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

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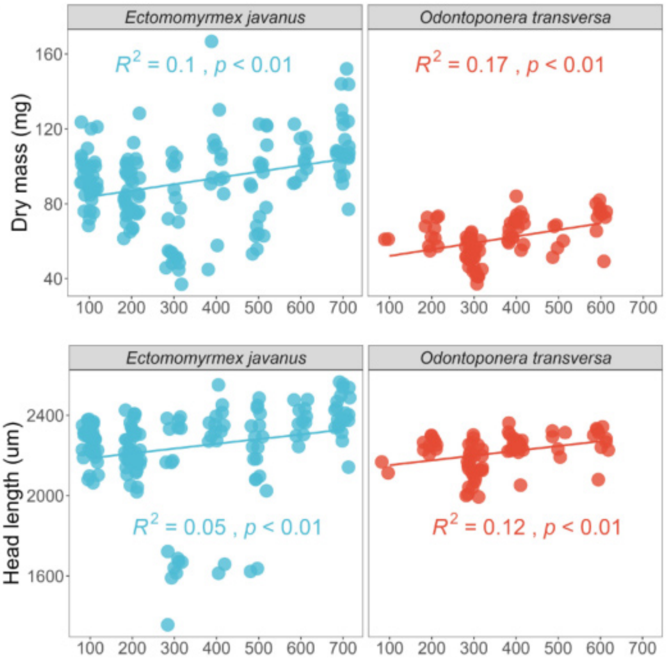
ABSTRACT



- Ant morphological traits (dry mass, head length, body size and leg length) increased with elevation.
- Ant $\delta^{13}C$ increased with elevation, whereas $\delta^{15}N$ did not.
- Ant $\delta^{13}C$ values correlated positively with soil C:N ratio.

Understanding the responses of species to changing climates is becoming increasingly urgent. Investigating the effects of climate change on the functional traits of species at the intraspecific level is particularly important. We used elevation gradients as proxies for climate change to explore the intraspecific responses of two ground-dwelling ant species, *Ectomyrmex javanus* and *Odontoponera transversa*, from 100 to 700 m.a.s.l. within a subtropical evergreen broadleaf forest. Our study addressed the specific 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. Using stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$), we quantified shifts in diets and trophic positions along the elevation gradients. Notably, our data revealed a significant elevation-related increase in Ant $\delta^{13}C$, whereas $\delta^{15}N$ 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 correlated positively with soil C:N ratio. Having revealed that the individual traits and $\delta^{13}C$ signatures of ground-dwelling ants exhibit significant negative correlations with temperature, our findings suggest that climate warming has the potential to cause intraspecific variation in the functional traits and diets of ground-dwelling ants and possibly other insect species.

Keywords altitude, ant, climate change, stable isotope, trophic position

1 Introduction

Functional traits include 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

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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 both natural and modified ecosystems (Gibb et al., 2023).

Ants exert global dominance as terrestrial ecosystem engineers, owing to their remarkable abundance and biomass. They play pivotal roles in various ecosystem processes, such as seed dispersal, nutrient cycling, and soil structure (Wiescher et al., 2012; Gibb et al., 2023). Alterations in ant functional traits resulting from environmental changes are therefore likely to impact terrestrial ecosystem functions and services (Joseph et al., 2019). Moreover, ants serve as an ideal study system for investigating the responses of functional traits to climatic 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 at higher altitudes and in colder environments. However, previous studies of ant functional traits along climatic gradients focused mostly on communities or assemblages, neglecting intraspecific climate–trait relationships (Gibb et al., 2023).

Species feeding traits encompass functional characteristics that govern potential food resources and trophic position. These traits play a crucial role in influencing a species fitness and its responses to environmental gradients such as changes in elevation (Moretti et al., 2017). Stable isotopes of carbon and nitrogen (δ^{13}C and δ^{15}N) can provide important insights into the diets and trophic levels 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 (δ^{13}C and δ^{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 elevational gradients (Pilar et al., 2020). Changes in the diet, especially shifts between plant-based and animal-based diets, are reflected in the δ^{13}C and δ^{15}N values of ants (Blüthgen et al., 2003; Joseph et al., 2019). Despite this, few studies have used stable isotopes of C (carbon) and N (nitrogen) to explore the intraspecific diet variation of ant species along environmental gradients.

Using elevation as a proxy for changing climatic conditions, we investigated the intraspecific responses of ants from 100 to 700 m in a subtropical evergreen broadleaf forest. First, we quantified the relationship among 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 would increase linearly with elevation 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 on 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 1927 mm. The vegetation is characterized as tropical monsoon forest and subtropical monsoon evergreen broadleaf forest. There is a classic shift in vegetation with elevation (Table S1; He et al., 2016). Mountain elevation ranges from 10 m to 1000 m.a.s.l. To avoid the impact of tourists at the foot of the mountain, our elevational transect started at 100 m.a.s.l. Due to the lack of sufficient numbers of ants collected at sites above 700 m.a.s.l., our highest elevation sample site was at 700 m. a.s.l. A total of seven sites were established along the elevational transect (Fig. S1), along which sampling sites were distributed at about 100-m elevation intervals (determined by GPS), ranging from 100 to 700 m.a.s.l. Basic site information for the seven sites is summarized in Table S1 and Fig. S2. The seven sites were situated on the southeast slope of Dinghu Mountain and roughly follow the fire lane. Selected sites were rarely impacted by humans. To reduce the influence of aspect, plots were positioned on the sunny side of any microtopography at each site along the transect.

2.2 Ant collection and morphological measurements

Ants were collected in September 2014 using pitfall traps (15 cm deep with 10 cm diameter) baited with a mixture of sugar and canned dace meat. A total of 12 pitfall traps were distributed randomly at each sampling site. Pitfall traps were emptied every three days for 15 days. We found four ant species across all transects (*Ectomomyrmex javanus, Odontoponera transversa, Diacamma rugosum, Leptogenys chinensis*), but only two of them (*E. javanus* and *O. transversa*) contained enough specimens (> 3 individuals) 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°C for at least 48 h until body weights became constant. We measured the dry mass and functional traits of each ant individual. Among the multiple traits 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 was enough to capture the variation of ant morphological traits at each sampling site.

Stable isotope measurements were taken from the same individual, from which functional traits were measured. Abdomens of individual ants were excluded to avoid the influence of partially digested food in their digestive systems. At least three samples were crushed and homogenized 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 δ^{13}C and δ^{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 δ^{15}N and Pee Dee Belemnite carbonate for δ^{13}C (Peterson and Fry 1987), and calculated δ^{13}C and δ^{15}N as follows (Peterson and Fry, 1987):

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

We measured environmental factors from soil cores (0−10 cm depth), and litter (20 cm × 20 cm), and quantified physicochemical properties, namely soil pH, soil organic carbon (SOC), soil total nitrogen (TN), litter carbon and nitrogen contents. Details of soil and litter sampling 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). At each elevation we also monitored hourly soil temperature at 10 cm depth from September to October 2014 with a temperature recorder (HOBO Onset U22-001, USA). Soil moisture content (g of water per 100 g of dry soil) of mineral soil at 10 cm depth was determined three times between October and November 2014 using a moisture probe meter (ICT International MPM-160B, Australia). Each soil moisture content measurement included 10 randomly distributed points in each plot.

2.3 Statistical analyses

We used univariate linear regression models to quantify elevational effects on functional traits and stable isotope signatures, and Pearson correlation analysis to investigate 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

Details of the elevational pattern of soil climate and soil physicochemical properties is published elsewhere (He et al., 2016) and presented in Supplementary Figure S2. Our data reveal intraspecific variation in the functional traits of ground-dwelling ants in response to elevational gradients, indicating that these species adapt certain traits to the 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 higher elevations suggest simpler forest floor habitats (Liu et al., 2018), in which larger ants are at an advantage when foraging, consistent with the size-grain hypothesis (trade-off between body size and locomotion costs) (Kaspari and Weiser, 1999). Larger body sizes under cooler temperatures can also be attributed to the temperature-size rule (larger body size at lower temperatures) which posits that insect maturation takes longer at cooler temperatures, resulting in larger body sizes of adult insects (Atkinson, 1994). Our observation of smaller ants with shorter legs at lower elevations agrees with Bishop et al. (2016), Silva et al. (2014) and Reymond et al. (2013).

Increasing elevation was associated with significant increases in the δ^{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 δ^{13}C signature. Elevation had no significant effect on the δ^{15}N values of the ants in the current study. 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 δ^{15}N values suggests that both ant species remained highly omnivorous and did not exhibit systematic changes (Fig. 1F). The large variation in δ^{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 δ^{13}C values of both ant species at higher elevations is probably linked with increasing plant δ^{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,

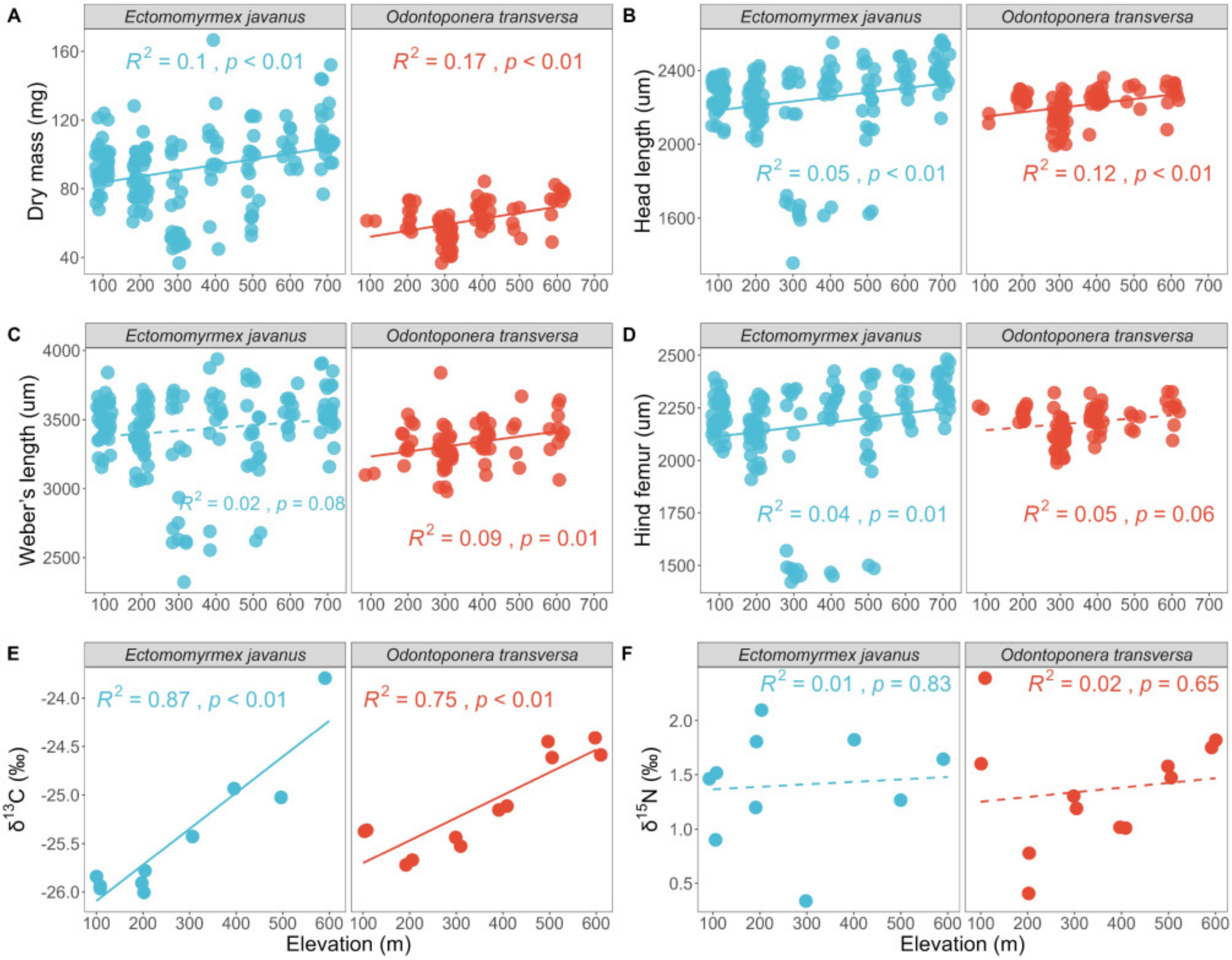

Fig. 1 Elevational patterns of body traits and stable isotope ($\delta^{13}C$ and $\delta^{15}N$) values of two ant species (*Ectomomyrmex javanus* and *Odontoponera transversa*). A. dry mass; B. head length; C. Weber's length; D. hind femur length. E. $\delta^{13}C$ value. F. $\delta^{15}N$ value. Solid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

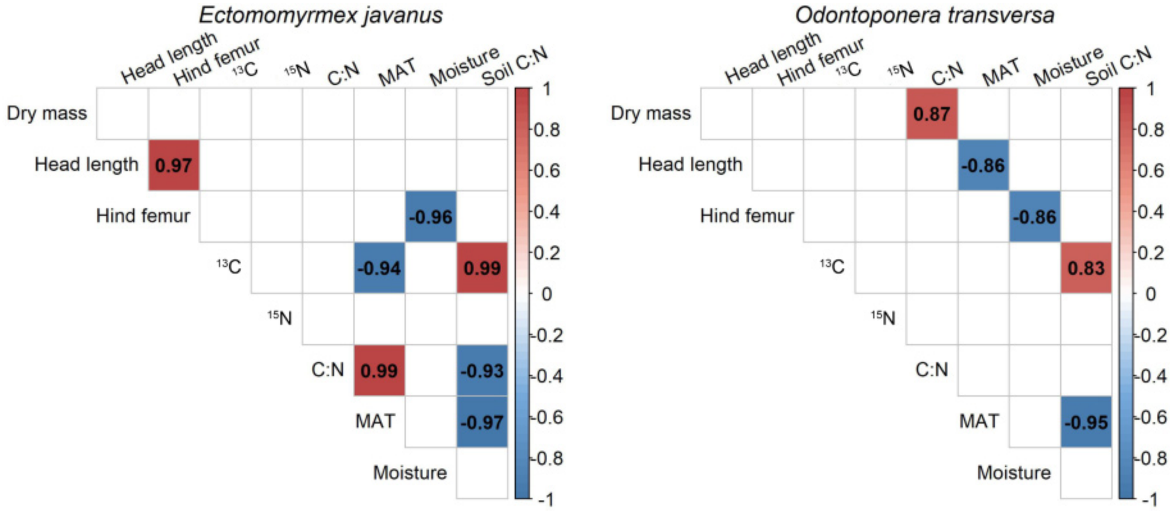

Fig. 2 Coefficients of Pearson correlations among ant body traits, stable isotope ($\delta^{13}C$ and $\delta^{15}N$) values and environmental factors for two ant species (*Ectomomyrmex javanus* and *Odontoponera transversa*). Coefficients with $P < 0.05$ are shown.

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 our knowledge, this is one of few attempts to systematically evaluate the impact of elevation gradients on intraspecific ant functional traits using stable isotope 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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Electronic supplementary material

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Compliance with ethics guidelines

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This paper does not contain any studies involving human participants performed by any of the authors.

Ethics declarations

The authors declare no competing interests.

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