

From cryptic herbivore to predator: stable isotopes reveal consistent variability in trophic levels in an ant population

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Abstract. Populations may collectively exhibit a broad diet because individuals have large diet breadths and/or because subpopulations of specialists co-occur. In social insect populations, the diet of the genetic individual, the colony, may similarly arise because workers are diet generalists or castes of specialists. We used elemental and isotopic methods to explore how the invasive red imported fire ant, *Solenopsis invicta*, achieves its status as a trophic generalist. In one 0.5-ha old field, 31 *S. invicta* colonies ranged from 1°-consumer to 2°-predator ($\delta^{15}\text{N}$'s 0.35–7.38‰), a range comparable to that shown in sampled ant communities. Moreover, a colony's trophic rank was stable despite $\delta^{15}\text{N}$ fluctuating 2.98‰ over the year. Colonies that fed at higher trophic levels were not larger, but consumed more C₃-based resources. Individual worker mass, however, did increase with $\delta^{15}\text{N}$ ($r^2 = 0.29$, $P < 0.001$). The ninefold variation in worker mass within a colony generated trophic variance approximately 15% of the population of colonies. Combined, we show how intraspecific trait variation contributes to the trophic breadth of *S. invicta*, and suggest mechanisms that further explain how their trophic signature varies across space, but remains stable over time.

Key words: fire ant; invasive species; niche breadth; stable isotopes; trait; trophic ecology.

INTRODUCTION

A key question in ecology remains the origin and maintenance of trait diversity among species, populations, and individuals (Nylin and Gotthard 1998, Bolnick et al. 2011). Increasingly, individual variation is quantified and found to be a significant component of niche variation (Bolnick et al. 2003, Sih et al. 2004, Roeder and Behmer 2014). In behavioral studies, for example, trait diversity can lead to a variety of behavioral types, personalities, or even syndromes (Sih et al. 2004, Pruitt and Ferrari 2011, Jandt et al. 2014). Likewise, individual-level niche variation may provide a key mechanism promoting coexistence (Clark et al. 2007). These variable traits play important roles in deciding the outcome of intra- and interspecific interactions that shape population and community dynamics (McGill et al. 2006, Agrawal et al. 2007).

Diet breadth is one common trait used to distinguish between species, as dietary generalists and specialists are frequently found among related species (Lee et al. 2006,

Smith et al. 2006). Furthermore, a population may collectively have a broad diet for two reasons: individuals have large diet breadths, or subpopulations vary widely in their diet preferences (Estes et al. 2003, Caut et al. 2008, Tinker et al. 2008). To measure this variance, trophic studies have adopted stable isotope techniques as one powerful tool to track resource assimilation and estimate a population's niche breadth (Bearhop et al. 2004, Newsome et al. 2007). A key challenge is quantifying this variation within populations (Fry 2006, Boecklen et al. 2011) and other traits underlying that variation.

Body size, an important functional trait (Kaspari and Weiser 1999, McGill et al. 2006), can covary with isotope signature within and across species (Kelly 2000). For example, insects that are more enriched in $\delta^{15}\text{N}$, and hence are higher-order consumers, are frequently larger (Warren and Lawton 1987) with higher concentrations of nitrogen (Fagan et al. 2002). Under these assumptions, we propose the trophic-size hypothesis, which posits that (1) as body size increases so does the requirement for more nitrogen per capita (e.g., to build more muscle mass) and (2) this acquisition of more nitrogen results in an enriched $\delta^{15}\text{N}$ signature and subsequent occupation of a higher trophic level.

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A second trait of interest is sociality. Social taxa such as ants (Hymenoptera: Formicidae) consist of often-specialized individuals that collectively forage as a “superorganism” for a variety of potential resources (Wheeler 1911, Hölldobler and Wilson 2009). Moreover, larger colonies forage over larger and more resource-diverse areas (Hölldobler and Wilson 1990, Tschinkel et al. 1995). Both worker and colony size thus have the potential to contribute to a social insect population’s diet breadth and trophic position.

Polymorphic ants like the red imported fire ant (*Solenopsis invicta*, hereafter RIFA) are a model system to explore worker and colony-level contributions to population variability. RIFA range in dry mass from 0.13 mg to 2.21 mg, and belong to colonies composed of 10 to over 250,000 workers (Tschinkel 2006). In addition, the monogyne form of RIFA (i.e., single queen) has discrete territorial boundaries, in which the foraging area is not only correlated with colony size but also rarely entered into by workers from other conspecific monogyne colonies (Tschinkel 2006). Here we test the trophic-size hypothesis by exploring the interactions of body mass, nitrogen content, and trophic level across workers within colonies and across colonies at different times of the year. Our ultimate aim was to quantify the trophic breadth of a population of this species across time and space. Our results suggest that whereas larger workers within colonies are enriched in $\delta^{15}\text{N}$, colony-level trophic position is independent of colony mass but highly variable among individual colonies, a pattern that remains constant over an annual cycle.

METHODS

Study site

All samples were collected in a 0.5-ha field at the University of Oklahoma Biological Station (Oklahoma, USA, 33.88° N, 96.80° W, 204 m elevation) in July and November of 2015 as well as March of 2016. Yearly air temperatures range from -16.1° to 44.4°C with a mean annual rainfall of 1,027.94 mm (Oklahoma Climatological Survey). This location, while undergoing secondary succession after decades of row crop agriculture, is dominated by the monogyne form of RIFA.

Using stable isotopes to quantify trophic variation in ants

Carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) stable isotope analyses were used to estimate the source of dietary carbon and relative trophic position. After collection in the field, each sample was frozen at -20°C . The petiole, post-petiole, and gaster were removed as they can contain residual food particles, potentially altering the isotopic signature (Tillberg et al. 2006). Replicates consisted of five homogenized workers with the same head width, measured in mm, which was necessary to attain the minimum weight required for analysis. For this reason, reported mass values are the actual measurements divided

by five. Samples were dried to constant mass at 60°C for 48 h and weighed in tin capsules to the nearest 0.001 mg on a Cahn microbalance (Cahn Instruments, Cerritos, California, USA). Delta values (δ) were calculated as

$$\delta = \left(\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right) \times 1000$$

where δ , reported in per mil notation (‰), represents the ratio of heavy to light isotopes within a sample (R_{sample}) relative to the ratio in an international standard (R_{standard}). Trophic position, TP, was determined as

$$\text{TP} = \lambda + \frac{(\delta^{15}\text{N}_{\text{ant}} - \delta^{15}\text{N}_{\text{base}})}{\Delta\text{N}}$$

where λ was equal to the trophic level of the basal food source (e.g., autotroph = 1), $\delta^{15}\text{N}_{\text{ant}}$ values were directly measured, and $\delta^{15}\text{N}_{\text{base}}$ was calculated by averaging the $\delta^{15}\text{N}$ values from vegetation in the 0.5-ha field. ΔN represents the standard enrichment per trophic level of 3.4‰ (Kelly 2000, Post 2002). All stable isotope analyses were performed at the University of California Davis Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon, Cheshire, UK).

Between-colony isotopic, trophic, and temporal variation

Thirty-one RIFA colonies were sampled to assess how carbon and nitrogen isotope values were affected by the size of a colony (estimated from mound volume as per Tschinkel [1993, 2006], Appendix S1). For each colony, two environmental variables that may affect a colony’s isotopic signature were also measured: (1) average daily temperature and (2) mean vegetation height (see Appendix S1 for supplemental methods). To evaluate how colony size affected a colony’s elemental and isotopic signature, worker δ -values and C:N ratios from three size classes—small (0.7 mm head width), medium (1.0 mm head width), and large (1.3 mm head width)—were averaged and compared to estimates of mound volume with environmental variables added to ascertain if temperature and vegetation height had an impact. Diet breadth (Bearhop et al. 2004) was then determined by plotting $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in isotopic space (δ -space) and calculating the relative trophic position for each colony using the $\delta^{15}\text{N}_{\text{base}}$ of the food web. Of the 31 RIFA colonies that were originally sampled, 23 were active the following November and March, and resampled to ascertain the amount of temporal isotopic and trophic variation (see Appendix S1 for supplemental methods).

Within-colony elemental and isotopic variation

To evaluate how body size affected an individual’s elemental and isotopic signature, the scaling exponents (hereafter b) of log-log regressions were calculated between an individual’s mesosoma dry mass (hereafter

body dry mass) and the amount of body carbon or nitrogen. Deviations from isometry were determined by comparing 95% confidence intervals around the calculated slope to the predicted scaling exponent for isometric growth ($b = 1$). If a relationship was allometric, isotope values were regressed against body mass to see if enriched δ -values were associated with increasing body size. The ratio of the amount of body carbon to body nitrogen (hereafter C:N ratio) was also compared to body mass.

Statistical analysis

All analyses were run in R version 3.2.2 (R Core Team 2015). Variables were checked for normality using the Shapiro-Wilks test and log transformed when non-normal. Regressions were tested for heteroscedasticity. Standardized major axis (SMA) regression was used to compare carbon and nitrogen dry mass to body dry mass while polynomial regressions were used to compare C:N ratios and $\delta^{15}\text{N}$ values to body dry mass. An information theoretic approach was used to rank all possible intercolonial regression models by Akaike's information criterion (AIC). ΔAIC values for each model were calculated from the difference of the AIC of i th model and the model with the lowest AIC value. Akaike weights (w_i) were then calculated and represent a weight of evidence that model i was the best fit. For multiple regression models, predictor variables were checked for multicollinearity using

a variance inflation factor (VIF) cutoff = 3. Ordinary least squares (OLS) regression was used to compare δ -values across colonies. Spatial autocorrelation of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and intercolonial predictor variables was examined using Moran's I . Finally, repeated-measures ANOVA was used to determine if $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and trophic position changed across time periods per colony.

RESULTS

The 31 colonies in this 0.5-ha old field fed across three trophic levels and assimilated nutrients acquired from C_3 and C_4 plants. During July, colonies varied from 0.35‰ to 7.38‰ in $\delta^{15}\text{N}$, from -24.69‰ to -16.69‰ in $\delta^{13}\text{C}$, and from 3.95 to 4.38 in their C:N ratio. Moreover, trophic position decreased linearly as colonies incorporated resources that contained C_4 plants in their diet (Fig. 1). We tested a variety of correlated but not collinear predictor variables (temperature VIF = 1.54, vegetation height VIF = 2.14, mound volume VIF = 2.18; Appendix S2: Fig. S1) that might account for this variation in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N ratio. Mean vegetation height was the only predictor found in each model within two AIC of the top model for colony $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (Appendix S2: Table S1).

Over three samples across the year, the isotopic signature of colonies varied by up to 2.98‰ in $\delta^{15}\text{N}$ (repeated-measures ANOVA, $F_{2,44} = 18.70$, $P < 0.001$;

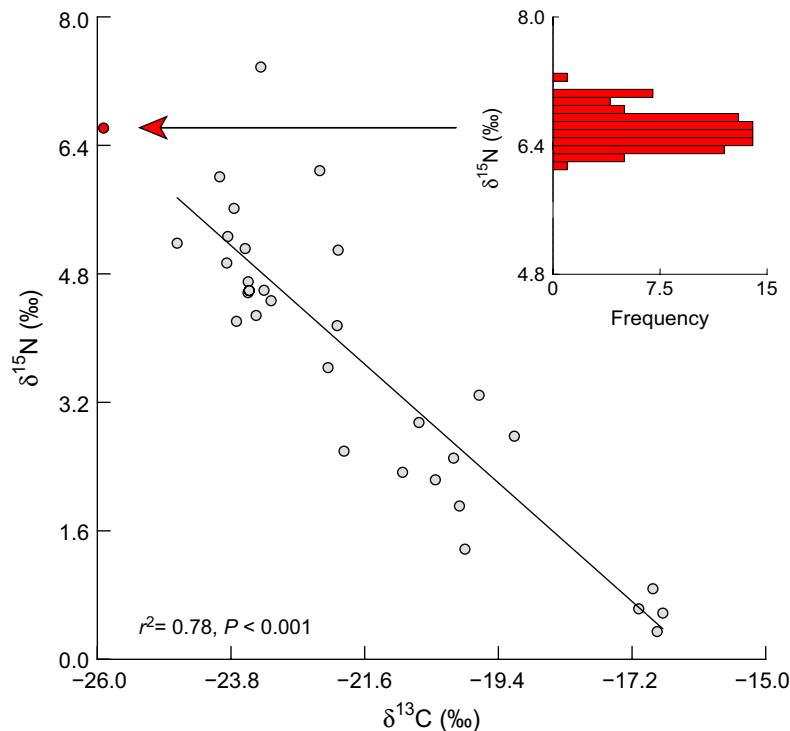


FIG. 1. Inter- and intracolony variation of red imported fire ant (*Solenopsis invicta*; RIFA) colonies in δ -space. The large panel shows across colony variation with an r^2 and P value corresponding to the linear relationship between colonial $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ($y = -0.67x - 10.83$). The inset bar plot represents the amount of variation across 90 polymorphic workers within a single colony, denoted by the red point, and scaled to the y -axis of the larger panel.

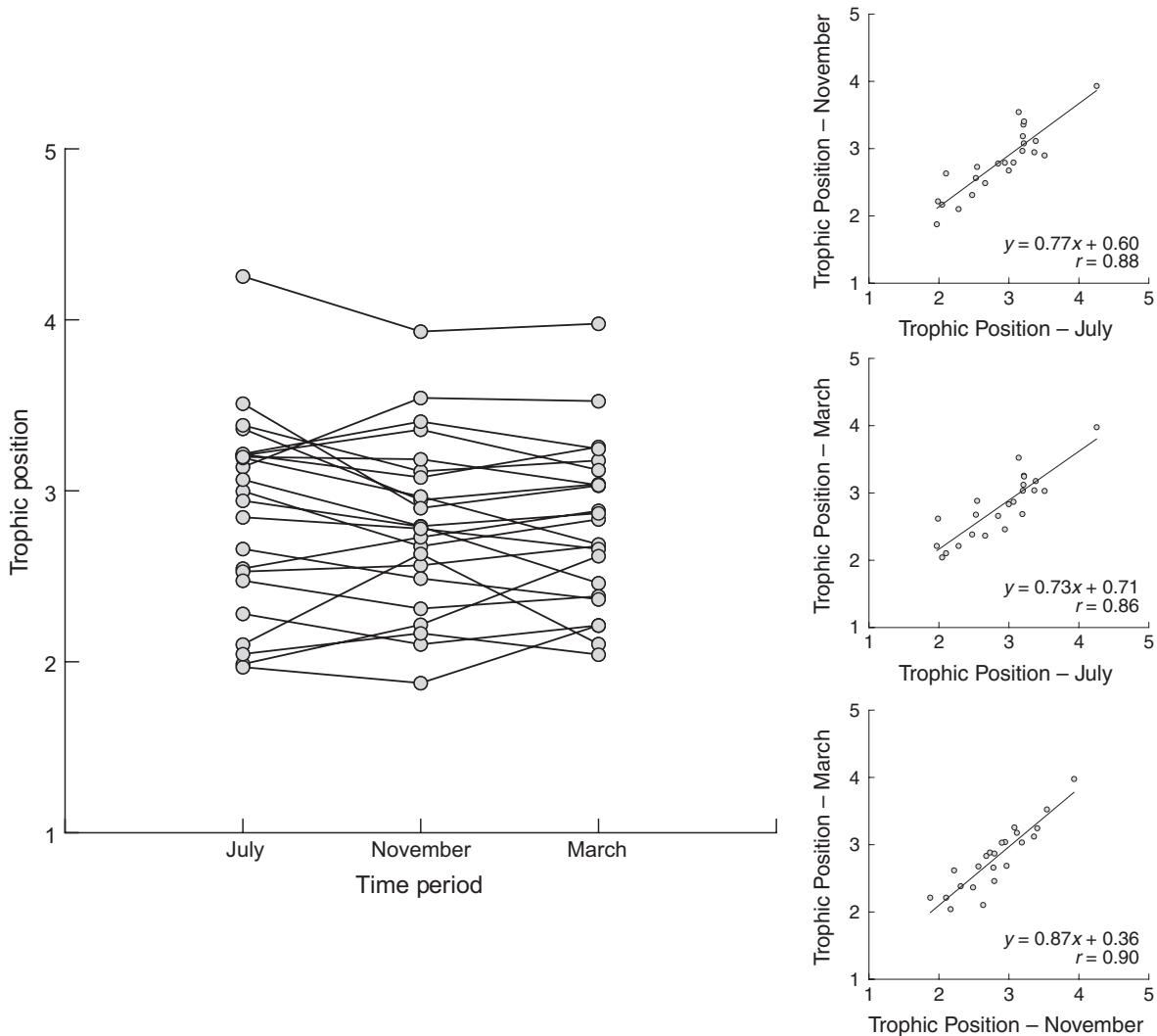


FIG. 2. Temporal variation in the trophic position of RIFA colonies. The large panel shows changes in trophic position of each colony, connected by lines, over three time periods. Small panels depict correlations of colonial trophic position between each combination of time periods with inset linear equations and r values.

Appendix S2: Fig. S2) and 4.58‰ for $\delta^{13}\text{C}$ (repeated-measures ANOVA, $F_{2,44} = 9.41$, $P < 0.001$; Appendix S2: Fig. S3). And while colonies isotopically fluctuated, their relative trophic position did not (repeated-measures ANOVA, $F_{2,44} = 1.16$, $P = 0.3216$; Fig. 2), primarily due to an enrichment in the $\delta^{15}\text{N}_{\text{base}}$ (July = -3.05‰ , November = -1.88‰ , March = -1.80‰). Within a colony, worker dry mass varied ninefold (range = 0.09–0.85 mg) with larger workers being elementally enriched in nitrogen and more isotopically enriched in $\delta^{15}\text{N}$ (Appendix S2: Fig. S4).

DISCUSSION

Here we confirm that a population of the red imported fire ant is, collectively, a trophic generalist. We show that the population achieves this by supporting a range of trophic specialists: colonies varied threefold in trophic

level, a ranking that persisted in the summer, winter, and spring of a seasonal cycle. The trophic-size hypothesis was not as useful at predicting between-colony differences; predators in this population did not represent the upper end of a 100-fold gradient in colony size. It was, however, useful in accounting for trophic variation across the ninefold variation in body mass within colonies; a colony's workers spanned, on average, only 15% of the $\delta^{15}\text{N}$ found among colonies in this old field. Combined, we show an unprecedented, consistent variation in trophic level among colonies as a chief cause for a commonly observed trait of a widespread, invasive species: its generalized diet.

Mechanisms underlying colony-level differences in trophic biology

We originally posited that, because larger colonies maintain larger territories, and thus have access to a

greater diversity of resources, a colony's size may be a good predictor of its isotopic signature. Yet despite a 100-fold difference within our population, colony size was not a good predictor. Moreover, the environmental variables of average daily temperature and mean vegetation height accounted for only a small amount of the isotopic variation across colonies. We propose two alternative, but not mutually exclusive, hypotheses to account for this variation across the 31 sampled RIFA colonies in this 0.5-ha old field.

The first states that colonies with low trophic positions feed on plant-derived honeydew. Many ants tend fluid-feeding hemipterans (Tillberg et al. 2007, Wilder et al. 2011). Honeydew, the by-product of these hemipterans, is rich in carbohydrates and only slightly isotopically enriched in $\delta^{15}\text{N}$ compared to the plant from which it was produced (Sagers and Goggin 2007). For this reason, hemipteran-tending ants have a lower $\delta^{15}\text{N}$ value and relative trophic position as they are essentially herbivores (Davidson et al. 2003). We predict that colonies near the trophic level expected for a fluid-feeding hemipteran (TP = 2) incorporate a large amount of honeydew in their diet.

The second states that colonial $\delta^{15}\text{N}$ tracks plant chemistry. Different types of vegetation vary in their elemental composition and nutritional value (Marschner 1995, Aerts and Chapin 2000). For example, forbs, which are mainly C_3 plants, have a higher nutritional value and lower C:N ratio than grasses, which are generally C_4 plants (Joern et al. 2012). As we observed a decreasing linear relationship between a plant's $\delta^{15}\text{N}$ and C:N ratio (Appendix S2: Fig. S5) perhaps differences in the elemental profiles of plants may account for a proportion of the variation in colonial $\delta^{15}\text{N}$ values, even though colony $\delta^{15}\text{N}$ values were not spatially autocorrelated (Appendix S2: Table S2). Furthermore, temporal shifts in the isotopic signature of baseline material may explain a parallel increase in the $\delta^{15}\text{N}$ of ants resulting in a constant relative trophic position for colonies through time.

Within-colony variation due to polymorphism

We have found only two studies (Smith et al. 2008, Smith and Suarez 2010), both using the Florida harvester ant, *Pogonomyrmex badius*, that tested how worker body size is related to its isotopic signature: both found larger workers had lower C:N ratios and were $\delta^{15}\text{N}$ enriched. In RIFA, worker size is determined during the 3rd larval instar where individuals that reach a critical size are physiologically reprogrammed to extend their development by 2–4 days (Tschinkel 2006). As only late instar larvae can digest solid food particles, this extended development time allows for the assimilation of more nitrogen from solid dietary items such as arthropod tissue (Petralia and Vinson 1978). However, while worker polymorphism accounted for worker

variance in trophic level, it was small relative to inter-colony variation.

CONCLUSIONS

Many studies have quantified the average isotope values of communities of ant species. Here, we reveal that much of a community's variance may be harbored by a generalist species that is in fact a collection of trophic specialists. When we reviewed 35 studies that incorporated ants and stable isotopes, 74% focused on across-species comparisons (details in Appendix S1 and Appendix S2: Table S3). Our results, a 7.03‰ difference in $\delta^{15}\text{N}$ across RIFA colonies, was broader than 58% of these studies that used more than one ant species (Appendix S2: Fig. S6) and was comparable to ranges of values for entire ant communities from agroecosystems (Ottonetti et al. 2008, Platner et al. 2012), grasslands (Ness et al. 2009, O'Grady et al. 2010), pastures and remnant woodlands (Gibb and Cunningham 2010), and tropical rainforests (Blüthgen et al. 2003, Kaspari et al. 2012). By quantifying within-population trait variation at multiple spatial and temporal scales we reveal a hidden biodiversity akin to that associated with cryptic species complexes (Smith et al. 2006). In addition, dietary specialization among conspecifics may allow for increased abundance due to decreased intraspecific competition for resources, as seen in sea otters (Estes et al. 2003). It is hard not to speculate that this flexibility in diet breadth may have aided RIFA in becoming one of the most widespread invasive species throughout the southeastern United States.

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