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RESEARCH ARTICLE

Differences in individual behavior and trophic niche of the red wood ant *Formica aquilonia* from urban and natural habitats: a case study in Novosibirsk region (West Siberia, Russia)

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Abstract

Behavior plays a key role in the ability of animals to adapt to human-induced environmental change, although the consistency of these behavioral changes and their links to ecological niche are less clear. In this study, we examined the effects of habitat type and season on behavioral variation, trophic isotopic niche, and their relationships in the ant *Formica aquilonia*, a keystone species of forest communities in North Eurasia, collected from an urban forest park and a natural forest in spring and autumn. We found that exploratory activity was lower in urban than in natural forest ants. Aggression scores towards competitors (*Lasius fuliginosus*, *L. niger*) were similar in both habitats, but urban ants interacted longer with *L. niger*. Individual ant responses were positively correlated as an aggression-boldness syndrome (more aggressive individuals are more exploratory) in the natural habitat, but not in the urban environment, where the association between aggression and exploration was negative in autumn and decoupled in spring. The trophic niche of the ants differed between the habitats, expressed by lower $\delta^{15}N$ and $\delta^{13}C$ values in urban ants. It indicates that ants in the urban park fed at a lower trophic level and presumably consumed less tree-related resources than in the natural forest. Exploratory activity was positively correlated with $\delta^{13}C$ value, suggesting a possible link between personality traits and ant diet. Our results on ants support findings on human-induced behavioral changes in vertebrates and highlight the need for further studies on the adaptation of wood ants to life in urban environments.

Keywords
Anthropogenic disturbance, conservation, behavior, nutrition, stable isotopes, animal personality, urbanization

Introduction

Urbanization greatly reduces biodiversity and alters animal communities through habitat transformation and disturbance, pollution, and microclimate change (Philpott et al. 2009; Faeth et al. 2011; Szabó et al. 2023). This leads to a homogenization and simplification of community structure relative to surrounding natural and rural areas, including the replacement of specialists by generalists and the spread of invasive species in cities (McKinney 2006; Piano et al. 2020a). While research has focused on the ecological success of urban exploiters and colonizers, the mechanisms of adaptation of native species to urban environments have been less well studied (Lowry et al. 2013; Sol et al. 2012; Tryjanowski et al. 2020). In particular, the links between behavioral traits, diet, and the ability to adapt to disturbance in urban green spaces in native species remain poorly understood.

Behavior plays a key role in the ability of animals to adapt to human-induced environmental changes, including urbanization (Wilson et al. 2020; Gunn et al. 2022). Successful urban species have been demonstrated to be less likely to avoid danger, more aggressive, and faster at exploiting new resources than closely related non-urban species (Carrete et al. 2010; Sol et al. 2013; Kralj-Fišer et al. 2017; Miranda 2017). At the species level, conspecifics from urban populations exhibit higher average activity, exploration, boldness, and aggression compared to non-urban populations (Lowry et al. 2013; Miranda et al. 2013; Ritzel and Gallo 2020). This behavioral phenotype is assumed to be adaptive, as it reduces the fear response to new objects and facilitates the acquisition of new food resources (Sol et al. 2013).

Individual variation in behavior can be consistent over time (‘animal personality’) and across situations (‘behavioral syndromes’; Réale et al. 2007). For example, more aggressive individuals demonstrate more exploratory and risk-taking behavior. Similar behavioral syndromes may differ among populations living in different environments and are associated with trade-offs, where beneficial responses in one context, e.g. food exploration, may be coupled with disadvantageous responses in another context, e.g. risk of predation (Sih et al. 2004; Jandt et al. 2014). Urbanization has been found to alter the relationships between personality traits, with decoupling of behavioral syndrome observed in birds (Evans et al. 2010; Scales et al. 2011; Bókony et al. 2012; Miranda et al. 2013), suggesting higher behavioral plasticity and greater among-individual variation in urban populations (Sih et al. 2010; Caspi et al. 2022). Fewer studies examined whether similar changes occur in invertebrates (Kralj-Fišer et al. 2017; Kaiser et al. 2020; Jacquier et al. 2023).

Urbanization significantly alters the trophic niche and affects the fitness of animals (Pollock et al. 2017; Gámez et al. 2022; Reid et al. 2024). Urban areas provide higher availability of anthropogenic subsidies, mainly carbohydrate foods, and lower availability of natural foods (Penick et al. 2015; Gámez et al. 2022). As a result, urban populations exhibited trophic plasticity and niche expansion (birds: Pagani-Núñez et al. 2019; mammals: Castañeda et al. 2019), but tend to decrease reproductive success (Pollock et al. 2017). Animal personality may drive variation in individual dietary specialization within a population, with more exploratory and bold individuals feeding on more diverse and higher quality food resources of (generalists) compared to less exploratory individuals feeding as specialists (Toscano et al. 2016). On a broader scale, links between trophic niche and animal personality traits have recently been addressed (Glon et al. 2016; Theódórsson and Ólafsdóttir 2022) and remain largely unexplored in the context of urbanization (Newsome et al. 2015; Herath et al. 2021).

Behavioral adaptations and their ecological consequences, including dietary changes in urban populations, have been studied primarily in birds and mammals (Newsome et al. 2015; Pollock et al. 2017; Sol et al. 2018; Ritzel and Gallo 2020). Invertebrates remain largely unexplored in this regard, although they are providers of important ecosystem functions and services, e.g. pollination, pest control, and decomposition (Kotze et al. 2022). Among arthropod taxa, ants are the most abundant group in urban areas and play an important role in the ecology of cities (Santos 2016; Perfecto and Philpott 2023). Ants are used as indicators of ecosystem change (Belskaya et al. 2017; Putyatina et al. 2017). Novel urbanized environments can provide habitat for heat-tolerant ant species (Menke et al. 2011) as well as generalist and opportunist species, especially those with large colonies and high aggression and competitive ability (Philpott et al. 2009; Santos 2016), including invasive ant species (Holway and Suarez 2006). Ant species that successfully colonize urban areas may exhibit changes in foraging and aggressive behavior, primarily related to heat tolerance (Philpott et al. 2009; Menke et al. 2011; Harris et al. 2024). For native ants, changes in the behavior and trophic niche of specialist species that allow them to adapt to life in disturbed habitats within cities remain poorly understood (Jacquier et al. 2023).

Here, we explore among-individual variation in worker aggressive and exploratory behavior and isotopic niche of the red wood ant *Formica aquilonia* from an urban forest park and a natural site in birch-dominated mixed forests in the Novosibirsk region, southern West Siberia. As ecosystem engineers and forest specialist species, red wood ants alter the composition and abundance of organisms within the community through seed dispersal, habitat construction, competition, and trophic interactions (Zakharov 2015; Stockan and Robinson 2016; Mikhaleiko et al. 2024), including close mutualistic relationships with tree-dwelling honeydew-producing aphids (Novgorodova 2005). The wood ant *F. aquilonia* is a behaviorally dominant species in temperate and boreal forest ant communities, characterized by large colony sizes, protected foraging territory, polydomy and supercolonial settlements with high nest density (Dlussky 1967; Zakharov 2015; Stockan and Robinson 2016).

Since studies on the effects of urbanization on behavioral traits and behavioral syndromes in arthropods are few and inconsistent (Kaiser et al. 2020; Magura et al. 2022; Jacquier et al. 2023), based on patterns across vertebrate taxa (Scales et al. 2011; Bókony et al. 2012; Miranda et al. 2013), we expected to find more aggressive and exploratory behavior, but a decoupled aggression-exploration syndrome, in urban *F. aquilonia* compared to rural conspecifics. In addition, we examine seasonal variation in behavior. In red wood ants, workers tend to be more active in searching for food and more aggressive toward competitors in early spring, when the colony's foraging territory is being re-established and food is scarce, than in summer and autumn (Rosengren 1977; Mabelis 1978). Next, we examined differences in ant trophic niche between sites and their relationships with behavior using stable isotope composition, as it reflects resource use over a prolonged period of time (1-2 months; Tillberg et al. 2006). Nitrogen and carbon stable isotope values ($\delta^{15}N$, $\delta^{13}C$) were used to assess ant trophic position and food resources, respectively (Feldhaar et al. 2010). We hypothesized that compared to a natural forest in an urban park with lower canopy cover, *F. aquilonia* ants consume resources (aphid honeydew, herbivorous insects) from the grass layer with lower $\delta^{13}C$ values to a greater extent than resources from the tree layer with higher $\delta^{13}C$ values due to the canopy effect (Iakovlev et al. 2017). We used the approach of combining field measures of trophic niche (stable isotopes) and laboratory-measured behavioral traits to investigate ecological relevance of personality (Glon et al. 2016).

We used a series of four behavioral tests to examine exploration of novel environments (in an open field test without and with novel artificial objects) and aggression toward two competitor ant species, *Lasius fuliginosus* as a facultative dominant in forest communities in the absence of red wood ants and *L. niger* as a potential competitor dominant in urban and open habitats (Seifert 2018). Our hypotheses were that (i) urban *F. aquilonia* ants are more aggressive and exploratory than their rural conspecifics; (ii) the among-individual correlation between aggression and exploratory behavior is weaker in urban than in rural sites; (iii) ants are more aggressive and exploratory in spring than in autumn; (iv) urban ants have lower $\delta^{13}C$ values than rural ants. Relationships between behavior and trophic niche were studied without predictions.

Materials and methods

Study sites

The study was conducted in the Novosibirsk region, West Siberia, Russia, in 2023–2024. The urban and natural study sites are located on the right bank of the Ob River on the border of forest and forest-steppe zones in the territory of mixed forests dominated by birch.

(1) The urban forest park "Dom uchyonyh" is located in the city of Novosibirsk (54°50.17'N, 83°6.23'E). The park was established about 65 years ago in a natural forest area and is now represented by a limited area of sparse mixed forest dominated by *Betula pendula*, inclusions of *Pinus sylvestris*, with plantings of *Picea abies*, *Prunus padus* and *Sorbus aucuparia*, with flower beds and lawns (canopy cover up to 60-70%, total area about 8 ha). High anthropogenic pressures include fragmentation (network of asphalt roads and paths, lawn patches), proximity to residential buildings (high human foot traffic, dog walking, human waste), mowing and harvesting of grass near paths, cutting and removal of dead trees. The study area is 1 ha. The nest density of *F. aquilonia* here is about 35 nests per ha. Nests of ants *L. niger* and *Myrmica rubra* were observed in the territory of the studied *F. aquilonia* colonies, while *F. cunicularia*, *F. fusca* and *L. flavus* are found near the territory of the studied colonies.

(2) The natural forest site is located 28 km from the urban park near the villages of Mostovaya and Gusiniy brod (55°3.76'N, 83°17.91'E). This site is a mixed forest dominated by *Betula pendula*, less represented by *Populus tremula*, with inclusions of *Pinus sylvestris* and predominance of *Aegopodium podagraria* in the grass layer (canopy cover 90-100%). Anthropogenic pressure is insignificant. The study area is 1 ha. The nest density of *F. aquilonia* is about 15 nests per ha. Nests of other ant species were not recorded in the territory of the studied *F. aquilonia* colonies; nests of *F. fusca*, *M. rubra* and *Leptothorax acervorum* were found in the vicinity of the territories of the studied ant colonies.

Five *F. aquilonia* colonies were selected at each study site. The nests were separated by more than 50 m so that there was no contact between them. Vegetation cover was similar in all five foraging territories of *F. aquilonia* colonies within the study sites. To minimize the factor of colony size and stage of sociogenesis, small, young and degenerating nest mounds were avoided. We selected focal colonies from the most active colonies without damaged nest mounds and heavy vegetation overgrowth, with a large growing dome and several foraging trail columns (Table 1).

Table 1. Characteristics of nest size and foraging territory of the studied colonies of *Formica aquilonia*

Site	Colony ID	Anthill diameter, cm	Dome diameter, cm	Anthill height, cm	Dome height, cm	No of foraging trails (columns)	No of foraging trees*
Urban park	U1	190	130	40	30	4	5
Urban park	U2	150	100	35	20	3	5
Urban park	U3	130	90	35	20	3	3
Urban park	U4	225	150	50	35	5	6
Urban park	U5	150	105	45	20	4	6
Natural forest	N1	220	110	60	30	5	4

Site	Colony ID	Anthill diameter, cm	Dome diameter, cm	Anthill height, cm	Dome height, cm	No of foraging trails (columns)	No of foraging trees*
Natural forest	N2	270	160	100	55	4	6
Natural forest	N3	240	170	65	45	4	4
Natural forest	N4	130	95	50	30	4	5
Natural forest	N6	230	120	55	20	3	4

Note: * – data on foraging trees over 10 cm in diameter are provided.

Laboratory maintenance of ant colonies

The behavioral study was conducted on eight *F. aquilonia* colonies, four colonies each from urban and natural sites (colonies U1-U4 and N1-N4, Table 1) in the autumn (August – September 2023) and spring (May – June 2024). Colony units consisted of 500-800 workers collected from foraging trails, foraging trees, the nest surface and inside the nest mound from a depth of 10 cm, without queens and brood. Each colony unit with nest material was placed in a container (40x30x15 cm) with a plaster bottom, access to water, periodic moistening and feeding twice a week with a 20% sugar solution. Laboratory colonies adapted to the conditions (12 hours daylight, 24-26^{0}C) for three weeks prior to the start of the experiments. This was done to reduce the influence of environmental factors on ant behavior.

Behavioral assays

For experiments in each colony unit, 30 workers were collected from individuals active outside the nest, marked with nitro dyes and placed in a separate container (25x15x15cm, with water, sugar feeder and shelter). After 24 hours, 15 individuals were randomly selected for behavioral testings. Workers were tested in four behavioral assays: (1) Aggression towards the competing ant *Lasius fuliginosus*; (2) Aggression towards the competing ant *Lasius niger*; (3) Exploration in an 'open field' test; (4) Response to a novel environment. Workers from the same colony performed all behavioral tests on the same day in random order, except for test (4), which always followed test (3). The interval between tests was at least 1 hour. In all tests, the adaptation period after placing the ant on the test arena was 1 minute (the ant was covered with a 35 mm Petri dish and then the dish was carefully removed). To prevent ants from escaping from the test arena, the walls were covered with a layer of talcum powder.

In tests (1) and (2), aggression was measured by confronting each worker with a freshly thawed dead worker of competitor collected from single colony more than 5 km away from the study sites. We used dead workers as opponents to maintain the intact odor of competitors and to eliminate behavioral differences between stimuli associated with response to live opponents. Ants were killed by freezing at -20°C and thawed 10 min before the experiments. A new corpse was used for each worker tested. The focal ant was placed in a 90 mm Petri dish, and during the adaptation period a competitor corpse was carefully placed at the edge of the Petri dish. The first encounter, defined as the first behavior of the focal ant with the corpse, was recorded and scored as follows: avoiding (rapid movement of the focal ant in the opposite direction) = 0; antennation with closed mandibles = 1; mandible opening or antennation with open mandibles = 2; short bite (not longer than 1 second) = 3; prolonged bite (2-5 seconds) = 4; death grip including formic acid spray (more than 5 seconds) = 5; seizure (the focal ant transports the competitor in the mandibles) = 6. In addition to the aggression score, the duration of the ant's contact with the competitor was recorded for a maximum of 1 minute.

(3) The exploration test was conducted similarly to the open field test by placing the ant in a circular test arena (160 mm diameter). The total time the ant spent in the central area of the arena (130 mm diameter) and the frequency of visits to the central area were counted for 3 minutes as indicators of exploratory activity. To examine (4) response to novel environments, at the end of test (3) the ant was covered with a shaded 35 mm Petri dish for 1 minute and new objects were placed in the test arena: an artificial shelter (50x25x5 mm white cardboard shelter with two entrances) was placed against the arena wall and an artificial plant object (5 cm ball of kraft paper strips) was placed in the center of the arena (Figure 1). The time and frequency of visits to the objects and the central area of the test arena were recorded for 5 min. Visits to the central area and the artificial plant were taken as an indicator of high exploratory activity, and visits to the shelter as an indicator of low exploratory activity.

A total of 964 individual tests were conducted on 241 ants collected from 8 colonies in spring and autumn.

Stable isotope analysis

For stable isotope analyses, worker ants were collected from the surface of the nest mound in early August 2023 (n=5 per colony). Thorax samples were used to eliminate effects of abdominal fat and crop content on isotopic signature of ants (Feldhaar et al. 2010). Sample preparation included drying at 50°C, weighing (200–300 μg) and packing in tin capsules. Stable isotope analysis was conducted using a Thermo Delta V Plus continuous-flow IRMS was coupled to an elemental analyzer (Flash 1112) equipped with a Thermo No-Blank device at the Joint Usage Center of the A.N. Severtsov Institute of Ecology and Evolution RAS, Moscow. The isotopic composition of N and C was expressed in the δ notation relative to the international standards (atmospheric nitrogen and VPDB, respectively): $\delta X\ (‰) = [(R_{\mathrm{sample}}/R_{\mathrm{standard}}) - 1] \times 1000$, where R is the molar ratio of the heavier isotope to the lighter. The samples were analyzed with a reference gas calibrated against the International Atomic Energy Agency (IAEA) reference materials USGS 40 and USGS 41 (glutamic acid). The measurement accuracy was ±0.2‰. Along with the isotopic analysis, the nitrogen and carbon content (as %) and C/N ratios were determined. In total, 50 ant samples were analyzed.

Mean δ^{13}C and δ^{15}N values of the most abundant plant species (leaves, leaf litter) are typically used as a baseline in isotopic studies of litter-dwelling macroinvertebrates, including ants (Korobushkin et al. 2014). To establish the isotopic baseline, two mixed leaf samples were collected from five birch trees at each study site. Significant differences between urban and natural habitats were observed in $\delta^{15}N_{leaf}$ values (mean±SD: 2.2±0.5 and 1.4±0.7, respectively; t(18)=2.7, p=0.014, t-test) and $\delta^{13}C_{leaf}$ values (mean±SD: -28.5±0.9 and -30.6±1.2, respectively; t(18)=4.4, p<0.0005, t-test). Therefore the mean values of δ^{13}Cleaf and $\delta^{15}N_{leaf}$ were used for baseline correction: $\delta^{13}C_{ant} = \delta^{13}C_{raw}$ ant – $\delta^{13}C_{average\ leaf\ in\ habitat}$, $\delta^{15}N_{ant} = \delta^{15}N_{raw\ ant} - \delta^{15}N_{average\ leaf\ in\ habitat}$.

Figure 1. Experimental setup for studying ant response to novel environment. The artificial plant object and the shelter are placed in the center and at the edge of the test arena, respectively. The black circle marks the central area of the test arena.

Statistical analysis

We used a generalized linear mixed model (GLMM) to examine whether site, season, or its interaction explained variation in individual behavioral variables. A Poisson distribution with log link function was used in these models for count data (aggression scores, number of visits). A Gamma distribution with identity link function was used for time variables. Colony ID was included as a random effect to account for potential differences between ant colonies within sites. This effect was not significant in all models. Site * season interaction was reported when significant. Post hoc pairwise comparisons using t-tests, corrected with Holm's sequential

Bonferroni procedure, were used to identify significant differences between sites (urban vs. natural) and seasons (autumn vs. spring). We used nonparametric Spearman rank correlations to test for correlations between behavioral traits measured in different behavioral assays. The results presented here are pooled data from all ant colonies for each site. Student's t-test was used to compare differences in isotopic composition of ants between sites. Spearman rank correlations were used to examine links between isotopic composition and behavioral traits of ant colonies. All analyses were performed with SPSS 22.0 (IBM, Armonk, NY, USA).

Results

Behavioral differences between sites and seasons

Aggression scores towards *L. niger* were higher in autumn compared to spring (F(3, 237)=2.66, p=0.049, Table 2, Figure 2A), but no difference between sites was observed (Table 2). Duration of contact with *L. niger* was higher in the urban park than in the natural forest (F(3, 230)=7.21, p<0.001; Table 2, Figure 2B), and it was higher in autumn than in spring (Table 2, Figure 2C). Variation in aggression scores towards *L. fuliginosus* was not significant (F(3, 237)=1.02, p=0.386), but duration of contact with *L. fuliginosus* was higher in autumn than in spring (F(3, 237)=5.33, p=0.001; Table 2, Figure 2D). Aggression scores of *F. aquilonia* ants were higher when interacting with *L. fuliginosus* compared to their aggression scores towards *L. niger* (median [Q1; Q3]: 3[2; 4] and 2[1; 3], respectively; Z=-5.3, p<0.0005, Wilcoxon signed rank test).

In the open field test, the total time spent by ants in the central area was higher in natural forest than in urban park (F(3, 234)=3.93, p=0.009; Table 2, Figure 2E) and did not differ between seasons (Table 2), while the number of visits to the central area was higher in autumn than in spring (F(3, 237)=12.57, p<0.0005; Table 2, Figure 2F). In the novel environment test, the time spent by ants in the central area was higher in spring than in autumn (F(3, 228)=8.94, p=0.009; Table 2, Figure 2G), and it was higher in natural forest than in urban park in spring but not in autumn (Table 2, Figure 2H). The number of visits to plant object was higher in autumn than in spring (F(3, 237)=4.51, p=0.004; Table 2, Figure 2I). Variation in number of visits and time spent in shelter was not significant.

The behavioral syndrome was found in the form of correlations between behavioral measures in different tests (Figure 3). In similar situations (interacting with different competitors or exploring open field and novel environment), correlations between behavioral measures were moderate and stronger (absolute value of Spearman correlation coefficient (r) ranged from 0.26 to 0.53) than correlations between behavior in different situations comparing aggressive and exploratory responses (absolute ranged from 0.25 to 0.34; Figure 3). For ants from natural habitats, correlations between measures of aggression (higher scores and longer duration of contact) and measures of exploratory activity (higher number of visits and total time spent by ants in the central area and novel plant object, and lower number of visits and total time spent in the shelter) were observed in both seasons (Figure 3A, B), i.e. more aggressive individuals are more exploratory. Urban ants did not show such correlations. In urban ants, relationships between measures of aggression and exploratory activity (number of visits and total time spent in the central area of the open field and novel environment tests) were negative in autumn with more aggressive individuals being less exploratory (Figure 3C), and these relationships were absent in spring (Figure 3D).

Table 2. Summary of results of generalized linear mixed model. Results are presented for behavioral variables when the overall model was significant. Significant p-values for fixed effects are in bold

Model / Fixed effect	Estimate	SE	t-value	p-value
Aggression score to *L. niger*				
Site	0.10	0.13	0.74	0.458
Season	-0.25	0.12	-2.10	**0.037**
Duration of contact with *L. niger*				
Site	9.63	4.53	2.13	**0.034**
Season	-5.68	1.92	-2.96	**0.003**
Duration of contact with *L. fuliginosus*				
Site	-4.07	3.97	-1.03	0.307
Season	-9.55	3.30	-2.90	**0.004**
Exploration: Total time in central area				
Site	-9.11	4.20	-2.17	**0.031**
Season	-4.61	4.43	-1.04	0.300
Exploration: Central area visits				
Site	-0.14	0.11	-1.34	0.183
Season	-0.19	0.07	-2.97	**0.003**
Novel environment: Total time in central area				
Site	-9.23	5.75	-1.61	0.109
Season	32.93	8.43	3.91	**<0.001**
Site * Season	-21.9	10.10	-2.09	**0.038**
Novel environment: Plant object visits				
Site	0.15	0.15	1.01	0.315
Season	-0.38	0.14	-2.68	**0.008**

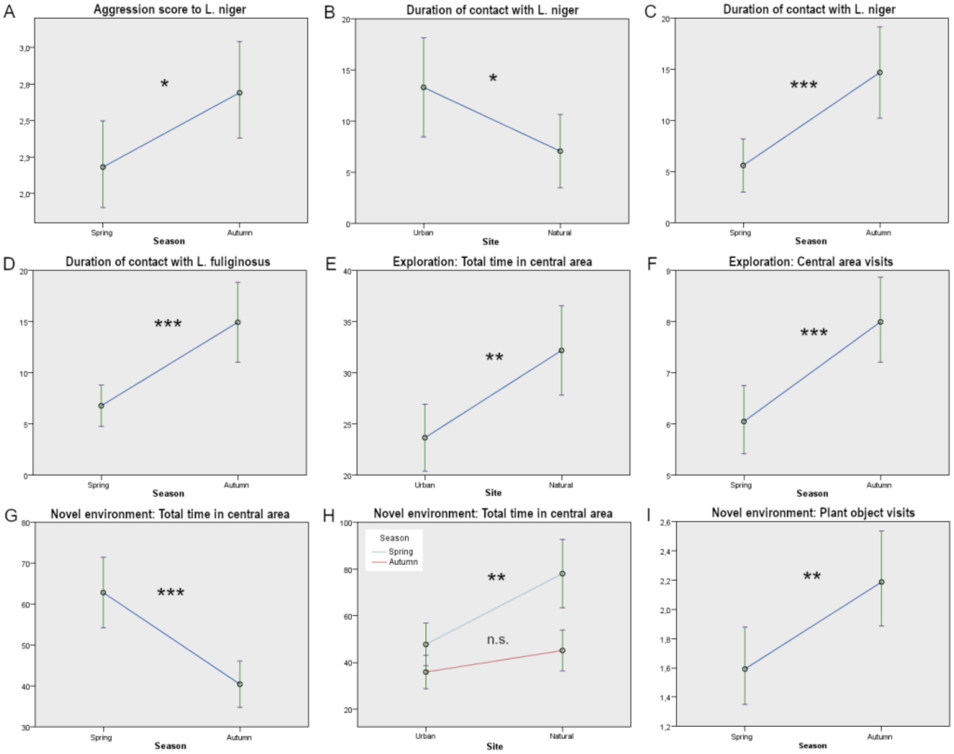

Figure 2. Habitat-dependent and seasonal differences in the behavior of F. aquilonia workers: aggression towards *L. niger* (A–C), aggression towards *L. fuliginosus* (D), exploration (E, F), response to novel environment (G–I). Significant differences in GLMM post hoc t-tests, corrected with Holm's sequential Bonferroni procedure, are shown: * – $p<0.05$, ** – $p<0.005$, *** – $p<0$. Estimated marginal means and 95% confidence intervals are shown.

Trophic isotopic niche and its relationship to behavioral traits

Ants from the natural forest had 1.0‰ higher $\delta^{15}N$ values compared to ants from the urban forest park (mean±SD: 3.8±0.4 and 4. 7±0.4‰, respectively; $t(48)=-8.0$, $p<0.0001$, t-test) and 0.5‰ higher $\delta^{13}C$ values (mean±SD: 4.2±0.4 and 4.7±0.4‰, respectively; $t(48)=-4.4$, $p<0.0001$, t-test; Figure 4A).

A positive correlation was found between the time spent by an ant in the central zone of the open field test arena and the average $\delta^{13}C$ value of the ant colony (Spearman's $r = 0.75$, $p=0.031$, $n=8$; Figure 4B) and, at the tendency level, the range of $\delta^{13}C$ values of the ant colony (Spearman's $r = 0.56$, $p=0.146$, $n=8$). Correlations between isotopic composition and other behavioral traits were not significant.

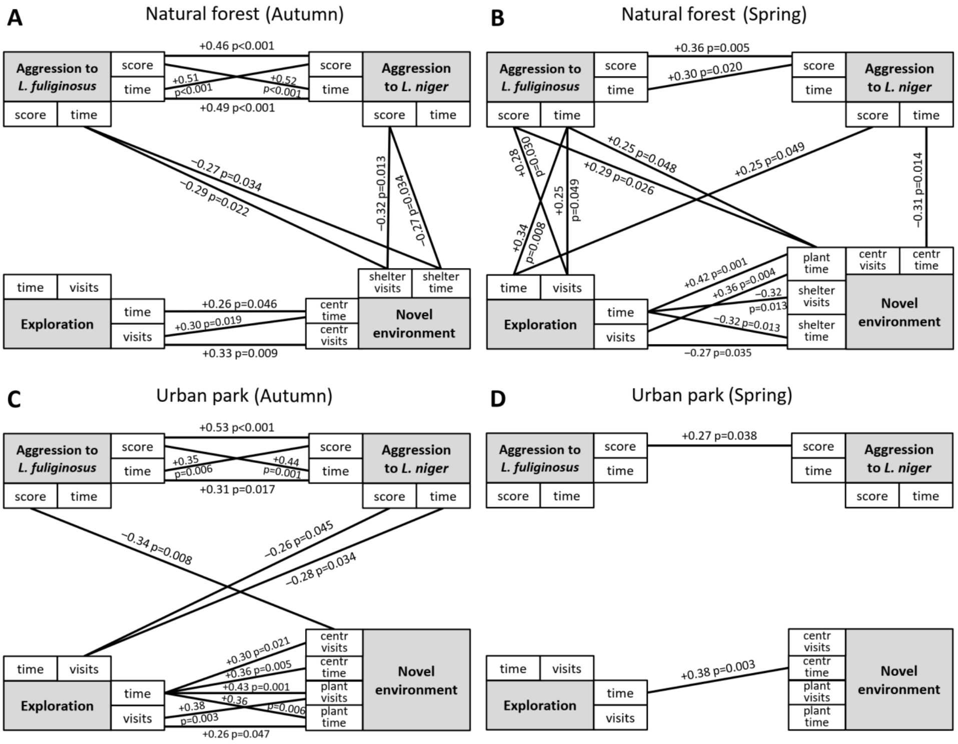

Figure 3. Correlations between behavioral traits measured in four behavioral assays for ants from natural forest (A, B) and urban forest park (C, D) in autumn (A, C) and spring (B, D). Black lines depict significant correlations. Spearman rank correlation coefficients and p-values are reported. In aggression tests, 'time' means duration of contact with competitor and 'score' means aggression score. In exploration test, 'visits' and 'time' mean number of visits and total time spent by ants in the central area of the test arena. In novel environment test, 'visits' and 'time' mean number of visits and total time spent by ants in a specific zone ('centr' is central area, 'plant' is artificial plant object, 'shelter' is artificial shelter).

Discussion

This case study shows differences in the behavior and ecological niche of red wood ants from urban and natural habitats. The results suggest that during adaptation to anthropogenic disturbance of a forest community in an urban park, the 'relict' population of red wood ants exhibited reduced exploratory activity, altered responses to the urban colonizer ant species *Lasius niger*, and decoupling of behavioral consistency between aggression and exploration, as well as reduced trophic position and changes in food resources.

Many studies have reported higher exploratory activity, bolder (when encountering a predator), and more aggressive behavior in urban populations than in rural conspecifics (Lowry et al. 2013; Miranda et al. 2013; Miranda 2017; Ritzel and Gallo 2020). Contrary to these studies on vertebrates, *F. aquilonia* ants in urban habitat appeared to be less exploratory than their rural conspecifics, with similar levels of aggression towards competitors. We attribute these differences in exploratory activity mainly to environmental differences between habitats, although genetic variation may also influence them (Mäki-Petäys et al. 2005). Since colony maintenance in the laboratory was the same during the experiments and three weeks before, environmental influences are likely to be due to early developmental conditions, nutrition and experience of workers in the wild. Environmental factors may include the modification of the park habitat (isolation and fragmentation of the forest area by the road network, high human traffic, periodic mowing and leaf collection) and the limited dispersal ability of red wood ants. Both study sites are inhabited by large settlements of *F. aquilonia* (supercolonies of about 100 nests). However, the 8 ha forest park has been restricted by urban infrastructure and buildings for more than 60 years. In contrast to urban colonizer ants such as *Lasius*, which have higher dispersal abilities due to independent colony establishment, the predominant mode of reproduction and dispersal in *F. aquilonia* is short-distant nest budding of new colonies (Hakala et al. 2019). The limited dispersal of *F. aquilonia* in the park is indicated by the low number of newly established bud-nests (Iakovlev, Maslov 2018) and higher nest density (35 vs. 15 nests per ha in natural forest). Together with human disturbance and lower canopy cover in the park than in the forest, this may increase competition and resource exploitation (Sorvari and Hakkarainen 2004; Salas-López et al. 2022) and alter the pattern of foraging activity of wood ants in the urban park, including less exploration of new areas but more defense and resource exploitation within colony territory. This hypothesis remains to be tested. The complication of competitive relationships in the urban park may be indicated by the colonization of open areas by *L. niger* near *F. aquilonia* colonies and by a higher duration of contact with *L. niger* in wood ants, including longer inspection and biting of the competitor, compared to wood ants from rural habitat, where *L. niger* did not occur in the territory of *F. aquilonia*. It is worth noting that, on average, the aggression score towards *L. niger* was one point lower than the aggression score towards *L. fuliginosus*, indicating a higher degree of competition between *F. aquilonia* and *L. fuliginosus*.

Data on the effects of urbanization on insect behavior are few and contradictory. In ground beetles, one study showed an urbanization-associated decrease in exploratory activity (Magura et al. 2021) and another study showed an increase in activity, but only in the first of a two-year study (Schuett et al. 2018). In rove beetles, urbanization had no effect on exploratory and risk-taking behavior (Magura et al. 2022). In butterflies, habitat type (urban, agricultural, or woodland habitat) had no effect on activity or boldness (Kaiser et al. 2020). In ants, urban colonies of *Temnothorax nylanderi* were less aggressive toward conspecifics and performed more foraging trips than forest colonies (Jacquier et al. 2023). Urban *L. niger* ants forage more actively at night (Trigos-Peral et al. 2024) and defend aphids from enemies more aggressively (Gaber et al. 2024) than conspecific colonies in rural habitats. In two out of three cavity-dwelling ants, urban populations showed a higher defensive response to threat relative to rural populations (Harris et al. 2024). It is suggested that increased exploratory behavior may not confer additional fitness benefits to good dispersers (flying beetles and butterflies) that are able to find suitable microhabitats even in urban environments (Magura et al. 2022). Conversely, for the wood ant *F. aquilonia*, it can be hypothesized that in the fragmented habitat of an urban park, the poor disperser species may reduce the costs of interacting with competitors and human traffic by decreasing the exploratory activity of workers.

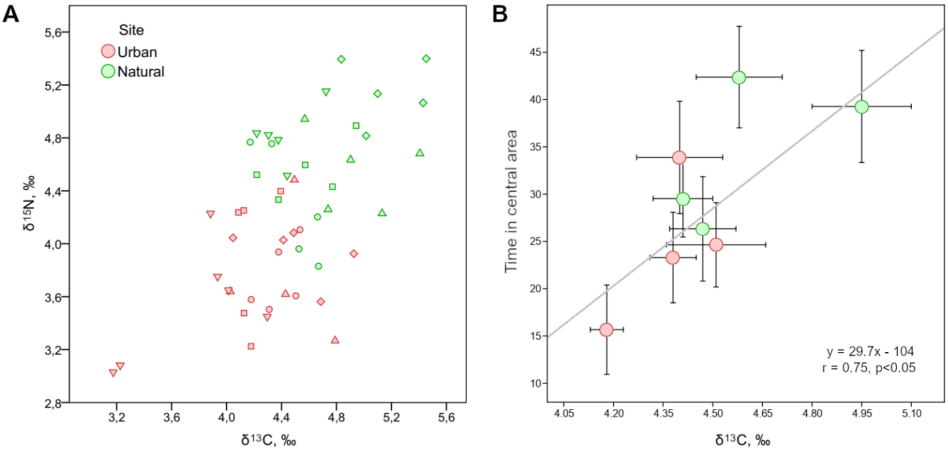

Figure 4. Carbon and nitrogen stable isotope composition of *Formica aquilonia* ants (A). Within each site, different colonies are represented by signs of different shapes. Correlation between carbon isotope composition of ant colonies and exploratory activity in the open field test (B). Mean±SEM are reported. Regression line and equation are shown (Spearman rank correlation, $p<0.05$).

Surprisingly, *F. aquilonia* ants were more aggressive and exploratory in autumn than in spring. These differences were evident in both urban and natural habitats. This does not support our hypothesis, which is based on the fact that red wood ants, as a territorial and dominant species, actively re-establish the foraging territory of the colony in spring and can show high levels of aggressiveness towards conspecifics (Rosengren 1977; Mabelis 1978). In other mound-building wood ant *F. exsecta*, seasonality has no effect on interspecific aggression (Katzerke et al. 2006). The explanation is probably related to the low food availability in spring and the high resource-holding potential of numerous colonies of aphids, whose honeydew the red wood ants collect in large quantities in autumn and process into fat reserves before hibernation (Zakharov and Zakharov 2022).

Analysis of among-individual behavioral variation revealed correlations in the behavior of *F. aquilonia* workers within similar contexts (more aggressive responses to different competitors; higher performance in the open field test and the novel environment test) and across different contexts (more aggressive individuals were more exploratory). This indicates the presence of an individual-level behavioral syndrome in *F. aquilonia* similar to the aggression-boldness syndrome found in various taxonomic groups (Sih et al. 2004; Jandt et al. 2014), including ants (Bengston, Dornhaus 2014; Reznikova 2021; Contala et al. 2024). According to our hypothesis, this behavioral syndrome was exhibited by ants from the natural forest in spring and autumn, but was uncoupled in spring and reversed in autumn (more aggressive individuals were less exploratory) in ants from the urban park. We suggest that this is related to the general aggressiveness of the species and more pronounced territoriality in the limited spatial conditions of the urban park.

Our findings of behavioral syndrome variation in ants are consistent with vertebrate studies showing behavioral correlations in rural but not urban populations (Evans et al. 2010; Scales et al. 2011; Miranda et al. 2013). The disruption of behavioral syndromes indicates an important role for behavioral plasticity in the adaptation of animals to life in urban environments (Miranda 2017; Caspi et al. 2022). Behavioral plasticity allows organisms to deal more efficiently with the variuos challenges of the novel urban environment, such as finding and exploiting of new resources and coping with potentially dangerous situations (Sol et al. 2013; Miranda 2017). Among invertebrate studies, evidence from spiders also supports an important role for behavioral plasticity in the adaptation of individuals to urban environments (Kralj-Fišer et al. 2017), but conversely, in the ant *T. nylanderi* and the butterfly *Pararge aegeria*, behavioral traits correlate in urban habitats but not in forest habitats (Kaiser et al. 2020; Jacquier et al. 2023).

Trophic niches of ants were measured using carbon and nitrogen isotope composition, which is formed in ants within 1-2 months and reflects a time-integrated assessment of their diet and trophic position in food webs (Tillberg et al. 2006; Feldhaar et al. 2010). As hypothesized, the urban population of *F. aquilonia* had a lower (by 0.5‰) δ^{13}C value than ants from the forest, presumably due to higher consumption of ^{13}C-depleted resources (aphid honeydew and herbivores from the grass layer) and lower consumption of ^{13}C-enriched resources (honeydew and prey from the tree layer; litter-dwelling arthropods) (Iakovlev et al. 2017). This could be related to the transformation of the plant community in the studied urban park (lower density of mature trees, planting of shrubs and saplings, litter removal, lawns) and possible changes in the urban arthropod community (decrease in abundance of saprophagous and predatory groups; Piano et al. 2020b; Kotze et al. 2022).

The lower δ^{15}N value (by 1.0‰) of urban ants compared to conspecifics from the natural forest indicates that *F. aquilonia* fed at a lower trophic level in the urban park. This is partly consistent with the variation in ant δ^{13}C values between sites. Presumably, differences in ant δ^{15}N values could be the result of a higher proportion of ^{15}N-depleted herbivores (e.g., hemipterans and caterpillars) than ^{15}N-enriched predatory litter-dwelling arthropods (e.g., spiders and beetles) in ant prey in the urban park compared to the natural forest (Iakovlev et al. 2017). Similarly, a lower trophic position for ant assemblage was shown in revegetation sites compared to natural remnant sites (Gibb and Cunningham 2010). This was attributed to the high availability of plant sugars, honeydew, and herbivorous arthropod prey in disturbed habitats and more complex food webs with ^{15}N-enriched omnivorous and predaceous arthropod prey in natural habitats (Gibb and Cunningham 2010).

However, differences in the trophic niche between urban and rural ant populations depend on ant species, habitat type, and local factors. In New York, ant species in traffic islands used human resources more than park species. Some ant species living in the most urbanized habitats, such as *Tetramorium* sp. had higher δ^{13}C values associated with human food (with ^{13}C-enriched corn and sugarcane), but no differences in δ^{15}N values, suggesting feeding at the same trophic level (Penick et al. 2015). In two cities in Taiwan, the ant *Pheidole megacephala* in urban forests had higher δ^{13}C and δ^{15}N values relative to periurban forest habitats, suggesting a higher trophic position and possibly higher intake of human food and animal-based resources in urban areas (Peng et al. 2023). In Novosibirsk, it is difficult to assess the contribution of human food to the diet of the urban population of *F. aquilonia* due to the low content of corn and sugarcane in human food, whereas the lower trophic position of ants in the city park seems to be determined by a more 'herbivorous' diet.

We applied an approach combining laboratory behavioral data with field data on long-term feeding (Glon et al. 2016) to ants at the colony level, using average measures for an ant colony instead of individual measures. Interestingly, the only significant and high correlation (r=0.75) was found between the average δ^{13}C value of an ant colony and an average measure of worker exploratory activity (time spent by ants in the central area of the open field test). Variation in δ^{13}C value between *F. aquilonia* colonies reflects long-term trophic differences in nature (Feldhaar et al. 2010), presumably related to persistent differences in the consumption of ^{13}C-enriched tree-associated resources and ^{13}C-depleted grass-associated resources, such as honeydew or herbivore prey (Iakovlev et al. 2017). Higher exploratory behavior in workers from colonies with presumably higher consumption of honeydew of tree-dwelling aphids may be related to the quantity or quality of differentially preferred honeydew (Völkl et al. 1999). The tendency for a positive association between the range of ant colony δ^{13}C values and exploratory activity may indicate more diverse food resources in more exploratory colonies. In ants, the positive effects of an abundant carbohydrate diet over several weeks to months on activity, exploratory behavior, and worker aggression, as well as colony productivity, are well established (Grover et al. 2007; Wittman et al. 2018). However, the effect of short-term variations in feeding and other environmental factors on the behaviour of *F. aquilonia* is likely to be minimal, as all colonies were fed a 20% sugar solution in the laboratory and kept under identical conditions for three weeks prior to the experiments. It remains unclear how long the effect of diet on ant behavior may last. It is necessary to test the relationships between ant personality traits and trophic niche indicators on *F. aquilonia* in other parts of its range, as well as on other ant species.

One of the main limitations of the present study is the use of one pair of urban and natural sites in one region of the species' range. However, the fact that patterns similar to those observed here have been found for other taxa in urban habitats supports our findings. A challenge for further expansion of the study is that red wood ants are not often found in urban forest parks, especially in the numerous settlements, as natural forest fragments with the original presence of these ant species in the city are rarely preserved (Putyatina et al. 2017; Balzani et al. 2022). Red wood ants, as keystone species providing important ecosystem services in forest communities (e.g., pest control; Zakharov 2015), need to be conserved, including in disturbed habitats where they are threatened with extinction (Iakovlev, Maslov 2018; Balzani et al. 2022).

Conclusion

The results of this case study suggest that urbanization not only influences the expression of exploratory activity and response to the competitor, but also, similar to vertebrates, may alter the relationship between exploration and aggression in ants. It is suggested that differences in the trophic niche of red wood ants between urban and natural habitats indicate the role of trophic plasticity in adaptation and highlight the ecological consequences of human-induced habitat change. From a conservation perspective, it is noteworthy that urban forest parks with high human traffic are able to maintain keystone specialist species, but urban planning and management should take into account wood ant habitat characteristics and implement practices to reduce anthropogenic pressure in urban forest areas.

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