

No effect of Zn-pollution on the energy content in the black garden ant

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Abstract Social insects may display a response to environmental pollution at the colony level. The key trait of an ant colony is to share energy between castes in order to maintain the existing adult population and to feed the brood. In the present study we calorimetrically measured the energy content per body mass (J/mg) of adults and pupae of workers, males and females of the black garden ant *Lasius niger*. The ants were sampled from 37 wild colonies originating from 19 sites located along the metal pollution gradient established in a post-mining area in Poland. The cost of metal detoxification seen as a possible reduction in energy content with increasing pollution was found neither for pupae nor adults. However, a considerable part of variance in energy content is explained by belonging to the same colony. These findings stress the importance of colony-specific factors and/or the interaction of these factors with specific site in shaping the response of ants to metal-pollution stress. Colony-related factors may constrain possible selfish decisions of workers over energy allocation in workers and sexual castes.

Keywords Ants · *Lasius niger* · Colony · Castes · Caloric value · Pollution gradient · Detoxification costs · Queen-worker conflict

Introduction

In ants each individual colony member can be described by recognizable morphological and physiological traits. Simultaneously, the colony is not just a random set of multiple individuals; a higher level of organization results in a new colony-specific traits that differentiate ants (as well as other social insects) from solitary non-social insects (Oster and Wilson 1978). Many examples of individual-level effects of industrial pollution have already been described (reviewed in Grześ 2010a; Sorvari and Eeva 2010; De La Riva et al. 2014). Effects at the colony-level have received relatively less attention. It has been shown that red wood ants (*Formica* s. str.) tend to develop smaller colonies in polluted areas compared to the reference sites (Eeva et al. 2004). Similar studies considering variable stress regimes as low temperature or high predation showed that certain colony-level traits are associated with the capacity of ant colonies to adapt to environmental stress. For example, an enhanced tolerance to disturbance in opportunistic ant species is underlined by the queen's short lifespan, polygyny and polymorphy as well as with frequent colony migration (reviewed in Linksvayer and Janssen 2009).

The key trait of an ant colony is the sharing of food resources between adults and the brood. Unlike larvae that require proteins and lipids, adults use mainly carbohydrates as an energy source (Blüthgen and Feldhaar 2010). In addition, the energy allocation pattern changes with the age of the colony. In the initial stage of a colony, new workers are produced intensively, contributing to an increase in the colony's growth and efficiency. Then, after crossing a certain colony size, a considerable part of the available energy is allocated to the sexual castes (Baroni-Urbani et al. 1978; Peakin and Josens 1978). Independently of the

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colony's age, the total amount of energy influx into the colony depends only on workers foraging for food outside the nest. Inside the nest, food is distributed among larvae by nursing workers. Among the larvae, the female-larvae have the highest energy requirements (Boomsma and Isaaks 1985; Baroni-Urbani et al. 1978). In turn, the adult females and males stay in the maternal colony till the mating flight and remain strongly dependent on resources brought into the nest by foraging workers.

This study concerns the colony-level energy allocation in workers and sexual castes of the black garden ant *Lasius niger* (L., 1758) inhabiting post-mining area polluted with high concentrations of zinc, cadmium and lead. We measured calorimetrically the amount of energy content expressed as caloric value per body mass unit (J/mg dry weight, d.w.) in six life stages of *L. niger* collected along the metal-pollution gradient. The life stages considered in the study are as follows: pupae and adult females, pupae and adult males, pupae and adult workers. We expected the energy content of all investigated life stages to decrease with increasing metal-pollution level of the site for three reasons. Metal-toxicity may cause (1) a decrease in the number of workers (Grześ 2010b), (2) the lower food supply caused by the decline in species diversity of many groups of arthropods (e.g., Kools et al. 2009; Skalski et al. 2010; Li et al. 2014), (3) increased energy expenses due to detoxification costs (Sibly and Calow 1989; Coustau et al. 2000; Bednarska and Stachowicz 2013).

It was proved previously that in the investigated species, workers that feed larvae are able to distinguish between worker- and sexual-larvae (Jemielity and Keller 2003) and may manipulate offspring numbers, sex ratio and resource allocation for their own genetic interests (Chapuisat and Keller 1999; Meunier et al. 2008). Workers by investing the resources into sexual castes may increase their inclusive fitness as males and females are the only means of transfer the workers' genes to the future generations (Trivers and Hare 1976). Therefore, we originally formulated the hypothesis that pollution affects workers and sexual castes differently. If the energy is indeed limited in reproductive colonies, feeding may be biased in favor of the females instead of workers. As a consequence, the caloric content along the pollution gradient should decrease more acutely in workers than in females and males.

Materials and methods

Study species

Black garden ants *L. niger* are monomorphic (no morphological subcastes of workers) and are strictly monogynous (each colony has one queen). This species builds

mineral nest mounds that usually contain from one hundred to more than ten thousand workers. They inhabit various anthropogenic environments and are widespread in the holoarctic area. The species is both carnivorous and aphidicolous. Nuptial flights take place from July to late August (Czechowski et al. 2012). Our previous study revealed that *L. niger* is the most abundant ant species inhabiting the investigated area; its relative abundance accounted for more than 70 % of all ant species (Grześ 2009a).

Study area

The study sites are located in the post-mining area of the Bolesław smelter in southern Poland. The area in the closest vicinity of the smelter is strongly polluted by Zn, Cd and Pb. Previous studies showed that metal total concentrations in the top soil in the meadow located 0.6 km from the pollution source was 2229 mg Zn/kg, 5.56 mg Cd/kg and 814 mg Pb/kg (Stefanowicz et al. 2008). Metal concentrations in the soil are highly correlated with each other (Zygmunt et al. 2006; Stefanowicz et al. 2014).

For the purpose of the present study nineteen sites (S1–S19) were established along the pollution gradient covering abandoned fields (14 sites), mown meadows (2 sites) and industrial wastelands (3 sites). The transect extended from 0.7 to 35 km from the pollution source. Metal concentrations in the soil at the sites decreased with increasing distance to the smelter, approaching background levels at the sites further than about 25 km from the smelter (Table 1). Ants in field conditions are exposed to environmental metal pollution via the trophic route (Maavara et al. 1994). Therefore, the pollution level of each of the 19 study sites was expressed as Zn total concentrations in a random sample of invertebrates, that potentially may consist the diet of the investigated species. Invertebrates were collected at each site in a preliminary study in the summer of 2011. The Zn concentration in invertebrates correlated positively with both the Cd concentration in invertebrates and the Zn total soil concentrations. The lowest and highest Zn concentrations detected in invertebrates accounted for 135 and 1545 mg/kg d.w. in the S19 and S14 sites, respectively (Table 1). Detailed Zn and Cd concentrations in soil and invertebrate samples as well as botanical description of all sites are reported in Grześ et al. 2015.

Sampling

In each of the 19 sites, 1–3 mature colonies were chosen. In total 37 colonies were investigated. Each colony was checked for the presence of males and females. Samples of life stages of the investigated ant (i.e. pupae and adult females, pupae and adult males, pupae and adult workers)

Table 1 Metal pollution at each study site expressed as Zn concentration in the random sample of invertebrates, the number of colonies sampled from particular site, colony size measured as the nest diameter, the number of replicates of each life stage for the particular colony, and the number of individuals in each sample

Site	Zn (mg/kg d.w.) ^a	Colony	Colony size (nest diameter) (cm)	Life stage	Number of replicates	Number of individuals per replicate
S19	135	A	65	MA	2	16, 15
				FP	3	5, 5, 5
				MP	2	20, 20
				WP	2	34, 31
S4	197	A	43	FA	3	2, 2, 2
				FP	3	5, 5, 5
				WP	2	35, 35
				MA	2	3, 3
		B	42	MA	1	7
				WA	2	24, 24
				FP	3	5, 5, 5
				MP	2	16, 17
		C	53	WP	2	36, 50
				FA	2	4, 4
				FP	2	5, 5
				MP	3	25, 25, 20
S2	252	A	54	WP	1	53
				FA	1	2
				FP	3	5, 5, 5
				MP	2	20, 19
		B	47	WP	1	49
				FP	3	5, 5, 5
				MP	1	25
		C	33	FP	3	5, 5, 5
				WP	1	29
		S7	253	A	36	FA
MA	2					32, 31
FP	3					5, 5, 5
MP	1					26
B	48			WP	2	35, 35
				FP	1	7
				MP	2	14, 13
				WP	2	30, 30
C	44			FA	3	2, 2, 2
				MA	3	30, 52, 30
				MA	1	1
				MA	1	10
S8	261	A	40	WA	2	40, 40
				FA	1	3
		B	49	FA	1	3
				MA	2	11, 10
S3	281	A	37	FA	3	2, 2, 3
				MA	1	36
				WA	2	35, 35
				MP	2	15, 16

Table 1 continued

Site	Zn (mg/kg d.w.) ^a	Colony	Colony size (nest diameter) (cm)	Life stage	Number of replicates	Number of individuals per replicate
S1	300	A	63	FA	1	2
				MP	3	25, 25, 25
				WP	1	33
		B	65	FA	1	3
				FP	3	5, 5, 5
				MP	1	33
S5	322	A	39	FA	1	1
				WA	2	40, 40
				FP	3	5, 5, 5
				MP	1	22
				WP	2	39, 40
				S9	331	A
S6	421	A	47	FA	3	2, 2, 3
				MA	1	37
				FP	3	5, 5, 5
				WP	2	34, 36
S17	480	A	60	FA	3	2, 2, 2
				FP	3	5, 5, 5
				MP	2	24, 26
		B	28	WP	2	33, 30
				FP	3	5, 5, 5
				WP	2	40, 50
S11	646	A	55	FA	3	2, 2, 2
				FP	3	5, 5, 5
				WP	2	32, 36
		B	68	FP	4	5, 5, 5, 5
				MP	2	20, 20
				MA	1	31
S12	687	A	56	FP	3	5, 5, 5
				MP	2	27, 28
				FA	2	2, 2
		B	58	MA	2	36, 36
				FP	4	5, 5, 5, 5
				MP	1	19
		C	48	WP	2	42, 38
				FA	1	3
				MA	2	33, 32
S16	747	A	38	WP	2	40, 40
				FA	3	2, 2, 2
				MA	1	29
		B	54	WA	2	29, 30
				FP	3	5, 5, 5
				MP	3	16, 15, 24
		C	48	WP	2	40, 40
				MA	2	36, 36
				FP	4	5, 5, 5, 5

Table 1 continued

Site	Zn (mg/kg d.w.) ^a	Colony	Colony size (nest diameter) (cm)	Life stage	Number of replicates	Number of individuals per replicate
S10	930	A	84	FA	2	2, 3
				MA	2	20, 23
				WA	2	45, 45
				FP	3	5, 5, 5
		B	45	MP	1	12
				MA	2	11, 12
				FP	3	5, 5, 5
S13	1121	A	80	WP	2	32, 31
				FA	3	2, 2, 2
				MA	1	9
				WA	3	32, 34, 34
		B	60	FP	3	5, 5, 5
				MP	1	11
				WP	3	28, 27, 26
S15	1141	A	58	FA	2	2, 2
				MA	2	25, 21
				FP	3	5, 5, 5
				MP	1	20
				WP	2	34, 31
		B	60	FA	2	2, 2
				MA	2	35, 33
				WA	1	23
				FP	3	5, 5, 5
				MP	2	20, 20
S18	1362	A	50	WP	2	35, 35
				FA	2	2, 2
				MA	1	28
		B	54	WP	2	40, 40
				WP	2	36, 36
S14	1545	A	55	FA	2	3, 3
				MA	1	9
				FP	3	5, 5, 5
				MP	2	16, 16
				WP	1	36
		B	70	FA	3	2, 2, 2
				MA	3	22, 21, 22
				WA	2	45, 45
				FP	3	5, 5, 5
				MP	2	19, 21
				WP	2	30, 29

^a After Grześ et al. 2015

FA female adult, MA male adult, WA worker adult, FP female pupa, MP male pupa, WP worker pupa

were collected, if present, from the colony from the uppermost nest chambers in July 2012. Because the time of pupation and the eclosion differed between the colonies, obtaining all life stages from every investigated nest was

difficult. Therefore, although we performed the sampling twice, most of the colonies were not represented by all investigated life stages and the number of collected individuals differed between life stages and the colonies (see

Table 1). In consequence, the study design was not orthogonal. This problem is inherent with the field studies where the life stages per colony, number of samples, number of replicates as well as the number of individuals per sample are not controlled by the researcher. However, as it is indicated in Table 1 the samples of each life stage do not gather round more polluted or less polluted sites, but they are distributed randomly along the gradient. As energy investments in castes may differ between small and large colonies, only colonies of large mounds were selected for the purpose of this study, based on their basal area diameter (Holec et al. 2006). In all colonies used in this study, the mound diameter ranged from between 0.4 and 1 m. Because in *L. niger* the nest volume grows almost proportionally to the colony size (Rasse and Deneubourg 2001), we used the nest basal area diameter as an estimator of the colony size.

The collected pupae and adults were transported to the laboratory. The pupae were separated carefully between workers, males and females based on their size (Boomsma and Isaaks 1985; Jemielity and Keller 2003). For the purpose of energy content measurements, different number of replicates, 1–4, depending on the presence of a particular life stage, were prepared for different colonies (Table 1). Life stages differed considerably in their body mass, therefore the samples were uneven between life stages in the number of individuals used. A given replicate contained approx. 5 female pupae, 20 male pupae, 35 worker pupae, 3 female adults, 24 male adults or 35 worker adults (Table 1). Samples were stored at $-5\text{ }^{\circ}\text{C}$ till the measurements of energy content.

Measurements of energy content

The energy content of each sample was measured using an oxygen micro-bomb calorimeter (MBC-3 type, see Klekowski and Bęczkowski 1973 for a description of the device). The calorimeter was calibrated with benzoic acid of a known caloric value, using a range of standard sample masses covering a range of readings obtained for the ants. Ants representing each replicate were lyophilized for 24 h. Due to the technical problems some samples were additionally dried by a halogene moisture analyser (Mettler Toledo) to dry weight before pellet preparation ($105\text{ }^{\circ}\text{C}$, 3 min). Dried samples were pressed into a small pellet weighing 5–40 mg depending on the life stage of the ants. The precisely weighed pellet ($\pm 0.1\text{ mg}$) was then placed in a small platinum capsule and connected to the micro-bomb electrodes with steel wire. Complete combustion was performed by ignition under high oxygen pressure (28 bar). The heat released from the pellet was digitally recorded with high resolution and the caloric value of the pellet was calculated per J/mg dry body mass against the calibration curve.

Statistical analysis

For the analysis of the energy content we used Generalized Linear Mixed Model (GLMM) procedures in Statgraphics Centurion XVI (version 16.11.1 for Windows). Energy content was used as a dependent variable in the model. The factors that were used as explanatory variables were: “caste”, “colony” nested in the “study site” [“colony (site)”], “pollution level” i.e. Zn concentrations in invertebrates and “colony size” i.e. mound diameter. The “caste” and the “colony” were of categorical character, while the “pollution level” and “colony size” were defined as quantitative factors. The “colony” and the “site” were entered as random factors to the model, while the “caste” was used as a fixed factor. Pupae and adults were analyzed separately by fitting the same model as described above. Hence, two independent analyses were performed (pupae, adults).

Results

Caloric values differed considerably between investigated life stages. The highest mean caloric value (28.2 J/mg d.w.) was found for adult females and the lowest (20.0 J/mg d.w.) for worker adults. Interestingly, the measurements of energy content indicated that unlike males and workers, adult females reached higher caloric values than their pupae (Table 2).

GLMM analysis performed on pupae showed similar results to the parallel analysis performed on adults. Testing the significance of each factor used in the model is summarized in Table 3. In both analyses, contrary to our expectations, no relationship was detected between the caloric value and Zn pollution of the sites (pupae: $F = 1.58$, $P = 0.23$ adults: $F = 0.18$, $P = 0.68$), meaning that the energy content of each developmental stage is insensitive to metal pollution. The relationship between pollution level and the colony size was also not detected (pupae: $F = 0.11$, $P = 0.75$ adults: $F = 0.01$, $P = 0.94$). Significant differences in caloric value between castes were only obtained in adults ($F = 5.70$, $P = 0.005$), among which females reached the highest values (post hoc LSD test, $P < 0.05$).

In turn, caloric value differed significantly between the investigated colonies in both pupae ($F = 2.30$, $P = 0.005$) and adults ($F = 2.57$, $P = 0.009$). The colonies differed significantly in caloric values even within the same study site (see Fig. 1 for the analysis on pupae). This demonstrates that energy content is determined primarily by colony-specific factors rather than by differences between sites. All of the second-order interactions tested in the analysis were insignificant in both analyses ($P > 0.2$ in each case).

Table 2 Means \pm SE of caloric value (J/mg dry weight) of pupae and adults of queens, males and workers of *L. niger* collected in the present study and compared with the values reported by Boomsma and Isaaks (1985, mean \pm SD)

Life stage	Caloric value (J/mg)	
	Present study	After Boomsma and Isaaks (1985)
Female (queen) pupae	22.2 \pm 0.26 $\left(\begin{smallmatrix} 80 \\ 401 \end{smallmatrix} \right)$	21.0 \pm 0.7 $\left(\begin{smallmatrix} 27 \\ 76 \end{smallmatrix} \right)$
Female (queen) adults	28.2 \pm 0.44 $\left(\begin{smallmatrix} 53 \\ 141 \end{smallmatrix} \right)$	27.5 \pm 1.0 $\left(\begin{smallmatrix} 10 \\ 5 \end{smallmatrix} \right)$
Male pupae	23.3 \pm 0.31 $\left(\begin{smallmatrix} 41 \\ 825 \end{smallmatrix} \right)$	22.5 \pm 0.8 $\left(\begin{smallmatrix} 29 \\ 171 \end{smallmatrix} \right)$
Male adults	20.6 \pm 0.62 $\left(\begin{smallmatrix} 33 \\ 805 \end{smallmatrix} \right)$	18.2 \pm 1.3 $\left(\begin{smallmatrix} 90 \\ 754 \end{smallmatrix} \right)$
Worker pupae	22.5 \pm 0.31 $\left(\begin{smallmatrix} 46 \\ 1651 \end{smallmatrix} \right)$	No data
Worker adults	20.0 \pm 0.57 $\left(\begin{smallmatrix} 18 \\ 640 \end{smallmatrix} \right)$	No data

The number of calorimetric samples and the total number of individuals used are presented in brackets

Table 3 Summary of the results of two independent GLMM analyses for caloric values in the pupae and adults of the ant *Lasius niger* collected along the metal pollution gradient (19 sites)

Source	d.f.	F	P
Pupae			
Cast	2	0.84	0.436
Colony (site)	16	2.30	0.005
Pollution level	1	1.58	0.228
Colony size	1	0.11	0.746
Cast \times pollution level	2	0.02	0.980
Cast \times colony size	2	1.4	0.250
Adults			
Cast	2	5.70	0.005
Colony (site)	10	2.57	0.009
Pollution level	1	0.18	0.682
Colony size	1	0.01	0.937
Cast \times pollution level	2	0.11	0.892
Cast \times colony size	2	0.81	0.450

The pollution level was expressed as Zn total concentrations in a random sample of invertebrates in a preliminary study. Colony was nested within the site. Cast and colony (site) are categorical factors, pollution level and colony size are quantitative factors. Significant factors are in bold

Discussion

The present study was performed to test the changes in energy content per body dry weight in the adults and pupae of workers and sexual castes originating from wild colonies of black garden ant *L. niger* along the metal-pollution gradient. Contrary to expectations, no caste responded significantly to an increase in metal pollution. As a consequence, no shifts in energy allocation between workers and sexual castes were found. Instead, the “colony (site)”

factor was found to have a strong explanatory influence on the energy content.

Averaged energy content for the pupae and adults of sexuals are consistent with values reported by Boomsma and Isaaks (1985, Table 2). Similarly to these authors, we found that females, in contrast to males, increased in weight between eclosion and nuptial flight. When comparing female pupae to female adults, it appeared that after eclosion females gain ca.30 % energy per body weight unit. In turn, males lost about 12 % of body energy content. This suggests that young females are fed much more intensively than males before their nuptial flight.

In this study we found that neither pupae nor adults of the investigated ant species decreased in their energy content along an increasing level of Zn-pollution. This result does not meet our expectation that energy content should decrease. This expectation emerged from the assumption that detoxification has a cost (Sibly and Calow 1989; Coustau et al. 2000) and from possible food limitation in the most metal-contaminated sites due to the decrease in abundance and diversity in some groups of invertebrates in the study area (Kools et al. 2009; Skalski et al. 2010; Li et al. 2014). It was previously suggested that workers of *L. niger* can regulate body Cd concentration once it reaches a certain critical level (Grześ 2009b). Thus, although ants are likely to eliminate excessive amounts of metals from their bodies it neither affects the energy content of pupae nor of adults. The existence of detoxification costs expressed as the decrease of energy content along pollution gradients are, in general, seldom observed in the wild populations of invertebrates (Zygmunt et al. 2006; Bednarska and Stachowicz 2013). On the other hand, the significant decrease of caloric value in invertebrates exposed to artificially contaminated food was found in laboratory experiments (Maryański et al. 2002; but

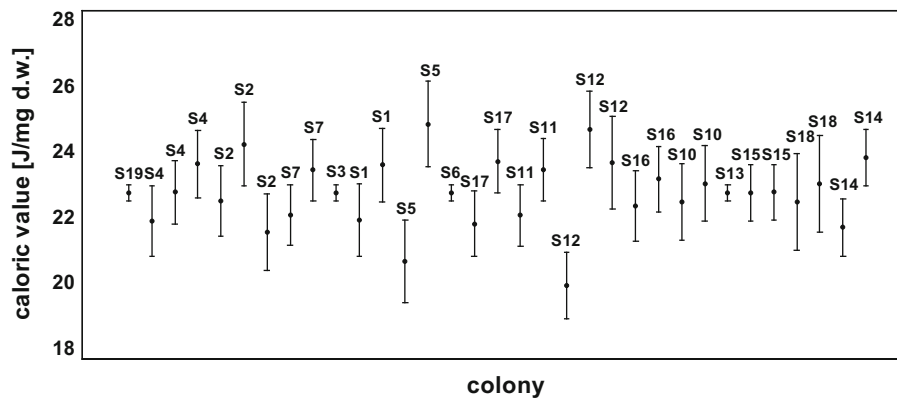


Fig. 1 Comparison of average caloric values (J/mg d.w.) between the colonies originating from the study sites S1–S19. The study sites are arranged according to increasing Zn concentrations in the samples of invertebrates used as an indicator of pollution. Each study site was represented by 1–3 colonies. Averages were calculated based on the

samples of female pupa, male pupa and worker pupa pooled within a given colony. The *dot* shows the average, the whisker shows the 95 % of LSD intervals. The colonies originating from S12 and S14 differ significantly between each other within the site

see Beaumelle et al. 2014). The difficulty to find detoxification costs in the organisms exposed in situ may emerge from the interactive influence of environmental factors that operate in the field that can potentially alter the effects of metal-pollution.

The lack of relationship between pollution level and energy content in *L. niger* indicates that the investigated ant can develop reproductive colonies in a highly polluted environment without any serious decrease of energy content in both workers and sexual castes. This may suggest the existence of a homeostatic mechanism. According to previous studies, homeostasis in ants may be mediated by foraging efficiency, which may compensate for the costs of living in a polluted environment. It was proved that foraging intensity can be adjusted to the current needs of the colony which can respond to environmental changes (Gordon 1987; Hölldobler and Wilson 1990; Bernadou and Fourcassiè 2008). In turn, queen-worker distinction in most ant species is determined by larval nutrition (reviewed in Anderson et al. 2008), confirming the strong relationship between ants and their environment.

Larval nutrition in the ant colony lies in the competences of nursing workers, namely sisters living in the same colony. In the case of the investigated species, the relatedness among sisters is particularly high: all workers constitute the offspring of the same mother (*L. niger* is monogynous) who usually mates only once or twice (Jemielity and Keller 2003). As predicted by kin selection theory (Hamilton 1964a, b), the high relatedness among sisters strengthen the queen-worker conflict. This is because workers may increase their inclusive fitness by allocating more resources into sisters ($r = 0.75$) than into much less genetically related brothers ($r = 0.25$). As a consequence, it can be expected that workers bias the

proportion of sex ratio towards females. Although workers of *L. niger* were proved to distinguish between males and females at the early stage of their larval development (Jemielity and Keller 2003), contrary to these expectations workers have weak control over sex ratio. Sex ratio was proved to be biased towards the queen's interest (females to males 1:1) rather than the workers' interest (3:1). It was also predicted that workers should have an interest in regulating the body size of sexuals, because females and males are the only means to transmit the workers genes to future generations (Fjerdingstad 2005) and larger females have greater fitness (Fjerdingstad and Keller 2004).

The detailed analysis on sexual size variation in *L. niger* of Fjerdingstad (2005) indicated that workers may have only partial control over the size of sexuals. This is because both genetic compounds and environmental factors explain a considerable part of variation in the body size of males and females in *L. niger*. These constrain (but do not exclude) the ability of workers to manipulate the size of sexuals towards the genetic interests of workers. Theoretically, it would also be beneficial for workers to control the energy content of the brood, thus its "quality". As a consequence, feeding sexuals should be favored over feeding workers especially in the stressful and potentially food-limited metal-polluted environment, in order to protect the males and females from a decrease in energy content. However, here we did not find any shifts in energy allocation between workers and sexual castes. In other words, sexual castes are not protected at the expense of worker larvae.

In this study the "colony (site)" factor was found to have a strong explanatory influence on caloric value. This observation suggests that similarly to the body size of sexual castes, the workers control over the energy allocation along the pollution gradient must be constrained by

colony-specific factors and/or by interaction of these factors with specific site.

However, it has to be emphasized that the nested model used in this study affects the interpretation of the significance of the “colony”. In this study we have nested “colony” (N = 37) in the “site” (N = 19), in order to avoid the pseudoreplication. In turn, nested designs do not allow to separate estimation of interaction variance (Ryan 2007; Schielzeth and Nakagawa 2013). The interaction variance inflates the main effect variance of the nested factor (Schielzeth and Nakagawa 2013). In consequence, in our study we can not fully discriminate if the variance explained by the nested factor “colony” is caused only by average differences between colonies and/or by between-colony differences in the effect of specific study site. In turn, factors explaining between-colony differences are complex. These theoretically involve: genes, maternal effects transmitted to the eggs by the queen and the colony-specific efficiency of workers to raise the brood. It is generally difficult to discriminate between maternal and direct genetic factors in ants (Meunier and Chapuisat 2009). To assess the explanatory importance of genetic compounds in *L. niger* wild populations a microsatellite analysis of each colony would be required in order to separate the effects of paternal lines that define genetically different subpopulations per colony of *L. niger* (Fjerdingstad 2005).

To summarize, we showed that the energy investments in the workers and sexual castes of black garden ant are not associated with metal-pollution level, but are influenced primarily by colony-related factors. Neither costs of detoxification nor energy-allocation shifts between castes were found. The results indicate that the investigated ant species can maintain reproductive colonies without any drastic decrease in energy content, hypothetically thanks to increased foraging efficiency.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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