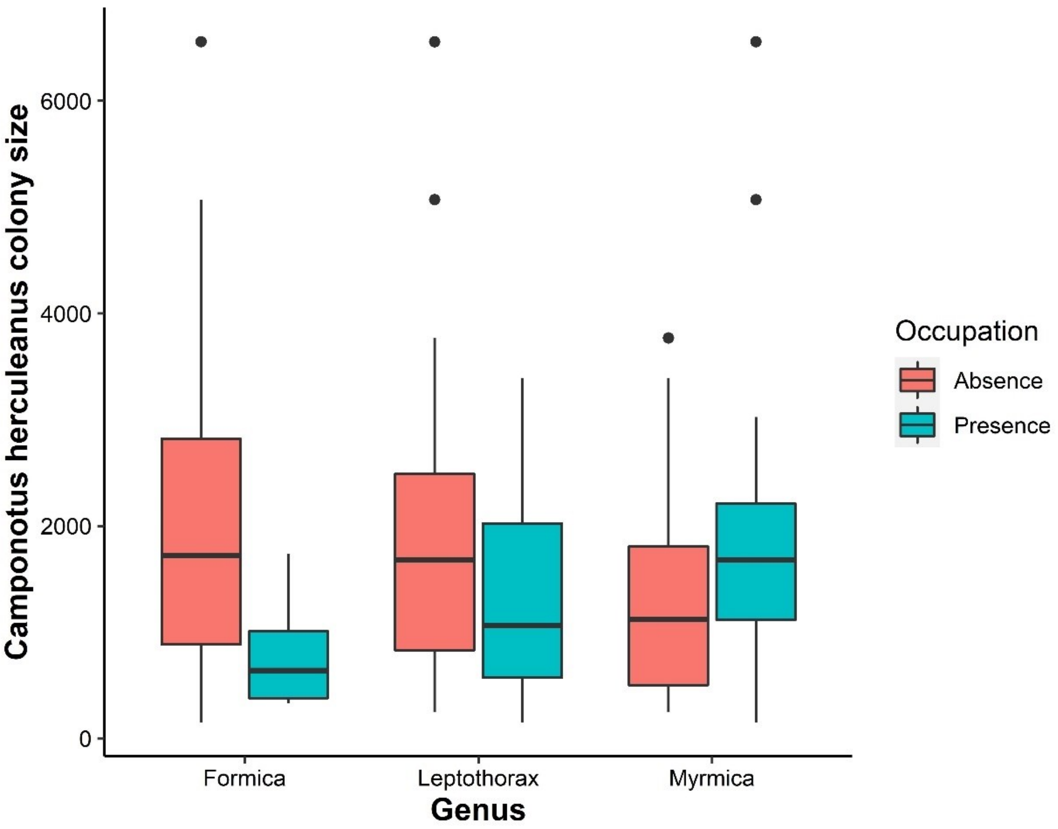


Figure S2. Size of *Camponotus herculeanus* colonies (number of workers) collected in 2021 along a latitudinal gradient in Quebec, Canada according to the presence or absence of nests of competitive ant genera in a radius of 10 meters ($n = 27$).

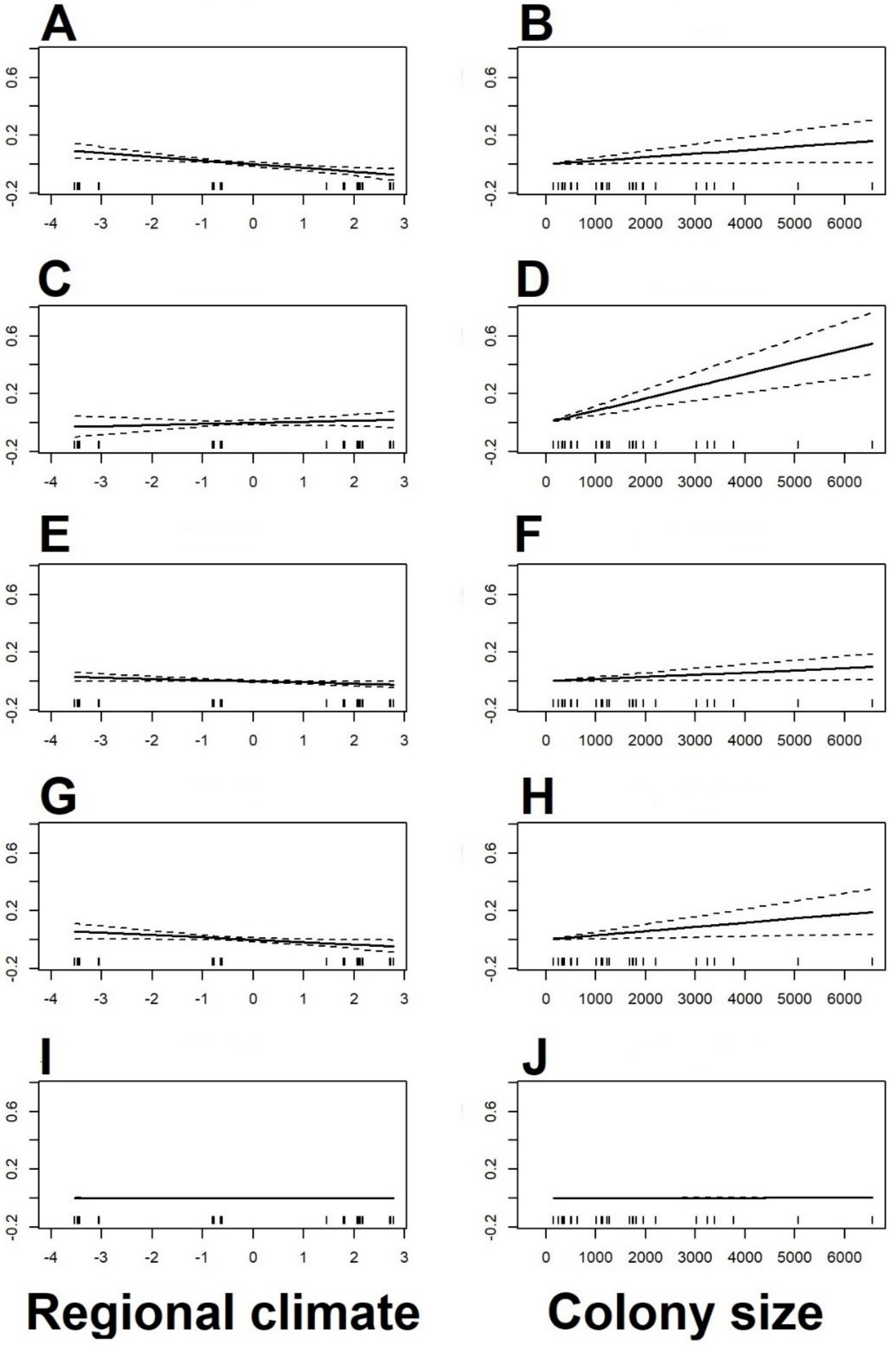


Figure S3. Partial residuals plots of a multivariate normal additive model of the colony traits *Camponotus herculeanus* collected in 2021 along a latitudinal gradient in Quebec, Canada according to colony size (total number of workers) and regional climate (PC1, positive values represent a colder and drier climate, while negative values represent a warmer and more humid climate) ($n = 27$). (A, B) Proportion of major workers; (C, D) Major worker mean body size (mm); (E, F) Worker body size variance (mm^2); (G, H) Worker head width variance (mm^2); (I, J) Worker head-to-body ratio variance (mm^2).

Table S1. Principal component analysis of the bioclimatic variables expected to affect worker polymorphism.

Bioclimatic variables	PC1 loading	PC2 loading
Mean diurnal range	-0.180	0.687
Isothermality	-0.345	0.388
Maximum temperature of warmest month	-0.377	0.222
Mean annual temperature	-0.399	0.002
Minimum temperature of coldest month	-0.391	-0.174
Annual precipitation	-0.390	-0.118
Seasonality	0.385	0.197
Temperature annual range	0.306	0.495

Table S2. Nine generalized additive models of the standard deviation, coefficient of variance and variance of worker body size, worker head size and head-to-body ratio according to biotic and abiotic predictors using gaussian family. All significant terms are shown in bold.

		Standard deviation		Coefficient of variation		Variance	
Trait	Predictor	Estimate	*P*	Estimate	*P*	Estimate	*P*
Worker body size	Intercept	3.5×10^{-1}	**< 0.001**	1.3×10^{1}	**< 0.001**	1.3×10^{-1}	**0.034**
	Regional climate	-1.2×10^{-2}	0.072	-3.0×10^{-1}	0.178	-1.1×10^{-2}	**0.045**
	Local climate	3.8×10^{-4}	0.524	1.0×10^{-2}	0.517	3.6×10^{-4}	0.466
	Nest diameter	-3.1×10^{-3}	0.668	-1.0×10^{-1}	0.614	-2.7×10^{-3}	0.650
	Colony size	2.8×10^{-5}	**0.036**	1.0×10^{-3}	0.170	2.1×10^{-5}	0.052
	Competition	-3.5×10^{-3}	0.936	9.3×10^{-1}	0.525	-9.2×10^{-3}	0.797
Worker head size	Intercept	4.4×10^{-1}	**< 0.001**	2.4×10^{1}	**< 0.001**	1.9×10^{-1}	0.055
	Regional climate	-1.8×10^{-2}	**0.047**	-4.7×10^{-1}	0.208	-2.0×10^{-2}	**0.037**
	Local climate	3.8×10^{-4}	0.646	2.2×10^{-2}	0.527	5.2×10^{-4}	0.539
	Nest diameter	-5.5×10^{-3}	0.581	-1.8×10^{-1}	0.667	-5.6×10^{-3}	0.586
	Colony size	4.0×10^{-5}	**0.030**	1.0×10^{-3}	0.127	3.8×10^{-5}	**0.041**
	Competition	-2.5×10^{-2}	0.674	8.9×10^{-1}	0.721	-3.5×10^{-2}	0.573
Head-to-body ratio	Intercept	7.3×10^{-2}	**< 0.001**	1.0×10^{1}	**< 0.001**	4.6×10^{-3}	**0.024**
	Regional climate	-2.3×10^{-3}	0.064	-2.8×10^{-1}	0.080	-5.1×10^{-4}	**0.016**
	Local climate	3.8×10^{-5}	0.732	1.0×10^{-2}	0.500	1.4×10^{-7}	0.420
	Nest diameter	-6.5×10^{-4}	0.630	-3.0×10^{-2}	0.863	-9.9×10^{-4}	0.624
	Colony size	4.0×10^{-6}	0.096	1.0×10^{-3}	0.093	6.9×10^{-7}	0.059
	Competition	-3.4×10^{-3}	0.674	3.6×10^{-1}	0.726	-6.8×10^{-3}	0.955

Table S3. Generalized additive models of major worker proportion, mean major worker body size, worker body size variance, worker head size variance and head-to-body ratio variance describing worker polymorphism according to biotic and abiotic predictors. Beta family was used for major worker proportion (z-values) and gaussian for the rest of the traits (t-values). Only the significant predictors (*P* < 0.05) were kept for further analyses. All significant terms are shown in bold.

Trait	Predictor	Estimate	Standard error	z/t-value	*P*
Major worker proportion	**Intercept**	-1.7×10^{-1}	5.5×10^{-1}	-3.1	**0.002**
	Regional climate	-0.2×10^{-1}	5.3×10^{-2}	-2.8	**0.005**
	Local climate	-1.0×10^{-3}	5.0×10^{-3}	-0.2	0.851
	Nest diameter	-4.0×10^{-3}	5.7×10^{-2}	-0.1	0.939
	Colony size	1.4×10^{-4}	1.0×10^{-4}	1.3	0.182
	Competition	-0.3×10^{-1}	4.0×10^{-1}	-0.6	0.526
Mean major worker size	**Intercept**	3.3	1.2×10^{-1}	28	**< 0.001**
	Regional climate	7.2×10^{-3}	1.1×10^{-2}	0.6	0.529
	Local climate	1.3×10^{-3}	1.1×10^{-3}	1.2	0.234
	Nest diameter	1.1×10^{-4}	1.3×10^{-4}	0.01	0.993
	Colony size	8.0×10^{-5}	2.2×10^{-5}	3.7	**0.002**
	Competition	-1.6×10^{-1}	7.7×10^{-2}	-2.0	0.056
Worker body size variance	**Intercept**	1.3×10^{-1}	5.6×10^{-2}	2.3	**0.034**
	Regional climate	-1.1×10^{-2}	5.2×10^{-3}	-2.1	**0.045**
	Local climate	3.6×10^{-4}	4.9×10^{-4}	0.7	0.466
	Nest diameter	-2.7×10^{-3}	5.9×10^{-3}	-0.5	0.650
	Colony size	2.1×10^{-5}	1.0×10^{-5}	2.1	0.052
	Competition	-9.2×10^{-3}	3.5×10^{-2}	-0.3	0.797
Worker head size variance	Intercept	1.9×10^{-1}	9.5×10^{-2}	2.0	0.055
	Regional climate	-2.0×10^{-2}	8.9×10^{-3}	-2.2	**0.037**
	Local climate	5.2×10^{-4}	8.3×10^{-4}	0.6	0.539
	Nest diameter	-5.6×10^{-3}	1.0×10^{-2}	-0.6	0.586
	Colony size	3.8×10^{-5}	1.8×10^{-5}	2.2	**0.041**
	Competition	-3.5×10^{-2}	6.1×10^{-2}	-0.6	0.573
Head-to-body ratio variance	**Intercept**	4.6×10^{-3}	1.9×10^{-3}	2.5	**0.024**
	Regional climate	-5.1×10^{-4}	1.9×10^{-4}	-2.6	**0.016**
	Local climate	1.4×10^{-7}	1.6×10^{-5}	0.8	0.420
	Nest diameter	-9.9×10^{-4}	2.0×10^{-4}	-0.5	0.624
	Colony size	6.9×10^{-7}	3.4×10^{-7}	2.0	0.059
	Competition	-6.8×10^{-3}	1.2×10^{-3}	-0.1	0.955

Table S4. Number of *Camponotus herculeanus* colonies where competitive species workers occurred within 10 meters around their nest ($n = 27$). *Formica* were considered strong competitors.

Species	Occurrence
Camponotus nearcticus	1
Dolichoderus mariae	1
Dolichoderus pustulatus	1
Formica neorufibarbis	4
Formica subaenescens	1
Lasius americanus	1
Leptothorax spp.	12
Myrmica alaskensis	11
Myrmica francticornis	2
Myrmica lobifrons	1
Tapinoma sessile	3