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Tolerance Induction and Life Cycle Changes in Cadmium-Exposed *Chironomus riparius* (Diptera) during Consecutive Generations

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Cultures of *Chironomus riparius* were exposed to cadmium during nine consecutive generations to determine whether cadmium tolerance could be induced. Selection for cadmium tolerance was assumed to influence the population dynamics of this species. Therefore, the responses and interactions of different population parameters (such as mortality, growth, and reproduction) were studied during the selection process. Exposure to cadmium during consecutive generations caused increasing effects on some life cycle parameters compared to a one-generation experiment. Tolerance to cadmium increased during exposure to 54.2 n*M* Cd and the tolerant population seemed to be stimulated by low cadmium concentrations (based on an acute growth experiment). Despite this tolerance development, mortality among cadmium-exposed tolerant chironomids remained high. These experiments illustrated that changes of the life cycle and tolerance can be expected as soon as single-generation NOEC values are exceeded, and in addition that "safe concentrations" based on a one-generation toxicity experiment could well underestimate the potential effects of a toxicant on midge populations. © 1995 Academic Press, Inc.

INTRODUCTION

Pollution can play an important role in natural selection among aquatic organisms. Macroinvertebrates originating from metal-polluted sites are often less susceptible to metals than their unexposed conspecifics (Klerks and Weis, 1987). Selection experiments in the laboratory revealed that an increased tolerance to metals may evolve within a few generations of *Daphnia magna* (e.g., Bodar *et al.*, 1990; Münzinger and Monicelli, 1992), *Limnodrilus hoffmeisteri* (Klerks and Levinton, 1989), and of *Tisbe holothuriae* (Moraïtou-Apostolopoulou *et al.*, 1983).

However, observations on chironomids have suggested contradictory trends. Wentsel *et al.* (1978) demonstrated that larvae of *Chironomus tentans* from a metal-polluted site were less affected by exposure to metal-polluted sediment than their conspecifics from a clean location (based on differences in survival, growth, and avoidance responses). In contrast, Klerks and Levinton (1993) did not detect any differences in tolerance between larvae of the chironomid *Tanypus neopunctipennis* from a polluted and an unpolluted area, while the oligochaete *L. hoffmeisteri*, originating from the same areas, demonstrated an increased tolerance at the polluted site.

Increased tolerance to metals in chironomids can be a result of a changed physiology. Krantzberg and Stokes (1989) observed, for example, that *Chironomus* species from an exposed field population had an increased capacity to regulate the body concentration of metals (Cu, Ni, and to some extent Mn). Metallothionein-like proteins could be involved, since both Yamamura *et al.* (1983) and Seidman *et al.* (1986) found that the *Chironomus* species (among which is *C. riparius*) can synthesize these proteins when exposed to cadmium.

Apart from physiological changes, changes in the life history can have an adaptive value (Maltby, 1991). Changes in growth rate or reproductive output have been demonstrated in both terrestrial (e.g., *Orchesella cincta*, Posthuma *et al.*, 1993; *Porcellio scaber*, Donker *et al.*, 1993) and aquatic species (e.g., *Asellus aquaticus*, Maltby, 1991). Both physiological and life-history changes may be expensive in terms of energy or nutrients and can, therefore, involve a diminution of the ability to invest in other activities (Holloway *et al.*, 1990), which will affect the population growth rate. Information on the responses of life-history characteristics during selection to toxicants is scarce. It seems, therefore, advisable that studies on tolerance induction should not only test for the net resulted tolerance but also, in addition, examine a possible shift in the population life cycle and growth dynamics.

The present study aimed to investigate the ways in which different parameters influencing the population growth rate would respond and interact during selection for metal tolerance. To do this, the chironomid *Chironomus riparius* (Meigen) was exposed during nine consecutive generations to environmentally realistic cadmium concentrations and the effects on mortality, growth, and reproduction were studied. In addition, acute tests were performed to see whether tolerance had increased. *C. riparius* was chosen because this is one of the species

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able to survive in metal-polluted rivers in the southern part of the Netherlands (unpublished results).

MATERIALS AND METHODS

Experimental Conditions

C. riparius was cultured in plastic aquaria ($l \times w \times h$ = $34 \times 17 \times 21$ cm^3) supplied with a 1-cm layer of shredded paper (14 g dry weight) as a substrate for the larvae. The overlying water (4 liters) was aerated constantly. A cage ($l \times w \times h = 39 \times 21 \times 30$ cm^3) was placed over each aquarium. Larvae of *C. riparius* originated from a laboratory culture that had been started about 4 years ago with larvae collected from one of the experimental ponds of the university. To reduce the potential effects of inbreeding, the stock culture was constantly maintained at a large population size and egg-ropes from this culture were exchanged with other laboratories. All experiments were carried out in a climate room at 20 ± 1 °C. A 16-hr: 7-hr light:dark regime was provided, with a twilight zone of 30 min before and after the light period, to stimulate reproduction.

Experimental Protocol

The experiments were carried out in water obtained from Lake Maarsseveen I, an oligomesothrophic lake in which trace metal concentrations are near detection limits, the pH was 7.8, and the Na and Ca contents were 18.8 and 63.6 mg/liter, respectively (Timmermans *et al.*, 1989). All metal analyses were performed with graphite furnace atomic absorption spectrometry (Perkin–Elmer 5100) equipped with Zeeman correction. Larvae were exposed to one of three concentrations of cadmium (added as a solution of cadmium chloride) or to an uncontaminated control 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 in the weekly samples during the experiment were 0.32 ± 0.43 (control), 17.0 ± 7.37, 54.2 ± 21.0, and 159.6 ± 61.5 n*M* Cd (mean ± standard deviation) (0.04, 1.9, 6.1, and 17.9 μg Cd/liter, respectively). There were no significant differences in the cadmium concentrations among the generations within one treatment (tested by ANOVA). The chosen concentrations were based on pilot experiments: 15 n*M* was found to be at or below the chronic NOEC (no observed effect concentration) depending on the parameter used (mortality, growth, or reproduction); 51 n*M* was a sublethal concentration, which had a negative effect on reproduction as well as on growth; and 139 n*M* was the chronic, one-generation LC_{50}.

In order to prevent a rapid decrease of the water concentrations during the experiments, the aquaria and the cellulose were equilibrated with the corresponding cadmium concentrations by soaking 14 g of dry cellulose in 4 liters lake water with 0.3, 17.0, 54.2, and 159.6 n*M* Cd before the experiments were started. Cadmium-loaded food was prepared by soaking 10 g of ground Trouvit and 0.5 g of Tetraphyll in 200 ml of the corresponding cadmium solution. Spiking of the aquaria, cellulose, and food lasted for 1 week, during which time all solutions were renewed twice. Before the beginning of the experiment all solutions were renewed once again. While the aquaria and the cellulose were fully loaded at the beginning of the experiment, the added food continued to bind cadmium, causing a gradual decrease of the water concentrations. During the experiment, twice a week 2.5 ml of food was added, providing an excess of food to the larvae.

Successive generations of midges were reared as follows. Every day egg-ropes were collected in the three replicates and placed in a petri dish. After hatching, three replicates of the next generation were started with 50 newly hatched, first instar larvae (less than 24 hr old) for each replicate. Normally, larvae originating from more than 10 different egg-ropes were randomly sampled. When small numbers of egg-ropes were produced, hatching was delayed by placing some of them at 4 °C, thereby ensuring that enough larvae originating from different egg-ropes hatched at the same time. In most cases it took about 1 month before the next generation could be started. Each generation was kept until all surviving larvae had emerged. This took about 4–8 weeks, depending on the cadmium concentration. The total experiment was conducted over nine consecutive generations and lasted about 1 year.

All materials used in the experiments and analysis were cleaned by soaking them in 0.1 *N* HNO_3 (Merck Pro Analysis) for at least 24 hr and then rinsed three times with double-distilled water. Lyophilized organisms were weighed and digested individually, in concentrated HNO_3 and H_2O_2, using a microdestruction 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.

Life Cycle Parameters

At the onset of emerging the following parameters were assessed daily:

—The number of newly emerged males and females, by sexing and counting the pupal exuviae. On the basis of these data the mortality and the mean larval development time were determined.

—The number of male and female midges in the cage, to estimate the mean life span of an imago.

—The number of dead male and female midges, to

check the first two parameters. Dead imagines were removed and for every concentration 10 males and 14 females were collected for analysis of dry weight and accumulated cadmium.

—The number of deposited egg-ropes as well as the number of eggs per egg-rope, to provide an estimation of the mean number of eggs per female midge. In addition, the hatchability of the egg-ropes was assessed after hatching in a petri dish 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.

Tolerance Tests

At the end of the experiment, acute tests for cadmium tolerance were performed by determining the body growth of first instar larvae. Larvae, less than 24 hr old, were exposed to cadmium concentrations ranging from 0.27 in the control to 101.4, 218.9, 409.3, 938.6, and 1699.3 n*M* Cd (actual values). All concentrations were tested on at least 30 larvae for each population. The initial length was measured on 30 additional animals using an ocular micrometer. After 96 hr the surviving larvae were counted and their lengths were measured. The percentage growth reduction relative to the control was calculated for the different cadmium concentrations.

Statistical Analyses

Routine statistical analyses (two-way ANOVA or Friedman method) were performed according to Sokal and Rohlf (1981), after the assumptions for ANOVA analyses were checked. However, statistical dependency of the data arising from the use of successive generations could have interfered with these analyses. In addition, the next generation was started using a subsample of the eggs deposited by the previous generation, thereby limiting statistical analyses based on repeated measurements on the same system. Differences between group means were tested using the Student–Newman–Keuls (SNK) procedure when possible.

Heritability of characteristics could not be estimated because offspring–parent regression analysis requires that a single couple will reproduce successfully, which is not the case for *C. riparius*.

RESULTS

Mortality

Mortality in the control group varied around 10% and differences among the generations were not significant (SNK procedure) (Fig. 1). Changes in mortality were, however, observed for cadmium-exposed midges, causing a significant interaction between the factors Cd concentration and generation ($F = 3.59$, $P < 0.001$) as well as significant main effects (Cd concentration: $F = 26.39$, $P < 0.001$; generation: $F = 5.05$, $P < 0.001$). Although mortality in the first generation was only increased by exposure to 159.6 n*M* Cd (50%), mortality increased significantly in all cadmium treatments during consecutive generations (SNK procedure). This increased mortality (together with a low reproductive output) caused the extinction of the population exposed to 17.0 n*M* Cd. In the population exposed to 54.2 n*M* Cd, mortality seemed to stabilize at the end of the experiment (around 35–40%).

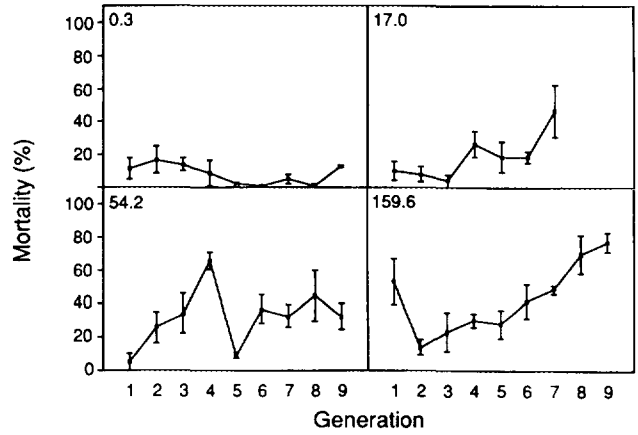

FIG. 1. Mortality (%) among the larvae during consecutive generations of *C. riparius* exposed to four different cadmium concentrations (number in upper left corner in n*M*). The mean values together with their standard errors are presented.

Larval Development Time

The mean larval development time of control larvae varied around 28 days for all generations, although it seemed to increase slightly in subsequent generations (Fig. 2). Exposing the midges to 17.0 n*M* Cd did not increase the larval development time in any generations;

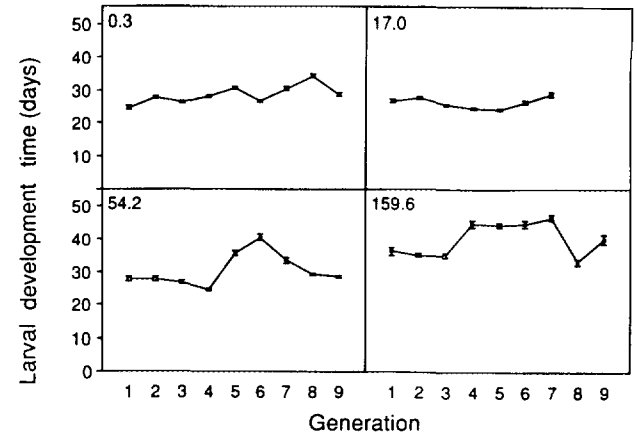

FIG. 2. The larval development time (days until emergence) during consecutive generations of *C. riparius* exposed to four different cadmium concentrations (number in upper left corner in n*M*). The mean values together with their standard errors are presented.

indeed, a slight tendency to shorter development times was observed. In the populations exposed to 54.2 and 159.6 n*M* Cd the larval development time was unaffected during the first generations, but increased significantly in later generations (Friedman's two-way ANOVA, $P < 0.001$). Thereafter, the larval development time decreased again. Especially for the population exposed to 54.2 n*M* Cd, this recovery was also observed in the pattern of emergence. During the fifth to seventh generation emergence was spread out in time, while in the eighth and ninth generation the characteristic mass emergence of chironomids was present again (as in the first four generations).

Eggs per Female

The mean number of eggs per female varied around 300 in the control population and did not differ significantly among the generations (SNK procedure) (Fig. 3). All cadmium treatments on the other hand reflected a small increase during the first few generations followed by a significant decrease in later generations (SNK procedures). This increase was caused by a higher number of egg-ropes deposited per female (up to 1.4 egg-ropes per female compared to 1.0 in the control). There was, therefore, a significant interaction between the factors Cd concentration and generation (two-way ANOVA; $F = 4.65$, $P < 0.001$) as well as a significant main effect of the factor generation ($F = 9.54$, $P < 0.001$). The main effect of the factor cadmium concentration was not significant ($F = 1.21$, $P = 0.30$). After this sharp decrease in fecundity, the populations exposed to 54.2 and 159.6 n*M* Cd seemed to recover as the average number of eggs per female increased and no longer differed from control values (SNK procedure). This recovery was partly caused by a higher number of egg-ropes deposited per female but especially by a higher number of eggs per egg-rope. While the control population produced on average 300 eggs per egg-rope, the last three/four generations of the 54.2 and 159.6 n*M* Cd populations produced egg-ropes containing on average 350 and 450 eggs, respectively.

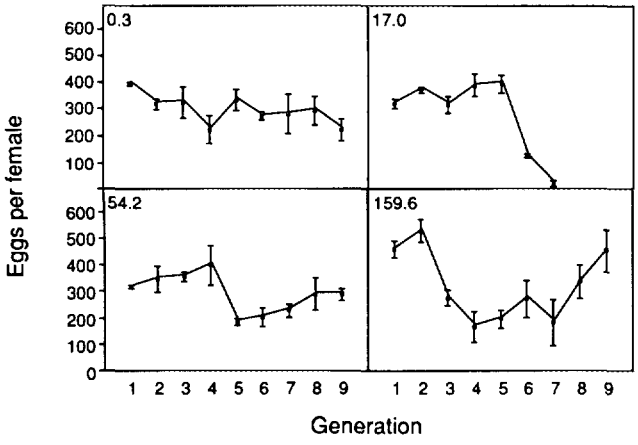

FIG. 3. The number of eggs deposited per female midge during consecutive generations of *C. riparius* exposed to four different cadmium concentrations (number in upper left corner in n*M*). The mean values together with their standard errors are presented.

Hatchability of the Egg-Ropes

The hatchability of the egg-ropes was estimated daily for all three replicates together and the data are only given as the mean value (Table 1). The hatchability of eggs of the control population was on average 86% (for all generations) and during the first four generations cadmium did not affect the hatchability negatively. In later generations, however, the hatchability decreased for all three exposed populations. The hatchability of egg-ropes in the population exposed to 54.2 n*M* Cd seemed to recover from the seventh generation onward.

Life Span of Imagines

Imagines, emerging from control larvae, lived an average 4.2 days, independent of the different generations (Fig. 4), but from exposed midges the mean life span increased significantly with consecutive generations (up to 6.5 days). The interaction term between the factor Cd concentration and generation was therefore significant ($F = 2.78$, $P = 0.001$) as were both main factors (Cd concentration: $F = 34.96$, $P < 0.001$; generation: $F = 7.20$, $P < 0.001$). In the population exposed to 54.2 n*M* Cd this initial increase was followed by a decrease and from the seventh generation onward the life span of these imagines no longer differed from the controls (SNK procedure).

Population Growth Rate

In most generations the population growth rate of the control population varied around 3.8, with a slight tendency to decrease (Fig. 5). Only the first generation differed significantly from the last two generations (SNK

TABLE 1

Hatchability of the Egg-Ropes of *C. riparius* (%) in the Different Generations

Generation	0.3 n*M* Cd	17.0 n*M* Cd	54.2 n*M* Cd	159.6 n*M* Cd
1	90.7	90.3	93.7	92.2
2	94.0	89.2	90.0	95.6
3	89.3	87.8	88.4	91.6
4	82.4	91.1	95.0	84.4
5	93.9	86.1	57.9	68.7
6	77.9	58.8	63.6	50.0
7	83.2	0.0	48.2	47.5
8	79.1		68.4	11.9
9	90.0		78.1	0.0

Note. Only the mean values are presented.

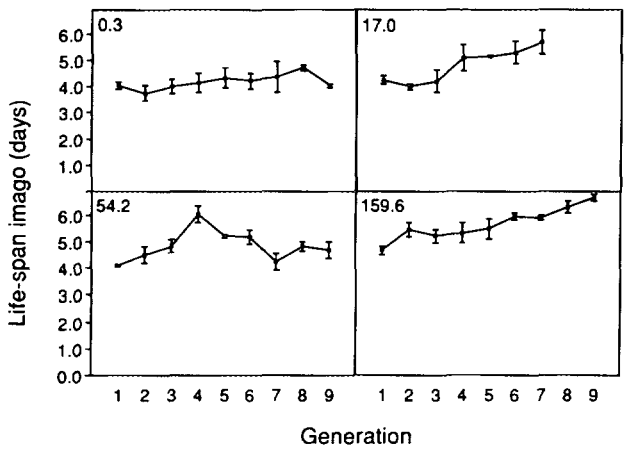

FIG. 4. The life span of imagines (days) during consecutive generations of *C. riparius* exposed to four different cadmium concentrations (number in upper left corner in n*M*). The mean values together with their standard errors are presented.

procedure). Changes were, however, observed in all cadmium treatments, causing both a significant interaction between the factors Cd concentration and generation (two-way ANOVA, $F = 6.91$, $p < 0.001$) and significant main effects (Cd concentration: $F = 52.39$, $P < 0.001$; generation: $F = 32.13$, $P < 0.001$). Increased mortality and reduced reproduction of the midges exposed to 17.0 or 54.2 n*M* Cd caused the population growth rate to decrease significantly (SNK procedure). The population exposed to 54.2 n*M* Cd, however, recovered and the population growth rate of the ninth generation was significantly higher compared to the fifth to seventh generations. The population growth rate of the population exposed to 159.6 n*M* Cd peaked in the second generation (with a value of 5.4, mainly due to a low mortality) but later it decreased continuously until all replicates were extinct by the ninth generation.

Dry Weight and Cadmium Accumulation

The dry weights of female midges varied, probably because some females had not deposited their egg-ropes. The cadmium accumulation in the female midges was consequently also rather variable and these data are therefore not presented. Since differences between dry weights of male midges were virtually absent among the generations at each cadmium concentration, differences among cadmium concentrations could be tested and the data are presented as the overall average (Fig. 6). The dry weight of a male midge from the population exposed to 159.6 n*M* Cd was, on average, 30 μg greater than from the other three populations (ANOVA; $F = 6.59$, $P < 0.001$; Fig. 6). The cadmium accumulation was not influenced by the consecutive generations but it increased clearly at higher exposure levels.

Tolerance Induction

At the end of the experiment tolerance induction could only be tested on the population exposed to 54.2 n*M* Cd, since the other two populations were already extinct. Cadmium clearly reduced the body growth of first instar larvae obtained from the ninth generation of the control population (Fig. 7). First instar larvae from the population exposed to 54.2 n*M* Cd, on the other hand, had a maximum body growth when exposed to 101.4 n*M* Cd. Even at 218.9 and 409.3 n*M* Cd, larvae from this population were less affected by cadmium than was the control population. This difference in cadmium tolerance among the populations is significant, as is demonstrated by the interaction term in a two-way ANOVA ($F = 8.46$, $P < 0.001$) and a significant main effect of the factor "population" (ANOVA; $F = 61.20$, $P < 0.001$).

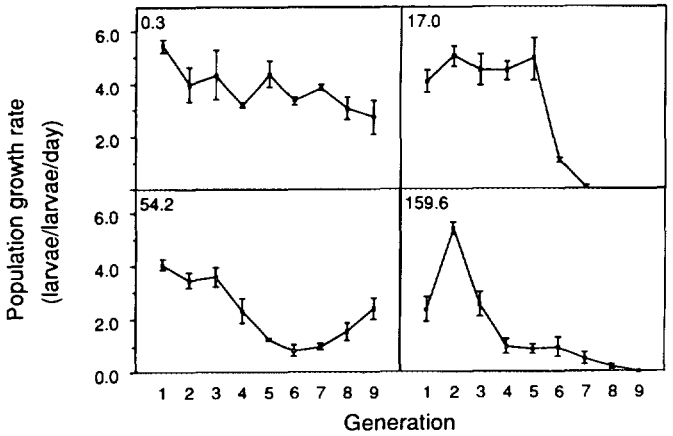

FIG. 5. The population growth rate (larvae/larvae/day) during consecutive generations of *C. riparius* exposed to four different cadmium concentrations (number in upper left corner in n*M*). The mean values together with their standard errors are presented.

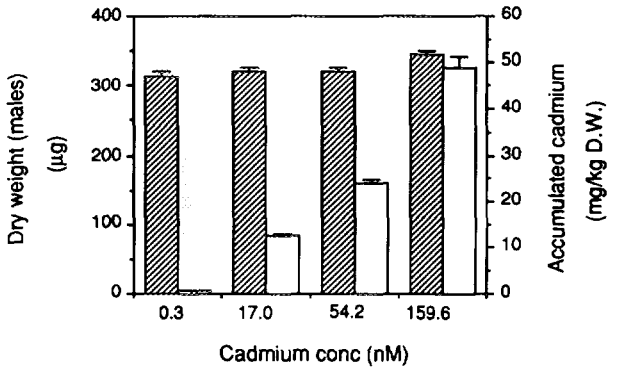

FIG. 6. The dry weight (μg) and the accumulated cadmium (mg/kg dry wt) of male imagines. As no large differences were observed during nine consecutive generations of *C. riparius* exposed to four different cadmium concentrations, only the overall averages (together with their standard errors) are presented. Hatched bars represents dry weight analysis and open bars represent the accumulated cadmium.

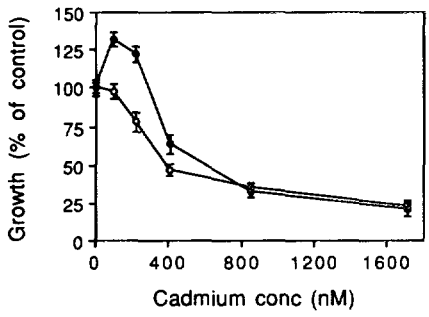

FIG. 7. The growth reduction by cadmium in first instar larvae of *C. riparius*. Data are expressed as a percentage reduction compared to the control. Open circles represent the ninth generation of the control population and closed circles represent the ninth generation of the population exposed to 54.2 n*M* cadmium. The mean values together with their standard errors are presented.

DISCUSSION

This study revealed considerable discrepancies among the response of *C. riparius* to cadmium during one generation compared to its response over consecutive generations. The results obtained in the first generation are in good agreement with other experiments on the toxicity of cadmium to *C. riparius* (Pascoe *et al.*, 1989; Timmermans *et al.*, 1992; Williams *et al.*, 1987) as well as with studies on the effects of metals on the reproduction of other chironomid species (Hatakeyama, 1987, 1988). However, in later generations deviations from this pattern were observed in most life cycle characteristics. The reproductive output, for example, decreased during the first generations, but after about six to seven generations a sudden increase in the mean number of eggs per egg-rope was observed in both populations exposed to 54.2 and 159.6 n*M* Cd (with 20 and 50% compared to the control, respectively), which persisted to the end of the experiment. Life history theory predicts that reduced adult survival or reproductive success will select for earlier maturation and increased reproductive effort early in life (Charlesworth, 1980; Michod, 1979). Changes in life cycle parameters can therefore be expected in both exposed field populations as well as in multigeneration selection experiments. Several authors have already demonstrated that changes in the life history are likely to occur in field populations of different animal species under toxicant stress (Donker *et al.*, 1993; Posthuma *et al.*, 1993; Tranvik *et al.*, 1993; Weis and Weis, 1989), although others have not found a significant change (Bengtsson *et al.*, 1992).

By exposing chironomids during nine consecutive generations an effort was made to see whether a change in life history could be induced by cadmium in the laboratory, which seemed to be the case for the reproductive output. A decrease of the larval development time was not found in this experiment, probably because the amount of genetic variation was rather low in laboratory-maintained cultures of *C. riparius*. In addition, the experimental setup should strongly favor midges with a short larval development time because the next generation was started by using egg-ropes which were deposited soon after the emergence started. The larval development time of the control did not, however, decrease, again probably indicating a low amount of genetic variation as a result of a laboratory culture, as has been demonstrated for *C. tentans* by Woods *et al.* (1989). As the laboratory stock culture was constantly maintained at a large population size, it was expected that inbreeding would especially play a role after the start of the experiment reported here, in which much smaller population sizes were used. Hence, the tendency of the population growth rate to decrease in the control population could have been caused by inbreeding, although normal fluctuations in population growth rates should not be ruled out. In addition, culturing midges during consecutive generations under strict conditions could possibly cause unknown selective forces. The absence of a clear response in most life cycle parameters of the control population seems to indicate that there were no selective forces or no genetic variance to act on. The changes in life-cycle parameters of cadmium-exposed populations resulted, therefore, most likely from the interaction between normal population fluctuations (maybe enhanced by inbreeding) and fluctuations caused by cadmium toxicity. To estimate the influence of cadmium as a causative factor, comparisons were made among the different cadmium concentrations.

In general, there was a good association between the generation in which an effect occurred and the level of cadmium exposure. Effects occurred at the highest cadmium concentration first, and later on in populations exposed to lower cadmium concentrations. It took seven generations before mortality reached a level of 50% in the population exposed to 17.0 n*M* Cd, while the same level was reached within four generations in the population exposed to 54.2 n*M* Cd. In addition, the sharp increase in larval development time occurred earlier in the population exposed to 159.6 n*M* Cd than in the population exposed to 54.2 n*M* Cd. The population growth rate, being a summation of the separate parameters, indicated this association with the cadmium concentration most clearly. The population growth rate started to decrease in the second, third, and fifth generations of the populations exposed to 159.6, 54.2, and 17.0 n*M* Cd, respectively. Based on this good association between the generation in which an effect occurred and the cadmium concentration, it was concluded that prolonged cadmium exposure, indeed, exerted a major influence and caused changes in most life-cycle characteristics during the experiment.

An overall decrease of the population growth rate in

this multigeneration experiment was observed at all cadmium concentrations, even at 17.0 n*M* Cd, a concentration which had no significant effect in the first generation on any of the life cycle parameters studied. This is in accordance with the findings of LeBlanc (1982), who determined increasing mortality when *D. magna* were exposed for three generations to sodium lauryl sulfate concentrations that were sublethal during the first generation. Observations on multigeneration effects of toxicants have rarely been published, but the present observations indicate that the current interpretation of single generation NOECs as "safe environmental concentrations" should be reconsidered. There is, therefore, a fundamental difficulty in extrapolating to natural populations the long-term, one-generation NOEC, EC_{50}, and other effects obtained in laboratory experiments. Comparing field-obtained data on tolerance development and laboratory-determined NOEC values in terrestrial insects, Posthuma *et al.* (1993) suggested that evolutionary modification can occur if NOEC values are exceeded, again questioning the ecological relevance of the currently used safe environmental concentrations.

Increased tolerance in addition to changes in the life-cycle characteristics indicated that the tolerance probably started to increase after about six generations. Both the larval development time and the mean number of eggs produced per female indicated a certain recovery at the end of the experiment. Also, the pattern of emergence confirmed the increased tolerance to cadmium. While emergence of the fifth to seventh generations exposed to 54.2 n*M* Cd was spread out over many days, the eight and ninth generations displayed again the mass emergence characteristic for chironomids. The population growth rate increased consequently, from the sixth generation onward. This population was maintained in the laboratory as a cadmium-adapted population and, although the experiment was ended, the population growth rate was monitored for an additional generation and continued to increase, reaching a level (3.24) in the tenth generation, which was comparable to the control population.

Although most life-cycle parameters (and consequently the population growth rate) seemed to resume normal values, mortality remained substantial throughout the experiment (about 40% from the sixth generation onward). In addition, LeBlanc (1982) found that mortality among *D. magna* exposed to copper remained rather variable during consecutive generations. An interesting detail in the present experiments is that while the effects on mortality remained substantial, the reproduction was not always affected to the same degree. This is in contrast with the commonly held view that reproduction is a more sensitive characteristic than mortality.

Most life-cycle parameters in cadmium-exposed cultures were gradually changing over consecutive generations, which is rather unlikely when only acclimation is involved. It seems therefore probable that genetic variance for several parameters was present, although a possible role of accumulating maternal effects cannot be ruled out. This aspect (adaptation versus acclimation) is the subject of current research. The physiological mechanism involved in this tolerance is not yet known. It is, however, likely that *C. riparius* can synthesize metallothionein-like proteins (Yamamura *et al.*, 1983; Seidman *et al.*, 1986). It is often suggested that metallothionein production increases the accumulation of metals, which could be an explanation for the observation that tolerant animals accumulate more metals than a nontolerant population (Bodar *et al.*, 1990; Brown, 1977; Bryan and Hummerstone, 1971). Accumulation of cadmium by *C. riparius* was, however, not affected. It neither increased nor decreased during nine generations, despite the increased tolerance to cadmium and changes in the effects on life-cycle parameters. It seems therefore likely that a direct correlation between the absolute amount of accumulated metal in the imagines and ecological relevant effects does not necessarily exist. The distribution of cadmium inside the body or the physiological state is probably also important.

Exposing chironomids during consecutive generations reveals that cadmium modifies the normal fluctuations in population growth rates. During these experiments even environmentally realistic concentrations, down to NOEC values, appeared to be important selective agents. The conclusion is that multigeneration experiments provide a better understanding of the processes involved in tolerance development. Even more importantly these experiments provide an insight in the population dynamics of species changing due to prolonged exposure to toxicants.

CONCLUSIONS

—The effects of cadmium on mortality, growth, and reproduction increased during consecutive generations (even at concentrations which had no negative effect during the first generation).

—Toward the end of the experiment, decreasing effects were observed on growth and reproduction, indicating an increased tolerance to cadmium. Effects on mortality remained, however, substantial.

—This increased tolerance was not reflected in a changed cadmium accumulation.

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