Biotic challenges in the city: Dietary restrictions and body fat content of female sexuals in urban ant populations

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Abstract

Urban habitats represent an important challenge for many organisms. Besides the abiotic changes, urban habitats are also characterized by changes in the biotic conditions, such as a more uniform species composition and declining population sizes. For urban ants this can result in dietary shortages. In our study, we tested whether urban ant colonies might suffer from dietary restrictions by carrying out a common garden experiment in which ant colonies from urban and rural habitats were exposed to high carbohydrate, protein, and fat / protein diets. We also investigated the body fat content of individuals from both habitat types. Our findings suggest a lower availability of high-quality carbohydrates in urban areas. Additionally, while not statistically significant, rural colonies exhibited a tendency to consume greater quantities of proteins and fat compared to urban colonies. This trend was in line with a higher body fat content observed in female sexuals (gynes) from rural colonies. These results might indicate the outcome of an evolutionary feedback process in which ant colonies adapt to nutritional constraints in urban environments. They achieve this by minimizing the investment in gynes, which might require fewer reserves for survival during colony foundation due to reduced competition for nesting sites within urban green spaces.

Keywords

Urbanization, homogenization, food preferences, macronutrients, body fat content, gyne

Introduction

During the last decades, urban ecology has received an increasing interest in the scientific community. Several studies have demonstrated how the anthropogenic transformation of the habitat has caused numerous behavioural and physiological changes in organisms (Ditchkoff et al., 2006; Calfapietra et al., 2015; Brans et al., 2018; Łopucki et al., 2021; Jacquier et al., 2023; Trigos-Peral et al., 2024; among others). The best-known consequences of urbanization are the biotic homogenization of the habitat and the establishment of invasive species (Vilà et al., 2011; Trigos-Peral et al., 2020, 2021; Brassard et al., 2021). Both affect species composition and habitat-niche partitioning, thereby triggering additional alterations in the local ecological networks. For example, the decrease in the availability or quality of food sources can disrupt fragile food webs (Shrewsbury & Raupp, 2006; Qu et al. 2022), influence food size preferences (Chen & Neoh 2023) and threaten the survival of species.

Low nutritional quantity and quality negatively affect growth, behavioural performance, reproduction, immunological defence, and lifespan (i.e., Spann & Schumann, 2009; McWilliams, 2011; Simpson & Raubenheimer, 2012; Nwaogu et al., 2020; Shik & Dussutour, 2020; Abellán et al., 2023) and thus endanger the survival of both species of plants and animals. Scarcity and low quality of nutrition particularly challenge organisms with low mobility, in which the chances of moving to new areas to find more suitable food sources are limited. For example, most ants are central-place foragers and their foraging efficiency is influenced by the distance between their stationary nest and the food source (Devigne & Detrain, 2006). Though many ant species have adapted to the ecological conditions in urban areas (Brassard et al., 2021), the potential nutritional deficiencies in this habitat type might nevertheless influence individual survival or brood development and consequently affect colony growth.

Ants obtain essential micronutrients, such as vitamins, salts, amino acids, or lipids, by consuming macronutrients from plant and animal sources. They gain proteins, fat, and salts by hunting or feeding on cadavers and in many species, carbohydrates and essential amino acids are provided in secretions from aphids (honeydew) (Csata et al., 2020). Honeydew is an easily accessible nutritional source due to the ubiquitous and cosmopolitan distribution of aphids (Alyokhin et al., 2022). Several aphid species appear to benefit from urban habitats, in which the number of their predators may be substantially reduced (Korányi et al., 2020). Nevertheless, honeydew composition heavily relies on the host plant species and its nutritional state (Fischer et al., 2005; Leroy et al., 2011). It is therefore possible that honeydew from urban aphids is of lower quality. The combined effects of a decline in ground-dwelling arthropods (Vergnes et al., 2013) and the potential impoverishment of aphid honeydew may jeopardize the variety and quality of the diet of ants.

In addition to carbohydrates and proteins, lipids are also important macronutrients (Blüthgen & Feldhaar, 2009), which influence the growth and survival of ant colonies. Ants can obtain fatty acids either directly from their diet or synthesise them from carbohydrates (Heath & Rock, 2002). Fat stored in the fat body of insects is crucial in numerous physiological processes. For example, it increases resistance to starvation (Dussutour et al., 2016), stimulates egg development (Legaspi & Legaspi, 1998; Legaspi & O'Neil, 1994), and affects the division of reproductive labour in queenless ants (Bernadou et al., 2020).

We therefore hypothesize that urban habitats represent an important challenge to the survival of ant colonies due to the potentially reduced quantity and quality of available natural food sources (i.e., Kaspari et al., 2008; Bujan & Kaspari, 2017; Kaspari et al., 2019). In a common garden study, we compared the foraging preferences of the ant *Lasius niger* from rural areas and urban parks to determine whether there is a nutritional shortage in the city and how it impacts ant colonies. Specifically, we investigated i) whether urban and rural colonies differ in their preference for three types of diet with different carbohydrate, protein, and fat content, and ii) if workers and female sexuals (gynes) from the two types of habitat differ in body fat content.

Material and Methods

Food source choice and consumption

The study was carried out in July 2021 using colonies of *Lasius niger* collected in three urban parks located in the city centre of Warsaw, Poland, and three rural areas (free of human influence) in the surroundings (Supplementary table 1). We collected three colony fragments in each of three urban and three rural areas (total 18 colony fragments). Each fragment consisted of a minimum of 500 workers, a few hundred larvae, and a few dozen pupae of female sexuals (gynes) and workers (differenced by the size and shape).

Immediately after collection, from each fragment we set up two experimental colonies consisting of 150 workers each. Brood was not included since it would have influenced both the food selection and the amount consumed depending on their developmental status, which could not easily be standardized (Dussutour & Simpson, 2008). The workers were placed in small plastic containers (18 cm × 12 cm × 6 cm) with a humid piece of sponge covered by a dark plastic plate serving as nest. Each container was connected to three different foraging arenas (10 cm x 6.5 cm x 5 cm) via silicone tubes. The 36 experimental colonies were deprived of food for one day, while the ants remaining in the18 original fragments were provided with sugar-water and mealworms *ad libitum* until they were used in the later experiments.

After starving the experimental colonies for one day, we started the nutrition choice experiment by placing into each foraging arena a feeder (a plastic plate of 3 cm diameter) with a different type of food: carbohydrates, protein, and fat / protein. The carbohydrate source was a saturated sucrose solution (1 M), protein was canned tuna packed in its own juice, and the fat / protein source was canned tuna in soybean oil (Supplementary table 2). To avoid differences in food retrieval caused by different food consistency, both protein and fat / protein were blended using mineral water (same that the one used for the sucrose solution) to achieve a liquid consistency similar to the carbohydrate solution. After placing the food sources, we opened the access to the arena and recorded the feeding activity of ants for half an hour using a video camera (Sony HDR-AS20 Full HD). Food sources were placed randomly across the observations.

One week later we repeated the experiment to determine whether food preferences are consistent or change after *ad libitum* feeding in the laboratory. The 36 experimental colonies were maintained at room temperature (approximately 24 ºC) under a natural light regimen and fed *ad libitum* by each day randomly providing new feeders with fresh prepared carbohydrates, protein, and fat / protein diets. After one week, we starved the colonies again for one day and then repeated the food preference experiment.

To estimate the food choice by the ants, we analysed the videos and counted the total number of ants present in each foraging arena every five minutes. To determine how much food was consumed by the ant workers, we weighed the amount of each type of food placed in the feeders before and after each observation using a laboratory balance (Ohaus Scout SKX123; error: ± 1 mg).

Body fat content and gyne size

To test if body fat content is higher in gynes of rural colonies due to the presumed richer nutritional availability, we collected five gynes and ten workers from five nests each located in the three urban parks and rural areas that had been sampled for the food choice experiment (Supplementary table 1). Samples were collected shortly before the massive nuptial flight of *L. niger*, which occur in Poland in mid-July (Van der Have et al. 2011 and pers. observations). We therefore can assume that all gynes collected for this experiment were produced approximately at the same time, since gynes that had been produced earlier should have already abandoned the nest during previous small nuptial flights. Each collected individual was placed in an individually labelled 2 ml vial and frozen at -25 ºC. To determine whether the differences in fat content reflected differences in the food consumed in the two different habitat types or differences in the genetic background of the two populations, we also measured body fat content in gynes and workers born and reared in the laboratory in the colonies collected one month before for the previous experiment and equally fed *ad libitum* with identical food (honey water and tenebrids/*Drosophila*).

Body fat content was measured following Bernadou et al. (2015). Individuals were dried at 60 ºC for five days and weighed individually to the nearest 0.0001 mg with a microbalance (Radwag MYA 5.4Y) to measure their dry mass. Subsequently, fat was extracted by soaking each worker for two days in 2 ml petroleum ether (boiling range 40–60 ºC, Merck, Darmstadt, Germany) at room temperature. After two days, the workers were transferred into new vials, the petroleum ether was renewed, and each worker was kept in petroleum ether again for two days. Afterwards, the ants were dried at 60 ºC for six days and weighed again to determine their lean mass. The percentage of fat was calculated according to the standard equation: (dry mass - lean mass) * 100/dry mass.

Statistical analyses

Food source choice and consumption

We tested whether urban and rural colonies were differently attracted by the different types of food (carbohydrates, protein, or fat / protein) sources by carrying out a GLMM (zero inflated Poisson) in which the number of foragers observed in each foraging arena was used as the dependent variable. The three-way interaction of habitat type, type of food, and the round of the experiment was included as explanatory factors, colony identity, and round of observation as nested random factors.

We checked whether the total amount of food consumed differed between habitat types and repetitions by carrying out a LMM (Gamma distribution) in which the total amount of food consumed was used as explanatory variable and the interaction between habitat type and round of the experiment as explanatory factors. We also tested for differences in the actual quantity of each type of food consumed by the colonies from each habitat type and across rounds with a LMM approach in which the *log+1* transformed quantity of carbohydrates, protein, fat / protein consumed was used as response variable, and the interaction between habitat type, type of food, and round of the experiment were used as the explanatory factors. In all LMMs, colony identity was included as random factor. Finally, paired t-tests were carried out on the consumption of each nutrient per each population to compare differences between the first and second round of observations.

Body fat content and gyne size

To test for differences in body fat content between ants from urban and rural habitats, we performed a LMM with fat content as response variable and the interaction of the habitat type, caste, and origin (field or laboratory) as explanatory factors. The origin of the individuals was included in the model to avoid an influence of genetic background. Finally, since larger gynes can potentially accumulate larger quantities of body fat, we tested for size differences in gynes between habitat types using dry mass as a proxy of individual size. Comparisons were performed using a LMM approach, in which individual dry mass was introduced as dependent variable, the interaction between habitat type and origin of the individual (field or laboratory) as explanatory factors, and colony identity was used as random factor.

Statistical analyses were performed using R v4.1.2 (R Core Team, 2021) and RStudio (Posit Team, 2023). Linear mixed models (LMM) were performed by using the function *lmer* from the package *lme4* (Bates et al., 2013). Models were tested for overdispersion by using the function *testdispersion* of the package DHARMa (Hartig, 2022). When overdispersion was found in LMMs, the dependent variable was transformed using the most suitable transformation approach obtained using the function *bestNormalize* from the R-package bestNormalize (Peterson, 2021). Zero-inflated Poisson regression was performed by using the *zeroinfl* function of the pscl package (Jackman, 2020). The significance of the explanatory factors was determined by performing the Anova of the LMMs and GLMMs using the function *Anova* from the car package (Fox & Weisberg, 2019). To assess differences in urban-rural trends for the tested factors, we performed post-hoc pairwise comparisons of all models using the *emmeans* function of the emmeans package (Lenth, 2023) for GLMMs. For LMMs we used the *posthoc_Pairwise* function of the grafify package (Shenoy, 2021). Graphical representations were performed using the packages ggplot2 (Wickham, 2016), magick (Ooms, 2023), png (Urbanek, 2022) and ggpubr (Kassambara, 2022). Mean and error values were calculated using the Rmisc package (Hope, 2022).

Results

Food source choice and consumption

Our results show that the interest of workers in exploring the different arenas was significantly influenced by food type ($\chi^2 = 41.64$, $p < 0.001$), habitat type ($z = 6.84$, $p < 0.001$), the round of the experiment ($\chi^2 = 15.06$, $p < 0.001$), and the interactions between these factors (Table 1). Just after collection (round 1), workers of urban and rural colonies did not differ in their interest in carbohydrates ($z = -0.09$; $p = 1$). In contrast, rural colonies were significantly more foraging for protein and fat / protein diets (protein: $z = 3.85$, $p = 0.006$; fat / protein: $z = 3.31$, $p = 0.043$). One week later (round 2), rural colonies were less interested in carbohydrates ($z = -5.21$, $p < 0.001$) and more interested in the fat / protein diet ($z = 4.83$, $p < 0.001$) than urban colonies (Figure 1).

Table 1. Influence of the study factors in the foraging interest of the workers in the food selection experiment.

Number of workers attracted by each type of food			
Factor	Df	χ^2	p-value
Type of food	2	41.64	< 0.001
Habitat type	1	6.84	< 0.001
Round of observation	1	15.06	< 0.001
Type of food * Habitat type	2	57.45	< 0.001
Type of food * Round of observation	2	15.96	< 0.001
Habitat type * Round of observation	1	26.84	< 0.001
Type of food * Habitat type * Round of observation	2	6.72	0.035

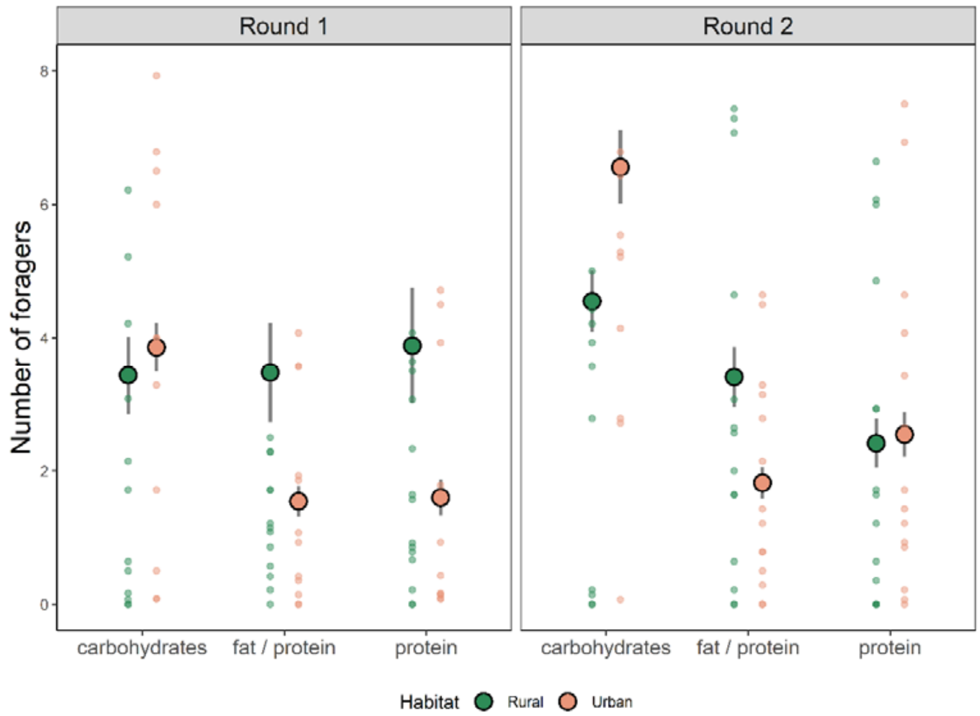


Figure 1. Mean and error bar plot showing the difference in the number of workers of the ant *Lasius niger* from rural and urban colonies present in the foraging arena containing different types of diet. The filled circles indicate the mean value of the consumption by the rural and urban colonies, the whiskers represent the standard deviation and the dots are the individual worker numbers for each colony.

When investigating the actual food intake, the total food consumed by the colonies decreased in the second round of the experiment ($t = -2.19$, $p = 0.028$; Supplementary figure 1). Colonies from urban and rural habitats consumed different quantities of each diet in the first round of observations. After collection, urban ants consumed more carbohydrates than rural colonies ($t = 3.75$, $p < 0.001$), but no clear differences were found in the consumption of protein ($t = 0.75$, $p = 0.675$) and fat / protein diets ($t = 0.05$, $p = 0.971$).

After one week of *ad libitum* feeding in the laboratory (round 2), no significant differences were found in food consumption between ants coming from different habitat types (carbohydrates: $t = 0.07$, $p = 0.971$; protein: $t = 0.41$, $p = 0.848$; fat / protein: $t = 0.137$, $p = 0.971$; Supplementary figure 2).

Finally, we observed a consistent decrease in the consumption of various types of nutrients the experimental colonies from both rural and urban populations between the first and second round of observations (Figure 2, Supplementary table 3).

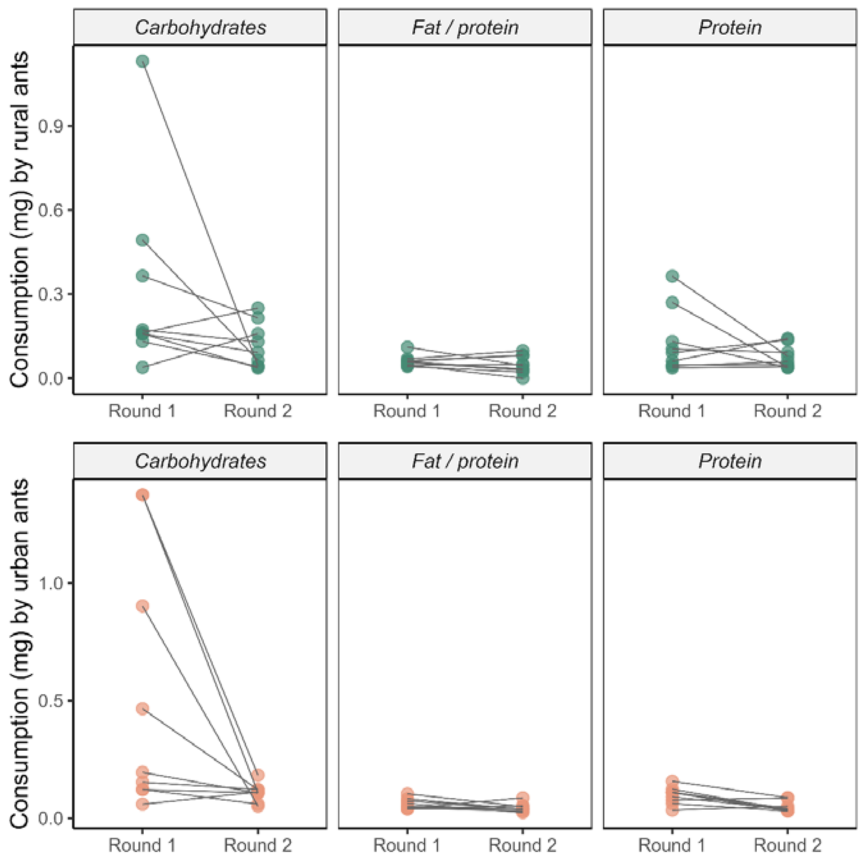


Figure 2. Paired dot plot showing the consumption of each diet (mg) by *Lasius niger* workers from urban and rural colonies during the two rounds of the experiment (one day after collection and one week after collection and feeding on a standard diet).

Body fat content and gynes size

Body fat content of gynes collected in the field differed between urban and rural habitats. Rural gynes had a significantly higher fat content than urban gynes ($t = 3.52$, $p = 0.003$), although laboratory-reared gynes from the two habitats did not differ ($t = -1.51$, $p = 0.262$). Workers from the two habitat types did not differ in body fat content, regardless of whether collected from the field (t = 0.78, p = 0.635) or reared in the laboratory (t = -1.76, p = 0.169). Finally, using the dry mass (mg) as a proxy of individual size, we found no significant difference between urban and rural gynes collected either in the field (t = -0.13, p = 0.895) or reared in the laboratory (t = -1.93, p = 0.072).

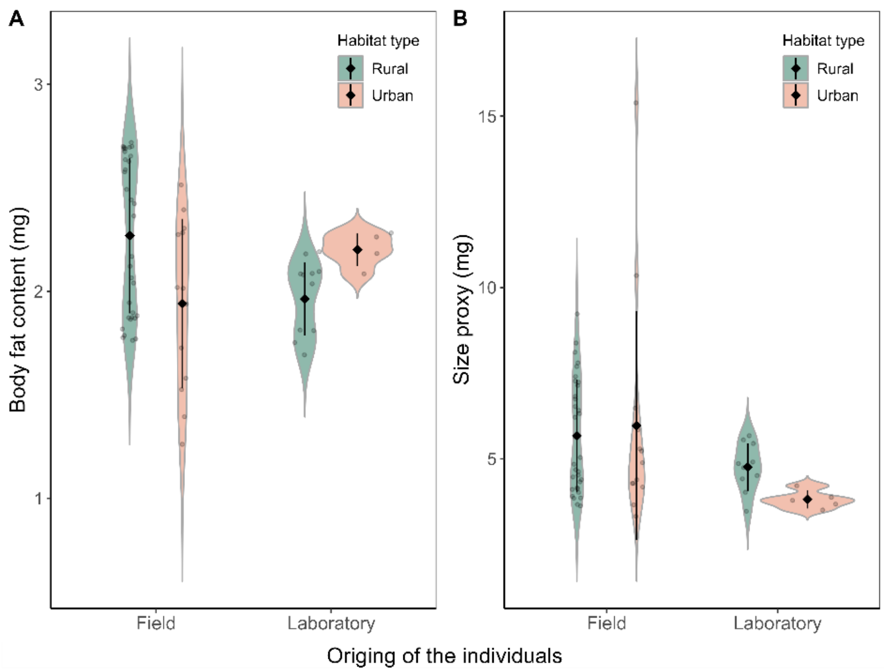


Figure 3. Violin plot and mean and error plot showing the body fat content and (in milligrams) size (using the dry mass as a proxy) of gynes of the ant *Lasius niger* reared under natural conditions in the field and under laboratory conditions. The black diamonds indicate the mean value of the consumption by the rural and urban colonies, and the whiskers represent the standard deviation.

Discussion

Workers of the ant *Lasius niger* from urban and rural colonies exhibited distinct preferences for three offered diets. Whereas the foraging activity in the arena containing carbohydrates did not differ between colonies from the two habitat types, workers from rural colonies showed significantly more interest in diets rich in protein or fat and protein compared to workers from urban ones.

Immediately after collection, workers from urban colonies showed a significantly higher intake of carbohydrates than workers from rural colonies, while no significant differences were found in the consumption of the other two diets. However, after one week of feeding on a standard diet in the laboratory, rural ants exhibited a significantly greater interest for the diet rich in fat / protein and less interest in carbohydrates, in contrast to urban ants. Ants from urban and rural habitats did not differ in the quantities they consumed from each diet.

Gynes from rural colonies were similar in size to those from urban colonies but had a significantly higher fat content. No differences in fat content were found when gynes from different habitat types were reared in the laboratory. Fat content was also identical in workers from urban and rural areas regardless of whether they were reared in the field or the laboratory.

We found a high general attraction of the ants for carbohydrates, which matches the well-known symbiotic association of *L. niger* with aphids (Czechowski et al., 2012; Seifert, 2018). The higher intake of carbohydrates in urban colonies immediately after collection suggests that carbohydrate sources, mainly honeydew, are less abundant and potentially of lower quality in urban habitats compared to sources in rural areas. Lower plant diversity and their growth in poor soil (Lescano et al., 2022) might negatively affect the honeydew production by aphids (Fisher et al., 2005) in urban areas, although it is worth noting that no specific studies have yet been carried out to test this assumption.

Contrary to our expectation that urban colonies would consume more protein and fat than rural colonies, we found that rural colonies were more interested in these diets, although differences were not significant. However, we attribute the lack of significance to the high concentration of nutrients in these diets, as found that ants the volume of food ingested by ants decreases with the increase of concentration in the diet (Dussutour & Simpson, 2008). Besides, we cannot exclude that recruitment during the initial observation round (one day after collection) still reflected natural factors, such as colony size and productivity (Mailleux et al., 2003). For example, colonies in rural areas might have been larger and more productive than urban colonies, explaining the unexpected, increased interest in protein and fat. While urban colonies can prolong their production period due to the elevated temperatures in urban environments (Trigos-Peral et al., 2024), rural colonies tend to have shorter reproduction periods and may need to optimize their productivity particularly during summer. As a result, rural colonies might have a greater demand for protein and fat to ensure an adequate brood development (Dussutour & Simpson, 2008; Rosumek et al., 2017).

Moreover, rural habitats present additional challenges in the search for proteins and fats. While greater biodiversity can theoretically provide a wider array of food sources, it also results in an increased number of neighbouring colonies competing for these resources. Penick et al. (2015) suggested that certain urban ant species can compensate the lack of natural food sources by feeding on human food waste. However, the observed consumption of leftover food varied with the ants' origin (native or invasive), location (parks or traffic islands), and trophic level (predator or herbivore). For example, consumption of food waste was only evidenced in the omnivorous, exotic ants *Tetramorium* spp. and *Nylanderia flavipes* in traffic islands, while *Lasius cf. emarginatus* appeared to avoid human food waste (Penick et al., 2015).

After being provided with similar diets for one week in the laboratory, rural colonies not only again foraged more for fat / protein but also exhibited an increased consumption of this diet. Though this might suggest that rural ants suffer more from protein and fat shortage, we found that rural gynes had a higher body fat content than urban counterparts. Body fat content plays a critical role in the success of solitary colony foundation, as it significantly affects queen survival and egg production during the development of the first workers (Heinze & Tsuji, 1995; Helms & Kaspari, 2015; Negroni et al., 2021). Body fat content increases in gynes after emergence due to feeding (Boomsma & Isaaks, 1985), so the differences might in principle result from a later emergence of urban gynes. However, the annual activity starts earlier in urban ants than in rural ones (GT unpublished data), which should lead to opposite results (higher body fat content in urban ants). Furthermore, prior to the massive nuptial flight in July, there are a few smaller nuptial flights in which the earlier emerged gynes leave the nest. Notwithstanding, further studies are needed to determine whether the lower fat content observed in urban gynes might result from different timing of emergence or indeed indicates an evolutionary adaptation to dietary constraints in their habitat. Moreover, although workers from both habitat types did not differ in body fat content, the nutritional shortage may not be apparent in this caste throughout our experiment. For instance, although Rosumek et al. (2017) did not find differences in body fat content of ant workers when testing diets with low and high fat content, they found a change of their fatty acid profile.

To summarize, our research offers new insights into the potential dietary shortages in urban areas and its consequences on ants. Further studies with controlled diets and genetic approaches are needed to accurately determine the interplay between the availability of food sources, foraging behaviour, and reproductive success in ants. In addition, our findings highlight once more the significance of diet quality in ants (Csata et al., 2020; Wendt & Czaczkes, 2020) and emphasize the importance of meticulously selecting the diets for use in baiting assays.

Data availability

The data used in this study are available through the link to figshare (https://figshare.com/s/20b4ecf6e7b571b9ca13) in the manuscript. Scripts and outputs are also available through the former link. Doi: 10.6084/m9.figshare.25513309

Literature

Abellán, M., Ruano, F., Rojo, S., & Martínez-Sánchez, A. (2023). Effect of two larval diets on the reproductive parameters of the housefly, Musca domestica L. (Diptera, Muscidae).

Journal of Insects as Food and Feed, 9 (11), 1473–1486. https://doi.org/10.1163/23524588-20220152

Alyokhin, A., Wenninger, E. J., & Jensen, A. (2022). Aphids: In Alyokhin, A., Rondon, S. I., & Gao Y. (2nd Eds.), *Insect Pests of Potato* (2nd ed., pp. 45–67). Academic Press.

Bernadou, A., Busch, J. & Heinze, J. (2015). Diversity in identity: behavioral flexibility, dominance, and age polyethism in a clonal ant. *Behavioral Ecology and Sociobiology*, 69, 1365–1375. https://doi.org/10.1007/s00265-015-1950-9

Bernadou, A., Hoffacker, E., Pable, J., & Heinze, J. (2022). Lipid content influences division of labour in a clonal ant. *Journal of Experimental Biology*, 223 (6), jeb219238. https://doi.org/10.1242/jeb.219238

Brans, K. I., Stoks, R., & De Meester, L. (2018). Urbanization drives genetic differentiation in physiology and structures the evolution of pace-of-life syndromes in the water flea *Daphnia magna*. P*roceedings of the Royal Society B,* 285, 20180169. http://dx.doi.org/10.1098/rspb.2018.0169

Brassard, F., Leong, C. M., Chan, H. H., & Guénard, B. (2021). High diversity in urban areas: how comprehensive sampling reveals high ant species richness within one of the most urbanized regions of the world. *Diversity*, 13 (8), 358. https://doi.org/10.3390/d13080358.

Blüthgen, N., & Feldhaar, H., (2009). Food and shelter: how resources influence ant ecology. Ant Ecology. https://doi.org/10.1093/acprof:oso/9780199544639.003.0007. Oxford scholarship online.

Boomsma, J. J., & Isaaks, J. A. (1985). Energy investment and respiration in queens and males of *Lasius niger* (Hymenoptera: Formicidae). *Behavioral Ecology and Sociobiology*, 18(1), 19–27.

Bujan, J., & Kaspari, M. (2017). Nutrition modifies critical thermal maximum of a dominant canopy ant. *Journal of Insect Physiology*, 102, 1-6. https://doi.org/10.1016/j.jinsphys.2017.08.007.

Calfapietra, C., Peñuelas, J., & Niinemets, Ü. (2015). Urban plant physiology: adaptation-mitigation strategies under permanent stress. *Trends in Plant Science*, 20(2), 72–75. https://doi.org/10.1016/j.tplants.2014.11.001

Chen, I. H., & Neoh, K. B. (2023) Urbanization causes shifts in the functional traits and foraging activity, and alters food particle size preference and biomass removal of urban-dwelling ants. *Frontiers in Ecology and Evolution*, 11, 1044485. https://doi.org/10.3389/fevo.2023.1044485

Csata, E., Gautrais, J., Bach, A., Blanchet, J., Ferrante, J., Fournier, F., Lévesque, T., Simpson, S. J., & Dussutour, A. (2020). Ant foragers compensate for the nutritional deficiencies in the colony. *Current Biololgy*, 30, 135–142. https://doi.org/10.1016/j.cub.2019.11.019

Csata, E., & Dussutour, A. (2019). Nutrient regulation in ants (Hymenoptera: Formicidae): a review. *Myrmecological News*, 29, 111–124. https://doi.org/10.25849/myrmecol.news_029:111

Csata, E., Bernadou, A., Rákosy-Tican, E., Heinze, J. & Markó, B. (2017) The effects of fungal infection and physiological condition on the locomotory behaviour of the ant *Myrmica scabrinodis*. *Journal of Insect Physiology*, 98, 67–172. https://doi.org/10.1016/j.jinsphys.2017.01.004

Czechowski, W., Radchenko, A., Czechowska, W., & Vepsäläinen, K. (2012) The ants of Poland with reference to the myrmecofauna of Europe. Fauna Poloniae 4. Warsaw: Natura optima dux Foundation.

Devigne, C., & Detrain C. (2006). How does food distance influence foraging in the ant *Lasius niger*: the importance of home-range marking. *Insectes Sociaux*, 53, 46–55. https://doi.org/10.1007/s00040-005-0834-9

Ditchkoff, S. S., Saalfeld, S. T., & Gibson C. J. (2006). Animal behavior in urban ecosystems: modifications due to human-induced stress. Urban Ecosystems, 9, 5–12. https:// 10.1007/s11252-006-3262-3

Dussutour, A., Simpson, S. J. (2008). Description of a simple synthetic diet for studying nutritional responses in ants. Insectes Sociaux, 55, 329 – 333. https://10.1007/s00040-008-1008-3

Fischer, M. K., Völkl, W., & Hoffmann, K. H. (2005). Honeydew production and honeydew sugar composition of polyphagous black bean aphid, Aphis fabae (Hemiptera: Aphididae) on various host plants and implications for ant-attendance. *European Journal of Entomology*, 102 (2), 155-160. https://10.14411/eje.2005.025

Fox, J., & Weisberg, S. (2019). An R Companion to Applied Regression, Third Edition, Sage. https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Hartig, F. (2022). DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.4.6

Heath, R. J, & Rock, C. O. (2002). The Claisen condensation in biology. *Natural Products Reports*, 19, 581–96. http://doi.org/10.1039/B110221B

Heinze, J., & Tsuji, K. (1995). Ant reproductive strategies. *Research on Population Ecology*, 37 (2), 135–149. https://doi.org/10.1007/BF02515814

Helms, J., Kaspari, M. (2015). Reproduction-dispersal tradeoffs in ant queens. – Insect. Soc. 62: 171–181.

Hope, R.M. (2022). Rmisc: Ryan Miscellaneous. https://CRAN.R-project.org/package=Rmisc

Jackman, S. (2020). pscl: Classes and Methods for R Developed in the Political Science Computational Laboratory. United States Studies Centre, University of Sydney. Sydney, New South Wales, Australia. R package version 1.5.5.1. URL https://github.com/atahk/pscl/

Jacquier, L., Molet, M., & Doums, C. (2023). Urban colonies are less aggressive but forage more than their forest counterparts in the ant *Temnothorax nylanderi*. *Animal Behavior*, 199, 11–21. https://doi.org/10.1016/j.anbehav.2023.02.004.

Kassambara, A. (2022). ggpubr: 'ggplot2' Based Publication Ready Plots. https://CRAN.R-project.org/package=ggpubr

Kaspari, M., Yanoviak, S., & Dudley, R. (2008). On the biogeography of salt limitation: A study of ant communities. Proceedings of the National Academy of Science USA, 105, 17848–17851. https://doi. org/10.1073/pnas.0804528105

Kaspari, M., Welti E. A. R., & deBeurs K. M. (2020). The nutritional geography of ants: gradients of sodium and sugar limitation across North American grasslands. *Journal of Animal Ecology*, 89, 276–284. https://doi.org/10.1111/1365-2656.13120

Korányi, D., Szigeti, V., Mezőfi, L. et al. 2021. Urbanization alters the abundance and composition of predator communities and leads to aphid outbreaks on urban trees. *Urban Ecosystems*, 24, 571–586. https://doi.org/10.1007/s11252-020-01061-8

Legaspi, J. C., & Legaspi B. C. (1998) Life History Trade-Offs in Insects with Emphasis on Podisus maculiventris(Say) (Heteroptera: Pentatomidae). , pp. 71-87 In M. Coll, J. R. Ruberson, and D. Rosen [eds.], Predatory Heteroptera: Their Ecology and Use in Biological Control . Entomological Society of America, Lanham, Md.

Legaspi, J. C., & O'Neil R. J. (1994). Lipids and egg production of *Podisus maculiventris* (Heteroptera: Pentatomidae) under low rates of predation. *Environmental Entomology*, 23, 1254–1259. https://doi.org/10.1093/ee/23.5.1254

Lenth, R.V. (2023). emmeans: Estimated Marginal Means, aka Least-Squares Means. R Package Version 1.8.4-1. https://CRAN.R-project.org/package=emmeans.

Leroy, P. D., Wathelet, B., Sabri, A., Francis, F., Verheggen, F. J., Capella, Q., Thonart, P., & Haubruge, E. (2011). Aphid-host plant interactions: does aphid honeydew exactly reflect the host plant amino acid composition? *Arthropod-Plant Interactions*, 5, 193–199. https://doi.org/10.1007/s11829-011-9128-5

Lescano, M. N., Quintero, C., Farji-Brener, A. G., & Balseiro, E. (2022). Excessive nutrient input induces an ecological cost for aphids by modifying their attractiveness towards mutualist ants. *Functional Ecology*, 36, 2661–2672. https://doi.org/10.1111/1365-2435.14163.

Łopucki, R., Klich, D. & Kiersztyn, A. (2021). Changes in the social behavior of urban animals: more aggression or tolerance?. *Mammalian Biology*, 101, 1–10. https://doi.org/10.1007/s42991-020-00075-1

Mailleux, A. C., Deneubourg, J. L., & Detrain C. (2003) How does colony growth influence communication in ants? *Insectes Sociaux*, 50, 24–31. https://doi.org/10.1007/s000400300004

McWilliams, S. R. (2011) Ecology of Vertebrate Nutrition. In: eLS. John Wiley & Sons, Ltd: Chichester. https://doi.org/10.1002/9780470015902.a0003211.pub2

Negroni, M. A., Feldmeyer, B. & Foitzik, S. (2021). Experimental increase in fecundity causes upregulation of fecundityand body maintenance genes in the fat body of ant queens. *Biology Letters*, 17, 20200909. https://doi.org/10.1098/rsbl.2020.0909

Nwaogu, C. J., Galema, A., Cresswell, W., Dietz, M. W., & Tieleman, B. I. (2020). A fruit diet rather than invertebrate diet maintains a robust innate immunity in an omnivorous tropical songbird. *Journal of Animal Ecology*, 89(3), 867–883. https://doi.org/10.1111/1365-2656.13152.

Ooms, J. (2023) magick: Advanced Graphics and Image-Processing in R. https://CRAN.R-project.org/package=magick

Penick, C. A., Savage, A. M., & Dunn, R. R. (2015). Stable isotopes reveal links between human food inputs and urban ant diets. *Proceedings of the Royal Society B: Biological Sciences*, 282(1806), 20142608. https://doi.org/10.1098/rspb.2014.2608.

Peterson, R. A. (2021). Finding Optimal Normalizing Transformations via bestNormalize. *The R Journal*, 13:1, 310-329. https://doi.org/10.32614/RJ-2021-041

Posit team, 2023. RStudio: Integrated Development Environment for R. Posit Software, PBC, Boston, MA. http://www.posit.co/.

Qu, J., Bonte, D., & Vandegehuchte, M. L. (2021). Phenotypic and genotypic divergence of plant–herbivore interactions along an urbanization gradient. *Evolutionary Applications*, 15, 865–877. https://doi.org/10.1111/eva.13376

R Core Team (2021). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rosumek, F. B., Brückner, A., Blüthgen, N., Menzel, F., & Heethoff, M. (2017). Patterns and dynamics of neutral lipid fatty acids in ants – implications for ecological studies. *Frontiers in Zoology*, 14, 36. https:// doi.org/10.1186/s12983-017-0221-1

Seifert, B. (2018). The ants of central and north Europe. Lutra Verlags-und Vertriebsgesellschaft

Shik, J., & Dussutour, A. (2020). Nutritional Dimensions of Invasive Success. *Trends in Ecology & Evolution*, 35, 8. https://doi.org/10.1016/j.tree.2020.03.009

Shrewsbury, P. M., & Raupp, M. J. (2006). Do top-down or bottom-up forces determine *Stephanitis pyrioides* abundance in urban landscapes? *Ecological Applications*, 16(1), 262–72. https:// doi.org/10.1890/04-1347

Simpson, S. J., Raubenheimer, D. (2012). The Nature of Nutrition: A Unifying Framework from Animal Adaptation to Human Obesity, Princeton University Press: Princeton, NJ, USA.

Spann T. M., Schumann, A. W. (2009). The Role of Plant Nutrients in Disease Development with Emphasis on Citrus and Huanglongbing. *Proceedings of the Florida State Horticultural Society*, 122, 169–171.

Trigos-Peral, G., Rutkowski, T., Witek, M., Ślipiński, P., Babik, H., & Czechowski, W. (2020). Three categories of urban green areas and the effect of their different management on the communities of ants, spiders and harvestmen. *Urban Ecosystems*, 23, 803–818. https://doi.org/10.1007/s11252-020-00949-9.

Trigos-Peral, G., Abril, S., & Angulo, E. (2021). Behavioral responses to numerical differences when two invasive ants meet: the case of *Lasius neglectus* and *Linepithema humile*. *Biological Invasions*, 23, 935–953. https://doi.org/10.1007/s10530-020-02412-4

Trigos-Peral, G., Maák, I. E., Schmid, S., Chudzik, P., Czaczkes, T. J., Witek, M., Casacci, L. P., Sánchez-García, D., Lőrincz, Á., Kochanowski, M., & Heinze, J. (2024). Urban abiotic stressors drive changes in the foraging activity and colony growth of the black garden ant Lasius niger. *Science of The Total Environment* 915, 170157. https://doi.org/10.1016/j.scitotenv.2024.170157

Urbanek, S. (2022). png: Read and write PNG images. https://CRAN.R-project.org/package=png

Van der Have, T. M., Pedersen, J. S., & Boomsma, J. J. (2011). Mating, hybridisation and introgression in Lasius ants (Hymenoptera: Formicidae). *Myrmecological News*, 15, 109–115. https://doi.org/10.25849/myrmecol.news_015:109

Vergnes, A., Pellissier, V., Lemperiere, G. et al. (2014). Urban densification causes the decline of ground-dwelling arthropods. *Biodiversity Conservation*, 23, 1859–1877. https://doi.org/10.1007/s10531-014-0689-3

Vilà, M., Espinar, J. L., Hejda, M., Hulme, P. E., Jarošík, V., Maron, J. L., Pergl, J., Schaffner, U., Sun, Y., & Pyšek P. (2011). Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters*, 14, 702–708. https://doi.org/10.1111/j.1461-0248.2011.01628.x

Wendt, S., & Czaczkes, T. J. (2020). Labeling effect in insects: Cue associations influence perceived food value in ants (*Lasius niger*). *Journal of Comparative Psychology*, 134(3), 280–292. https://doi.org/10.1037/com0000212

Wickham, H. (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.

Supplementary material

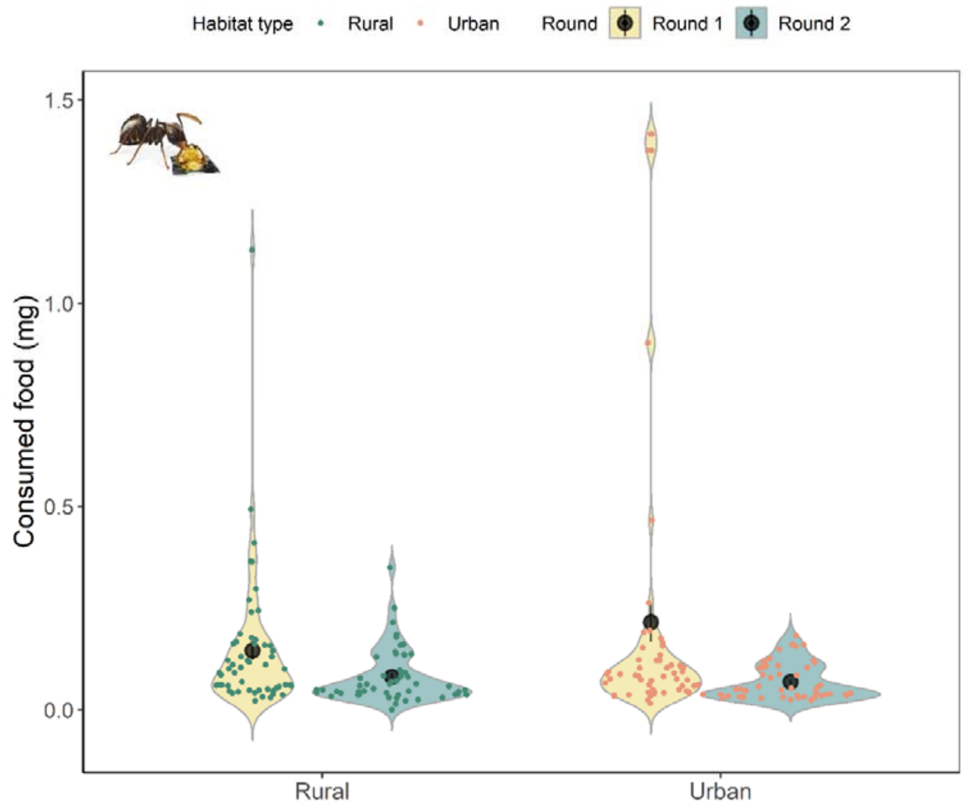
Supplementary table 1. Locations for the collection of the fragments of urban and rural colonies in Warsaw (Poland).

Urban areas	
Location	**GPS coordinates**
Ogród Saski Park	52.240482, 21.008921
Morskie Oko Park	52.205943, 21.026827
Marshal Edward Rydz-Śmigły Park	52.225446, 21.033746
Rural areas	
Location	**GPS coordinates**
Gmina Michałowice	52.159636, 20.873293
Klaudyn	52.272952, 20.861246
Warsaw Airport	52.146279, 20.987392

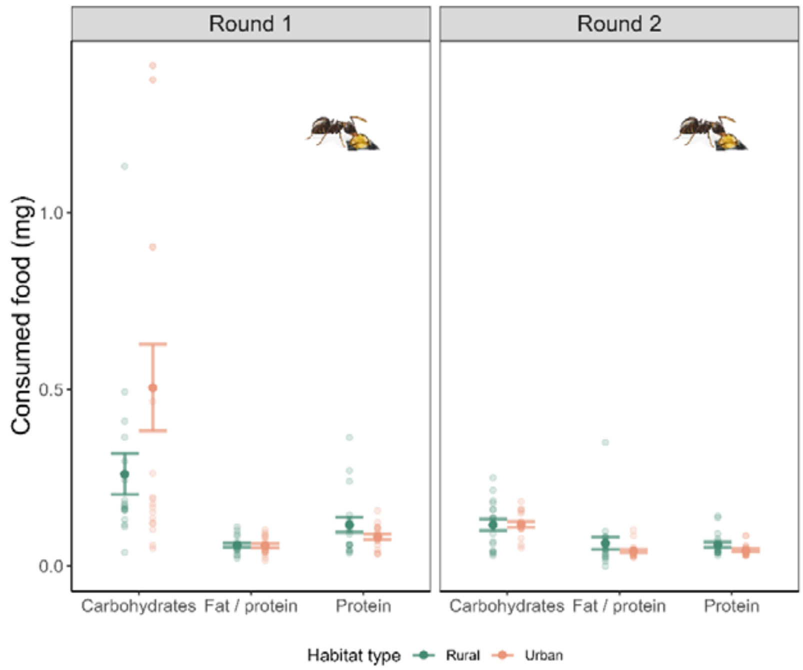
Supplementary table 2. Nutritional information in 100 gr of canned tuna in own juice (protein) and tuna in soybean oil (fat / protein) used in the experiments.

Proteins (tuna in own juice)	
Nutrient	**In 100 gr of product**
Proteins	23 gr
Fat	0.6 gr
Salt	1.05 gr
Carbohydrates	< 0.1 gr
Fat / proteins (tuna in soybean oil)	
Nutrient	**In 100 g of product**
Proteins	23.5 gr
Fat	9.5 gr
Salt	1.05 gr
Carbohydrates	< 0.1 gr

Supplementary figures



Supplementary figure 1. Violin plots showing the amount of food consumed (mg) by the colonies from urban and rural habitats during the first round of observations (one day after colonies collection) and the second round of observations (one week after collection and feeding at the laboratory under the same type of diets). The black circles show the average consumption per each population, whereas coloured dots represent the individual values for each urban (in pink) and rural (in green) experimental colony.



Supplementary figure 2. Mean and error bar plot showing the difference in the amount of food (mg) consumed by *Lasius niger* workers from urban and rural colonies during the two rounds of the experiment (one day after collection and one week after collection and feeding on a standard diet). The full circles indicate the means of the consumption by the rural and urban colonies, and the whiskers represent the standard deviation. Open circles represent the individual values per each experimental colony.

Data availability statement - The data used in this study are available through the link to figshare (https://figshare.com/s/20b4ecf6e7b571b9ca13) in the manuscript. Scripts and outputs are also available through the former link. Doi: 10.6084/m9.figshare.25513309