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 therewith the level of actual selection pressure, shows only slight temporal fluctuations (cf MacNair 1997; Weis et al 1999). When animal species with low dispersal rates were also tested, the impact of gene flow is supposed to be weak (cf Klerks & Levinton 1989; Posthuma 1992) 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 Brandon 1990; Posthuma & vanStraalen 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. riparius. 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
combination of the strength of the natural selection and the heritability ($h^2$) of characters involved. So far in invertebrates, estimations of such heritability values in metal tolerance are only available for the springtail *Orchesella cincta* ($0.33 < h^2 < 0.48$; Posthuma et al. 1993) and the oligochaete *Limnodrilus hoffmeisteri* ($h^2 > 0.9$; Klerks & Levinton 1993). Indeed, those values illustrate good responses to the selection process and indicate that adaptation to metals can develop very rapidly within a few generations. Accordingly, a high rate of adaptation is also suggested by the development of metal tolerance in metal-exposed cultures of *C. riparius* (Postma & Davids 1995). The possibility of a quick regeneration of the genetic ability to cope with metal stress is indicated in the present set of observations also. The studies on temporal variation in adaptational characteristics showed large differences in responses of the metal-exposed midges over time. On certain sampling dates no tolerance and hence no interpopulation differences could be traced, whereas, during following sampling events highly significant interpopulation differences were found. These repeated observations suggest that restoration of the metal tolerance must have taken place in the intermediate periods. This is likely to have been facilitated by the species' short generation time during the reproductive season. Thus, hypothetically the newly built up metal adaptation can develop quickly in non-tolerant midge larvae, but can also emerge from rare metal-adapted genotypes which are likely to be present all year round in low frequencies at metal-exposed sites.

Short-term adaptation to strong and consistent selection may lead to a substantial loss of genetic variation influencing an individual's developmental stability negatively. Fluctuating asymmetry is used as an indicator for developmental disturbances and the increased asymmetry levels in metal-exposed chironomids suggest that the chironomids have been under severe stress. The major part of this increased asymmetry could be attributed to the increased metal concentrations, because it disappeared after one generation culturing under clean conditions. Accordingly, it has recently been argued that increased asymmetry levels could reasonably serve as indicators of the strength of natural selection (Swaddle et al. 1994; Möller &
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Thornbill 1997). Indeed, experimental work has shown that *Drosophila melanogaster* populations, after several generations under selection for high temperatures, exhibited increased fluctuating asymmetry levels (Bradley 1980). The increased levels of asymmetry of downstream metal-exposed midge populations therefore support the notion of a strong present day selection by metals in exposed chironomids.

In summary, it is concluded that, although no experimental estimations of the heritability of characters involved in metal tolerance can be presented (cf Postma & Groenendijk 1999), it is highly likely that adaptation to metals in *C. riparius* can occur within a few generations. These high micro-evolutionary rates are facilitated by the strong selective force, the severe metal pollution, as indicated by the increased asymmetry levels.

**Influence of gene flow**

It is known that gene flow among populations will prevent complete fixation of an adaptive trait. There can be no doubt that gene flow from non-adapted subpopulations act as force counteracting selection in adapted populations. On the other hand, gene flow must exceed a certain level to prevent substantial genetic differentiation such as put forward in the shifting balance model of evolution (cf Wright 1931; 1982). The present observations did not indicate genetic isolation, due to distance or different reproductive mechanisms, between metal adapted and non-adapted chironomids. In support, even midge populations which were situated over ten kilometres apart, showed a complete lack of population substructuring. High gene flow rates between the outermost midge populations in the River Dommel were calculated on one occasion (Raijmann & van Grootveld 1997). The allozyme analyses were carried out with larvae sampled in October 1995 and first generation offspring of simultaneously sampled larvae showed no population differentiation based on several life history characteristics, confirming the results of the allozyme analysis. Other indications for long distance migration were suggested by the equal values for morphological markers for both metal exposed and non-exposed chironomids in early spring 1995. Due to heavy rainfall and the resulting spate of the River
Dommel, fluctuating asymmetry values and mentum gap percentages, normally increased due to metal stress on metal exposed sites, were on this occasion equal to values for chironomids sampled at upstream reference locations. This suggest a displacement of non-exposed larvae over more than seven kilometres (cf Hemsworth & Brooker 1979), and such massive displacement agrees with simultaneously measured high larval drift rates.

It is argued that because of the similarity in population dynamics of midges at reference sites and metal-exposed sites, drifting, non-tolerant larvae most probably are in similar growth stages as the metal-exposed populations. It is therefore put forward that drifting non-adapted chironomids can interbreed with metal-adapted midges present at downstream sites. Even if the rate of interbreeding is lower than the estimated values based on the drift measurements indicated, the influence on the level of cadmium adaptation will be serious. In the crossbreeding experiments it was shown that a rapid loss of metal adaptation in the first generation hybrid progeny occurred. Values of life-history characteristics indicating metal adaptation, were reduced to levels comparable with reference populations suggesting an almost complete loss of metal adaptation. It seems therefore, that the influence of downstream transport of midge larvae and the resulting gene flow is quite high, considerably affecting both population structure and cadmium-tolerance levels.

dynamics of adaptational processes

The influence of gene flow will vary both in time and space and depend upon several factors, such as seasonal population dynamics and current velocity. Furthermore, the effects of the second shaping force on the local chironomid fauna, the selective pressure of toxic metal concentrations, will depend on several biotic and abiotic factors. Field observations of disappearance of first and second instar C. riparius larvae, for example, showed a correlation, on several occasions, with a sudden increase in metal concentrations in detritus. This strongly suggests that selection pressure may act particularly on the sensitive younger instar larvae. Furthermore, the highly variable metal concentrations in conjunction with rather strict
phasing of the chironomid generations render the selection process highly unpredictable. Therefore, fluctuations in factors such as seasonal instar dynamics, rainfall and discharges might directly influence the level of tolerance present in field populations. Consequently, it is put forward that adaptation to metals in river-inhabiting chironomids should be regarded as a dynamic state in which the actual level of tolerance gradually fluctuates and is sometimes even very low or absent (figure 8.1).

**FIGURE 8.1**: Schematic view of *Chironomus riparius* subpopulations (ovals) in the River Dommel close to the zinc factory. The strength of the influences of metal concentrations (black arrows) and drifting non-tolerant larvae (white arrows) are indicated by the width of the arrow. Darker ellipses indicate better adapted chironomid populations. The influence of drift of non-tolerant larvae is supposed to be weaker at more downstream located sites.

A) high levels of metal pollution will increase selection pressure and increase the amount of adapted individuals and will promote metal adaptation further upstream

B) high drift rates of non-tolerant larvae reduce the degree of metal adaptation close to the point of discharge
Support for this dynamic model is displayed in the present thesis. Both studies in which seasonal measurements on field populations were carried out, demonstrated that several characteristics indicating metal adaptation, recorded for the reference populations, showed stable responses during the complete set of observations. In contrast however, field sampled metal-exposed populations showed considerable fluctuations in seasonally repeated measurements of control mortality (up to a factor 80), larval growth under cadmium exposure (up to a factor 8) and short term EC₅₀ values for larval growth (up to a factor 5), which were all tested in clean cultured F₁ larvae. Furthermore, those high temporal fluctuations will also be responsible for differences among metal exposed midge populations. For example, significant interpopulation differences were recorded in the shedding capacity of accumulated zinc. The downstream population demonstrated a higher metal handling capacity compared with the chironomids inhabiting the river bed close to the zinc factory. Based on these differences, it was argued that population differentiation due to metal stress is a gradual process, rather than an all-or-nothing response, confirming the differences in response shown by metal-exposed populations in the temporal studies. Furthermore, it should be noted that the impact of gene flow at downstream sites close to the zinc factory is most likely stronger and, hence, better adapted midge populations are to be expected on most occasions further downstream. It is therefore concluded that no uniform response of metal-exposed chironomids under experimental conditions can be expected, because the actual level of adaptation to metals is subject to strong fluctuations and can sometimes even be temporally absent.
References


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