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Pre-adaptive cadmium tolerance in the black garden ant

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HIGHLIGHTS

- *Lasius niger* showed high Cd-tolerance.
- Even a concentration of 1300 mg/kg Cd did not increase ant mortality.
- Mortality was not correlated with Cd-pollution of sites of origin.
- *L. niger* is pre-adapted to Cd environmental pollution.

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ABSTRACT

The black garden ant *Lasius niger* is a common component of habitats subjected to anthropological stress. The species can develop very abundant populations in metal-polluted areas. In this study, we raised the question of its tolerance to Cd pollution. Workers of *L. niger* were collected from 54 colonies, originating from 19 sites located along an increasing gradient of Cd pollution in Poland. Ants were exposed to a range of dietary Cd concentrations in a controlled 14-day laboratory experiment in order to test Cd-sensitivity in the investigated ants. The level of ant mortality was recorded as the endpoint of the experiment. We used much higher concentrations of dietary Cd than those the ants are most likely exposed to in field conditions. The investigated ants were highly Cd-tolerant; even a very high dietary Cd concentration of approx. 1300 mg/kg did not affect mortality of workers when compared to the control. Mortality was unrelated to Cd-pollution along the pollution gradient, meaning that high Cd-tolerance can be found even in ants from unpolluted areas. The results stress the importance of pre-adaptive mechanisms in the development of metal tolerance in ants.

1. Introduction

Many ant species can develop abundant and reproductive populations in areas subjected to environmental stress. Ants can be recorded even in highly metal-polluted areas, where the existence of many other more sensitive invertebrates is constrained or impossible (Rabitsch, 1995; Grześ, 2009a; Satta et al., 2012; Żmudzki and Laskowski, 2012). The black garden ant (*Lasius niger* L., 1758) can be abundant in harsh environmental conditions such as prolonged periods of hot weather (Zakharov and Zakharov, 2014), urban pressure (Vepsäläinen et al., 2008; Ślipinski et al., 2012), crop-field conditions and metal-polluted areas (reviewed in Pisarski, 1978; Satta et al., 2012), often being a dominant species. *L. niger* is also the most common species in the post-mining area of the Bolesław smelter in southern Poland. In the study area, mining has taken place since the eleventh century (Coppola et al., 2009), which has resulted in high soil contamination with zinc, cadmium and lead. In the vicinity of Bolesław, *L. niger* inhabits a range of different habitat types, reaching an abundance of 70% in relation to other ant species (Grześ, 2009a; Grześ et al., 2015). Therefore, the main question that should be asked is whether *L. niger* originating from metal-polluted areas is metal-tolerant due to long-term selection pressure. High metal tolerance, expressed as better survival, might explain (at least partially) the phenomenon of extraordinarily high abundance of this species in the investigated area.

The response of exposed populations of invertebrates may arise with heritable genetic change (adaptation), or by means of phenotypic plasticity within a given genome. Evidence for adaptations that can evolve in invertebrates chronically exposed to metal pollution have been found in many studies, including alterations in life histories, sustained growth or metallothionein gene duplication (Klerks and Weis, 1987; Posthuma and van Straalen,

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1993; Janssens at al., 2009). Research performed on laboratory-reared organisms has demonstrated that significant fitness-related changes may occur after a relatively short time (Guan and Wang, 2004; Łukasik and Laskowski, 2007). In turn, ants being social insects have evolved unique traits to survive even in very demanding habitats. These include traits, which are of a pre-adaptive character that make an ant colony an outstandingly flexible system when faced with environmental perturbations. Previous studies have revealed that colony-level traits in ants can respond to environmental stress. For example, the degrees of polydomy (number of nests per colony) and polygyny (number of queens per colony) might be shifted by means of colony fusion or budding (Hölldobler and Wilson, 1990). Ants may also adjust their foraging activity to current food availability, weather conditions or actual needs of the colony (Pętal, 1978; Gordon, 1987; Holec et al., 2006; Bernadou and Fourcassie, 2008). Furthermore, the distribution of body size may also be flexible. For example, the production of large, winged males, at the expense of wingless males, increases in *Cardiocondyla* ants in response to suboptimal temperatures, even though the production of such males requires much higher energetic investment than producing wingless males (Cremer and Heinze, 2003).

In the present study we performed a laboratory experiment on adult workers of the black garden ant *Lasius niger* originating from the pollution gradient in the vicinity of Bolesław smelter. Ants in field conditions are exposed to environmental metal pollution via the trophic route (Maavara et al., 1994); therefore in the present study the collected workers were exposed to artificially contaminated food. Three dietary Cd concentrations were used. The mortality of ants under Cd-exposure was recorded and used as an estimator of metal sensitivity. A similar method was applied previously to assess the tolerance of the ant *Myrmica rubra* to Zn (Grześ, 2010). We used high dietary Cd concentrations, because *a-priori* we expected low sensitivity of *L. niger* to Cd. Our previous study showed no effect of 150 mg/kg Cd on the mortality of *Myrmica* ants. In the present study we expected that ants originating from the most polluted sites, hence pre-exposed to pollution, would display better survival after being exposed to additional pollution. In other words, tolerance of ants exposed to dietary Cd was expected to correlate positively with the Cd-pollution gradient. The determination of which mechanism is responsible for Cd-tolerance in the investigated ant species was beyond the scope of the present study.

2. Materials and methods

2.1. Study species

The black garden ant (*Lasius niger* L., 1758) is one of the most common species in the Palearctic area, with an unusually wide ecological flexibility and great biological plasticity. *L. niger* colonies contain from a hundred to more than ten thousand workers (Czechowski et al., 2012). *L. niger* inhabits various anthropogenic habitats including farmlands, pastures, ruderal places or green spaces in urban areas. The species is both aphidicolous and carnivorous (Czechowski et al., 2012).

2.2. Study area

The study area is located in the vicinity of Olkusz in southern Poland. Metal concentrations in the humus layer at the most polluted sites of this region exceed 9600 mg/kg for Zn, 1500 mg/kg for Pb, and 80 mg/kg for Cd (Stone et al., 2001). Previous studies performed at the area showed that metal concentrations in the soil are highly correlated with each other (Zygmunt et al., 2006; Stefanowicz et al., 2014). Nineteen sites (S1–S19) were established along the pollution gradient covering abandoned fields and fresh meadows. The study sites were represented mostly by 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 of Cd in 15 cm-topsoil samples was correlated negatively with the distance from the smelter ($p < 0.05$, Table 1), approaching background levels at the sites located about 25 km or more from the smelter.

2.3. Pollution level

The pollution level of each site was expressed as Cd concentrations in a random sample of invertebrates. We decided to use the Cd concentrations in invertebrates rather than those detected in the soil samples or in the ant tissues to operate on more biologically realistic metal levels. It has been shown that ants were not relevant to discriminate polluted sites (Grześ, 2009b) because they were able to regulate their Cd body concentrations. As the investigated ants are carnivorous and aphidicolous (Czechowski et al., 2012) the concentrations of Cd in invertebrates that potentially may be eaten by ants seems to be more relevant metal-pollution indicator. The invertebrates were collected by sweep-netting at each site in a preliminary study in the summer of 2011 and included mainly Orthopterans, spiders, caterpillars, and beetles. Cd concentration in invertebrates correlated positively with the Cd total soil concentration (simple regression, $p < 0.01$, $R^2 = 0.32$). The lowest and highest Cd concentrations in invertebrates were 3.03 and 39.70 mg/kg dry weight (d.w., Table 1). Detailed Cd and Zn concentrations in soil samples, are reported in Grześ et al. (2015).

2.4. Sampling

In each of the 19 sites, three large (mound diameter 40–80 cm) and mature colonies were marked. Each colony was checked for the presence of males and females. In total, 54 colonies were investigated. Three colonies were not included in the final analysis due to damage caused by animals or humans or due to high mortality of ants during transport. A random sample of approximately 300 workers was collected directly from each colony from the uppermost nest chambers in July 2012. The correctness of species identification was checked in the laboratory according to Czechowski et al. (2012).

2.5. Experimental setup

The experiment was performed on queenless cultures containing 20 adult workers. Three cultures were established from each of 54 colonies and workers from each culture were exposed to one of the following dietary Cd nominal concentrations: 226, 800, 2400 mg/kg d.w. Because in our previous study no effect on mortality under 150 mg/kg Cd was recorded in the ant *Myrmica rubra*, we decided to use the Cd-concentrations that could be considered relatively high. Two additional cultures were established per colony in order to validate the experimental procedure. One of these was exposed to uncontaminated food and served as a negative control. The second culture was exposed to very high dietary concentration of Zn at nominal concentration of 16000 mg/kg Zn. Based on our previous studies performed on *M. rubra*, we expected that this concentration of Zn should significantly affect the mortality rate of the exposed workers (Grześ, 2010). Therefore, this culture served as a positive control used to ensure that the experimental protocols were actually capable of detecting the effects on ant mortality (Festing and Altman, 2002). Thus, the experiment was performed on 270 cultures (54 colonies × 3 Cd-treatments = 162, 54 negative