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CHAPTER VII

Loss of metal adaptation in *Chironomus riparius* (Diptera: Chironomidae) by simulating gene flow in adapted field populations

ABSTRACT

The ability of the non-biting midge *Chironomus riparius* to survive and reproduce in metal polluted lowland rivers facilitates the opportunity to study micro-evolutionary processes in situ. However, due to larval drift, adapted midge populations are subject to regular immigration of non-adapted specimens from clean upstream river reaches. To examine the influence of non-adapted genes in adapted midge populations on the level of metal adaptation, a reference and a metal-exposed chironomid population were both experimentally crossbred to mimic high gene flow rates under laboratory conditions. Several life-history characteristics, indicating adaptation to metals, were followed in the parental strains as well as in the reciprocal crossings. Such crossings were repeatedly done for over a year, to examine the heritability of metal adaptation in chironomids. Results confirmed the presence of adaptation to metals in exposed chironomids. However, a rapid loss of metal adaptation in the first generation hybrid offspring was clearly demonstrated. Consequently, the large temporal variation in metal adaptation in midge populations from the studied lowland river can be explained by the influence of gene flow from upstream non-polluted areas. In addition, the responses of the reciprocal first generation crosses showed no clear indications of maternal effects indicating a major genetic component for the increased metal tolerance in the exposed midge populations.

Introduction

Worldwide, many areas are contaminated to a greater or lesser extent by metals originating from the mining and smelting of metal ores. This has resulted in the elimination of several plant and animal species and a subsequent drastically changed species composition (Bradshaw et al 1965; Klerks & Levinton 1993; Clements 1994; Kiffney & Clements 1994). However, some plant (Macnair 1997; Schat & ten Bookum 1992) and animal species (reviewed in Klerks & Weis (1987) for aquatic organisms and in Posthuma & van Straalen (1993) for terrestrial invertebrates) have shown their adaptive strength by surviving and reproducing in metal polluted environments. Amongst terrestrial invertebrates, conclusive evidence for genetically based metal adaptation has been presented for a few species only (Posthuma & van Straalen 1993), and also among aquatic organisms extensively studied examples of metal adaptation are still scarce: Brown (1976) with the isopod *Asellus meridianus*, Nevo et al (1984) with the gastropod *Monodonta turbinata*, Klerks & Levinton (1989) with the oligochaete *Limnodrilus hoffmeisteri* and Postma & Groenendijk (1999) with the non-biting midge *Chironomus riparius*.

In general, the actual level of metal adaptation is the residual effect of the dynamic interaction between the selective pressure of elevated metal concentrations and gene flow (Brandon 1990). Due to this interaction the presence or absence of a certain species in a contaminated habitat is not only influenced by its sensitivity or ability to adapt, but also by the rate of immigration from non-polluted sites. Hence, high levels of gene flow will reduce the speed of adaptation to toxicants (Comins 1977; Taylor & Georghiou 1979; Roush & McKenzie 1987). A reverse effect of gene flow however, is the introduction of essential new genes for a further increase in tolerance (Slatkin 1987). As a consequence, regular immigration of non-adapted specimens will push a given animal population to either sides of the balance, resulting in variable levels of adaptation. Recently, indications for a fluctuating level of metal adaptation were presented for the non-biting midge *Chironomus riparius*, inhabiting a metal polluted lowland river in

Belgium (Groenendijk et al 1999a). In addition, it was shown that migrating *C. riparius* larvae entering the polluted zone will most likely interbreed with the specimens already present at the polluted sites because of their simultaneous development (Groenendijk et al 1999b). Although not yet demonstrated for metal-adapted invertebrates, gene flow has been shown to lower metal tolerance amongst offspring in copper tolerant plants (McNeilly 1968). The main factor responsible for the fluctuations in metal adaptation in *C. riparius* below the point source of metals is therefore most likely to be the gene flow by drifting chironomids from non-polluted upstream environments. To validate this hypothesis, a reference and a metal-exposed *C. riparius* population were crossbred to simulate gene flow under laboratory conditions. Several life-history characteristics, indicating metal adaptation were followed in the parental strains as well as in the reciprocal crossings repeatedly for more than a year, to examine the heritability of metal adaptation in chironomids and the possibility of maternal effects.

Materials and Methods

study sites

The populations of *C. riparius* used in this study originated from the River Dommel which is situated in the northern part of Belgium. The Dommel is a second to third order stream fed by rainwater, and is one of the numerous lowland rivers in the Meuse River basin. The river is characterised by a sandy substrate and neutral waters with a naturally high iron content. Visibility is often limited to 10-20 cm due to considerable amounts of suspended organic material, but seasonal variation does occur. Part of the Dommel is heavily loaded with cadmium and zinc, which originates from a nearby zinc factory situated on the banks of a small stream. This enters the Dommel close to the Dutch-Belgian border, thereby creating a distinct point source of metal pollution in the river. The factory began producing zinc and cadmium from ores in 1888. During the 1980s yearly production averaged 120,000 tons of zinc and 600 tons of cadmium. In 1992,

production of zinc and cadmium was stopped and the factory switched to the recycling and production of zinc alloys. Since then and the present day, no change in metal concentrations in the River Dommel has been measured, because metal input in the river is mainly derived from seepage from wasted ores. 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. Dissolved metal concentrations downstream from the point source varied during the sampling period between 6.0-290 nM Cd (average: 112 nM) and 1.9-11.3 μ M Zn (average: 5.8 μ M). More detailed information on water characteristics and concentrations of trace metals in both detritus and water in the River Dommel is given in Postma (1995), Postma & Groenendijk (1999) and Groenendijk et al (1999b). The metal pollution present in the River Dommel still acts as a strong selective force on the benthic fauna for over a century now and has resulted in locally adapted *C. riparius* populations (Postma & Groenendijk 1999). The present situation in the river, therefore, provides a suitable test case for studying micro-evolutionary processes in *C. riparius* populations in situ.

Two closely situated sampling stations were selected in the Dommel on either side of the point source of metal pollution. The reference location (R) was situated only some tens of metres upstream from the inlet of the zinc factory and the metal-polluted sampling station (P) was situated in the polluted downstream area near the village Neerpelt, circa 400-500 metres downstream from the inlet of the zinc factory.

field sampling and crossbreeding

Fourth instar *C. riparius* larvae were collected at approximately bimonthly intervals at the two field sites on eight occasions between August 13, 1996 and October 7, 1997. Larval sampling took place in sediment banks in the bed of the Dommel which are a suitable habitat for larvae of *C. riparius*. The upper mud layer was scraped off over several metres, using nylon nets with a mesh size of 300 μ m. Sediment was sieved (400 μ m) 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. The aquaria were filled with circa 5 litres of clean Dutch Standard Water (DSW) (NPR 6503 1980), a standardised synthetic analogue of common Dutch surface waters. For all field sampled populations, cultures were started with circa 300 fourth instar larvae, which were fed ad libitum a suspension 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. Crossbreeding of midge populations was carried out with a newly designed emergence trap, which is described in detail in Groenendijk & Lückner (1998). In this trap, adult, newly emerged midges were caught separately in small plastic tubes (25 ml; 34 per aquarium), which were placed at the water surface. These plastic tubes were checked at least twice a day and tubes in which both male and female midges were present, were discarded from further use. All individually caught male and female midges were placed in two different flight cages: newly emerged males (m) from the reference population together with newly emerged females (f) from the metal-exposed population and vice versa. Both parental populations were cultured without emergence trap. Hence, this set-up produced egg masses from four different, first generation (F1) *C. riparius* strains: mR*fR; mR*fP; mP*fR and mP*fP. It was shown that by using this technique, high numbers of fertilised egg masses were produced (10-80) to ensure sufficient genetic variability at the start of the toxicity experiments. In addition, no indications of parthenogenesis in *C. riparius* could be traced, permitting application of this technique for numerous conceivable possibilities (Groenendijk & Lückner 1998). The egg masses produced were collected and allowed to hatch in clean water (DSW). Randomly caught male imagoes were collected from each population and control identification using Pinder (1978), confirmed that these all belonged to *C. riparius*.

experimental protocol

Short term experiments for testing metal adaptation and possible effects of crossbreeding on the level of metal adaptation, were performed by determining mortality and growth rate of first instar larvae under cadmium exposure. First generation laboratory-reared animals (F1) were used in the experiments to examine the presence of a genetic component for population differentiation in metal adaptation. All experiments were started with newly hatched first instar larvae, less than 24 hours old, from the first generation egg masses. Twenty-five larvae per treatment (plastic box, 500 ml) were exposed during four days to six concentrations of cadmium (added as CdCl_2 ; Titrisol, Merck) in 400 ml of Dutch Standard Water. Depending on the cadmium concentration, samples were analysed by air-acetylene Flame (Perkin-Elmer 1100B equipped with an impact bead) or Graphite Furnace Atomic Absorption Spectrometry (Perkin-Elmer 5100PC/HGA600/AS60 equipped with Zeeman background correction) after acidification to calculate actual concentrations in each experimental treatment. The average actual concentrations during the experiments were 0.2 (control treatment), 59, 108, 220, 600 and 1200 nM cadmium. The food, 1 ml of the suspension of ground Trouvit and Tetraphyll[®], was provided at the start of the experiments and constituted a suitable substrate for tube building so therefore no additional sediment was added. The initial length of larvae from each of the four strains was measured in an additional sample of 25 larvae, using an ocular micrometer. After 96 hours, the surviving larvae were counted and their lengths determined. Population differences in the growth rates of larvae in the unexposed control treatments were observed. Therefore, growth in each of the four strains exposed to cadmium, was expressed by calculating the percentage reduction (or occasionally stimulation) of the growth relative to the corresponding control.

data analysis

Relative growth (to corresponding control level) was plotted against the actual cadmium concentration in the water and from these dose-response

plots EC_{50} values with 95% confidence limits were calculated using a non-linear curve fitting procedure within the computer programme Kaleidagraph (Synergy Software) using the model for logistic response described in Haanstra et al (1985). When a subtoxic stimulus was present an extended model for this logistic response (van Ewijk & Hoekstra 1993) was used to calculate the EC_{50} value and its accessory 95% confidence limits. In addition, for every curve fitting procedure a regression coefficient (r) and an estimate of the parameter describing the hormesis (H) was calculated.

Routine statistical analyses were applied according to Sokal & Rohlf (1995). Assumptions for ANOVA were, however, repeatedly violated, even after logarithmic transformation of the data. Therefore, it was decided to perform non-parametric Kruskal-Wallis (KW) tests to analyse the data in all cases to facilitate mutual comparisons. Mortality percentages were tested after angular transformation of the data. The significance was tested at the $p < 0.05$ level.

Results

mortality under control conditions

When grown under clean conditions, the laboratory cultured F1 generation larvae from the polluted location showed a large temporal variation in mortality (figure 7.1A). Control mortality was clearly increased from November 1996 until January 1997 (circa 25-80%) and again in October 1997 (circa 40%) compared with the reference population. On the other hand, low control mortality rates ($< 10\%$) were found during the remaining sampling dates and, consequently, the factor 'sampling time' showed a significant influence (KW = 15.5; df = 7; $p < 0.05$). The reference population showed low and stable mortality values (0-12%) throughout the full sampling period. The average over the total experimental period for midges from the polluted field site was nearly 25%, circa five times higher than for the reference population (figure 7.1B). Both reciprocal crosses showed an intermediate response (10-15%), however, no significant difference could be

traced among populations ($KW = 4.59$; $df = 3$; $p = 0.20$), most likely due to the observed high temporal variation especially in the polluted population.

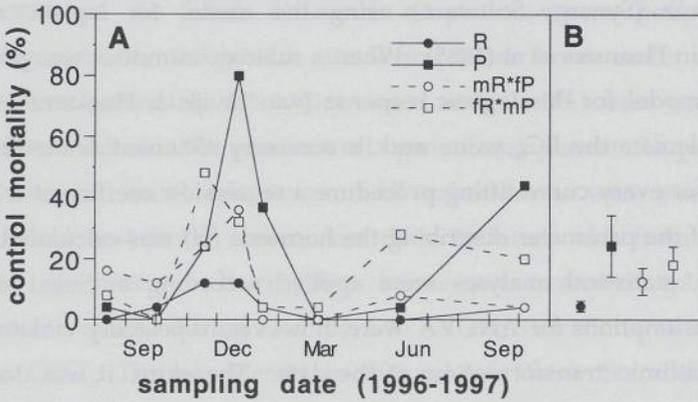


FIGURE 7.1: Panel A: temporal variation in control mortality (%) in the reference (R) and polluted (P) populations of *Chironomus riparius*, as well as in both reciprocal crosses. Panel B: average values with standard errors based upon the temporal data.

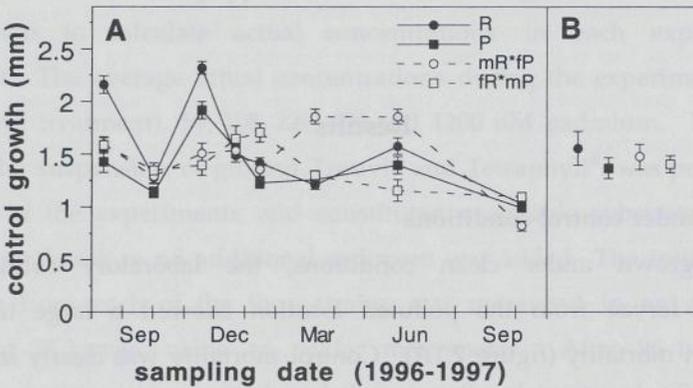


FIGURE 7.2: Panel A: temporal variation in larval growth (mm) after 96 hours in the reference (R) and polluted (P) population of *Chironomus riparius*, as well as in both reciprocal crosses, reared under control conditions. Mean values together with standard errors are presented. Panel B: average values with standard errors based upon the temporal data.

larval growth under control conditions

Growth of 96 hours old F1 larvae from the reference population, reared under clean control conditions, showed a high temporal variation with extreme values of circa 1.0 and 2.2 mm (figure 7.2A). Larvae from the polluted population showed a similar temporal variation, but responded on

five occasions with a lower larval growth than the reference site. As a consequence, both the factor 'sampling time' ($KW = 191.8$; $df = 7$; $p < 0.001$) and the factor 'population' ($KW = 15.5$; $df = 3$; $p = 0.001$) showed a highly significant influence. On the other hand, no differences were visible in the average values based upon the temporal data (figure 7.2B), and no clear responses in the reciprocal crosses could be traced either.

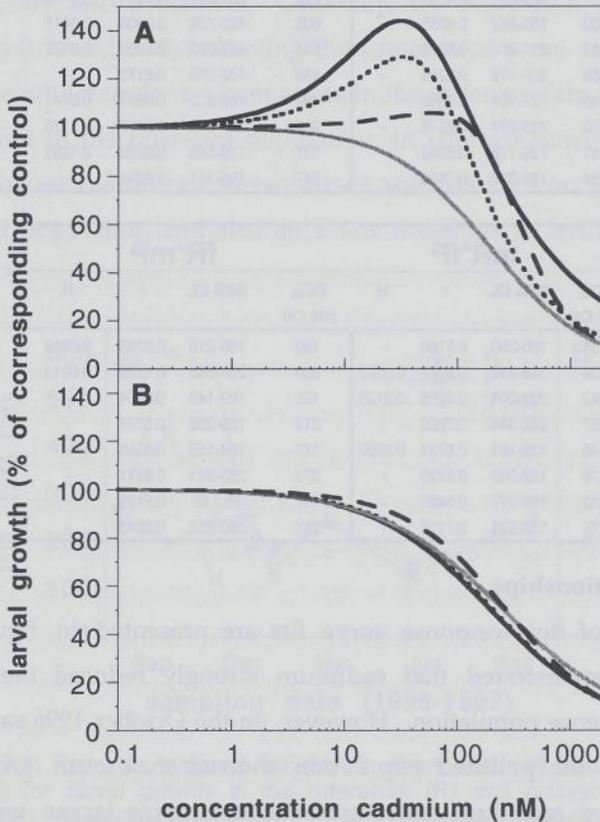


FIGURE 7.3: Larval growth (as percentage of the corresponding control) of first instar *Chironomus riparius* larvae exposed to cadmium. F1 generation larvae were obtained from both the non-polluted reference (R) site (grey solid line) and the polluted (P) downstream site (black solid line). In addition, both populations were interbred resulting in two reciprocal crosses males R * females P (dashed line) and females R * males P (dotted line). Curve fitting was done using a model for logistic response as outlined in the materials and methods section. For a clear interpretation of the dose-response relationships, the original measurements were omitted from the graphs. However, an indication of the level of interpopulation variability can be obtained by judging the regression coefficients and the EC_{50} values including the 95% confidence limits as shown in table 7.1. Panel A shows the results for the sampling date in October 1996 and panel B for the sampling date in December 1996.

TABLE 7.1: Short term EC_{50} values (96 hours) in nM cadmium for *Chironomus riparius* larval growth, with their 95% confidence limits (CL) at each sampling date for both the reference (R) and the polluted (P) population as well as for the reciprocal crosses. For every curve fitting procedure a regression coefficient (r) and an estimate of the parameter describing hormesis (H) is also presented.

R					P			
sampling date	EC_{50} nM Cd	95% CL	r	H	EC_{50} nM Cd	95% CL	r	H
13-8-96	271	236-311	0.8723	-	246	203-297	0.7515	0.1730
2-10-96	200	165-242	0.8356	-	603	492-739	0.8408	0.0411
19-11-96	247	211-289	0.8913	-	564	469-680	0.8559	0.0129
23-12-96	258	208-319	0.8245	-	189	132-270	0.8173	-
16-1-97	145	127-164	0.7999	-	182	165-202	0.8857	0.0741
13-3-97	250	213-294	0.8378	-	240	207-279	0.8117	0.1170
4-6-97	151	129-178	0.8642	-	131	116-149	0.8276	0.1253
7-10-97	254	191-338	0.7528	-	247	196-311	0.8224	-

mR*fP					fR*mP			
sampling date	EC_{50} nM Cd	95% CL	r	H	EC_{50} nM Cd	95% CL	r	H
13-8-96	234	196-280	0.8160	-	190	166-218	0.8788	0.0919
2-10-96	439	368-524	0.8072	0.0020	300	254-356	0.8286	0.0213
19-11-96	243	201-294	0.8055	0.0128	121	102-143	0.8804	-
23-12-96	267	208-344	0.7800	-	219	169-285	0.8297	-
16-1-97	145	129-163	0.8724	0.2209	127	104-155	0.8258	-
13-3-97	176	152-203	0.8720	-	273	233-321	0.8371	-
4-6-97	233	196-277	0.8490	-	112	85-146	0.8136	-
7-10-97	171	130-224	0.7125	-	215	180-257	0.8248	-

dose-response relationships

Two examples of dose-response curve fits are presented in figure 7.3. Results clearly demonstrated that cadmium strongly reduced the larval growth of the reference population. However, on the October 1996 sampling date (figure 7.3A), the polluted population showed maximum growth at somewhat increased cadmium concentrations and midge larvae were less affected by cadmium compared with the reference larvae, even at the highest concentrations, resulting in significantly different EC_{50} values (table 7.1). Results of the two crossbred populations were more identical to each other and showed intermediate responses to cadmium compared to growth patterns of the two parent populations (figure 7.3A). In contrast, no population differentiation was found in larvae sampled in the River Dommel in December 1996 (figure 7.3B) indicating a large temporal

variation in metal adaptation present in the metal-exposed midge population.

The EC_{50} values and their accessory 95% confidence limits (95% CL) calculated for both populations and reciprocal crosses at each sampling date are presented in table 7.1. Furthermore, regression coefficients (r) for each curve fit and values of the parameter describing the subtoxic stimulus (H), if present, are also presented. Generally, the regression coefficients varied between 0.80 and 0.90, indicating the presence of some variation in growth response whilst under toxicant stress in the different data sets. No hormesis was found in the reference population, in contrast however, a stimulation in the lowest cadmium concentrations was recorded on six occasions in the polluted population and also on a few occasions in both reciprocal crosses (table 7.1).

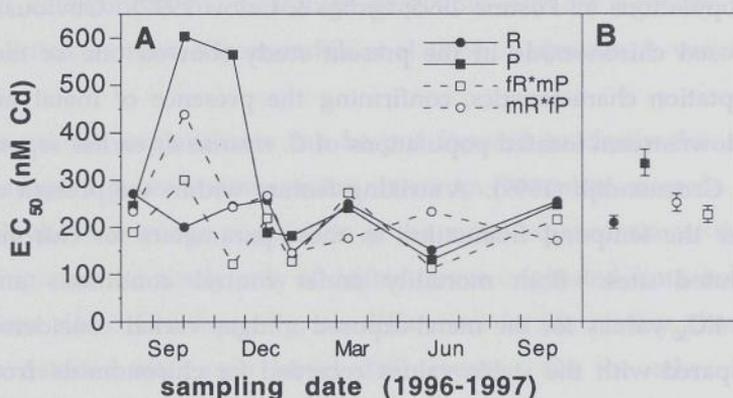


FIGURE 7.4: Panel A: temporal variation in short term (96 hours) EC_{50} values (nM cadmium) for larval growth in the reference (R) and polluted (P) populations of *Chironomus riparius*, as well as in both reciprocal crosses. For clarity, 95% confidence limits were omitted from the graph, but are shown in table 7.1. Panel B: average values with 95% confidence limits based upon the temporal data.

Temporal variation in short term EC_{50} values is presented in figure 7.4. The EC_{50} values recorded for the reference populations showed a stable level which was maintained throughout the year and varied between circa 145 and 270 nM cadmium. No clear differences between the two field populations and the reciprocal crosses were present in August 1996 or again between December 1996 and October 1997. In contrast however, a clear and highly significant difference in the larval response between the reference

and the polluted site was noticed in October and November 1996. On these sampling dates, the crossbred strains showed intermediate EC_{50} values between the two parent populations (October 1996) or displayed an equal or even lower response compared with the reference parent population (November 1996) (figure 7.4).

Discussion

metal adaptation

In general, a high control mortality, a lower larval growth under clean conditions and an increased EC_{50} value observed in clean cultured first generation animals, are reliable indicators for local adaptation to pollutants in field populations (cf Postma 1995; Forbes & Calow 1997). Obviously, the metal-exposed chironomids in the present study showed one or more of these adaptation characteristics, confirming the presence of metal adapted genes in downstream located populations of *C. riparius* as earlier reported by Postma & Groenendijk (1999). A striking feature within the present dataset is however the temporal fluctuation in these parameters for chironomids from polluted sites. Both mortality under control conditions and the calculated EC_{50} values for the metal-exposed midges varied considerably in time, compared with the stable values recorded for chironomids from the reference site. This observation showed consistency with earlier measurements of metal adaptation in *C. riparius* from the River Dommel (Groenendijk et al 1999a) and corroborate the conclusion that gene flow from non-polluted sites has a large impact on the level of metal adaptation in *C. riparius* at metal-exposed sites in the river. This hypothesis is also clearly supported by the results of the crossbreeding experiments. During the period that interpopulation differentiation was recorded (October-November 1996), crossbreeding of the field collected parent population resulted in a strong decrease of metal adaptation in the crossbred offspring. After one generation of intense gene mixing, the adaptation present in metal-exposed chironomids was decreased to intermediate levels or became

even comparable with the reference midges. The loss of metal adaptation in *C. riparius* after crossbreeding with a non-adapted midge population, showed a large resemblance with results obtained in comparable experiments using insecticide resistant dipterans. After crossbreeding a pyrethroid resistant strain of Hornflies (*Haematobia irritans*) with a susceptible population, the F1 hybrids showed intermediate LC_{50} values compared with both parental populations. However, like in the present November experiment, the hybrid sensitivity to the pyrethroid showed values closer to the susceptible strain, indicating a rapid loss of tolerance to the toxicant within one generation (Roush et al 1986; McDonald et al 1987). It should be noted that the present crossbreeding technique is the result of mixing circa 50% reference together with circa 50% metal-exposed midges, mimicking a high gene flow rate. However, it was estimated that under natural field conditions, these high gene flow rates are regularly reached at the polluted location (Groenendijk et al 1999b; Raijmann & van Grootveld 1997).

Two related explanations can be put forward to clarify the absence of metal-adapted genes at downstream sites from December onwards. It is well possible that the midge population at the metal-exposed site at the time of sampling, consists nearly only of drifted larvae originated from non-exposed upstream sites. Consequently, no differences in life history characteristics can be detected after such larval drift events. On the other hand, the reproductive season for *C. riparius* start in March and last well into November (Groenendijk et al 1999b), and gene mixing in the field could, therefore, also be responsible for the decline in EC_{50} values. The rapid loss of metal adaptation by gene flow as shown in the October and November experiments under artificial conditions, therefore, is most likely to occur also in the field situation and this explains why metal adaptation in the field is regularly absent (cf Klerks et al 1997). This is confirmed by the recorded temporal variation in life-history characteristics in the metal-exposed midge population.

heritability of metal tolerance

In the present study, differences in metal tolerance between the reference and metal-exposed midges were demonstrated using larvae from a clean cultured laboratory-reared F1 generation. Therefore, the presence of a major genetic component for the heritability of metal tolerance was assumed. However, the influence of maternal effects, recently described as the final individual phenotype as affected by the environmental experiences of the mother (Mousseau & Fox 1998), could not be ruled out completely. It was for instance reported that the freshwater snail *Brotia hainanensis* collected from two separate sites along the same river, showed marked different acute responses to cadmium even after acclimation of a period of one week to laboratory conditions. Similar interpopulation differences were recorded for the F1 juveniles, but these faded away when cultured in the laboratory for longer periods. Therefore, it was concluded that both environmental and maternal effects had a major influence on cadmium tolerance in the snails and this was supported by the results of semi-quantitative genetical analysis (Lam 1996). In the present study, it was assumed that indications of the presence of maternal effects (or sex-linked inheritance of the metal tolerance) on metal adaptation could be properly detected by comparing the level of metal tolerance in the two reciprocal crossbred strains. Indeed, in some experiments a slight indication of maternal effect or sex-linked inheritance of metal adaptation could be traced. This is for instance visualised in the significantly increased EC_{50} value in the October experiment for the mR*fP strain compared with the reciprocal mP*fR strain. However, no persistent pattern was observed during the sampling period and differences between both reciprocal crosses were small and in most cases insignificant, indicating a major genetic component for the increased metal tolerance in the polluted population.

In many arthropods, resistance to pesticides is commonly thought to be monogenically controlled, especially when the selection pressure is intense (Hoffmann et al 1995; Walker et al 1996). This major gene response shows in many cases a simple inheritance, with the resistant gene being dominant and heterozygotes showing intermediate responses compared to the

homozygote strains (Taylor 1986). However, many exceptions are reported (cf Shaw 1999), indicating the absence of uniform genomic responses in adaptation to toxicants. The response shown by *C. riparius* in the October experiment strongly suggest a single gene response, with both the reciprocal crosses almost similar and intermediate compared with the parent populations. This is highly comparable with the results of Martínez & Levinton (1996), who studied metal adaptation in the oligochaete *Limnodrilus hoffmeisteri* and presented evidence for metal adaptation controlled by one single gene. However, the temporal response in the present set of experiments is far from uniform and, in addition, the studied characteristics showed only relatively small differences between the adapted and the non-adapted reference *C. riparius* population. This is highly congruent with earlier research on this subject (reviewed in Postma & Groenendijk 1999). Therefore, adaptation to metals in the midge *C. riparius* seemed to be a gradual process (cf Posthuma & van Straalen 1993), and it is tentatively hypothesised that in such gradual cases the metal tolerance of an individual is determined by several, partly, additive factors. This probably involves a polygenic rather than a monogenic response. Consequently, differences in the level of adaptation may arise between populations depending on the genetic variation present for each of the additive factors. However, although the net process seemed to be gradual, a discrete and sudden increase in one of the factors is still possible.

synopsis

The present set of observations showed that metal adaptation in riverine chironomids is subject to considerable fluctuations. If, however, at certain moments, population specific differences are recorded, experimental simulation of high gene mixing rates showed a rapid loss of metal adaptation in the first generation hybrid offspring. It is therefore concluded that the often observed absence of metal adaptation in *C. riparius* populations from the River Dommel can be reliably explained by the influence of gene flow from non-polluted areas. Judging by the response of the studied set of parameters in the reciprocal first generation crosses, no

clear indications of maternal effects could be traced. It is therefore suggested that a major genetic component for the increased metal tolerance in the metal-exposed midge populations is present.

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CHAPTER VIII

Concluding Remarks

Virtually all research on adaptation to metals is carried out in relatively stable environments. Indeed, in most soils the level of contamination, and therefore the level of actual selection pressure, shows only slight temporal fluctuations (cf. McNair 1977; Wata et al. 1993). When natural species with low dispersal rates were also tested, the impact of gene flow is supposed to be weak (Klarke & Lewinton 1988; Postburg 1993) and, consequently, stable adaptation to metals can be attained. Clearly, this explains why the dynamic influence of gene flow and selection is often poorly or even not documented in cases of metal adaptation (cf. Beaudou 1990; Postburg & van Duijn 1993).

However, both components (gene flow and selection pressure) were expected to change rapidly in riverine environments. The general aim of this thesis was therefore to identify key factors influencing the dynamics of metal adaptation in *C. nigricornis*. This section reviews the present observations on temporal and spatial components of metal adaptation in the River Dommel.

Speed of micro-evolution

Because selection through metal contamination can be very strong, the speed of micro-evolution can be many orders of magnitude higher than the average rates estimated over macro-evolutionary time scales (Kirkpatrick 1996). Insight in the rate of adaptation can be obtained by analysing the

