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Los Angeles

The Relationship between Food Preference and Invasibility in Argentine ants

A thesis submitted in partial satisfaction

of the requirements for the degree Master of Science

in Biology

by

Ana Rubio

2020

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ABSTRACT OF THESIS

The Relationship between Food Preference and

Invasibility in Argentine ants

Master of Science in Biology

University of California, Los Angeles, 2020

Professor Noa Pinter-Wollman, Chair

Urbanization creates novel environments that constrain the availability of natural resources, like food, which can often be a limiting factor in determining where species may establish and survive. Compared to natural habitats that have supported native fauna for hundreds of years, urban areas provide novel habitats and food sources that native species may not be able to exploit. Instead, we see the proliferation of invasive species in these areas, because they can adapt and survive on a wide variety of food sources that most native species cannot. One species of concern is the Argentine ant (Linepithema humile), an invasive species that has been able to outcompete native ants throughout the world, by displaying plastic responses to novel environments. Yet, little is understood about how urbanization affects behaviors such as food preference, which may be disproportionately supporting invasive species in urban landscapes. To determine food preference in the Argentine ant, I examined the differences in food preference between two invasive populations of Argentine ants - from natural and from urban environments. Because these two habitat types provide divergent food sources, we may be able to uncover how urban areas skew food availability and affect preference in invasive species.

The thesis of Ana Rubio is approved.

Peter Nonacs

Nathan Kraft

Noa Pinter-Wollman, Committee Chair

University of California, Los Angeles

2020

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Introduction

Urban habitats often have detrimental effects on the health and behavior of local wildlife (Fischer et al., 2015; Lee et al., 2006; Russo & Ancillotto, 2015). Urbanization affects habitat and wildlife both directly and indirectly, often causing rapid shifts in how animals behave within their local environment (Theobald et al., 1997). For example, large carnivores living near humans have altered their activity patterns to minimize human contact by hunting at night (McClennen et al., 2001; Tigas et al., 2002). The type and quality of food in a particular habitat plays a large role in species distributions and can differ between urban and natural environments (Johnson & Sherry, 2001; Layme et al., 2004)

The food preferences of animals are constantly and heavily impacted by a variety of locality-specific issues that influence spatial and temporal scales, including but not limited to seasonality, food availability, and urbanization. The food that individuals consume might be restricted to what is available to them. Animals might over-consume certain nutrients because they are abundant in their environment (Rodewald et al., 2011). For example, raccoons in urban areas primarily use and choose den sites based on the availability of anthropogenic food resources (Prange et al., 2003, 2004). Alternatively, individuals might selectively search for nutrients that are scarce in their environment to balance their diet (Hasse & Lathrop, 2003). For example, seasonal food scarcity prompts long distance foraging in the social bee, *Bombus vosnesenskii* (Pope & Jha, 2017). Finally, animals might actively select certain foods based on their immediate nutritional and physiological needs. For example, peruvian spider monkeys try to maintain a high protein intake, despite the diversity of resources available to them (Felton et al., 2009).These three scenarios are not mutually exclusive.

Ants are faced with nutritional challenges that often requires them to make tradeoffs of food selection for survival and reproduction. Ants require different ratios of Protein:Carbohydrate throughout the life of a colony (Dussutour & Simpson, 2008 ; Dussutour & Simpson, 2009; Csata & Dussutour, 2019). Ant colonies require protein for egg production and larval development, and carbohydrates for energy for the workers (Feldhaar et al., 2009; Lach et al., 2010). Thus, the abundance of protein or carbohydrates can result in changes in behavior (Kay et al., 2010). Ants primarily obtain essential nutrients from animal tissues (protein) and insect or nectar secretions (carbohydrates) (Lach et al., 2010). However urban areas have impacted the availability of these resources in some places by providing an overabundance of macronutrients, including meats that raise the ants' trophic level (Penick et al., 2015). Stable isotopes have been used to uncover the diets of a variety of invertebrates, including ants (Post, 2002; Blüthgen et al., 2003; Tillberg et al., 2006; Penick et al., 2015). Although both habitat types (i.e., urban and natural) house invasive Argentine ant populations, they may differ in food sources, where previous work has shown an overabundance of protein sources in urban habitats provided by overflowing trash cans with human waste (Youngsteadt et al., 2015)

Ants provide a great model to study what determines food preference, as they are sensitive to variability in the abundance of foods. Further, because ants require specific nutritional mixes for optimal fitness, their behaviors might be skewed by the introduction of new resources. Various ant species in Los Angeles occupy a wide range of habitats both urban and natural, that differ in food availability and access to human refuse. This would allow me to manipulate and observe differences in diet under experimental conditions, where foods can be introduced or removed, and experiences can be created. By conducting such research, I can further uncover the relative importance of external factors on food preference in ants.

One ant species that dominates metropolitan areas in Mediterranean climates is the Argentine ant (*Linepithema humile*), an invasive species originating from South America (Newell & Barber, 1913; Suarez et al., 2001; Walters, 2006). Along with its high population density and generalist behavior, the Argentine ant outcompetes native ant species leading it to become a dominant species in many Californian habitats (Holway, 1999; Suarez et al., 2000; Sanders et al., 2001; Daane et al., 2007; Wetterer et al., 2009). These invasive ants exhibit plastic responses to novel resources which may have facilitated their proliferation in many places (Lach et al., 2010), including urban and natural habitats in California (Holway 1995).

Argentine ants are a generalist species (Kay et al., 2010) and their ability to consume a wide array of foods (Feldhaar, 2014) may explain their invasive success, amongst other behaviors (Ingram, 2002; Holway & Suarez, 2004; Buczkowski & Bennett, 2008; Carpintero & Reyes-López, 2008). Past research has focused on the Argentine ants' ability to create large colonies that lack intraspecific competition (Helanterä et al., 2009; Gordon & Heller, 2014) as it's primary mechanism for its success. However, the diversity in type and abundance of food between habitats might explain how Argentine ants occupy habitat types ranging from urban and disturbed, to natural and undisturbed; whereas many native species require specific foods that only certain habitats provide (Deslippe & Savolainen, 1994; Chapman & Bourke, 2001). Alternatively, because invasive species show high behavioral plasticity (Wright et al., 2010) they can also quickly adapt to and exploit novel environments effectively (Hobbs et al., 2009), possibly giving them an advantage when faced with food shortages or novel resources. Thus, their ability to find and exploit certain resources rapidly might impact their invasive success. Although ants generally display a generalist strategy of regulating total diet and consumption by over-consuming both protein and carbohydrates (Lihoreau et al., 2015; Krabbe et al., 2019), they can also regulate their nutritional needs when food is patchy or temporarily unavailable (Grueter & Leadbeater, 2014; Lihoreau et al., 2015; Giraldeau & Caraco, 2018). Thus their food

preferences are locality specific, as each habitat has its own environmental constraints (e.g. food availability, water access) (Baumberger et al., 2019; Favero et al., 2020), providing further evidence that ants might be heavily impacted by their recent experience.

To uncover how food preference of Argentine ants is affected by local conditions, I used both in-lab food preference tests and isotope analysis. Because food preference is highly impacted by the local conditions faced, I hypothesize that food preference can be determined by either (1) habitat type, based on the food that they are exposed to in either urban or natural environments (2) resource limitation which means the ants are most attracted to foods not in their environments (natural sites tend to have less access to processed meats) or (3) recent experience, meaning that the ants exploit what is is available to them at any given time. Studying food preference is important, because food plays a critical role in the establishment of invasive species (Banko et al., 2002)) and thus is an important variable to test when studying invasion success.

Materials and Methods

Defining "urban" and *"natural" environments & collection sites*

To select collection sites, I used google maps to identify natural locations throughout Los Angeles county. I defined 'natural' sites as green spaces and state parks. I defined 'urban' as areas with densely concentrated housing or human infrastructure. To confirm my colony site choices made with google maps and to visualise the land cover in LA, I used QGIS to create a projected map of urban and natural land cover throughout Los Angeles County (Figure 1). GIS layers were obtained from online sources including: CalVeg, Los Angeles County Department of Regional Planning, and the LA City Planning GIS Data Portal.

I collected Argentine ants from six urban and natural locations in Southern California. Urban ants were collected from areas surrounding the UCLA campus and Koreatown, whereas natural ants were collected from the Santa Monica mountains (figure 1). 500-1000 workers were collected from each site (N=6 sites) during December 2018-March 2019 using an aspirator. After collection, ants were transported to the lab and were placed in artificial nests made from plastic containers.

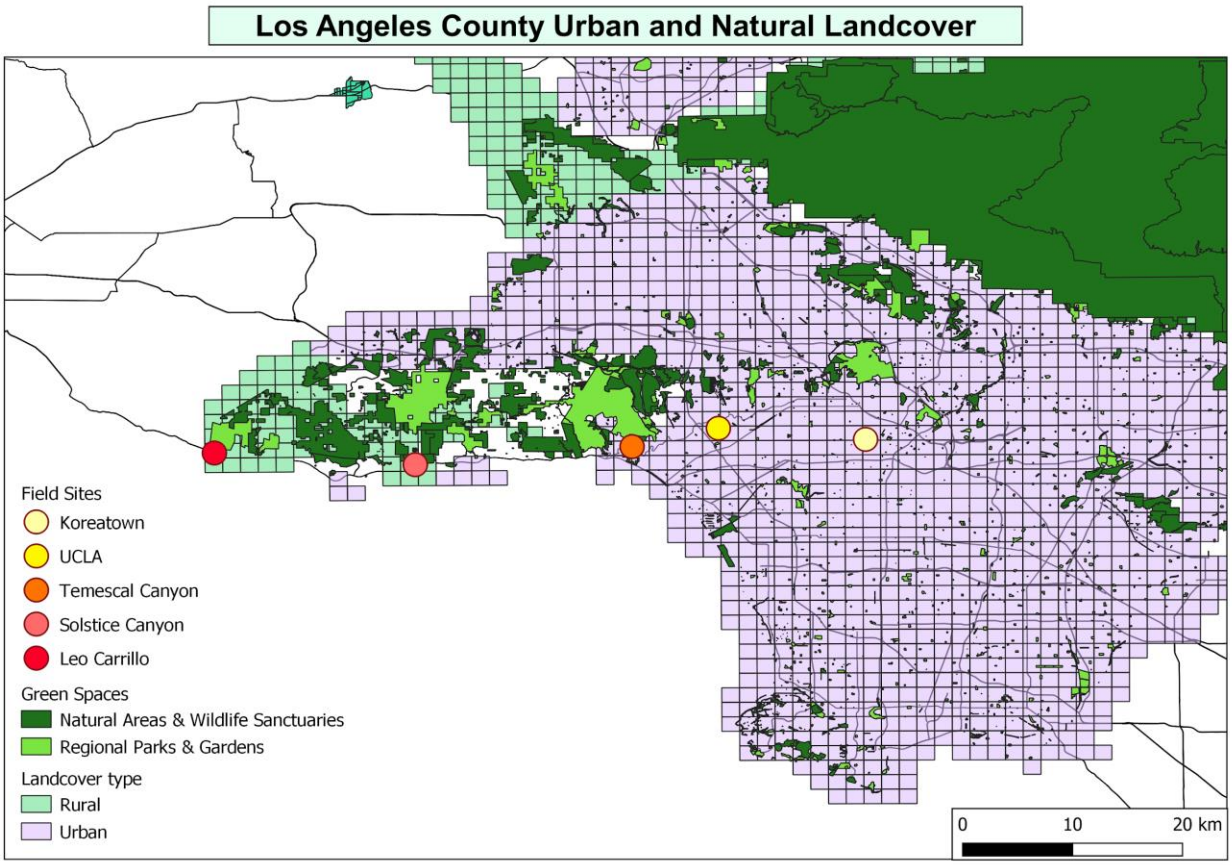


Figure 1: Map of field sites in Los Angeles County. Geographic data from CalVeg, Los Angeles County Department of Regional Planning, and the LA City Planning GIS Data Portal was used to identify natural and urban land cover. Two 'urban' sites (botanical gardens and UCLA campus) are combined and represented by one point labeled UCLA due to geographic proximity.

Choice Tests

To test the food preference of Argentine ant workers, I created a five-armed maze from clear tubing and fluon-coated plastic containers (figure 2). Each container had food with a different protein:carbohydrate ratio (table 1) and I counted the number of ants in each container at certain time intervals to determine food preference. I ran one experimental trial with ants from each of the 6 sites within 24 hours of collection (N=6). In each trial,100 workers were haphazardly chosen from the 500-1000 individuals that I collected. The workers were placed into the central container (CC) and were allowed 5 minutes to acclimate. During these 5 minutes, I blocked off entrances to the containers holding food, blocking their ability to explore the arms of the maze. After the acclimation period, I removed the tape to allow ants to explore the and choose food in the five-armed maze for two hours. I counted the number of ants in each container, both touching and not touching the food, for two hours at the following time intervals: For the first 30 minutes of the trial, I recorded the number of ants in each chamber every five minutes. After the first 30 minutes, I counted the number of ants every 30 minutes until two hours had elapsed. The reduction in sampling effort is based on preliminary observations that showed slower ant accumulation at the food after the first 30 minutes. After the trials, ants were aspirated back into their artificial nests.

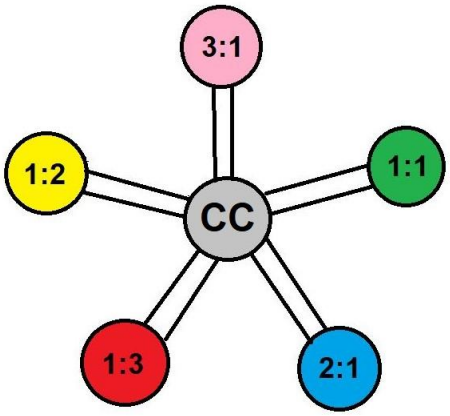


Figure 2: Schematic diagram (left) and image (right) of the 5-armed maze for testing ant food preference

Food Preparation

Food was prepared by placing in one dish a dry mix of protein powder, egg powder, and sugar (table 1). In a separate bowl, I added boiling water and agar to activate the gelatinous properties of the agar. After about 5 minutes I mixed the dry powder with the agar. Once all the ingredients were mixed, I poured the liquid into a container where the solution solidified and was stored in the refrigerator for later use. During experiments, the food was removed from the refrigerator and sectioned off into 5x5cm^2 squares and placed into the chambers of the 5-armed maze (Figure 2). I used handmade colored paper flags to denote each food type, so that the observer (myself and two other students) were blind to the food composition in each arm of the maze but could still distinguish between the choices that the ants made.

PC	Whey Protein Concentrate (g)	Calcium caseinate (g)	Whole Egg Powder (g)	Sucrose (g)	Water (mL)	Agar (g)
3 : 1	22.3 (18.6)	20.4 (18.6)	16 (7.8)	15	300	4
2 : 1	19.3 (16.1)	17.7 (16.1)	16 (7.8)	20	300	4
1 : 1	13.3 (11.1)	12.2 (11.1)	16 (7.8)	30	300	4
1 : 2	7.3 (6.1)	6.7 (6.1)	16 (7.8)	40	300	4
1 : 3	4.3 (3.6)	4 (3.6)	16 (7.8)	45	300	4

Table 1: Composition of diets the ants were presented with in the 5-armed maze - from (Dussutour & Simpson, 2008b)

Stable Isotopes

I examined the stable isotopes of ants collected in the field during December 2018, following procedures from (Feldhaar et al., 2009; Penick et al., 2015; Tillberg et al., 2006) to

determine what ants ate in their source habitats. Approximately 50 ants of the ones collected at each site were frozen at -20C until preparation for isotope analysis. Specimens were prepared for isotope analysis by placing them in a drying oven at 50C for 48 h. After specimens were dried, I weighed out 0.1–1.5 mg of tissue from each sample into a 5x9 mm tin capsule using a laboratory scale. I pooled 5-10 ants into each sample to reach the minimum required weight (1mg+-0.02) and had three samples per site (N=18). The capsules were crushed using sterilized forceps and placed individually into a 96-well plate for shipment to the Stable Isotope Facility at the University of California, Davis.

Data Analysis

To quantify preference, I used the proportion of ants in each container defined as choice. The proportion of ants in each container was calculated as the number of ants on the food inside the chamber out of 100 ants that were originally placed in the apparatus (including ants that remained in the CC and did not forage). To run several analyses, I used only the last time point (t=120), as ant proportions were highest during the second hour of the experiment.

To test the effects of habitat type on food preference, "*Habitat Hypothesis*" I compared the proportion of ants on each food type between the three urban and three natural sites. Argentine ant food preferences were analyzed with a GLM with a log link function, using the proportion of ants in each five food containers as the dependent factor, and food type, habitat type, and trial number as independent factors.

To test the relationship between food preference and resource limitation, "*Resource Limitation Hypothesis",* I compared the proportion of ants on each food type (1-5) to isotopic composition of ants throughout all six sites. To measure the strength of the association between

lab food preference and isotope content in ants collected from the field I used a Pearson's correlation test.

To test if recent experiences affect food preference, "*Recent Experience Hypothesis",* ants were starved for one-week. The starvation period was meant to create a recent experience in which ants are deprived of carbohydrates. I provided ants with water to increase survival throughout the one-week starvation period. After the week-long starvation period, I ran a second-choice test to determine if starvation impacted the ants' food preferences. To test the 'Recent Experience Hypothesis' I compared the proportion of ants before and after starvation by including a 'starvation period' effect in the GLM detailed above.

Finally, to determine if ant preference, which was measured based on the proportion of ants at each maze container in the last time point of the experiment, was based on the order in which they discovered the food, I looked at the change in number of ants throughout the entire experiment and calculated a spearman's correlation between the proportion of ants in each container during the first and last 30 minutes of the experiment.

Analyses of all data were carried out in R version 3.6.3 for Windows (*R Core Team (2017*).

Results

I found that recent experience had some (non-significant) impact on ant food preference, and neither source habitat nor food composition had any impact on food choice. The generalized linear model showed no significant effect of starvation, habitat type, or food type on the number of ants at the food (table 2). The ants' recent experience accounted for the largest shifts in food choice during laboratory tests (figure 3).

Variable	Estimate	Standard Error	T-value	P-value
Intercept	-2.60704	1.14780	-2.271	**0.0528**
Trial (before/after starvation)	-0.13841	0.45912	-0.301	0.77
Habitat (urban vs natural)	0.28546	0.45912	0.622	0.55
Food (Protein vs Carbohydrate)	0.03638	0.28115	0.129	0.90

Table 2: Output of the generalized linear model testing the relationship between ant trial preferences against habitat type, trial (before/after starvation), and food type.

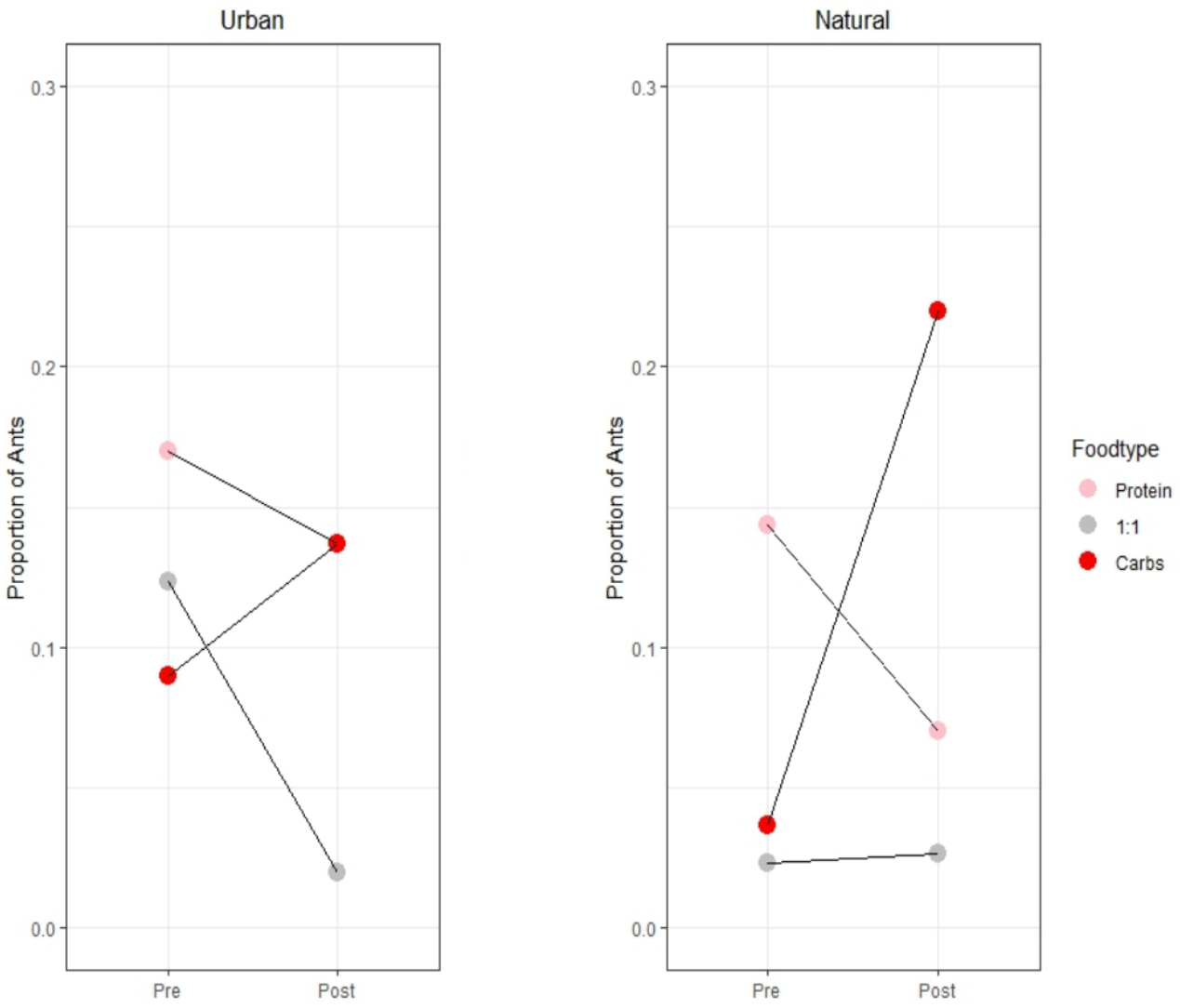


Figure 3: Food preference of ants from urban (left) and natural (right) environments. The proportion of ants at the different types of food were aggregated by high protein options (3:1, 2:1) in pink, and high carb proportions (1:3, 1:2) in red. Grey points represent equal proportions of protein and carbs (1:1). Each plot shows preference data before and after experimental starvation.

Isotope analysis showed little difference between habitat types, with some overlap between isotopic composition of Urban and Natural environments (Figure 4). The three samples from each site were very similar to one another and did not cluster by habitat type

(natural/urban). I did not detect a relationship between food isotope composition (C:N) of ants in the urban and natural sites and their food preference in the lab. When examining the correlation between protein preference in the lab and 15N and 13C isotopes extracted from ants collected in the field I did not detect a significant relationship (Pearson's correlation results in (table 3 and figure 5).

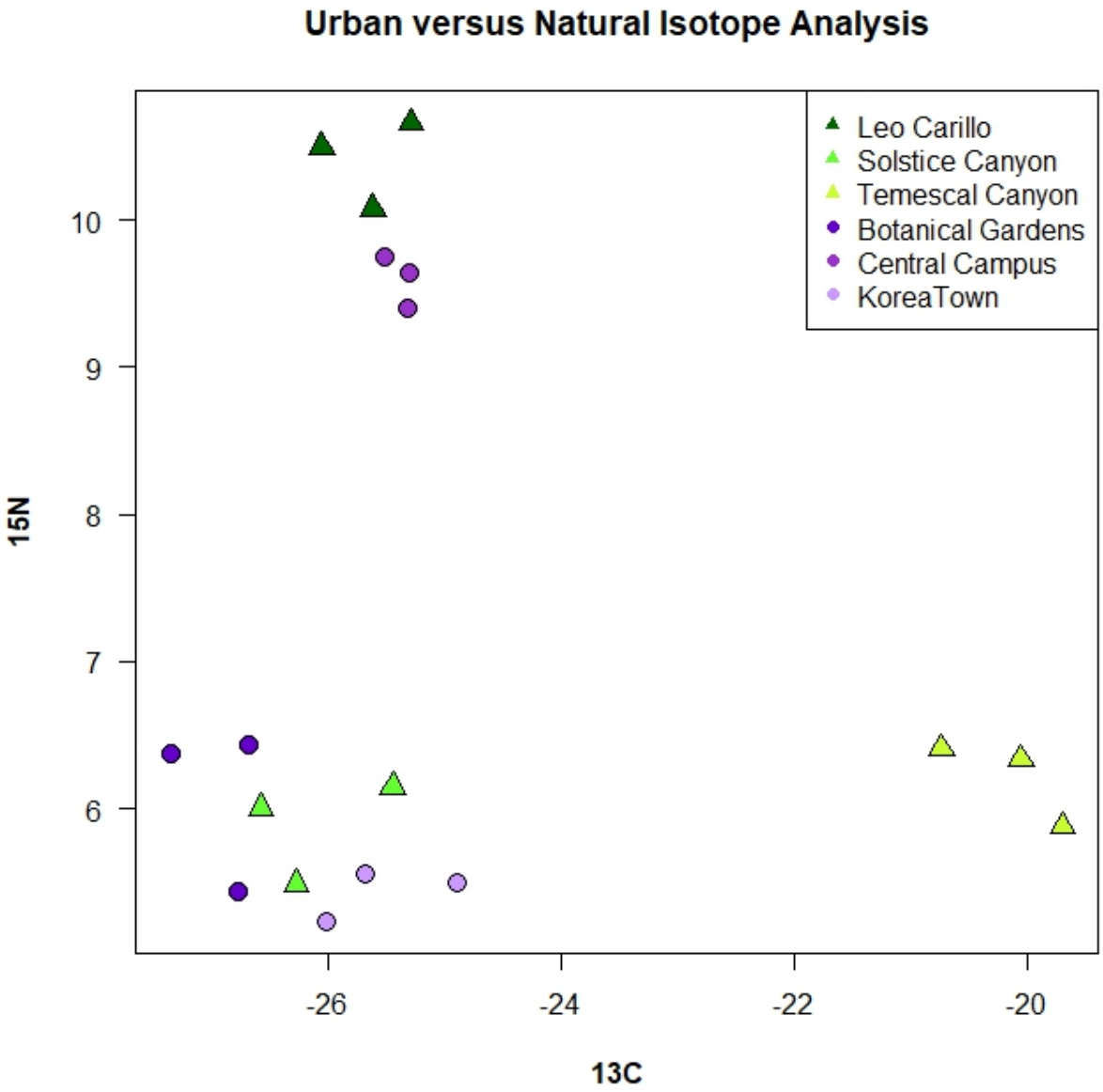


Figure 4: The relationship between 13C and 15N isotopes from ants collected from three urban and three natural environments. Habitat type is indicated by the shape and color, where green triangles represent natural sites, and purple circles represent urban sites. Shape shade difference represents unique site data.

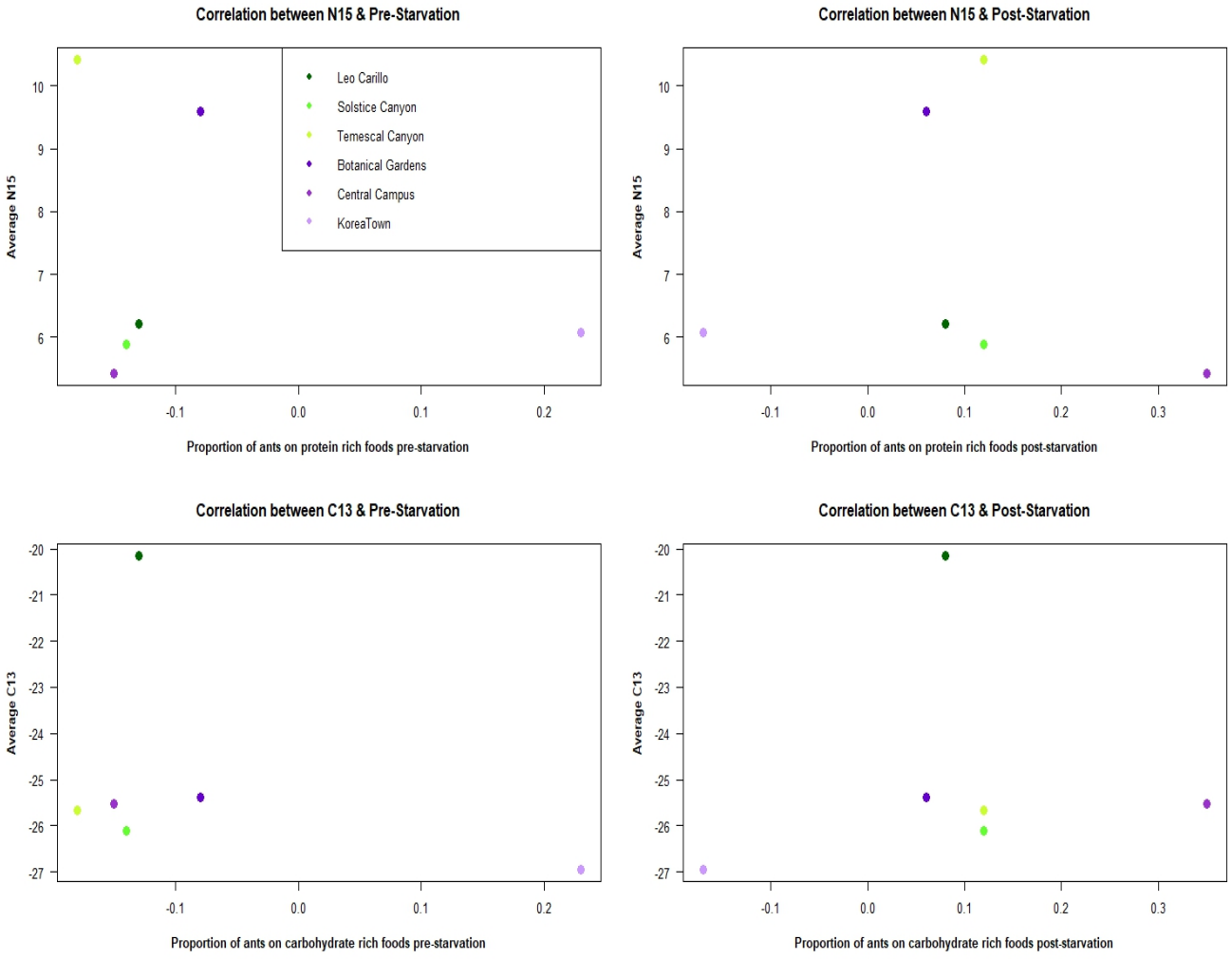
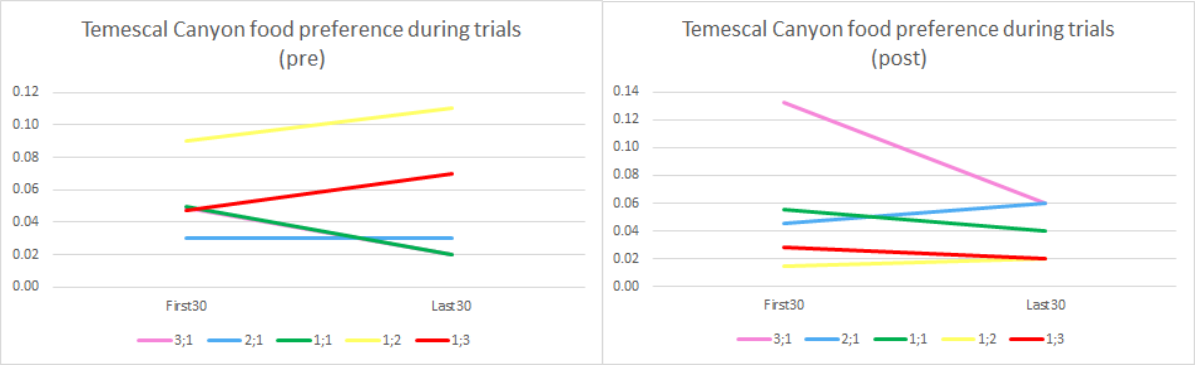


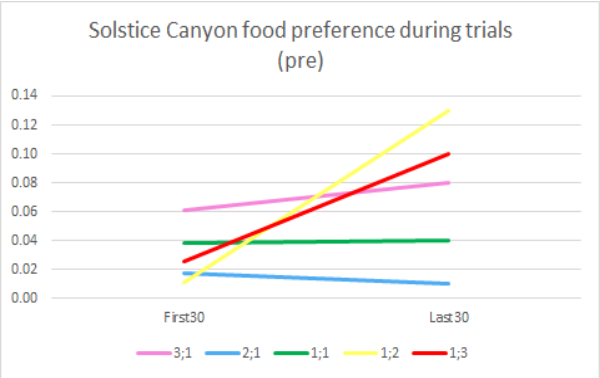
Figure 5: Correlations between N15 & C13 isotopes against protein bias food preference before and after experimental starvation. Unique site data and habitat type represented by color, where green is natural sites, and purple represents urban sites.

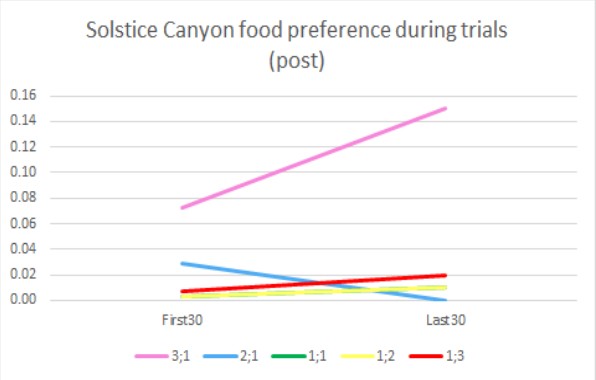
Variables	R-value	P-value
C13 & Pre-starvation carb preference	0.3651915	0.47
C13 & Post-starvation carb preference	-0.39653	0.43
N15 & Pre-starvation protein preference	-0.253849	0.62
N15 & Post-starvation protein preference	-0.09837053	0.85

Table 3: Pearson's correlation results for relationship between isotope composition and food preference in the lab.

The total number of ants at each container of the maze only slightly changed throughout experimental trials, where ants would continuously recruit to the food they visited the most during the first 30 minutes (figure 6). The spearman's correlation showed a strong positive, but not statistically significant, correlation after starvation and weak relationships in both positive and negative directions before starvation - with only the strongest of those being statistically significant (table 4).




Solstice Canyon food preference during trials (pre)
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
First30
Last30
3;1
2;1
1;1
1;2
1;3


Solstice Canyon food preference during trials (post)
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
First30
Last30
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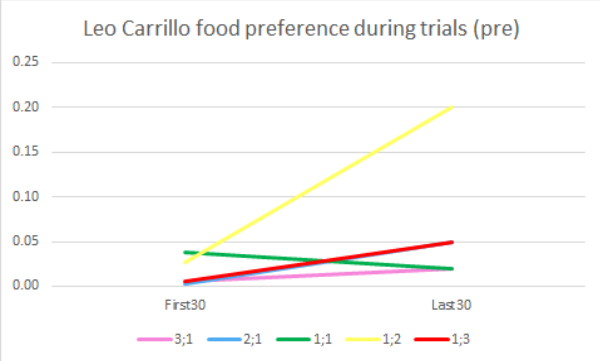
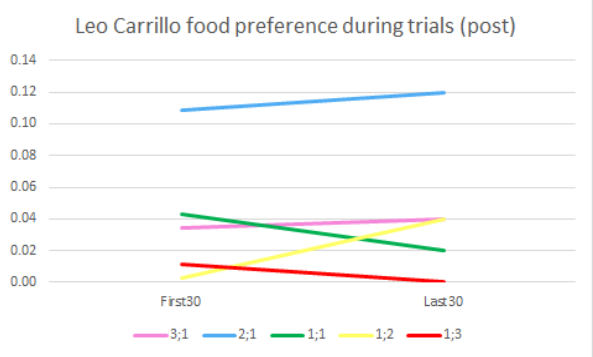
2;1
1;1
1;2
1;3


Leo Carrillo food preference during trials (pre)
0.25
0.20
0.15
0.10
0.05
0.00
First30
Last30
3;1
2;1
1;1
1;2
1;3


Leo Carrillo food preference during trials (post)
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
First30
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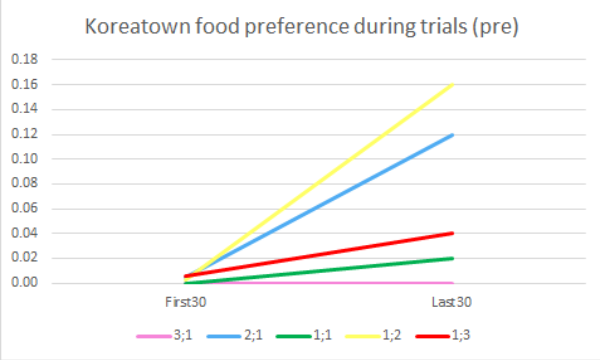
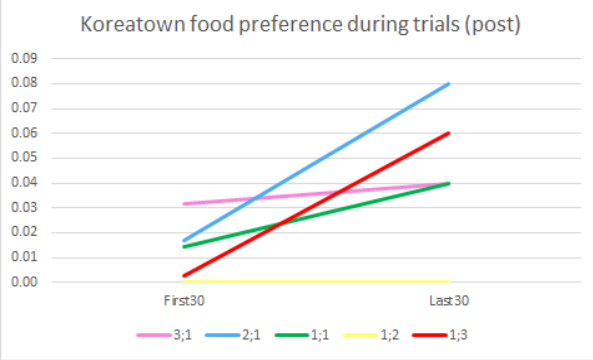
1;1
1;2
1;3


Koreatown food preference during trials (pre)
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
First30
Last30
3;1
2;1
1;1
1;2
1;3


Koreatown food preference during trials (post)
0.09
0.08
0.07
0.06
0.05
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Last30
3;1
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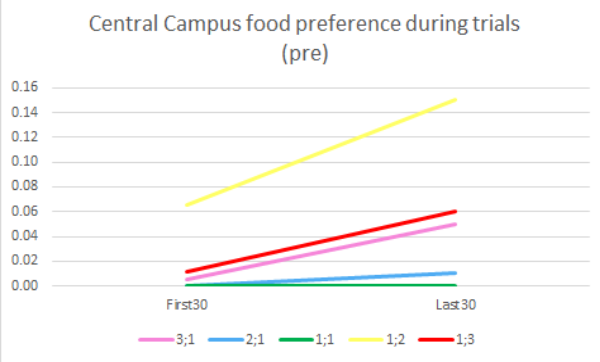
1;1
1;2
1;3


Central Campus food preference during trials (pre)
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
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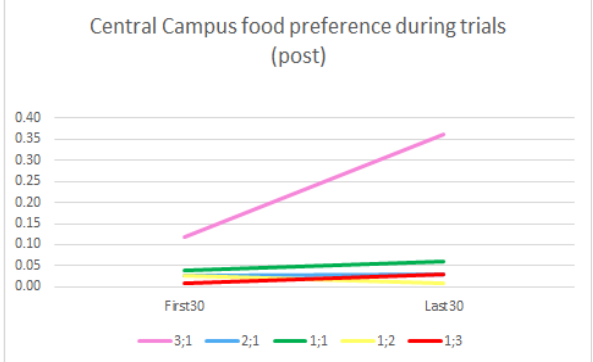
Last30
3;1
2;1
1;1
1;2
1;3


Central Campus food preference during trials (post)
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0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
First30
Last30
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2;1
1;1
1;2
1;3

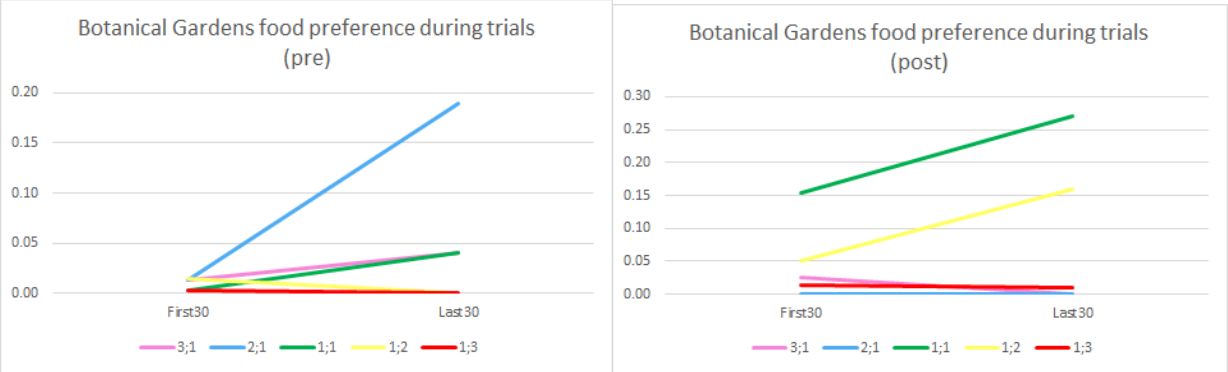

Figure 6: Proportion of ants on each food type (e.g. 3:1, 2:1, etc) during experimental trials with the average proportion during the first 30 minutes, and the total proportion during the last 30 minutes.

	Pre-Starvation		Post-Starvation	
Site	**Rho**	**P-value**	**Rho**	**P-value**
Temescal Canyon	0.46	0.44	0.79	0.11
Solstice Canyon	-0.3	0.68	0.37	0.54
Leo Carrillo	-0.16	0.79	0.41	0.49
Koreatown	0	1	0.26	0.67
Central Campus	0.95	**0.01**	0.76	0.13
Botanical Gardens	0.3	0.62	0.82	0.08

Table 4: Spearman's correlation coefficients and p-values for the relationship between the proportion of ants on the 5-food types during the first 30 and last 30 minutes of the experiment.

Discussion

I did not find evidence that Argentine ants from urban environments show a different food preference compared to workers from natural environments in Los Angeles county. Furthermore, workers did not prefer foods that were abundant (or unavailable) to them in their

environments. Instead, the ants' food preference was most influenced by their recent experience during the experimental trials, i.e., by their immediate nutritional needs, due to starvation, and the speed at which they discovered a particular food type.

My inability to detect a statistically significant difference in food preference between ants from urban or natural sites may be explained by the wide range of urbanization seen throughout Los Angeles County. It is possible that locations which seem natural, still provide the ants with similar resources to those in areas that seem urban. Previous research has shown that urbanization in areas like Los Angeles is pushing city limits and encroaching on green spaces by the use of transportation (Bekele, 2005). Indeed, the isotope analysis (figure 4) revealed little difference between the nutrients available to ants in the areas I defined as urban or natural. In fact, the two sites that were closest in geography (UCLA botanical gardens and main campus) were among the most distinct in isotope composition, suggesting that local human activity might determine the nutrients that ants consume rather than larger global geographical patterns of urbanisation. Furthermore, the sites that were selected as urban or natural in this study represented environments which have been modified by humans and contain human waste, for example at garbage cans of picnic sites in the 'natural' sites.

Resource availability did not significantly affect Argentine ant food preference. While both habitats might present different nutrients, with urban areas often showing an overabundance of proteins (Hoornweg & Bhada-Tata, 2012; Penick et al., 2015), the nutrients at the urban and natural sites in this study were not distinct in the isotope profiles of the ants (table 3). I did not detect a relationship between ant isotopes and food preference. This means that the ants' food preferences in the lab were not affected by the lack of resources in their source environment. The fact that urban and natural sites did not differ in food availability, suggests that

ants in these habitats might have access to similar resources. Although natural areas may provide fewer reliable protein sources than urban sites (Birnie-Gauvin et al., 2017), I still found evidence that protein is available in natural sites at similar levels to those at urban sites (Figure 4). Argentine ants tend to forage at trash cans, and therefore may be eating the same human waste as their urban counterparts. Future research on ant diets should consider collecting colonies on a larger spatial scale, as individuals' isotopic values can vary considerably both within and among colonies of the same species (Blüthgen et al., 2003; Tillberg et al., 2006).

The recent experience of workers was the best predictor of their food preferences. Ants from both urban and natural environments increased their preference for carbohydrates after starvation (figure 3). This change in preference can be explained by the use of carbohydrates as an energy source for workers (Dussutour & Simpson, 2009). Ants after starvation are likely deficient in energy stores and would be expected to seek out carbohydrates to replenish these stores of energy to maintain their activities.

I further found evidence that starved ants foraged on the food item they found first. Figure 6 and table 4 show that after starvation the food preference, i.e., the rank order of the food options, during the first 30min of the trial is maintained in the last 30min of the trial. Thus, it is possible that the first food the ants encounter might be where ants accumulate throughout the experiment because of recruitment, further supporting the recent experience hypothesis. Ants that were not starved spend more time looking at food, increasing the accuracy of their choices, whereas starved ants generally find one food choice and accumulate on it, prioritizing speed. Infact, previous research looking at speed-and-accuracy tradeoffs in ants, has shown that colonies use positive feedback to make accurate decisions for the colony that often deal with time-sensitive requirements, such as nutrition (Chittka et al., 2009; Hui & Pinter-Wollman, 2014).

Thus there is always contention between speed-and-accuracy. Further analysis of the temporal dynamics of arrival to the food items would be necessary to confirm this hypothesis. Indeed, previous research has shown that Argentine ants use recruitment to food through positive feedback (Flanagan et al., 2013). Consequently, ants might have strong evolutionary pressures to quickly choose, and use their most recent experience to make accurate choices. Future studies should examine the trends seen, as the type of food chosen over time could illuminate important aspects of Argentine ant food preference.

One important aspect to consider is the effect of seasonality on ant food preference, as ants are highly impacted by changes in food availability, temperatures, and the presence of brood. Seasons have a large impact on ants' energetic requirements (Cook et al., 2011), and thus food preferences of ants may change, based on whether or not the colony is producing brood, which require protein for survival and growth (Weeks et al., 2004; Dussutour & Simpson, 2008a, 2009). Further, because there were no brood kept with experimental colonies during starvation, the ants may have not had a need for protein.

It is possible that Argentine ants display good preference that is affected by locality rather than specialisation that emerges from large-scale geographical patterns (Segev & Foitzik, 2019). As I've shown, despite the difference in source habitat, both urban and natural populations of Argentine ants shared similar food preferences. Ants from both environments preferred carbohydrates over proteins post starvation, which is in line with the physiological needs of ants (carbohydrates=energy) (Dussutour & Simpson, 2009). Indeed, nutritional geometry theory states that social insects often have trade-offs to maintain colony fitness (Lihoreau et al., 2015), which means that ants are constantly making active-decisions on what the colony requires. Therefore, the variation among ant groups in food preference during the

pre-starvation trials can potentially be explained by the need to acquire either proteins or carbohydrates in different manners based on the locality specific constraints where they were collected (e.g., access to trash, human presence).

Future research could examine differences in preference between generalist native ant species in California and invasive ants population in food preference to reveal whether Argentine ants' success as an invader is due to their generalist behavior or to aggression, colony structure, unique mating system etc. Future work should take into account the effect of seasonality on the food preferences of ants, as it can impact colonies' lifecycle and therefore their nutritional needs (Dussutour & Simpson, 2009). The needs of the colony change with seasons both because of changes in food availability in the environment and changes in the presence of brood throughout the year. Such questions can be addressed by testing multiple colonies throughout all seasons and at different reproductive stages. Furthermore, increasing the sample size might reveal if the lack of observed differences in food preference between urban and natural environments was a result of low statistical power. Finally, future work might consider examining ants from extremely rural environments (e.g., national parks) with little human presence and strict waste management policies, to ensure that ants from 'natural' environments have minimal access to human waste.

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