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Alterations in Life-History Traits of *Chironomus riparius* (Diptera) Obtained from Metal Contaminated Rivers

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**Abstract.** Cadmium tolerance in field populations of the midge *Chironomus riparius* was studied by comparing the effects of chronic cadmium exposure on several life-history parameters using first generation, laboratory-reared animals. Differences between populations of *C. riparius* were therefore assumed to have a genetic basis. Field populations naturally exposed to metals were less sensitive to cadmium compared to unexposed populations, when larval development time and hatchability of the egg masses were measured. However, larval mortality still increased with cadmium exposure and no differences between exposed and unexposed populations were observed. Furthermore, life-history patterns differed between metal tolerant and nontolerant populations grown under control conditions. Metal tolerant populations were characterized by a high control mortality (50%) or an increased larval development time (with 30%). The results, therefore, indicated the presence of costs of tolerance, while a direct selection on certain life-history characteristics due to metal pollution was absent.

An increase in metal tolerance of exposed populations is well documented for bacteria (Trevors *et al.* 1985), plants (Antonovics *et al.* 1971; Macnair 1993), and several animal species (Klerks and Weis 1987; Posthuma and Van Straalen 1993). Among Diptera, metal tolerant populations are well known for the fruit fly *Drosophila melanogaster* (Chapco *et al.* 1978; Magnusson and Ramel 1986; Nassar 1979), but to a less extent for chironomids. Both Postma *et al.* (in press) and Wentsel *et al.* (1978) demonstrated that chironomid larvae obtained from metal-exposed sites were less affected by exposure to metals than their conspecifics from clean locations (based on differences in survival and growth responses). However, as these experiments on chironomids studied larvae only, it is still unknown to what extent the population growth rate of exposed populations can benefit from such an increased tolerance, especially since studies on *Drosophila melanogaster* demonstrated alterations in life-history parameters correlated with metal exposure (Bajraktari *et al.* 1987b).

Expectations for such consequences of a prolonged metal exposure on life-history characteristics are often formulated using life-history theories. These theories predict that disturbances in a habitat resulting in reduced adult survival will select for earlier maturation and increased reproductive effort, while reduced juvenile survival will select the opposite (Charlesworth 1980; Sibly and Calow 1989). In experiments with both terrestrial (Bajraktari *et al.* 1987b; Donker *et al.* 1993; Posthuma *et al.* 1993; Tranvik *et al.* 1993) and aquatic animals (Maltby 1991), such changes in growth rate or reproductive output have been demonstrated. For semelparous organisms like chironomids, the problem of finding the optimum life-history is reduced to finding the optimal age for reproduction (Gadgil and Bossert 1970). In this case, disturbances that reduce the survival of chironomids (such as metal pollution) will tend to lower the age of reproduction. Whether or not such a selection pressure modifies life-history patterns substantially will be influenced by several factors, such as the amount of genetic variation available, trade-offs among life-history characteristics, and matings with non-tolerant individuals (Falconer 1981; Mulvey and Diamond 1991).

In this study, larvae of *Chironomus riparius* were collected at several field sites, differing in metal contamination. Laboratory-reared offspring were exposed to a range of cadmium concentrations during a complete life-cycle to study the presence of cadmium tolerance for all life-history parameters. Special attention was given to chironomids grown under control conditions to test the hypothesis that in populations exposed to metals, selection favors midges with shorter development time.

**Materials and Methods**

**Site Description**

In September 1993, *Chironomus riparius* larvae were collected from two small lowland rivers in the Netherlands and Belgium. In the river Dommel, flowing from Belgium to the Netherlands, three sampling sites were selected, each located about 100 km east of the city of
Experimental Protocol

Field-sampled larvae were cultured in the laboratory using plastic aquaria with a cage on top. Larvae were kept in clean sediment (fine sand) and were fed a solution of ground Trouvit and Tetraphyll®. After emerging, egg masses were collected and allowed to hatch in clean water. Experiments were started by using larvae originating from at least 10 different egg masses. Male imagoes were collected and identified as C. riparius using Pinder (1978).

First generation laboratory-reared animals were used in the experiments to examine the presence of a genetic component for population differentiation, although maternal effects cannot be ruled out. Culturing more than one generation, however, was avoided to minimize inbreeding and artificial selection by laboratory conditions (Hoffman and Fisher 1994). Experiments were carried out in plastic aquaria supplied with a 1 cm layer of shredded paper as a substrate. The overlying water was aerated constantly and a cage was placed over each aquarium. Experiments were started by adding 50 first instar larvae less than 24 hours old to each aquarium. All experiments were made in a controlled environment room at 20°C ± 1°C. A 16:7 h light:dark regime was provided, with a twilight zone of 30 min before and after the light period to stimulate mating.

The experiments were made in water obtained from the oligo-mesotrophic lake Maarsseveen I (pH = 7.8, Na and Ca content were 18.8 and 63.6 mg/L, respectively; Timmermans et al. 1989). Metal concentrations in this lake water were as follows: <0.2-1.8 nM Cd, <0.03 μM Zn, <1.0 nM Pb, and <4.7-12.6 nM Cu (van Hattum et al. 1991). Three concentrations of cadmium (added as a solution of cadmium chloride) and an uncontaminated control were tested, and each concentration was tested in triplicate. The water was renewed once a week and water samples were taken before and directly after water renewal. The actual concentrations of cadmium during the experiment were 0.62 ± 0.12 (control), 18.8 ± 1.05, 53.0 ± 3.1, and 175.8 ± 11.8 nM Cd (mean ± standard error) (0.07, 2.1, 6.0, and 19.8 μg Cd/L, respectively). There were no significant differences in the cadmium concentrations between corresponding treatments in the different populations (tested by ANOVA). In order to prevent a rapid decrease of metal concentrations in the water during the experiments, the aquaria, the cellulose paper, and the food were spiked with the corresponding cadmium concentrations by pre-exposure during one week, in which the solutions were renewed three times. While the aquaria and the shredded paper were saturated at the beginning of the experiments, the food continued to bind cadmium, causing a gradual decrease of the water concentrations. Twice a week during the experiment, 2.5 ml of suspended food was added, providing an excess of food.

All materials used in the experiments and analysis were cleaned by soaking in 0.1 N HNO3 (Merck) for at least 24 h and rinsing three times with double distilled water. Lyophilized organisms were weighed and digested individually, in concentrated HNO3 and H2O2, using a micro-destruction method (Timmermans et al. 1989). Quality control of the trace metal analysis was carried out by analyzing destruction blanks and reference material (IAEA MAA-3/TM shrimp homogenate). Measured values were in good agreement with certified values (less than 10% deviation) and destruction blanks were near detection limits (0.4 nM Cd).

After the onset of emergence, the following parameters were assessed daily: (1) the number of newly emerged males and females; (2) the total number of male and female midges in the cage; (3) the number of dead male and female midges (dead imagoes were removed daily to check 1 and 2. For every treatment, 10 dead males—less than 24 h old—were collected for analysis of dry weight and accumulated metal; and (4) the number of deposited egg masses as well as the number of eggs per egg mass. In addition, the hatchability of the egg masses was assessed after hatching at 20°C. These parameters were integrated into a population growth rate, calculated as the mean number of larvae of the next generation which were produced per larva of the previous generation per day.

Statistical Analyses

Statistical analyses were applied according to Sokal and Rohlf (1981). When assumptions for ANOVA were violated, data transformations were executed logarithmically. If assumptions were still violated, non-parametric tests were performed (Kruskal-Wallis, KW). Differences between groups were tested using the Student-Newman Keuls (SNK) procedure when possible. Unless stated otherwise, the significance was tested at the p < 0.05 level.

Results

Control Conditions

Mortality: When grown under clean control conditions, larvae from the exposed population Neerpept (Pu) had a higher mortality rate than larvae of the other populations, including those from the other exposed site, Borkei (Oneway; F = 28.5, p = 0.001) (Figure 1a). This increased mortality most likely occurred in the early instar larvae, since the number of dead fourth instar pupae found was not increased. Mortality of larvae from the unexposed population Neerijze (C2) also seemed to be increased, but 20% mortality was within the range observed for a laboratory population during nine generations (Postma and Davids 1995).

Larval Development Time: Larvae form the exposed site Borkel (Pd) had an increased development time (about one week) compared to the unexposed populations, while no differences were observed for Pu (Figure 1b) (KW = 195.2, p < 0.001).

Eggs per Female: Both exposed field populations (Pu + Pd) produced about 60–70 eggs per female less than the unexposed populations (due to a reduced number of deposited egg masses), but these differences were not significant (ANOVA, p > 0.05) (Figure 1c).

Hatchability of the Eggs: The hatchability of the egg masses did not differ between populations and varied between 55% (C2) and 68% (Pd) (Figure 1d).
Life-Span of Imagoes: Imagoes from the unpolluted population Neerijse (C2) lived about one day less than the imagoes from the other populations (One-way; F = 12.6, p = 0.002) (Figure 1e).

Population Growth Rate: Although no significant differences were found in the population growth rates between populations (ANOVA, p > 0.05), the large difference between Neerpelt (Pu) and Peer (C1) should be noted (Figure 1f).

Accumulation of Metals: The dry weight of male imagoes was, on average, 330 μg and did not differ between populations (Figure 1g). Imagoes from Pu and Pd contained more zinc than imagoes from C1 (One-way; F = 8.0, p < 0.001 and SNK analyses) (Figure 1h), while imagoes from the population Neerijse (C2) seemed to have intermediate concentrations (500 mg Zn/kg D.W. was, however, within the range observed for a non-adapted laboratory population; Postma and Davids 1995). Furthermore, no differences were found in the cadmium concentrations between imagoes of the four populations.

Cadmium Exposure

Mortality: Mortality of larvae from the exposed Neerpelt (Pu) population increased less due to cadmium exposure compared to all other populations, including Pd (Figure 2a), and a significant two-way interaction between the factors Cd concentration and population origin was found in statistical analysis (ANOVA; F = 3.47, p = 0.004). In addition, SNK analyses confirmed that Pu was the only population in which mortality by exposure to 175.8 nM Cd did not differ from mortality in the control and 18.8 nM Cd treatment (caused by the high control mortality). Furthermore, both main factors of the ANOVA
were also significant (Population: F = 17.1, p < 0.001; Cd conc: F = 31.0, p < 0.001).

Larval Development Time: Significant differences in larval development time were found between cadmium concentrations (KW = 429, p < 0.001), as well as between populations (KW = 74.2, p < 0.001) (Figure 2b). Both the 53 and 175.8 nM Cd treatments increased larval development time for all populations, but less for the exposed populations, Pu and Pd, than for the unexposed populations, C1 and C2, in which the development time doubled. Furthermore, the larval development time of the Borkel (Pd) population decreased in the first two treatments, and larvae exposed to 53 nM Cd emerged, on average, 3 days earlier than the control.

Eggs per Female: The average number of eggs deposited per female did not differ significantly between populations (ANOVA; F = 2.55, p = 0.07) and no significant interaction term was found (ANOVA; F = 1.06, p = 0.4). The main effect of the Cd concentration was, however, significant (ANOVA; F = 3.9, p = 0.017), probably caused by a gradual decrease with increasing cadmium concentrations, but this effect is not clear (Figure 2c). Furthermore, it should be noted that female midges from the exposed Borkel (Pd) population produced about 100 eggs per female more when exposed to 53 nM Cd compared to the control situation.

Hatchability of the Eggs: Cadmium exposure significantly influenced the hatchability of the eggs (ANOVA; F = 6.6, p = 0.001), but both the factor population and the interaction term were not significant. However, the reduction of the hatchability as observed in the 175.8 nM Cd treatment was significantly less for the two exposed populations (Pu and Pd) than for the unexposed populations (C1 and C2), where hatchability was
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reduced to less than 5% (Figure 2d). The hatchability even increased about 20% when midges from Pu were exposed to 18.8 nM Cd compared to the control. Furthermore, the observed decrease in hatchability was to a large extent caused by a reduced number of fertilized egg masses instead of a reduced hatchability per egg mass.

Life-Span of Imagoes: The average life-span of imagoes was not influenced by cadmium exposure, but differences did exist between populations (KW = 16.45, p < 0.002), as imagoes from Pd showed a slightly longer life-span relative to the other populations (Figure 2e).

Population Growth Rate: Cadmium exposure lowered the population growth rate for all populations (ANOVA, F = 13.6, p < 0.001), and no significant differences between populations were found (ANOVA, F = 0.5, p = 0.69). Still, it should be noted that both exposed populations treated with 175.8 nM Cd produced some viable offspring, while the population growth rate of both unexposed populations was reduced to almost 0 (Figure 2f).

Accumulation of Metals: The dry weight of male imagoes was, on average, 330 µg and was not influenced by either the cadmium concentration or the population origin (data not shown). The concentration of cadmium in imagoes also did not differ between populations (Figure 2g). However, the concentration of zinc in imagoes showed significant main effects of both the cadmium concentration (ANOVA: F = 39.2, p < 0.001) and population (ANOVA: F = 6.4, p < 0.001), as well as a significant interaction between these factors (ANOVA: F = 5.8, p < 0.001). For most populations, the concentration of zinc in imagoes was lowered by cadmium exposure, and differences in zinc concentrations between exposed and unexposed populations, as observed under control conditions, were not found (Figure 2h). C2 was the only population with a different pattern, as the zinc concentration increased again in the 175.8 nM treatment.

Discussion

The present observations on both the chronic toxicity of cadmium and the life-history characteristics of unexposed field populations of C. riparius are in good agreement with those based on laboratory populations (Pascoe et al. 1989; Postma and Davids 1995; Timmermans et al. 1992). The hatchability of egg masses was, however, exceptional as it was low for unexposed populations (about 50-60%, compared to 80-100% for laboratory populations) and decreased significantly under cadmium exposure. This difference in fertility was possibly caused by an artificial selection pressure, which has modified the emergence patterns of laboratory cultures. As most laboratory cultures are started with larvae or eggs from the same age, some imagoes will emerge without the presence of imagoes from the other sex, due to the presence of protandry (Danks 1978; Kureck 1979, 1980). In field situations, however, these imagoes mate with midges originating from other egg masses. This is consistent with the observed reduction in hatchability being caused mainly by egg masses, which did not hatch at all, instead of a reduced number of eggs hatching per egg mass. The low fertility of field populations was, therefore, probably due to the experimental set-up.

Populations from polluted and unpolluted sites differed in their response to cadmium. Notably, the effects of 175.8 nM Cd on the larval development time as well as on the hatchability of the egg masses were less severe for midges obtained from exposed field populations compared to unexposed populations. This also holds for midges from the exposed Borkel (Pd) population, although short-term experiments did not detect any cadmium tolerance in these midges (Postma et al. in press), indicating differences in sensitivity between short- and long-term experiments. The present observations accord with Wentzel et al. (1978), who illustrated that chironomids directly obtained from metal-exposed sites were less affected by metal exposure than conspecifics from a clean location. However, increased tolerance to cadmium, as found in the present study, did not suppress effects on larval mortality, although all tested concentrations were below the average cadmium concentration found at the studied field sites (270 nM Cd). This persistent high mortality, where increased tolerance did influence larval growth, accords with results of selection experiments for cadmium tolerance carried out in the laboratory (Postma and Davids 1995). Whether this high mortality is correlated with an increased frequency of lethal mutations as observed in Drosophila melanogaster populations exposed to severe industrial contamination (Bajraktari et al. 1987a) is not yet known.

The high control mortality (about 50%) as observed in the present experiments for larvae of the exposed Neerpelt (Pu) population also accords with results for cadmium-tolerant midges obtained from selection experiments (Postma et al. 1995). However, a high control mortality was absent in the other exposed population, Pd. This difference could be caused by the location of these two field populations. The population Neerpelt (Pu) is situated only a few 100 m away from the outlet of a zinc factory and, therefore, rather close to the unpolluted, upstream part of the river. As the river Dommel is rather fast-flowing, this population could well be affected by a high immigration rate of non-tolerant midges from sites located upstream (Davies 1976). Such a high gene-flow can slow down the rate at which tolerance increases (Comins 1977; Croft and van de Baan 1988; Roush and McKenzie 1987; Taylor and Georghiou 1979) and can keep the genetic composition of a population rather heterogeneous. Consequently, sexual reproduction can produce non-viable offspring or offspring with a lowered fitness due to an ‘unfavorable’ combination of genes. This can increase the mortality rate, but the larvae who do survive are likely to have an increased cadmium tolerance. Furthermore, gene flow from non-tolerant populations can lower the metal tolerance of offspring, as is demonstrated in metal-tolerant plants (McNeilly 1968). Seasonal differences in both metal tolerance and life-history characteristics can, therefore, be expected for the exposed population Neerpelt (Pu), since the drift of chironomids fluctuates during the year (depending on, for example, the larval instar present, the population density, and catastrophies like a high river spate).

The Borkel (Pd) population, on the other hand, is probably less affected by a high flow of non-tolerant genes, since it is situated 7 km downstream of the factory, while no reductions of metal concentrations were observed in this part of the river. In addition, up to now we did not find large populations of C. riparius in other streams running close to Borkel. This population has had the opportunity, therefore, to reach a genetically
stable situation, due to the continuous selection for cadmium tolerance and a low gene flow. This accords with the high control mortality observed in cadmium tolerant midges, obtained from a selection experiment in the laboratory, since it can be expected that the genetic composition of this laboratory population was not yet stable (Postma and Davids 1995). High control mortality has also been found in metal tolerant collombola (Posthuma et al. 1993), and a lack of nutrients (for example, zinc) was suggested as one of the possible reasons, since an increased accumulation of zinc has been found (Posthuma et al. 1992). For chironomids, this is less likely, since mortality remained high when cadmium tolerant midges (cultured in the absence of cadmium) were supplied with additional zinc (Postma et al. 1995). It seems likely that a high control mortality in cadmium-tolerant midges indicates the presence of non-tolerant genotypes.

In addition, differences in life-history characteristics between the populations Pd and Pu are likely to be influenced by the high flow of non-tolerant genes into the population Pu. Therefore, life-history patterns as found in the population Pd are more likely to represent the effects of a prolonged exposure to cadmium, since it can be expected that alterations in life-history patterns are most pronounced in populations with a stable genetic composition.

Commonly held views on life-history evolution suggest a lowered larval development time in metal stressed chironomid populations. Such a lowering was not found in the present study. On the contrary, the larval development time even seems to be increased in the metal-tolerant population Pd. A discrepancy, therefore, exists with some terrestrial invertebrates, where metal-adapted populations have a shorter life-cycle and a higher reproductive effort (Bajraktari et al. 1987b; Donker et al. 1993; Posthuma et al. 1993). The present findings of an increased larval development time for the metal tolerant population, Pd, accords with a similar increase observed in selection experiments in the laboratory (Postma et al. 1995). These results demonstrated that in the case of metal-tolerant chironomids, modifications in life-history seemed to be associated with a reduced overall fitness, probably caused by some extra “costs of tolerance.”

Extra “costs of tolerance” and consequently a trade-off between increased tolerance and, for example, larval development time, can be expected when metal-tolerant genotypes have to invest large amounts of energy in, for example, the syntheses of metallothionein-like proteins. Experiments with several dipteran species (Kasai et al. 1993; Maroni et al. 1987; Otto et al. 1986), among which chironomids (Yamamura et al. 1983; Seidman et al. 1986) demonstrated that these proteins may be involved. Furthermore, both the present observations and those by Postma et al. (1995) demonstrated an increased accumulation of zinc when cadmium-tolerant midges were cultured in the absence of cadmium. This supports a possible role of metal binding proteins, since these proteins often have an affinity for several metals (Roesjadi 1992). Experiments on interactions between cadmium and zinc metabolism in cadmium-tolerant laboratory populations of C. riparius further demonstrated that the lowered larval growth rate of cadmium-tolerant midges cultured in the absence of cadmium was indeed partly caused by an increased need for zinc. In addition, the stimulation by low cadmium concentrations as observed in the present experiments for several parameters (larval develop-

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References

Kureck A (1979) Two circadian eclosion times in Chironomus thummi (Diptera), alternately selected with different temperatures. Oecologia 40:311–323


Mulvey MC, McIntosh AW (eds) Metal Ecotoxicology: Concepts and applications. Lewis Publishers, Boca Raton, FL


Pinder LCV (1978) A key to adult males of British Chironomidae. Freshwater Biological Association, Scientific Publ, No 37, Vols 1 and 2


Postma JF, Kyed M, Admiraal W (in press) Site specific differentiation in metal tolerance in the midge Chironomus riparius (Diptera: Chironomidae). Hydrobiologia


