Pollution diminishes intra-specific aggressiveness between wood ant colonies
Jouni Sorvari *, Tapio Eeva

Department of Biology, Section of Ecology, FI-20014 University of Turku, Finland

1. Introduction

Increased concentrations of heavy metals, such as copper and lead, may cause hyper- and hypoactivity in vertebrates in laboratory conditions (Delville, 1999; Li et al., 2003; Lisboa et al., 2005), but corresponding behavioural changes in wild animal populations have remained unknown. Aerial pollutants, such as aerosols and dust particles, are widely spread from tropics to the Arctic (Veron et al., 1992; Colbeck and Lazaridis 2010), and emissions still remain at high level (Gong and Barrie, 2005). Therefore, possible effects on behavioural traits on animals can be widespread. Behavioural changes have been noted in invertebrates exposed to sub-lethal concentrations of toxic compounds such as pesticides (Helliövaara and Väisänen, 1993), but studies on the effects of industrial pollutants on insect behaviour are very scarce, and no effects have been detected (Pan et al., 2006).

Colonial ants are favourable study subjects for assessing pollution-induced changes in behaviour because they easily accumulate pollutants such as heavy metals (e.g. Migula and Glowacka, 1996; Eeva et al., 2004; Grzes, 2009) and display a variety of aggressive behaviour against their conspecifics (Katzerke et al., 2006; Rosset et al., 2007; Thomas et al., 2007; Sorvari et al., 2008; Thurin and Aron, 2008). Ants recognise their nestmates with chemical cues, and nestmate recognition is a fundamental character to keep up the colony integrity (Crozier and Pamilo, 1996). A failure in nestmate recognition might therefore risk the unity of a colony and negatively affect its reproduction and survival. In addition, ants show a variety of inter- and intra-specific dominance behaviours, ranging from submissive non-territorial ants that defend only their nests, to aggressive territorial ants that defend their nests, food sources and the territories surrounding their colonies (Savolainen and Vepsäläinen, 1988).

We tested with pair-wise aggression tests whether heavy metal emissions of a copper smelter affected the aggressive behaviour of an aggressive and territorial wood ant, Formica aquilonia collected from wild populations in polluted and unpolluted environments. F. aquilonia is the most important keystone wood ant species in boreal forests because of its high frequency, abundance and wide distribution (Punttila and Kilpeläinen, 2009). Changes in habitat quality, e.g. in terms of clear-cutting of forests, have a potential to modify the intensity of aggressive behaviour in this species (Sorvari and Hakkarainen, 2004). F. aquilonia is known to easily accumulate heavy metals in polluted environments (Eeva et al., 2004), but whether elevated levels of industry released heavy metals have potential to change aggressive behaviour in its populations has not been studied.

2. Materials and methods

We collected the study material from the surroundings of a copper smelter in the town of Harjavala (61°20’ N, 22°10’ E), SW Finland in summer 2004. Heavy metals (especially Cu, Ni, As, Pb and Zn) are common pollutants in the area and they have been shown also to accumulate in the wood ant F. aquilonia (Eeva et al., 2004). Elevated heavy metal concentrations occur in the polluted area due to current and long-term (since 1940s) deposition, and metal contents decrease exponentially with increasing distance to the smelter approaching background levels at sites further than 5 km from the smelter (Eeva and
Lehikoinen, 1996; Sorvari et al., 2007). We used ten nests in the polluted area near the copper smelter (mean distance 1.2 km ± SD 0.18) and ten nests in the background area (mean distance 8.8 km ± SD 2.68) in our experiment. All nests were spatially well separated (>200 m) from each other and sampled from several separate forests to avoid pseudoreplication (five forest stands in polluted area and five forest stands in unpolluted area). This ensured that closely related nests of the same multiset colony were not used in our experiment. *F. aquilonia* is known to have very limited dispersal (Pamilo et al., 2005); most of the queens and males mate within the group of neighbouring nests (Rosengren et al., 1993) and thus, ants from different forest patches are likely not closely related. Special attention was paid in selecting study patches representing the same habitat type, i.e. relatively barren pine dominated forests typical of the study area.

Aggressive behaviour in ants can be classified from mild to strong aggression as follows: ignoring, avoidance, investigation (antennation), attack-like movements and biting (Carlin and Hölldobler, 1986; Holzer et al., 2006; Sorvari et al., 2008). Here we used two behavioural classes: aggressive behaviour which consisted of attack-like movements and biting, whereas other types of interactions were classified as non-aggressive. We collected c.a. 50 workers at the top of the nest mound, kept them in glass jars overnight (at 18 °C) in the laboratory and performed aggression tests the day after the field collection. To measure the level of aggression between the individuals from the target nests we placed two workers (always one individual from polluted and one individual from unpolluted nest) on a Petri-dish, i.e. one worker from each target nest. It was possible to distinguish the individuals by always selecting opponents of different body size for a trial (i.e. one small and one large worker). The source nest for the larger worker was changed between each trial. Using body size for identification was necessary because e.g. colour marking may disturb the normal behaviour of ants. An earlier study has shown that body size does not correlate with the level of aggressive behaviour in this species (Sorvari et al., 2008). Average worker body size and fat content were similar in polluted and unpolluted sites (Eeva et al., 2004; Sorvari et al., 2007). The relative size difference of the workers was assessed by eye.

During the first ten interactions between the two workers we counted the number of attacks of each worker towards the alien worker. It was easy to detect which ant was the attacking one in each confrontation: the attacker moved with speed towards the opponent ant and bit this. While these ants are known to use formic acid in battles, formic acid projections did not occur in observed pairs. Biting exceeding 10 s was scored anew for each period of 10 s. This procedure was repeated six times for each nest pair, each time with novel workers (n = 10 colony pairs; 6 aggression tests each pair, total number of tests was 60). All aggression tests were performed by the same observer (JS). To prevent potential chemical contamination of the test arena, we used new clean Petri-dish in each new test. The distance between colonies might affect the level of aggressiveness due to higher degree of kinship between close than remote nests (Beye et al., 1997, 1998; Katzerke et al., 2006). Therefore, separate within-site analyses (i.e. polluted vs. polluted and unpolluted vs. unpolluted) were not conducted. We wanted to ensure that spatial distribution of focal nest does not affect the result, i.e. the combating individuals were always from colonies far away from each other.

We have earlier measured heavy metal levels in workers of 37 *F. aquilonia* nests in the same study area (Eeva et al., 2004). 17 of these nests were used to test the aggression and we use here the previously measured heavy metal values to assess the level of heavy metal exposure in our experiment. Heavy metal concentrations were therefore known for 17 (n = 9 polluted and 8 unpolluted colonies) out of 20 experimental nests. Metal concentrations were measured from workers collected at the top of the mounds. The samples (c.a. 80 workers each) were dried at 50 °C for 48 h and analyzed for contents of As, Cd, Cu, Ni, Pb and Zn. Two millilitres of supra-pure HNO₃ and 0.5 ml of H₂O₂ were added to the samples (0.15–0.20 g) into TeFon bombs for digestion of samples with a microwave system. After the digestion, the samples were diluted to 50 ml with de-ionized water (Elgastat Maxima). The determination of concentration of the analytic elements was done with ICP-MS (Elan 6100 DRC+ from Perkin Elmer-Sciex) (Montaser, 1998). The calibration of the instrument was done with certified solution (Claritas PPT, Multi element solution 2A) from Spex Certiprep.

Statistical analyses were made with statistical software SAS version 9.1 (SAS Institute, Cary, NC, USA). The heavy metal burden of each nest was used as an explanatory variable in the analyses. For six separate heavy metal concentrations, we used principal component analysis (procedure Princomp in SAS) to reduce the number of intercorrelated variables and to avoid multicollinearity of explanatory variables in the models. First principal component (PC1) had positive loading from all the measured heavy metals, and it describes well the general level of heavy metal exposure (eigenvectors: Cu 0.53, As 0.53, Ni 0.51, Pb 0.35, Cd 0.20 and Zn 0.11). Therefore, PC1 was used as an independent factor describing heavy metal burden in the following analyses. We used a mixed model ANOVA (unbalanced design) with Satterthwaite approximation of degrees of freedom to analyze the difference between heavy metal burden (PC1) between polluted and unpolluted zones. Differences in attack intensity were analyzed with generalized linear models (GLM; procedure Genmod in SAS) using Poisson error distribution. Nest of origin was used as a repeated factor. We further tested with GLM whether the number of aggressive attacks was related to the worker size.

### 3. Results

Ants from the colonies near (~2 km) the copper smelter showed higher concentrations of heavy metals (Cu, As, Pb, Ni, Cd and Zn; Mixed ANOVA for PC1: n = 17, F₁,₁₅ = 40.4, p < 0.0001) as compared with more distant (~5 km) colonies. For example, As concentrations were 4.8 times higher in the polluted area than in the unpolluted one (see Fig. 1 for actual differences in each metal). Pair-wise aggression tests between nest pairs showed that ants from unpolluted colonies were more aggressive towards the member of a foreign nest than ants from polluted colonies (model based mean number of attacks ± 95% CI: polluted: 1.62 ± 0.53; unpolluted: 3.38 ± 1.06; n = 20, generalized linear model: χ² = 12.7, df = 1, p = 0.0004). There was also an association between the number of attacks and heavy metal burden of nest: increase in heavy metal concentrations decreased aggression (generalized linear model: n = 17, χ² = 9.57, df = 1, p = 0.002; Fig. 2). Worker size did not affect aggressive behaviour, and the results were the same between ants from polluted and unpolluted nests (generalized linear models: worker size: n = 120, χ² = 1.11, df = 1, p = 0.29; worker size × pollution: χ² = 0.62, df = 1, p = 0.43).

### 4. Discussion

Ants from the polluted area behaved more passively towards a member of a remote foreign nest than ants from uncontaminated areas. Such a pollution-related change in behaviour could either be produced by direct physiological effects of heavy metals on ants or by indirect effects e.g. via changed resource levels. In general, polluted environments tend to be less productive and increased resource competition could be expected to increase the level of aggressive behaviour in ants (Sorvari and Hakkarainen, 2004; Boulay et al., 2007; Sorvari et al., 2008). Food resources for wood ants may, however, be actually better in the polluted than unpolluted environment. The most important food resource for wood ants is tree-dwelling aphids and the honeydew they produce (Domisch et al., 2009). The numbers of aphids typically tend to increase in the polluted areas and this is the case also in our study area (Heliövaara and Väisänen, 1990). Therefore, increased primary food resources produced by abundant aphid populations in the polluted area might decrease the
aggressiveness of wood ants via relaxed competition for food. In the honeybee, *Apis mellifera*, it was found that the absence of food availability resulted in higher aggression towards non-nestmates to prevent inter-colonial food robbing (Downs and Ratnieks, 2000).

Two factors related to the toxicity of heavy metals could also account for decreased aggression. High concentrations of heavy metals can impair immune functions (Sorvari et al., 2007) and make ants more prone to infections and/or put the animal under physiological stress and thereby decrease its disposable resources for aggression. There are, however, no studies to support this hypothesis. Alternatively, heavy metal poisoning could result in sensory or neurological impairment which decrease the ant’s ability to recognize a non-nestmate and initiate aggressive interactions. A number of studies have shown that relatively low, non-toxic concentrations of chemicals, such as heavy metals, can disrupt the transfer of chemical information, inducing maladaptive responses in both the signaler and the receiver (Lürling and Scheffer, 2007). For example pollutants inhibit the sex attractant perception in males of the lepidopteran species *Laspeyresia pomonella* and *Ephesia kuehniella*, as well as activate the pheromone perception in males of *Archips podana* (Savinov, 1996). So far, most work on pollution-related disruption of signals relates to aquatic environments and there are only very few studies on this topic in terrestrial environments.

Although environmental factors are known to modify aggressive behaviour among wood ant colonies (Sorvari and Hakkarainen, 2004) geographic and genetic distances between colonies might also affect the aggressive behaviour (e.g. Beye et al., 1998, Katzerke et al., 2006). For example, due to spatial configuration of sampling sites around the point source of pollutants the nests in the polluted sites might also be genetically more closely related than colonies from the unpolluted sites. The study of Boulay et al. (2007), however, showed that workers of *Camponotus cruentatus* were equally aggressive towards alien ants irrespective of whether they were sympatric neighbours, sympatric non-neighbours, or allopatric. Similar independence between geographical distances and aggression was also noticed in *Cataglyphis ibéricus* (Dahbi et al., 1996). The effects of genetic relatedness and environment, in this case, pollutants, remain to be tested separately with a series of laboratory experiments. Possible genetic effects do not however, bias our results since in our trials we always tested aggression between far distant colonies.

So far there is no information on the possible fitness effects of reduced aggressiveness of wood ants or its possible consequences to insect communities of polluted environments. Reduced aggression might lead to lower competitive ability and thus lower fitness, when a more aggressive competitor is present. Concentrations of heavy

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**Fig. 1.** Concentrations (µg/g, dry mass) of heavy metals (As, Cd, Cu, Ni, Pb and Zn) in ants located close to the copper smelter (polluted, n = 9 colonies) and in the background area (unpolluted, n = 8 colonies), and the results of ANOVAs.
Fig. 2. Association between the number of attacks and 1st principal component calculated from the concentrations of six heavy metals in worker ants (heavy metal burden of colonies). Dots denote colony means, the fitted line (±95% CI) is based on original metals in environment may vary considerably within a short distance (Salminen and Haimi, 1999), and thus affect differently the aggressive behaviour of neighbouring nests. In other words, the natural dominance order in ant nests could be biased by heavy metals. Likewise, inter-specific dominance order could be altered in polluted environments if the susceptibility of species to behavioural changes varies, which is likely.

F. aquilonius is one of the dominant forest-dwelling predatory insects in boreal forests affecting the assemblage of other invertebrates (Niemelä et al., 1992; Punttila et al., 2004) and breeding success of insectivorous birds (Aho et al., 1999; Jäntti et al., 2007). They also have an important role in short term and local nutrient cycling of boreal forest ecosystems (Domisch et al., 2009). If predation activity is lowered together with decreased intra-specific aggressive behaviour, it may potentially affect the structure of invertebrate community of boreal and temperate forests, which may have unpredictable cascade effects on the forest ecosystems exposed to pollution. Additional studies on the effects of pollution on aggressive behaviour and predation activity in variety of taxa are needed to assess how universally the wildlife is affected by aerial pollution.

Acknowledgments

We thank Paul Ek (Åbo Akademi) for making the heavy metal measurements and Sophie Miller (University of East Anglia) for comments on the manuscript. This work was funded by the Emil Aaltonen Foundation and Academy of Finland (project 8119367).

References


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