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Susceptibility of a Carabid Beetle, *Pterostichus oblongopunctatus* Fab., from a Gradient of Heavy Metal Pollution to Additional Stressors

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Abstract Insects inhabiting contaminated areas show increased susceptibility to other stressors, the purpose of this study was to investigate whether this phenomenon had a genetic basis. We investigated changes in susceptibility to food deprivation and insecticide (dimethoate) treatment of the ground beetle *Pterostichus oblongopunctatus* originating from four populations situated along a metal pollution gradient. To determine whether the increased susceptibility to additional stressors found in field-exposed animals from chronically metal-polluted sites had a genetic basis, our research was conducted on the second generation of laboratory-reared animals. There was no difference in susceptibility to the additional stressors indicating that the differences between populations observed in earlier studies do not have a genetic basis.

Keywords Metal pollution · Multiple stress susceptibility

In many polluted areas organisms are exposed to mixtures of toxic chemicals, rather than to a single toxicant only (e.g. metals and pesticides). Natural stressors, such as drought, elevated temperatures, parasitism, seasonal reductions in the availability of food, and many others, are also common in natural environments. Although the influence of these stress factors separately may not seriously affect survival of populations, the interaction between stressors may have detrimental effects (Holmstrup 1997; Holmstrup et al. 2000). A study by Stone et al. (2001) showed that carabid beetles exposed to chronic pollution exhibit elevated susceptibility to additional stressors. In that study, the ground beetles (*Pterostichus oblongopunctatus* F.) were trapped from five sites along a gradient of heavy metal pollution and then subjected to food deprivation or insecticide (dimethoate) treatment. The authors argued that the beetles from polluted areas incurred physiological or genetic costs, which made them more susceptible to natural and other anthropogenic stressors. However, it is not known whether the cost is a result of acclimation (physiological) or adaptive (genetic) processes.

Genetic adaptations to metal pollution have been demonstrated in many plants and aquatic invertebrates, but relatively few terrestrial invertebrate species (Posthuma and van Straalen 1993). Heavy metals are non-biodegradable, accumulating in the soil and are toxic at high concentrations. As a result, they are persistent in the environment and may subject local populations to strong and directional selection pressure. Costs related to adaptation to pollutants are evident from reduced fitness of metal-adapted individuals in unpolluted environments (Van Straalen and Hoffmann 2000). To distinguish between physiological acclimation and genetic adaptation evidence is needed that the characteristics involved in divergence between populations are heritable. To exclude maternal effects, population comparison studies with second or later generation offspring reared in identical conditions are preferred (Klerks and Levinton 1989; Walker et al. 1996).

The purpose of this study was to determine whether the higher susceptibility of the ground beetle *P. oblongopunctatus* inhabiting contaminated areas has a genetic basis. The second laboratory generation of the beetles was

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subjected to food deprivation and insecticide (dimethoate) treatment. We hypothesised that, if the cost of survival in polluted areas has a genetic basis, the progeny of carabids from the most polluted sites would also be less tolerant to additional stressors. The alternative hypothesis was that ground beetles inhabiting chronically metal-polluted environments are more susceptible to additional environmental stressors as a result of either cost of physiological acclimation or direct toxic effects of the pollutants.

Materials and Methods

Pterostichus oblongopunctatus were sampled with pitfall traps from the same five sites along the gradient of heavy metal pollution in the vicinity of Olkusz city in southern Poland as were used in the study by Stone et al. (2001) (sites named OLK2, OLK3, OLK4, OLK6 and OLK7). The most polluted sites OLK2 and OLK3 were located in close proximity to the zinc-and-lead smelter (2–3 km), while distances from the smelter to other sites were: 4 km (OLK4), 8.0 km (OLK6), and 32 km (OLK7—the unpolluted reference site). The levels of metal pollution at the sampling sites ranged for Zn from 150 to 10,450 mg/kg dry humus; Pb, 140–3,590 mg/kg; Cd, 0.8–100 mg/kg; and Cu, 11–74 mg/kg (Stone et al. 2001).

From each sampling site we collected 18–28 individuals (sex ratio 1:1). Males and females were paired accordingly to the site of origin and kept on uncontaminated medium (moist grounded peat) and offered uncontaminated food (*Lucilla* sp. larvae). All animals were kept in a controlled temperature room, at 20 ± 2°C, approx. 60% relative humidity, and a light:dark regime of 16:8 h. Eggs laid by the females were collected and the hatched larvae were reared until maturity on the same uncontaminated medium and food. For further reproduction 40 young F_1-generation adults (20 females and 20 males) originating from each of the sites/breeding lines were randomly chosen and paired accordingly to the site of origin. The rearing of the F_2-generation of animals was conducted in the same way as described above.

Adult animals from the second laboratory generation were used to determine differences in susceptibility to additional stressors. Two experiments, insecticide treatment and food deprivation study, were performed according to the method described by Stone et al. (2001). For the pesticide treatment we used dimethoate dose of 0.2-µg active ingredient (a.i.) per beetle. The dimethoate was diluted with acetone from the initial concentration of 400 g a.i. per litre to the desired concentration. The acetone was used as a diluent instead of water as it evaporates much faster which allows shortening the time during which animals have to be immobilised. The beetles were dosed topically using a Hamilton gas-tight syringe with 1 µL drops, which were dried for 3–5 s. Four individuals were dosed with 1 µL of acetone as a control for diluent effect. Prior to the start of the experiments all animals were starved for 48 h to clean the gut contents. After that, they were weighed to the nearest 0.0001 g on an electronic balance (Precisa, Switzerland) and dosed with the insecticide. Following the dosing, the beetles were observed constantly for 2 h. After that survival was recorded after 3, 6, 12, 24, 26, 48 h and at 24-h intervals thereafter. In the food deprivation study carabids were treated in a similar way, but without the insecticide and acetone applications. The animals were not fed during both experiments and were kept separately in transparent plastic boxes with perforated lids. The boxes were randomly assigned for storage in large closed terraria with a layer of wet sponge at the bottom to retain humidity. The terraria were placed in controlled temperature of 20 ± 2°C and a light:dark regime of 16:8 h. In each of the two experiments we used 120 individuals: 15 females and 15 males from each of the following sites/breeding lines: OLK2, OLK3, OLK6 and OLK7. OLK4 was excluded from the study because we could not obtain sufficient animals from our stock culture.

One-way analysis of variance (ANOVA) was applied to compare body mass of the beetles between the sexes and between the sampling sites within the sexes. To find the differences in times to death between individuals originating from the four sampling sites we used survival analysis module of the CSS-Statistica software (v. 6.0, StatSoft Inc.), where comparisons are conducted with a log-rank test (Mantel 1966). Following the methodology described by Stone et al. (2001), we pooled together the data for males and females and performed separate survival analyses for the food deprivation and pesticide treatment experiments. For each treatment, the median survival time after the treatment was calculated. At the next stage, we performed similar survival analyses for males and females separately, for both experiments. To look for the difference in survival between females and males we pooled together the data from all sites and performed Cox's F test (Gehan and Thomas 1969) comparing survival times of the two sexes, again for the two experiments separately.

Results and Discussion

The average body mass for females was 62.95 ± 1.34 and 59.38 ± 1.32 mg (average ± SD) in the food deprivation and pesticide application experiments, respectively. Males were approximately 10% lighter, with body masses amounting to 52.16 ± 1.16 and 51.46 ± 1.03 mg, respectively. Both females and males originating from the site OLK6 were significantly lighter than their counterparts from other sites ($P < 0.00001$). The values observed in our study are similar to these reported by Stone et al. (2001) for his three experiments performed on field-collected animals: 61.6, 57.8 and 60.9 mg averages for females and 51.7, 50.5 and 52.0 mg for males.

Survival analyses did not reveal statistically significant differences in time to death between the animals originating from different sampling sites and which were subjected to food deprivation or pesticide application ($P = 0.9691$ and $P = 0.6821$, respectively; Fig. 1). Median survival times of the carabids from the three polluted sites were 1,656, 1,440 and 1,452 h in the food deprivation experiment, and 21, 21 and 27 h for the dimethoate treatment.

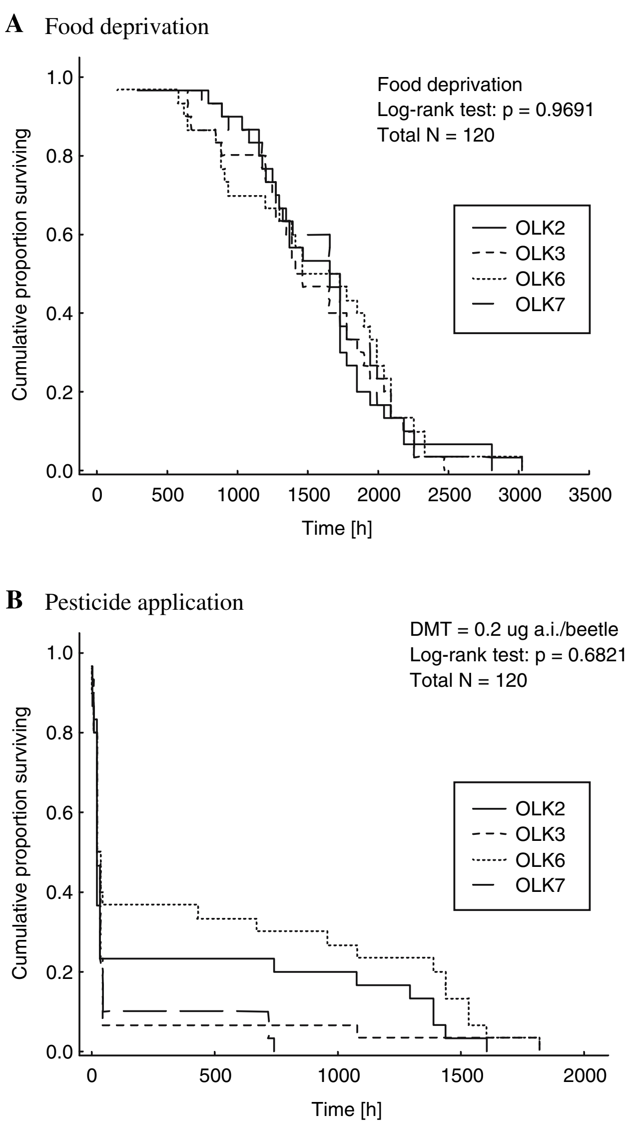

Fig. 1 Time to death analysis of the second laboratory generation of *Pterostichus oblongopunctatus* originating from polluted (OLK2, OLK3, OLK6) and unpolluted (OLK7) sites: (**a**) in the food deprivation experiment, (**b**) in the pesticide application experiment

The median survival times for the beetles from the uncontaminated site were 1692 and 27 h for the food deprivation and dimethoate experiments respectively. When calculations were performed for both sexes separately, we also did not find significant differences in time to death between the animals originating from the four sampling sites (all $P > 0.4$). It should be noted that survival times in the food deprivation experiment in our study were an order of magnitude longer than those reported by Stone et al. (2001).

When the data for females and males were compared, we found a tendency for females to survive longer than males ($P = 0.0701$). The median time to death for the females was 1,728, while for the males 1,560 h. In the pesticide application experiment we observed that the females were also surviving longer than the males—the median survival time was 33 h for the females in comparison to 21 h for the males ($P = 0.00005$). Such difference in resistance may be attributed to the difference in body mass between the sexes, which in the pesticide treatment results in lower dose per unit mass in larger beetles, while during food deprivation more energy reserves are available to larger animals. On the other hand, smaller size of the carabids originating from the site OLK6 did not seem to have any influence on their performance in our study. Thus, observed differences in survival time might be associated with the sex-specific physiology.

Earlier research on *P. oblongopunctatus* was mainly focused on finding effects of chronic, multigenerational exposure to metals on life-history characteristics and resistance to toxic metal concentrations. Lagisz et al. (2002) showed that laboratory-reared F_1 males whose parents originated from the polluted sites had shorter development time and smaller body mass than males originating from less contaminated sites. No such effects were found for F_1 females. These results may indicate that there were genetically based differences between the local populations studied. However, it cannot be ruled out that the presence of some between-population differences in the first generation laboratory animals may be also a result of maternal effects (Mousseau and Fox 1998). No differences in resistance to metals were found in the experiments conducted by Mozdzer et al. (2003) when the F_1-generation laboratory-reared larvae originating from sites OLK3 (highly polluted) and OLK7 (reference) were offered food contaminated with Zn and/or Cd. Similar results were obtained when the same metal-accumulation experiments were performed on the first laboratory generation adult stage (Lagisz et al. 2005). These two studies show that, in terms of metal assimilation and excretion, adaptation has not occurred in the beetles inhabiting sites chronically polluted with metals.

We conclude that the differences observed earlier by Stone et al. (2001) between field populations from polluted and unpolluted sites do not have genetic basis and are only the result of physiological acclimation and/or direct toxic effects of metal pollutants because there was no decrease in resistance to additional stressors in the second laboratory generation of the ground beetle reared in a uniform non-polluted environment.

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