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Serpentine soils affect heavy metal tolerance but not genetic diversity in a common Mediterranean ant

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HIGHLIGHTS

- Ants in serpentine soils are affected by the presence of heavy metals.
- Here we test the effect of exposure to nickel on the ant *Crematogaster scutellaris*.
- Chronic exposure to intermediate metal levels increases ants' tolerance to nickel.
- Microsatellites analysis reveals no detectable effect on genetic diversity.

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ABSTRACT

Natural habitats with serpentine soils are rich in heavy metal ions, which may significantly affect ecological communities. Exposure to metal pollutants results, for instance, in a reduction of population genetic diversity and a diffused higher tolerance towards heavy metals. In this study, we investigated whether chronic exposure to metals in serpentine soils affect accumulation patterns, tolerance towards metal pollutants, and genetic diversity in ants. In particular, we studied colonies of the common Mediterranean ant, *Crematogaster scutellaris*, along a contamination gradient consisting of two differently contaminated forests and a reference soil with no geogenic contamination. We first evaluated the metal content in both soil and ants' body. Then, we tested for tolerance towards metal pollutants by evaluating the mortality of ants fed with nickel (Ni) solutions of increasing concentrations. Finally, differences in genetic diversity among ants from different areas were assessed using eight microsatellite loci. Interestingly, a higher tolerance to nickel solutions was found in ants sampled in sites with intermediate levels of heavy metals. This may occur, because ants inhabiting strongly contaminated areas tend to accumulate higher amounts of contaminants. Additional ingestion of toxicants beyond the saturation threshold would lead to death. There was no difference in the genetic diversity among ant colonies sampled in different sites. This was probably the result of queen mediated gene flow during nuptial flights across uncontaminated and contaminated areas of limited geographical extent.

1. Introduction

Metal pollution due to anthropogenic activity is a widely documented phenomenon of environmental and medical relevance worldwide (Duruibe et al., 2007; Agarwal, 2009; Förstner and Wittmann, 2012). High metal concentrations also occur naturally in areas with specific rock compositions, such as serpentine soils. These terranes originate from ultramafic rocks and are characterized by low nutrient content and high nickel, cobalt, and chrome concentrations (Brooks, 1987; Angelone et al., 1993). Not surprisingly, they harbor particular and often highly specialized biological communities (Prasad, 2001). For example, nickel hyperaccumulator plants and high-nickel herbivorous insects are well-known examples of extreme adaptations to these harsh environments (e.g.

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Brooks, 1987; Reeves and Baker, 2000; Boyd, 2009). Additionally, arthropod predators such as spiders or mantids that feed in these areas accumulate considerable amounts of nickel in their tissues with different responses to heavy metal poisoning (Boyd and Wall, 2001).

In the absence of compensative systems limiting ion absorption, the effects of heavy metal toxicity can be severe and may ultimately have behavioral, functional, or genotoxic consequences (e.g. Reinecke and Reinecke, 2004; Sánchez, 2008; Agarwal, 2009; Boyd, 2010; Grześ et al., 2015). Avoidance of polluted resources can be an efficient method for preventing poisoning (Tyler et al., 1989; Lefcort et al., 2004). However, when this is not possible other physiological mechanisms may allow for improved tolerance to metal exposure (Morgan et al., 2007; Janssens et al., 2009). These mechanisms may include, for example, overexpression of genes related to metallothionein production (Amiard-Triquet et al., 2011; Isani and Carpenè, 2014) or the ability to store metals in a non-soluble form within intra-cytoplasmic membrane-bound granules (Jeantet et al., 1977; Carneiro et al., 2013). Moreover, prolonged exposure to contaminated soils may work as a selective drive through the survival of tolerant genotypes (e.g. Klerks and Weis, 1987; Shaw, 1989; Maes et al., 2005). It is also known that in many taxa, from plants to crustaceans and fishes, populations living in contaminated areas show a lower genetic diversity than populations from uncontaminated sites (Nadig et al., 1998; Mengoni et al., 2000; Ross et al., 2002; Fratini et al., 2008).

Ants are found in virtually every habitat type except permanently frozen soils and some oceanic island's forests, such as the Azores (Ribeiro et al., 2005; Bolton et al., 2006). Due to their key role in ecosystem functioning, high abundance, ease of identification, and the spatial stability of colonies, ants are widely used as bioindicators to assess biological effects of degraded or polluted habitats (e.g. Majer, 1983; Madden and Fox, 1997; Hoffmann et al., 2000; Ottonetti et al., 2006; Nummelin et al., 2007; Ribas et al., 2012). Some species exhibit considerable resistance to metal pollution and maintain viable populations even in contaminated areas (Migula and Głowacka, 1996; Eeva et al., 2004). However, different species show different responses to metal contamination exposure. For example, in *Myrmica rubra*, Zinc tolerance is higher for ants living in polluted areas, suggesting an adaptation to heavy metal exposure (Grześ, 2010a). Conversely, in *Formica aquilonia* there is no evidence for adaptation to metal pollution, implying damage occurs to the immune systems of exposed individuals (Sorvari et al., 2007). Notwithstanding that the accumulation of metals in ants is well documented (e.g. Rabitsch, 1995, 1997; Del Toro et al., 2010; Gramigni et al., 2013), the long-term consequences of prolonged exposure are still poorly known (Grześ, 2010b), and to our knowledge, there is no data on the adaptation of ants to ophiolitic soils.

In this study, we analyzed the effects of chronic exposure to geogenic heavy metal contamination due to serpentine soils on populations of the acrobat ant *Crematogaster scutellaris*, a dominant Myrmicine species found widespread throughout the Mediterranean basin. This species has been previously used to evaluate metal pollution in urban areas (Gramigni et al., 2013), and it is known that it may selectively accumulate some metallic ions in different body parts and tissues (Gramigni et al., 2011). This species is a top-ranked competitor found in both natural, semi-natural, and urban areas (Morris et al., 1998; Schatz and Hossaert-Mckey, 2003; Santini et al., 2011). Similarly to many other ants, it has a variety of feeding strategies including preying, scavenging, and homopteran tending (Ottonetti et al., 2008). The wide resource spectrum used by *C. scutellaris* may facilitate the uptake of metal ions (Gramigni et al., 2013).

We sampled ants from three sites across a gradient of contaminated soils: an uncontaminated oak-pine forest used as a reference site, an oak-pine forest located at the margin of an ophiolite outcrop, and a pine forest in the middle of another ophiolite outcrop. We first quantified the metal content both in the soil and in the ants in order to assess which elements were accumulated or metabolically regulated by ants. Second, we performed mortality experiments to test for evidence of some form of adaptation to chronic contamination. This was conducted by feeding ants with nickel-contaminated food. Finally, we genotyped ants using nuclear DNA microsatellite markers to assess whether long-term exposure to metals resulted in a reduced genetic diversity. In particular, we expected that ants from contaminated sites showed a lower mortality and genetic diversity with respect to ants from uncontaminated areas.

2. Materials and methods

The study was carried out between May 2013 and September 2014 in late spring and summer. Ants were sampled in three sites in Tuscany, Italy: the Casaglia oak-pine forest near the town of Florence (CS, the reference site), an oak-pine forest located at the margins of the Monterufoli Nature Reserve ophiolite outcrop near Pomarance (PO, the intermediate contaminated area), and a pine forest located over the ophiolitic Monteferrato Protected Area outcrop, near Prato (MF, the most contaminated area). The reference site is far from other sources of human induced pollution (urban habitats, heavy traffic roads) and has no geogenic contamination. None of the three areas is actively managed for economic purposes. Boundaries for the ophiolite outcrops were defined from the Geological Map of Tuscany (http://www502.regione.toscana.it/geoscopio/cartoteca.html, accessed on March 29, 2017). For each site, ants were sampled from six *C. scutellaris* nests located at least 50 m apart. Each sample to be used for subsequent metal content analysis was formed by a total of 100 ants from six out of the ten nests. Ants were immediately taken to the laboratory and kept without food for three days to allow gastric emptying. Water was available to ants during this time. Ants were then stored at −80 °C. Soil samples were randomly sampled within a 5 m radius from each nest. Approximately 3 cm of soil layer were first removed in order to discard temporary atmospheric depositions (Carter, 1993) and 100 cm^3 of soil were then sampled and stored in a plastic container. In summary, we collected a total of 18 ant (six replicate nests x three sites) and 18 soil samples.

The metal content in ants and soil was assessed in mg/kg by an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) analyzer (IRIS Intrepid II XSP Radial, Thermo Fisher Scientific, USA). Analyses were conducted on six of the most important elements found in ophiolitic soils: Cd, Co, Cr, Cu, Ni, and Zn. Ants were first cleaned with double distilled water and then dried at 60 °C in an oven for 48 h. Soil samples were purified from debris and visible organic material, such as roots or invertebrates, dried at 60 °C in an oven for 48 h, and then ground using a powder mill (Pulverisette 6, Fritsch GmbH). Approximately 0.5 g of pulverized soil and 0.1 g of dried ants were diluted in 10 ml and 5 ml nitric acid, respectively. Each sample was mineralized using a MARS microwave reaction system (CEM Corporation, Matthews, NC) according to the protocol provided by the manufacturer. The pH of soil was also measured. For each metal and site, we computed a biota-to-soil accumulation factor (BSAF, Cortet et al., 1999), which is the ratio of metal in ant tissues to that in the soil. Metal concentrations assessed by the ICP-OES and BSAF values were log-transformed prior to analysis due to the wide difference in the relative abundance of metals. Differences in the metal contents (ants and soil) and BSAF among sites were assessed using ANOVA and the Tukey post-hoc test for multiple comparisons. We employed non-metric multidimensional scaling