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Intraspecific functional traits and stable isotope signatures of ground-dwelling ants across an elevational gradient

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Abstract

Understanding the responses of species to changing climates is becoming increasingly urgent with global change impacts. Of particular importance is the impact of global environmental change on species functional traits at intraspecific level. Utilizing elevation gradients as a surrogate for climate change, we explored the intraspecific responses of two ground-dwelling ant species, *Ectomomyrmex javanus* and *Odontoponera transversa*, across elevations ranging from 100 to 700 meters within a subtropical evergreen broadleaf forest. Our study specifically addressed the relationships among environmental factors, trait variations, and trophic levels. Key functional traits such as dry mass, head length, body size, and leg length exhibited a general increase with elevation. By employing stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$), we successfully quantified shifts in diets and trophic positions along elevation gradients. Notably, our data revealed a significant elevation-related increase in Ant $\delta^{13}C$, while $\delta^{15}N$ exhibited no such correlation. Moreover, Ant $\delta^{13}C$ values of E. javanus demonstrated a negative correlation with mean annual temperature (MAT), and the $\delta^{13}C$ values of both species positively correlated with soil C:N ratio. This study underscores that the individual traits and $\delta^{13}C$ signatures of ground-dwelling ants exhibit a significant negative correlation with temperature along elevation gradients. Consequently, our findings suggest that future climate warming has the potential to induce intraspecific variation in the functional traits and diets of ground-dwelling ants.

Introduction

Functional traits are morphological, behavioral and physiological characteristics of a species, which govern the performance of individuals and their responses to environmental gradients (Moretti et al., 2017). In the face of global environmental change, understanding the responses of species to changing climates is an urgent requirement of modern ecology (Moretti et al., 2017; Classen et al., 2017). Nowhere is this more urgent than in the case of ecosystem engineers such as the ants (Insecta: Hymenoptera), which are fundamental to the functioning of the world's ecosystems. Ants are globally dominate terrestrial ecosystem engineers in terms of their high abundance and biomass, and play critical roles in many ecosystem processes, including seed dispersal, nutrient cycling, and soil structuring etc. (Wiescher et al., 2012; Gibb et al., 2023). Consequently, changes in ant functional traits due to environmental change are reasonable to affect the terrestrial ecosystem functions and serveries (Joseph et al., 2019). In addition, ants are ideal study system to investigate the responses of functional traits to elevational gradients given their extensive morphological variation and wide ecological distribution (Gibb et al., 2023). Bishop et al. (2016) found that ants were larger and darker in higher and, therefore colder environments. However, previous studies on ant functional traits along elevational gradients mostly focused on communities or assemblages level, neglecting intraspecific climate–trait relationships (Gibb et al., 2023).

Feeding traits are dimension of species functional traits that related to the species potential food resource and trophic position, which are likely to impact species fitness and regulate species' responses to environmental perturbations like elevational change (Moretti et al., 2017). Using stable isotopes of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) can provide important insights into the diets and trophic level of organisms (Diniz-Reis et al., 2022). This is particularly useful in the case of omnivorous species such as many ant species, which feed on both animals and plants. Different stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) reflect different proportions of plant-based and animal-based diets (Tillberg et al., 2006; Feldhaar et al., 2009), providing an effective way to quantify the shifts in diets along elevation gradients (Pilar et al., 2020). Changes in the diet, especially shifts between plant-based and animal-based diets, would be reflected in the $\delta^{13}C$ and $\delta^{15}N$ values of ants, and higher $\delta^{13}C/\delta^{15}N$ ratio indicate species rely on large amounts of plant-based resources (Blüthgen et al., 2003; Joseph et al., 2019). Despite this, few studies have used stable isotopes of C and N to explore intraspecific diet variation of ant species along environmental gradients.

Using elevation gradients as a proxy for changes in climatic conditions, we investigated the intraspecific responses of ants across elevations from 100-700m in a subtropical evergreen broadleaf forest. First, we quantified the relationship between trait variation, diets, and elevation in two widely distributed and dominant ground-dwelling ant species (*Ectomomyrmex javanus* and *Odontoponera transversa*). Second, we determined the extent to which environmental factors explain these elevational patterns. We hypothesized that ant functional traits exhibited linear increases along with increasing elevation gradient due to the changing climatic gradients and soil nutrients (He et al., 2016).

2. Materials and methods

2.1 Study sites

Our study took place in Dinghu Mountain (23°09′–23°11′ N, 112°30′–112°33′ E) in Guangdong Province, China. This area has a typical subtropical humid monsoon climate, with a mean annual temperature (MAT) of 21°C, and a mean annual precipitation (MAP) of 1927mm. The vegetation is characterized as tropical monsoon forest and subtropical monsoon evergreen broadleaf forest. There is a classic elevation-caused vegetation change along the elevational gradients (Table S1; He et al., 2016). The elevation of the mountain ranges from 10 m to 1000 m asl. To avoid the impact of tourists at the foot of the mountain, our elevational transect starts from an elevation of 100 m asl. Due to the lack of a sufficient number of ant samples collected at sites above 700 asl, our highest elevation sample site reaches a maximum elevation of 700 m asl. A total of seven sites were established in the elevational transects (Figure S1), within which sampling sites were distributed at about 100-m intervals in elevation (determined by GPS), with elevations ranging from 100 to 700 m asl. The basic site information for the 7 sites is summarized in Table S1. These 7 sites are distributed on the southeast slope of Dinghu Mountain and roughly follow the fire lane. The selected sites are rarely impacted by humans. To reduce the influence of aspect, plots were positioned on the sunny side of any microtopography at each point along the transect.

2.1 Ant collection and morphological measurements

Ants were collected in September 2014 using pitfall traps (15 cm deep with 10cm diameter) baited with sugar and tuna oil. A total of 12 pitfall-traps were distributed randomly in each sampling site. Pitfall traps were emptied every three days for 15 days. We found four ant species across all the transects (*Ectomomyrmex javanus, Odontoponera transversa, Diacamma rugosum, Leptogenys chinensis*), but only two of them (*E. javanus* and *O. transversa*) contained enough specimens at each elevation for the measurement of functional traits and stable isotopes.

Ants were killed by freezing for six hours and cleaned using an ultrasonic cleaner. All ants were dried in a ventilated oven at 35℃ for at least 48h until its weights became constant over time. We measured the dry mass and functional traits of each ant individual. Among the multiple traits we measured, three body traits were relevant to ant trophic level and resource use (Liu et al., 2016): (i) head length: measured as the maximum length of the head; (ii) Weber's length: the length of the mesosoma, as an indicator of body size; (iii) leg length: measured as hind femur length. Functional trait measurements were taken from 4-10 adult workers of each species within each elevation, which is enough to show the variation of ant morphological traits in each sampling site.

Stable isotope measurements were taken from the same ant individual, from which functional traits were measured. Abdomens of ants were excluded to avoid the influence of partially digested food in their digestive systems. At least three samples were crushed and homogenised using tweezers for measuring. We then measured C, N, ^{13}C, and ^{15}N contents of the samples using a Stable Isotope Ratio Mass Spectrometer (ThermoFisher Scientific, 253 Plus, USA). Amounts of ^{13}C and ^{15}N were recorded as $\delta^{13}C$ and $\delta^{15}N$, being a measure of the ratio of the two stable isotopes of carbon or nitrogen—$^{13}C/^{12}C$ or $^{15}N/^{14}N$—reported in parts per thousand (‰). We used the international standards of atmospheric N for $\delta^{15}N$ and Pee Dee Belemnite carbonate for $\delta^{13}C$ (Peterson and Fry 1987), and calculated $\delta^{13}C$ and $\delta^{15}N$ as follows:

δ_{sample} (‰) = $[R_{sample}/R_{standard} - 1] \times 10^3$ (Peterson and Fry 1987).

We measured environmental factors from soil cores (0-10 cm depth), and litter (20 × 20 cm), and quantified physicochemical properties, namely soil pH, soil organic carbon (SOC), soil total nitrogen (TN), litter carbon and nitrogen contents. Detailed sampling details of soil and litter can be found in our previous paper (He et al., 2016). Soil pH was measured using a PHS-3C pH acidometer (soil-water ratio of 1:5). The C and N concentrations in soil cores and litter were determined by dry combustion with an elemental analyzer (Perkin Elmer 2400 Series II). We also monitored soil temperature at 10 cm depth hourly from September to October 2014 with a temperature recorder (HOBO Onset U22-001, USA). MAP at each sampling site was extracted based on the geographic coordination record from the global climate layers of WorldClim (http://www.worldclim.org/).

2.3 Statistical analyses

We used univariate linear regression models to examine elevational effects on functional traits and stable isotope signatures. We used Pearson correlation analysis to examine the relationships between environmental factors, functional traits, and stable isotope signatures. All statistical analyses were performed in R (R Core Team 2018), and graphs were generated with the ggplot2 package.

3. Results and Discussion

The elevational pattern of soil climate and soil physicochemical properties is detailed in a prior publication (He et al., 2016) and can be referenced, along with supplementary Figure S2, for additional information. Our data reveal that the functional traits of ground-dwelling ants responded to elevational gradients at intraspecific level, indicating that certain traits of these ant species are adaptable under the particular environmental conditions of different microhabitats (Wiescher et al., 2012). Individuals of both *E. javanus* and *O. transversa* were larger at higher elevations (Fig. 1). Both species exhibited significant linear increases in dry mass and in the length of the head and the hind femur, while increases in the length of the hind femur of *O. transversa* showed marginal significance (Fig. 1d). This may be explained by the fact that there are fewer plants at higher elevations, a trend observed in our study and elsewhere (He et al., 2016). Fewer plants at high elevations suggest simpler forest floor habitats (Liu et al., 2018), in which larger body sizes are advantageous for foraging, consistent with the size-grain hypothesis (trade-off between body size and locomotion costs) (Kaspari and Weiser, 1999). Larger body sizes under cooler temperature can also be attributed to the temperature-size rule (larger body size at lower temperature) which posits that the insect maturation takes more time under cooler temperature, resulting in larger body sizes of adult insects (Atkinson, 1994). Our observation of smaller ants with shorter legs at lower elevations in agreement with Bishop et al., (2016), Silva et al., (2014) and Reymond et al., (2013).

Increasing elevation was associated with significant increases in the $\delta^{13}C$ values of both ant species (Fig. 1e). Elevational changes in functional traits may allow ants to use different plant-based food resources, altering their $\delta^{13}C$ signature. Elevation had no significant effect on $\delta^{15}N$ values for ants. Ants are opportunistic scavengers and predators, and are thus highly omnivorous, being able to balance food resources (the proportion of arthropod prey to plant-based foods) (Kjeldgaard et al., 2022). The lack of any significant correlation between elevation and $\delta^{15}N$ values suggests that the trophic level of both ant species remained highly omnivorous and did not exhibit systematic changes (Fig. 1f). The large variation in $\delta^{15}N$ values could also suggest that the trophic level of these species was influenced more by microhabitat conditions than by food resources.

The fact that increasing elevation correlated with significant increases in the $\delta^{13}C$ values of both ant species at higher elevations is probably linked with increasing plant $\delta^{13}C$ at higher elevations (Zhou et al., 2011; Yan et al., 2013). Our results showed that

δ^{13}C values were negatively correlated with mean annual temperature (MAT) and significantly positively correlated with soil C:N ratio (Fig. 2). The δ^{13}C of *E. javanus* decreased significantly with increasing MAT. Low temperatures can constrain stomatal conductance, leading to higher plant δ^{13}C values (Panek and Waring, 1995). Given that higher elevations with lower air temperature result in higher δ^{13}C values of plant-based food resources, and thus higher δ^{13}C values of ants, this would explain the negative relationship between MAT and the isotopic ^{13}C signature of ants.

The δ^{13}C of both ant species increased significantly with increasing soil C:N ratio, albeit only marginally for *O. transversa*. Previous studies have noted the positive effect of soil C:N ratio on soil δ^{13}C values (Feng et al., 2020), so it is unsurprising that the body tissues of ground-dwelling ants are enriched in isotopic ^{13}C through nutrient transfer along the food chain (Penick et al., 2015).

To the best of our knowledge, this study stands as one of the few endeavors that systematically evaluates the impact of elevation gradients on intraspecific ant functional traits using stable isotopes techniques. In conclusion, our study of the intraspecific changes in body traits and δ^{13}C signatures reveals that these ground-dwelling ant species are able to adapt their body traits and food resources to the specific environmental conditions of different microhabitats along an elevation gradient. Subsequent studies should corroborate our results by examining a wider range of ant species and additional elevational transects globally, aiming to provide a more comprehensive understanding of how climate change influences the morphological traits and dietary patterns of ants.

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Figure 1. Elevational patterns of body traits and stable isotope (δ^{13}C and δ^{15}N values) of two ant species (*Ectomomyrmex javanus* and *Odontoponera transversa*). A. dry mass; B. head length; C. Weber's length; D. hind femur length. E. δ^{13}C value. F. δ^{15}N value. Solid and dashed lines indicate significant ($p < 0.05$) and non-significant ($p > 0.05$) linear regression relationships.

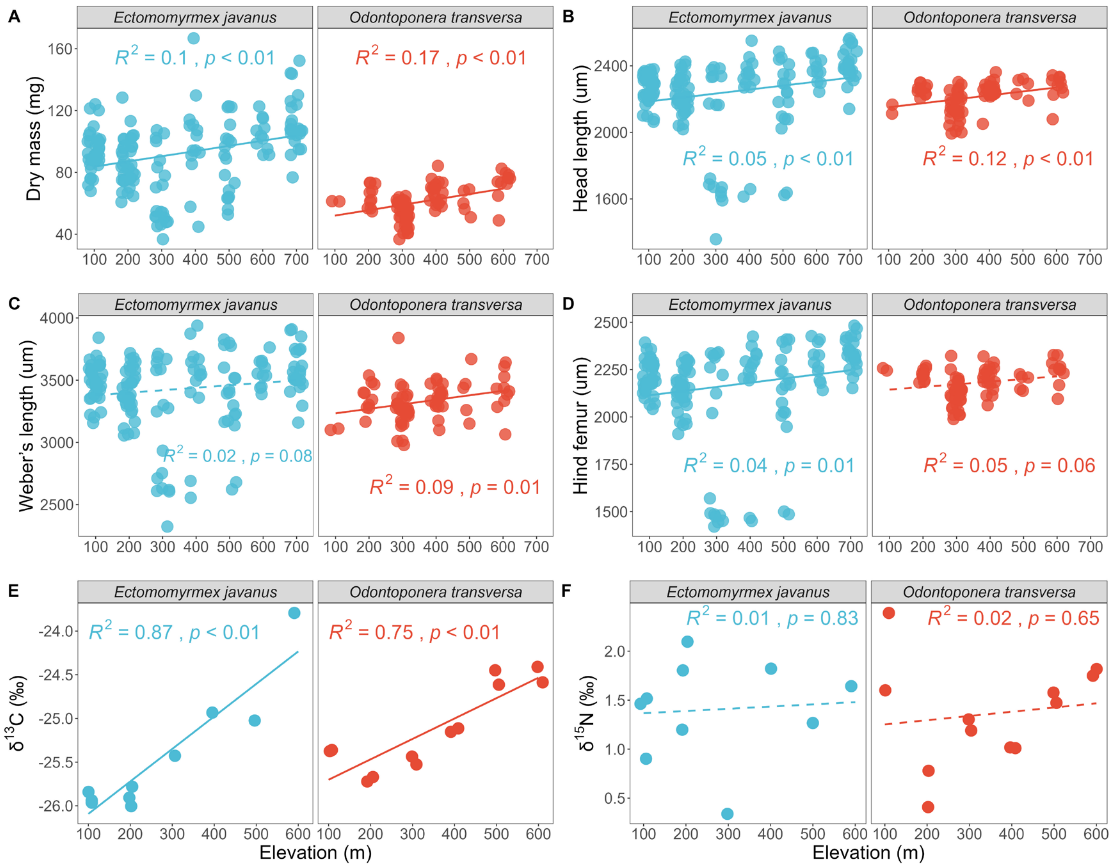

Figure 2. Coefficients of Pearson correlations among ant body traits, stable isotope ($\delta^{13}C$ and $\delta^{15}N$ values) and environmental factors for two ant species (*Ectomomyrmex javanus* and *Odontoponera transversa*). Coefficients with *P* < 0.05 are shown.