Dynamics of metal adaptation in riverine chironomids.
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Fluctuating life-history parameters indicating temporal variability in metal adaptation in riverine chironomids

ABSTRACT

Adaptation to toxicants in animal populations is influenced primarily by two counteracting forces. Firstly, the intensity and duration of peak concentrations of toxicants is responsible for the actual level of selection pressure on the population. Secondly, the process of adaptation can be disrupted by gene flow as a result of crossings with non-tolerant individuals. These counteracting forces were analysed in riverine insects where we expected that the level of metal adaptation is subject of considerable fluctuations, due to variable dilution of metals and a variable transport of non-tolerant individuals in river water. To this purpose, the stability of metal adaptation in different *Chironomus riparius* populations was analysed during a five-month period in a heavily polluted lowland river. This was examined by measuring mortality, larval dry weight and accumulation of zinc under laboratory conditions. The results showed that in midge populations originating from metal contaminated field sites several life-history parameters (like control mortality and growth response under cadmium exposure) of the laboratory reared F1 generations showed considerable temporal variation. In addition, the presence of metal-adapted midge populations was indicated on several occasions on the metal-exposed field sites. Reference populations on the other hand, showed stable life history patterns throughout the sampling period and no signs of metal adaptation were found. These observations showed that the actual level of metal adaptation varies considerably, both in time and space. Adaptation to metals in riverine chironomids, therefore, should be looked upon as a highly dynamic process.
Adaptation is one of the core principles in evolutionary biology and natural selection is universally regarded as the primary cause of evolutionary changes. Adaptation has recently been defined as a heritable attribute of a certain character that offers advantages in survival and reproduction of that character in a given environment (cf Vermeij 1996). Analysing a case of metal-adapted chironomids inhabiting a polluted lowland river (see Postma & Groenendijk (1999) for a review of this case) in the light of this definition, reveals that the characteristics involved in metal adaptation are indeed hereditary. In addition, populations of chironomids exposed to metals in the field were less sensitive to cadmium compared to unexposed populations when laboratory experiments on larval development and hatchability of the egg masses were carried out (Postma et al 1995).

It is virtually unknown how the stability of adaptation is maintained in a certain population, because it may be strongly affected by an influx of non-adapted specimens. In general, the process of gene flow determines the spatial scale of adaptation (Slatkin 1987) and the higher the mobility of a species, the higher the influence of gene flow can be expected. It was shown before that in riverine metal-adapted chironomids, drift of larvae can introduce large amounts of genotypes into environments to which they are not adapted (Groenendijk et al 1999). Consequently, gene flow can reduce the rate of adaptation to pollution very strongly (Comins 1977; Taylor & Georghiou 1979; Roush & McKenzie 1987), but on the other hand it may provide essential new genetic material and can also reduce the effect of inbreeding by introducing new genes essential for a further increase in adaptation (Slatkin 1987). The residual effect of gene flow on adaptation will differ per situation and, furthermore, will interfere with other structuring forces like a fluctuation in selection pressure, due to changing levels of toxicants.

It is hypothesised that in dynamic stream communities the actual level of metal adaptation will strongly fluctuate. The main objective of this study,
therefore, was to examine if temporal variability in the actual level of metal adaptation exists, occurring as a result of immigration of non-exposed midge larvae or a fluctuating selection pressure. This was analysed by following several life-history characteristics, indicating metal adaptation in *Chironomus riparius* populations over a five-month period. The present study was carried out in a lowland river in the northern part of Belgium, where a zinc factory causes a continuous high, but fluctuating metal load in the river.

### Materials and Methods

**Chironomus riparius**

The species used in the present study, *Chironomus riparius*, is a member of the dipteran family Chironomidae or non-biting midges. Its life cycle comprises an egg stage, four larval stages and a pupal stage, all in the aquatic environment, and a terrestrial adult stage. At 20 °C, the life cycle can be completed within three to four weeks (cf Mackey 1977). At temperate latitudes *C. riparius* displays multivoltine life cycles with repeated settling of larvae during April to November (Groenendijk et al 1999). The feeding mode of *C. riparius* larvae can be characterised as collecting-gathering (Armitage et al 1995) and the main food source for the larvae is sediment-deposited detritus (Rasmussen 1984).

**Sampling sites**

Populations of *C. riparius* were obtained from the lowland river Dommel, rising in the northern part of Belgium, which is part of the River Meuse catchment area. The Dommel is characterised by a sandy bottom, a limited width and depth, a current velocity roughly varying between 0.3 and 1.0 m s⁻¹, and neutral waters with a naturally high iron content (Postma 1995; Groenendijk et al 1999). Furthermore, this river is affected by a diffuse domestic and agricultural input, which creates a somewhat increased background for many toxicants. In addition to this diffuse contamination,
the River Dommel is heavily loaded with metals, especially cadmium and zinc, coming from a distinct point source. This metal pollution acts as a strong selective force on the benthic fauna for more than a century (Postma & Groenendijk 1999). The metal pollution in the River Dommel, therefore, provides a suitable test case for studying metal adaptation processes in situ.

In the present study three sampling locations in the Dommel were examined. Two sites are heavily polluted with metals and are situated downstream from the point source: Neerpelt (P1), situated only 400-500 metres downstream and Borkel (P2), situated circa seven kilometres downstream from the point source of pollution. The reference site was located just upstream from the metal input (< 100 m) and is abbreviated in both text and figures as R2. Metal analyses of both water and detritus demonstrated that at the sites P1 and P2, cadmium and zinc were the dominant pollutants. Dissolved metal concentrations were similar at both polluted sites and varied during the sampling period between 56-470 nM Cd (yearly 1995 average: 475 nM) and 5.3-17.1 μM Zn (yearly 1995 average: 12.8 μM). Concentrations of cadmium are circa two orders of magnitude higher and concentrations of zinc are about one order of magnitude higher at the metal-exposed sites compared with the reference site R2. For more detailed information on water characteristics and concentrations of trace metals in both detritus and water in the River Dommel is referred to Postma (1995), Janssens de Bisthoven (1995) and Groenendijk et al (1999). In addition to the midge populations obtained from the field, the laboratory culture (LAB) of C. riparius, started in 1986 from a small experimental pond on the campus of the University of Amsterdam, was used as an extra non-adapted reference population (R1).

field sampling and laboratory culturing

Fourth instar C. riparius larvae were collected at the three field sites on seven occasions between July 25 and November 21, 1995. Due to low densities, the number of larvae was too low for successful breeding on a few occasions, so that on five occasions a complete set of observations could be made. Larval sampling took place in sediment banks in the bed of the
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Dommel, which are a suitable habitat for larvae of *C. riparius*. The upper mud layer was scraped over several metres, using nylon nets with a mesh size of 300 μm. Sediment was sieved (400 μm) the next day in the laboratory, and larvae belonging to the genus *Chironomus* were collected. Field sampled larvae were cultured in plastic aquaria with a flight cage (35*20*30 cm) on top. All aquaria were filled with circa 5 liters of Dutch Standard Water (DSW) (NPR 6503 1980), a standardised synthetic analogue of common Dutch surface waters. Larvae were kept in clean, fine sand (grain size < 300 μm). For all field sampled populations, cultures were started with circa 300 fourth instar larvae and they were fed ad libitum a solution of 10.0 g ground Trouvit and 0.5 g Tetraphyll® in 200 ml water. A 16 : 7, light : dark regime, with a twilight zone of 30 minutes before and after switching, was provided. The water temperature was maintained at 20.0 ± 1 °C. After a few days, adult midges started to emerge. The egg masses produced were collected and allowed to hatch in clean water (DSW). All experiments were started by using first instar larvae from at least ten different egg masses. Randomly caught male imagoes were collected for each population and identified as *C. riparius* using Pinder (1978).

**Experimental Protocol**

In the present experiments, first generation laboratory-reared animals were used to examine the presence of a genetic component for metal adaptation. At the start of the experiments, 20 newly hatched first instar larvae were added to plastic 0.5 l containers, containing 400 ml DSW, circa 1 cm layer of shredded cellulose fibres and 1.0 ml food suspension. The overlying water was aerated constantly and all experiments were carried out in a controlled environment room (20.0 ± 1 °C and a 16 : 7, light : dark regime, with a twilight zone of 30 minutes before and after switching). Two concentrations of cadmium, added as a solution of cadmium chloride (nominal concentrations: 178 and 267 nM Cd) and a control were tested in triplicate. The water was renewed once a week and water samples were taken before and directly after water renewal. Three times a week 1.0 ml of
suspended food was added, providing an excess of food. The average actual cadmium concentration (nM Cd ± 1 SE) during the experiment in the controls was 0.27 ± 0.09. The average cadmium concentration in both treatments was 90.9 ± 5.2 and 131.9 ± 9.7 nM Cd, values representative for cadmium concentrations at the polluted field sites. In order to prevent a significant loss of metals in the test medium during the experiments, the plastic containers, the cellulose fibres and the food was spiked with the corresponding cadmium concentration by pre-exposure during one week, in which the solutions were renewed three times.

Experiments lasted 14 days and the following parameters were assessed for each population: 1) mortality; 2) dry weight to the nearest µg of the surviving larvae and 3) accumulated zinc under control conditions. The latter parameter was measured because it was shown that cadmium-tolerant midges, cultured in the absence of cadmium, showed an increased accumulation of zinc (Postma et al. 1995) and this parameter, therefore, can be used as an additive indicator of adaptation to cadmium. For the accumulation of zinc, ten larvae were randomly sampled from the control treatments of every population and used for trace metal analysis. Lyophilised organisms were weighed and digested individually, in concentrated HNO₃ (Baker Ultrex) and H₂O₂ (Merck, 30%), using the micro-extraction method described in Timmermans (1989). Quality control of the zinc analysis was carried out by analysing extraction 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 (0.3 µM zinc). All materials used in the experiments and analysis were cleaned by soaking in 0.1 N HNO₃ (Merck) for at least 24 hours, and were rinsed three times with double distilled water. Cadmium measurements were performed by using Graphite Furnace Atomic Absorption Spectrometry (Perkin Elmer 5100) equipped with Zeeman background correction. Zinc samples were analysed using air-acetylene Flame Atomic Absorption Spectrometry (Perkin Elmer 1100B).
statistical analyses

Routine statistical analysis were applied according to Sokal & Rohlf (1995). Assumptions for analysis of variance were, however, repeatedly violated, even after logarithmic transformation of the data. Therefore, non-parametric Kruskal-Wallis tests were performed to analyse the data. Mortality percentages were tested after angular transformation of the data. The significance was tested at the $p < 0.05$ level.

Results

mortality under clean conditions

When grown under clean control conditions, the laboratory cultured first generation *C. riparius* larvae from both polluted locations showed a higher mortality (20-50%) between July and September, compared with the low and stable mortality values (0-20%) of the reference field population (R2) and the LAB culture (figure 5.1A). Mortality levels for both polluted populations in October and November were, however, comparable to, or even slightly lower than mortality rates in both reference populations. These fluctuations in control mortality in midges originating from polluted field sites were responsible for a highly significant influence of the factor 'sampling time' ($KW = 18.2; p < 0.01$). The seasonal average for midges from the polluted field stations (P1 and P2) was around 20%, twice as high as compared to the reference midge populations. However, this difference was not significant ($KW = 0.43; p > 0.05$), most likely due to the observed high temporal variation (figure 5.1B). Because the maximum cadmium concentrations tested are sublethal for *C. riparius* larvae, no differences in mortality rates among populations under cadmium exposure were present (data not shown, $KW = 1.74$ for the 90.9 nM Cd treatment and $KW = 0.73$ for the 131.9 nM Cd treatment respectively; $p > 0.05$).
FIGURE 5.1: Panel A: temporal variation in control mortality (%) in four populations of *Chironomus riparius* in 1995. R1 represents the LAB population and R2 the upstream located reference population. P1 and P2 represents the polluted downstream located populations. Panel B: average values based on the temporal data.

**growth under clean conditions**

Weight of 14 days old first generation larvae reared under clean control conditions was approximately 500 µg in each population, although some temporal variation was present. The influence of the factor ‘sampling time’, therefore, was found to be highly significant (KW = 229.0; p < 0.001), and was most prominent in the P2 location (figure 5.2A). However, no differences among populations could be detected (KW = 3.23; p > 0.05).

FIGURE 5.2: Panel A: temporal variation in dry weight (µg) after 14 days in four populations of *Chironomus riparius* in 1995 reared under control conditions. R1 represents the LAB population and R2 the upstream located reference population. P1 and P2 represents the polluted downstream located populations. Panel B: average values based on the temporal data.
response to cadmium exposure

When exposed to cadmium, both reference populations showed a reduction in larval dry weight (around 50%) compared to their corresponding control level (figure 5.3A & 5.3C). In contrast, populations from both polluted locations showed stimulated growth during July and August. This stimulation even reached a factor 3-4 at the beginning of August at the P2 site (figure 5.3A & 5.3C). The stimulation faded away during autumn and the degree of inhibition reached values equal to reference populations during September-November. In the 131.9 nM cadmium treatment, both the factor ‘sampling time’ (KW = 144.0; p < 0.001) and the factor ‘population’ (KW = 75.4; p < 0.001), showed a highly
significant influence. In addition, the responses of the *C. riparius* larvae growing in the 90.9 nM Cd treatment (figure 5.3A) were congruent with the responses in the highest cadmium concentration. The seasonal average of the responses during cadmium exposure, relative to the corresponding control, showed an inhibition in larval growth of approximately 50% for both reference populations. However, the growth responses in the cadmium treatments were clearly higher for both polluted populations and increased to an overall value equal to the corresponding control (100% level) in the P2 population. However, the large variability should be noted (figure 5.3B & 5.3D).

accumulation of zinc in larvae grown under clean conditions

During the first two sampling dates, first generation fourth instar *C. riparius* larvae from both metal-exposed populations grown under control conditions, contained more zinc compared with larvae from the reference populations (figure 5.4A) and a significant difference among populations was detected (KW = 47.5; p < 0.001), even if the LAB population was excluded from the calculations (KW = 6.54; p < 0.05). This difference between exposed and non-exposed populations was, however, less pronounced during the remaining sampling dates. Although especially in the field populations some temporal fluctuation seemed to be present, there was no significant influence by the factor 'sampling time' (KW = 5.97; p > 0.05). During the whole sampling period, the LAB population (R1) showed a lower larval zinc concentration compared with the three field populations. This is also apparent in the average value presented in figure 5.4B, where larval zinc content increased from 3.3 for the LAB population to 5.0 μmol g⁻¹ dry weight for the P2 location.
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FIGURE 5.4: Panel A: temporal variation in zinc concentration (μmol g⁻¹ DW) in larvae of four populations of *Chironomus riparius* in 1995 reared under control conditions. R1 represents the LAB population and R2 the upstream located reference population. P1 and P2 represents the polluted downstream located populations. Panel B: average values based on the temporal data.

Discussion

population differentiation

The observations on mortality under control conditions, present in the metal-exposed chironomids, showed a high temporal variation. The low mortality rates during October and November, are in contrast to the high values recorded in July, August and September. High control mortality has been observed previously in toxicant-exposed arthropod populations (Donker & Bogert 1991; Posthuma et al 1993; Postma et al 1995), but is explained in different ways. In some studies control mortality is hypothetically explained in terms of 'costs of tolerance'. The maintenance of an adaptive mechanism may be energetically expensive (Sibly & Calow 1989; Holloway et al 1990) and an increased investment in the tolerance mechanism may initiate a trade-off between the benefits of the adaptation and the decreased investments into other processes like growth and reproduction (cf Harper et al 1997a). This may result in individuals with a reduced fitness in an environment to which they are not adapted, explaining for instance the observed high control mortality values.
Although in some studies indications for this trade-off hypothesis were found (Jones & Hopkin 1996), no clear signs of costs of tolerance could be traced in other investigations (Harper et al 1997a; 1997b). Alternatively, a lack of essential micronutrients, like zinc and copper, was suggested by Posthuma et al (1992) as an explanation for the high control mortality in metal-tolerant Collembola. Metal-adapted organisms often show indications for an increased metal-handling capacity, resulting in an increased efficiency to store and/or eliminate metals (Posthuma et al 1992; Postma et al 1996; Donker et al 1996). Possessing such a mechanism could well be responsible for an increased dependency on essential metals by adapted specimens placed in a clean environment (cf van Capelleveen 1987). In support of this explanation, a significant increased zinc concentration was observed in control larvae from the P1 and the P2 location, which coincided with a low larval weight under control conditions and the increased control mortality. Furthermore, later during the sampling period low control mortality values were recorded in the metal-exposed chironomids and no clear differences in larval zinc content could be traced either. Therefore, the increased mortality in metal-exposed chironomid populations seemed to be caused by an increased need for essential metals, like zinc, and is reflected in an increased larval zinc content, being rather 'consequences of tolerance' instead of 'costs of tolerance'.

The significantly enhanced larval growth under cadmium exposure in both metal-exposed chironomid populations compared to their corresponding controls provided further evidence for the presence of adapted specimens on the first sampling dates. Yet, we conclude that there is a considerable temporal variation in life-history characteristics associated with metal adaptation in metal-exposed chironomids in the River Dommel.

**cause of temporal variation in metal adaptation**

The question may arise why certain characteristics associated with cadmium adaptation do show such a high variation. Significant differences in certain life history characteristics were measured during July, August and September, demonstrating the presence of metal-adapted chironomids in
the River Dommel. In contrast however, no clear differences were found in October and November, indicating a lack of population differentiation. At first glance, this variation in population characteristics seems highly unlikely. Either there is a contrasting metal adaptation present which is genetically based, implying local selection and predominance of certain genotypes of C. riparius, or there is a uniform chironomid population indicating a lack of selection pressure and, consequently, no local variation in metal adaptation. However, this situation is expected only in situations where 1) the selection pressure is rather stable due to a constant level of contamination in combination with 2) a low level of gene flow due to a low dispersal rate.

Indeed, the actual cadmium and zinc concentrations in the River Dommel both exceed EC_{50} and NOEC-values for C. riparius regularly (Pascoe et al 1989; Timmermans et al 1992), suggesting a direct selective effect on local chironomid populations (Postma & Groenendijk 1999). However, the actual selection pressure in the River Dommel is not constant because the metal contamination is subject to regular changes. During the present study, both the concentrations of zinc and cadmium in sediment compartments, as well as the dissolved metals showed a high temporal variation by comparing two-weekly measurements (differing up to more than a factor 10 between the observed outermost values). Furthermore, these observations indicated that during late summer 1995 at both polluted locations peak concentrations were recorded in both water and sediment compartments (Groenendijk et al 1999). This suggests an increased selection pressure on the C. riparius populations at the polluted sites and this observation correlates with the increased metal adaptation in July and August in both metal-exposed chironomid populations.

Secondly, the presence or absence of an adapted C. riparius population is influenced by the rate of immigration of animals from upstream non-polluted sites as well. Immigration of non-adapted specimens will quickly lower the speed of local adaptation (Comins 1977; Taylor & Georghiou 1979; Flexner et al 1996), and will keep the composition of a population heterogeneous. Slatkin (1987) however, suggested that with a low level of
gene flow, local adaptation can increase further due to the reduction of the chance of inbreeding. Furthermore, the introduction of new genes are of vital importance for a viable heterogeneous population. A tentative quantitative model in which aspects of this ‘shifting balance’ theory were incorporated indeed suggested that low amounts of gene flow can increase local resistance to insecticides (Caprio & Tabashnik 1992). On the other hand, if gene flow of non-resistant individuals exceeds 10% per generation of the receiving population, the speed of developing insecticide resistance will decrease strongly (Caprio & Tabashnik 1992).

In the case of *C. riparius*, gene flow is relatively easy to estimate because drifting larvae normally represent a major part of the total gene flow compared to the dispersal of the short living and weakly flying imagoes (cf Davies 1976). Measurements of larval drift of *C. riparius* at the R2 location during 1995 showed a large amount of drifting larvae during August and September entering the metal-exposed zone (Groenendijk et al 1999). Based on these drift measurements and the actual larval densities at the polluted sites, estimates were made of the percentages of larvae entering the metal-exposed population per day. Although roughly estimated, it was calculated that during this period the daily input of non-exposed larvae into the metal-exposed sites regularly exceeded 5-10% of the local midge population present. Because of the similarity in population dynamics of *C. riparius* at the R2 and the PI site, it was concluded that drifting non-tolerant larvae most likely interbreed with the PI population (Groenendijk et al 1999). The estimated input of *C. riparius* larvae to downstream sites is therefore most likely higher than the mentioned 10% per generation in the model of Caprio & Tabashnik (1992). Consequently, this drift and the resulting crossbreeding can quickly lower the level of adaptation at downstream, metal-exposed sites and this ‘dilution’ with non-adapted genotypes could therefore well explain the lack of population differentiation between upstream and downstream sites in October and November. Furthermore, genetic evidence supporting this explanation was found in a study using allozyme polymorphisms, carried out with fourth instar *C. riparius* larvae sampled at the beginning of October 1995 at the R2 and both the PI and P2 site. This study demonstrated
a complete lack of substructuring between metal exposed and non-exposed midge populations (Raijmann & van Grootveld 1997). Furthermore, high values of gene flow parameters were measured and this correlates strongly with the total absence of interpopulation differences in October in the present study.

We conclude therefore, that the observed variation in metal adaptation present in river-dwelling C. riparius populations is most likely explained by two important factors. Firstly, the actual level of metal adaptation is influenced by fluctuations in selection pressure and secondly, the impact of immigration of non-tolerant larvae which can easily result in gene flow is most probably even larger. This view is supported by the near absence of variability in life-history parameters in the C. riparius laboratory population because of the lack of gene flow in this stable, non-contaminated population in a controlled environment.

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References


CHAPTER V


FLUCTUATING LIFE-HISTORY PARAMETERS


