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CHAPTER I

General Introduction

Local adaptation, causing population differentiation, is one of the most important basic principles of modern evolutionary biology. Natural selection, the mechanism widely regarded as the primary cause of phenotypic evolutionary changes, can be seen as the driving force of such adaptational processes (Brandon 1990; Amundson 1996). An adaptation can be defined as a trait that enhances an individual's fitness and that arose historically as a result of natural selection (Lauder 1996). Consequently, adaptation as a state is defined in part by the action of a mechanistic process, natural selection, acting directly on that trait currently deemed to be an adaptive one. For example, a high predation pressure in natural populations of Guppies (*Poecilia reticulata*) acted as a strong directional force and resulted in Guppy populations genetically different from those with a lower predatory rate. Besides, it was shown that estimated micro-evolution rates were up to seven times faster than rates inferred from paleontological records (Reznick et al 1997).

The intensity of selection pressure caused by pollution may often exceed the level of selection under natural circumstances. Hence, micro-evolutionary changes can be expected not only to be more stringent in response to a particular anthropogenic directive force, but also become apparent in a much shorter period of time (Shaw 1999). Indeed, rapid micro-evolution of tolerance to toxicants has been demonstrated in a number of arthropods, genetically responding to the use of pesticides (cf Taylor 1986; Caprio & Tabashnik 1992). Other examples of a swift genetic adaptation can

be found in cases of anthropogenic metal input. It was demonstrated in benthic invertebrate populations that adaptation to metals can occur within a few generations only (Klerks & Levinton 1987; Postma & Davids 1995), clearly demonstrating natural selection. An increased tolerance to metals has been demonstrated in a number of different taxa as shown in reviews on bacteria (Trevors et al 1985), vascular plants (Macnair 1993; Shaw 1999) and animals (Klerks & Weis 1987; Posthuma & van Straalen 1993), although not in all cases a proven genetic alteration was responsible for the increased tolerance.

It may, however, be questioned how the persistence of a population is affected during the process of metal adaptation, especially when such rapid evolutionary changes are involved. It has been often hypothesised that selection for metal tolerance may lead to a substantial loss of genetic information that reduces a species capacity to cope with other natural stress factors (cf Kopp et al 1992; Theodorakis & Shugart 1999). However, spatial inhomogeneity has only rarely been included in such studies, even though it is well known that drifting genes from surrounding non-adapted environments may affect and shape the genetic structure and evolution of metal-adapted populations (cf Slatkin 1985; Slatkin 1994; Boecklen & Mopper 1998). Indeed, the interaction of gene flow and adaptation in metal-tolerant animal populations is only rarely described, although this interaction is likely to be essential in understanding the persistence of species in metal-contaminated habitats (cf Brandon 1990). The present study, therefore, aims to examine the dynamics of metal adaptation in natural populations of an invertebrate. Understanding the adaptational processes is assumed to be fundamental to any further study of the ecological consequences in metal-exposed invertebrates and the communities involved.

This study focused on the non-biting midge *Chironomus riparius* (Meigen, 1804), because it is present in high densities at both metal polluted and non-polluted sites in the severely contaminated lowland river Dommel, thereby providing a suitable test case. Furthermore, metal-exposed midge populations from this river have shown to be genetically adapted to cadmium (Postma 1995), and larval drift and hence gene flow are

obvious natural features of riverine chironomids. This case, therefore, provides the opportunity to study the seasonal population dynamics in relation to processes of adaptation to metals and gene flow, that have not been quantified in metal-exposed natural invertebrate populations so far. At first, an introduction to the ecology of the test species *C. riparius* (box 1.1) and a detailed description of the research area will be provided. Next, a summary of the current knowledge of metal adaptation in *C. riparius* will be presented. Finally, the objectives will be elaborated and the methodology discussed.

research area

Field studies were conducted in the River Dommel, a second to third order lowland stream situated in the Kempen area in northern Belgium and the southern parts of the Netherlands. The Dommel is a tributary of the River Meuse and mainly fed by rainwater. The Dommel is characterised by a sandy bottom, a width of 5-7 m, a depth of 0.4-1.5 m, a current velocity varying between 0.3 and 0.8 m s⁻¹, and neutral waters with a naturally high iron content. The visibility is often limited to 10-20 cm due to suspended organic material, but seasonal variation does occur. Detailed information on principal water characteristics of the Dommel is provided in Postma (1995) and Janssens de Bisthoven (1995). The source of metal contamination in this river is a zinc factory of Union Minière on the banks of a small tributary of the Dommel, the Eindergatloop, situated close to the Dutch-Belgian border (cf figure 2.1; 3.1 & 4.1). This factory started producing zinc and cadmium from ores in 1888. Yearly production during the 1980s was on average 120,000 tons zinc and 600 tons cadmium per year. In 1992, production of zinc and cadmium was stopped and the factory switched to recycling and the production of zinc alloys. In addition to the severe metal input, a diffuse domestic and agricultural input in the catchment area is responsible for slightly elevated background levels of some contaminants like zinc, even at sites located upstream from the Eindergatloop (cf figure 2.2; 4.2; 4.3; table 2.1; 3.1 & 3.2). Average amounts of metals transported through the Dommel downstream from the Eindergatloop are 1-3 kg cadmium per

day and 50-200 kg zinc per day with an average water discharge of $4.5 \text{ m}^3 \text{ s}^{-1}$. The metal pollution has acted as a strong selective force on the benthic fauna for over a century making it a suitable test case for studying micro-evolutionary processes in *C. riparius* populations.

In the present study, a total of nine different sampling locations in the River Dommel were selected, which are introduced in detail in later chapters. Two sampling sites, however, are used in all experiments. The first one is located only some tens of metres upstream from the inlet of the zinc factory and is used as a reference location (abbreviated as DEG). The second is a metal-polluted sampling station, situated in the polluted downstream area near the village Neerpelt (abbreviated as NP), circa 400-500

BOX 1.1: *Chironomus riparius*

World-wide, the dipteran family Chironomidae or non-biting midges consists of at least 15,000 different species inhabiting a wide variety of freshwater lakes and streams (Armitage et al 1995). The life cycle of chironomids comprises an egg stage, four larval stages and a pupal stage, all in the aquatic environment, followed by a terrestrial adult stage (figure 1.1). After swarming and mating, females deposit egg masses at the water surface where they are attached to natural substrates such as plants or wood litter, or to artificial substrates, such as bridges or stony walls. Each egg mass may contain up to 600 eggs which hatch after a few days. First instar larvae are predominantly planktonic, while older instars in most species migrate to the sediment and build tubes constructed from detritus, algae and sediment particles (Armitage et al 1995).

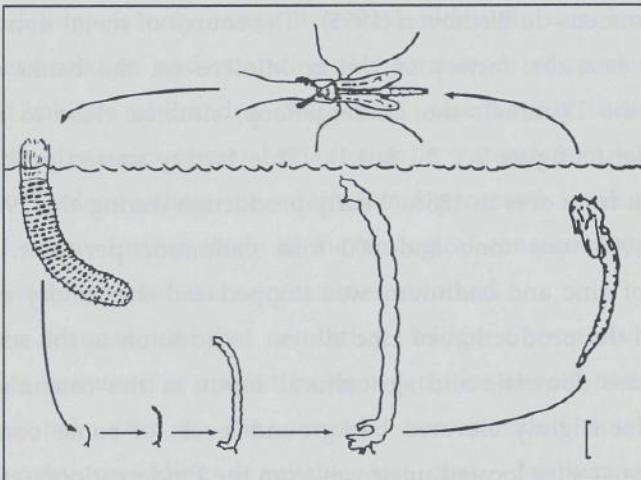


FIGURE 1.1: Life cycle of Chironomidae displaying the egg stage, the four larval instars, the pupal stage and the terrestrial imago. The scale bar indicates a length of approximately 1 mm (adopted from Timmermans 1991).

metres downstream from the confluence with the Eindergatloop (cf figure 2.1; 3.1 & 4.1). Those closely situated sampling stations showed a high congruence in both biotic and abiotic characteristics, except for the highly increased metal pollution at the downstream site, descending from the Eindergatloop. Due to the limited distance and the year round presence of dense populations of *C. riparius*, comparative studies are facilitated. In addition to the midge populations obtained from the Dommel, a laboratory culture 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.

BOX 1.1: *Chironomus riparius* (continued)

The species used in the present study, *Chironomus riparius*, is widely distributed in the northern hemisphere, most commonly at temperate latitudes in the Western Palearctic and probably in the Nearctic as well (Janssens de Bisthoven 1995). *Chironomus riparius* favours eutrophic or organically enriched conditions and healthy populations, reaching densities up to 50,000 individuals per square metre, are frequently found in waters under the influence of effluents from sewage works (Learner & Edwards 1966; Köhn & Frank 1980; Davies & Hawkes 1981; Armitage et al 1995). Furthermore, *C. riparius* is known as a species able to survive extreme environmental conditions such as low pH values and an increased salinity (Parma & Krebs 1977; Havas & Hutchinson 1982). Besides its general tolerance to extreme conditions *C. riparius* can be characterised as an opportunistic species (Pinder 1986), quickly invading newly raised, suitable habitats (Gower & Buckland 1978; Matěna 1990). At temperate latitudes, this opportunistic behaviour is facilitated by the swift succession of life cycles. For example, in rivers affected by organically polluted water at least five generations per year were produced (Learner & Edwards 1966; Gower & Buckland 1978). Wintering of *C. riparius* occurs in the third or fourth larval instar and the diapause is characterised by a developmental stop of the imaginal discs in the fourth subphase of the last larval instar. However, a small part of the population in Belgian lowland rivers exhibited diapause phenomena, including the development of imaginal discs, already in the third instar (Vermeulen 1998). Synchronised emergence of the wintering cohort takes place in spring and settlement of first instar new generation larvae occurs within a few weeks thereafter. At temperate latitudes *C. riparius* displays multivoltine life cycles with repeated settling of larvae during April to November (Groenendijk et al 1996). However, because larval development is strongly related to the water temperature (Mackey 1977), the number of consecutive generations per year is strongly restricted by climatic conditions. In the boreal zone of Canada for example, *C. riparius* exhibit an univoltine life history in prairie ponds where the ice-free season lasted only six months and where food supply was limited (Rasmussen 1984a). The feeding mode of larvae of *C. riparius* can be characterised as collecting-gathering and the main food source for the larvae is sediment-deposited detritus (Rasmussen 1984b; 1985).

adaptation to metals in *Chironomus riparius*

Recently, the state of the art on metal adaptation in *C. riparius* is reviewed in Postma & Groenendijk (1999), mainly based on the results of Postma (1995) and part of the research described in the present thesis. Metal adaptation in *C. riparius* is analysed in Postma & Groenendijk (1999) using Brandon (1990), who argued that an ideal demonstration of genetic adaptation to metals should consist of five kinds of information. The analysis of these five criteria is summarised below.

- 1) evidence that selection for metal tolerance has occurred in the past
- 2) demonstration that, based on life-history characteristics, some individuals are better adapted than others
- 3) evidence that the characters involved in metal tolerance are heritable
- 4) information on the possible influence of gene flow
- 5) phylogenetic information to enable distinction between the original and the derived state of the character

criterion 1: evidence that selection has occurred in the past

Establishing that selection has occurred over a relevant time interval might be problematic. According to Brandon (1990) there are two basic reasons for this: 1) Selection might have driven the selected trait to fixation and selection in action can not be observed; 2) In cases where actual selection is observed, it should be extrapolated back through time and claimed that the present, selective environment is similar to past selective environments. However, circumstantial evidence can be obtained from indications of present-day selection and the presence of other tolerant species. Posthuma & van Straalen (1993) concluded that combining data on the actual exposure in the field with dose-effect relationships for reference populations, including estimation of parameters such as EC_{50} or NOEC (No Observed Effect Concentration), is crucial in obtaining circumstantial evidence for selection in the past. Comparing populations along a gradient around a point source can be informative, but attention should be paid to differences in habitat characteristics. In addition, information is needed for

the extrapolation of laboratory derived toxicity data to the actual exposure in the field. In the case of the Dommel and the midge *C. riparius*, circumstantial evidence based on the presence of other tolerant species is not available, although several other (midge) species inhabit polluted sites in the Dommel. Consequently, attention must be focused on either a comparison between the actual exposure in the field with dose-effect relationships obtained in the laboratory, or on indications of present day selection.

Toxicity experiments performed by Postma et al (1994; 1995a) combined with literature data (Pascoe et al 1989; Timmermans et al 1992; Miller & Hendricks 1996) showed that effects on field populations of *C. riparius* are to be expected when cadmium or zinc concentrations in the water regularly exceed 20 nM Cd or 0.12 μ M Zn. Circumstantial evidence for selection in-situ can be obtained by comparing these laboratory-derived toxicity data with measured concentrations in the field (cf figure 2.2; 4.2; 4.3; table 2.1; 3.1 & 3.2). At polluted sites in the Dommel, NOEC-values for both larval growth and even survival are regularly exceeded by a factor of 10-30 and 50-80 for cadmium and zinc respectively. Even at upstream reference locations, estimated NOEC-values for zinc were exceeded. Selection for tolerance is therefore highly likely to occur. Indications for present day selection were found in acute toxicity experiments, in which first instar larvae of *C. riparius*, obtained from a laboratory culture, were exposed to surface water obtained from several locations in the Dommel (Stuijzand et al 1996; Stuijzand 1999). These experiments demonstrated that when larvae were exposed to polluted surface water from Neerpelt, growth was about half that of larvae exposed to surface water from the upstream reference location DEG. It can therefore be concluded that although evidence for selection in the past is missing, actual cadmium and zinc concentrations in the River Dommel directly affect local chironomid populations.

criterion 2: demonstration that, based on life-history characteristics, some individuals are better adapted than others

Differences between metal-exposed and non-exposed populations can be detected by comparing life-history parameters such as survival, growth or reproduction. Furthermore, insight into the physiological mechanisms causing an increased tolerance would benefit the interpretation of differences in life-history parameters. In the case of the Dommel midges therefore, chronic toxicity experiments were started with non-exposed laboratory-reared offspring (F1) obtained from field-sampled larvae and demonstrated significant differences in the effects of cadmium between populations. Increased tolerance to cadmium in the metal-exposed field population manifested itself in a reduction of the adverse effect on larval growth rate, while effects on larval mortality remained substantial (Postma et al 1995b).

It is hypothesised that disturbances in a habitat will select for earlier maturation and increased reproductive effort (Charlesworth 1980; Sibly & Calow 1989). However, in experiments on cadmium-adapted chironomids cultured in the absence of cadmium no indications were found for a lowered age of reproduction (Postma et al 1995b). On the other hand, results demonstrated a reduced fitness under clean conditions, which is commonly explained as costs of being tolerant (Cook et al 1972; Cox & Hutchinson 1981; Holloway et al 1990). Both larval mortality and larval development time were shown to be increased compared to reference populations (R1 & R2), when cadmium-tolerant midges (P1 & P2) were cultured in a clean environment (figure 1.2). Also in experiments using a cadmium-adapted laboratory-reared population, such indications of 'costs of tolerance' were found (Postma et al 1995a). However, differences existed among cadmium-adapted populations. For example, control mortality of unexposed larvae from an adapted field population (P1) as well as from a cadmium-adapted laboratory-derived population was increased, but was absent in another adapted field population (P2) (figure 1.2).

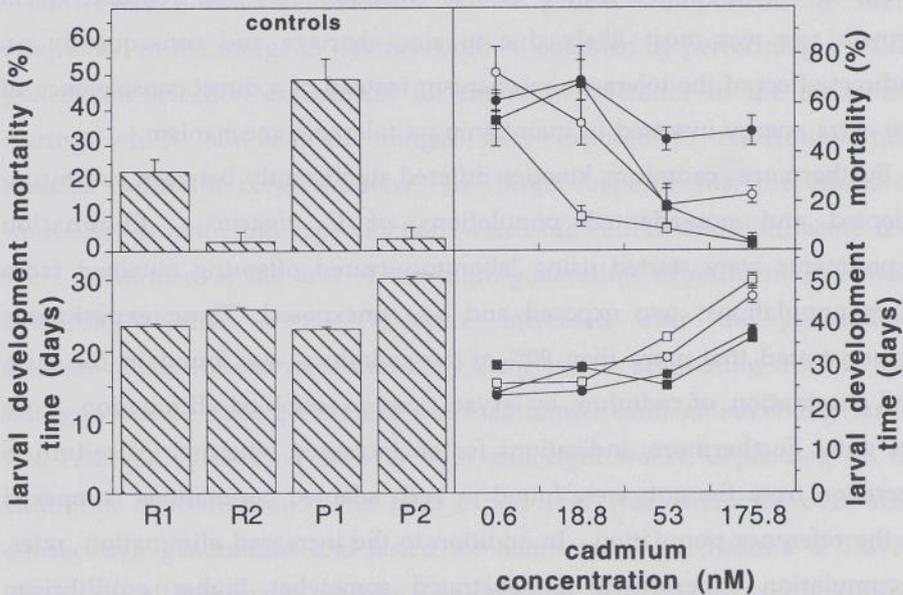


FIGURE 1.2: Mortality (%) and larval development time (days) of two reference (○ = R1; □ = R2) and two metal-exposed (● = P1; ■ = P2) field populations of *Chironomus riparius*. Presented are average values together with standard error in both the controls (hatched bars) and the treatments with three different cadmium concentrations (in revised form adopted from Postma et al 1995b).

High mortality under clean conditions has also been found for metal-tolerant Collembola (Posthuma et al 1993). A lack of metabolically available essential metals such as zinc was suggested as one of the possible reasons because an increased accumulation of zinc has been found in these organisms (Posthuma et al 1992). An increased accumulation of zinc has also been found in metal-adapted chironomids (Postma et al 1995a; 1995b), but a causal relationship with the high control mortality seemed less likely, because mortality remained high when cadmium-tolerant midges were supplied with additional zinc. These experiments, in which cadmium-tolerant midges were supplied with additional zinc, further demonstrated that the increase in larval development time, as observed in some tolerant chironomid populations when cultured under control conditions, was at least partly due to an increased need for essential metals such as zinc (Postma et al 1995a). Interpreting the reduced fitness of metal adapted populations reared in a clean environment as 'costs of tolerance', can

therefore be questioned in the case of chironomids. The reduced larval growth rate was most likely due to zinc shortage and consequently an indirect effect of the tolerance mechanism instead of a direct consequence of the extra energy invested in maintaining a tolerance mechanism.

Furthermore, cadmium kinetics differed significantly between cadmium-adapted and non-adapted populations of *C. riparius*. Elimination experiments were started using laboratory-reared offspring obtained from three populations: two exposed and one unexposed. These experiments demonstrated that more than 90% of the cadmium was found in the guts, and elimination of cadmium by larvae closely resembled elimination from the guts. Furthermore, indications for an increased efficiency of cadmium excretion from the guts were found in both adapted populations compared to the reference population. In addition to the increased elimination rates, accumulation experiments demonstrated somewhat higher equilibrium values in cadmium-adapted larvae (Postma et al 1996).

Apparently, metal-tolerant larvae of *C. riparius* were capable of maintaining high growth rates when exposed to cadmium, even when larval mortality remained high. This increased tolerance was at least partly based on a higher excretion efficiency from the gut epithelium.

criterion 3: heritability of metal tolerance

Because differences in metal tolerance between field populations of chironomids were demonstrated using larvae from a laboratory-reared F1-generation, the presence of a genetic component for metal tolerance was assumed. However, the presence of maternal effects could not be fully excluded. Further proof that the increased metal tolerance was at least partly caused by a genetic component could not be obtained using quantitative genetics based on parent-offspring relations, because these analyses require that offspring can be related to individual parents. It is therefore necessary that a single couple reproduces successfully. For *C. riparius* this is not the case, because swarms need to be formed at twilight, from which paired insects drop out.

However, additional evidence for a genetic component in metal adaptation in the midge *C. riparius* could be obtained by performing a multi-generation selection experiment for cadmium tolerance in the laboratory, during which non-adapted midges were exposed to environmentally realistic cadmium concentrations. In chronic experiments, the effects on mortality, growth, and reproduction were studied. In addition, acute tests were performed at the end of the multi-generation experiment to further establish whether or not tolerance increased over the generations. Successive generations were started by using larvae originating from at least ten egg masses. Each generation was maintained until all surviving larvae had emerged. This took between four and eight weeks, depending on the cadmium concentration. The total experiment was conducted over nine consecutive generations and lasted for about one year (Postma & Davids 1995). For all treatments, results demonstrated that the effects of cadmium significantly increased over the generations based on an integrated measure for population growth rate. The population exposed to 54 nM cadmium, on the other hand, recovered and the population growth rate of the ninth generation was significantly higher than that of the fifth to seventh generations. The population growth rate of the population exposed to 160 nM cadmium peaked in the second generation, but later it decreased continuously until the ninth generation when all replicates were extinct. Also the population exposed to 17 nM cadmium became extinct, indicating that adaptation does not always occur. Based on these results, additional short-term experiments were conducted in which the effects of cadmium on the growth of unexposed larvae (obtained from the control population) was compared to the growth of larvae obtained from the ninth generation of the population which had been continuously exposed to 54 nM cadmium. Based on these chronic and short-term experiments it appeared that life-history characters strongly correlated with both the cadmium concentration and generation and confirmed that adaptation to metals can occur within a few generations. Furthermore, it was concluded that the increased tolerance to cadmium in the midge populations studied was mainly due to genetic factors.

criterion 4: influence of gene flow

The fourth criterion refers to the dynamic interaction between the selective pressure by elevated metal concentrations and gene flow, as determined by two spatial components, namely pollution and population heterogeneity (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. Gene flow can reduce the speed of adaptation to pollutants (Comins 1977; Taylor & Georghiou 1979; Roush & McKenzie 1987) but on the other hand it can reduce the effect of inbreeding and can introduce new genes that are essential to a further increase in tolerance (Slatkin 1987).

A possible influence of gene flow in the Dommel chironomids was indicated by the differences found between two metal adapted midge populations. The first metal-exposed population (P1), sampled near the zinc factory, was characterised by a high control mortality, while larval development time did not differ from control populations. The second metal-exposed population (P2) on the other hand, which was sampled seven kilometres further downstream, was characterised by a low control mortality, but showed a reduced larval growth rate (figure 1.2). It was argued that one of the main factors responsible for these differences was the influence of gene flow (cf Raijmann & van Grootveld 1997). Drifting, non-tolerant larvae originating from sites upstream from the point source of cadmium can easily reach the metal-exposed population just downstream from the zinc factory, whereas the effect on the second metal-exposed population will be much smaller. This would decrease not only the level of metal adaptation, but also the genetic stability of the most upstream adapted population, because sexual reproduction can produce offspring with a lowered fitness, due to an unfavourable combination of genes. This can increase mortality rate, but the surviving larvae would be likely to have an increased metal tolerance (Postma et al 1995b). The hypothesis that gene

flow, as a result of larval drift, influences the dynamics of metal adaptation will be studied in several chapters of the present thesis.

criterion 5: phylogenetic information to enable distinction between the original and the derived state of the character

Obtaining evidence about the original state of characters involved in metal adaptation is often difficult, like obtaining evidence for selection in the past (criterion 1), especially in situations where the actual exposure is rather constant. It is therefore, not surprising that no conclusive information judging this criterion could be presented by Postma (1995) concerning the metal adaptation in *C. riparius*.

In the River Dommel however, studying the actual evolution of tolerance seems possible. The selective pressure will depend on the actual metal concentrations and on gene flow from sites upstream. It is hypothesised that fluctuations in factors such as population dynamics, rainfall and discharge will directly influence the level of tolerance present in the field populations, as will be examined in the present thesis.

outline of this thesis

The synopsis of the current knowledge on metal adaptation in *C. riparius* using Brandon's (1990) criteria, has shown extensive evidence for genetic life-history differences in metal-exposed chironomids. However, substantial knowledge about the dynamics of metal adaptation in natural chironomid populations, influenced by spatial components such as fluctuating selective force (cf indications for present day selection, criterion 1) and population heterogeneity (cf the possible role of gene flow, criterion 4; the original state of midge populations, criterion 5) is virtually absent (Posthuma & van Straalen 1993). In the present study therefore, an approach was chosen in which attention was focused on the following objectives.

- 1) to relate seasonal instar dynamics with the amount of drifting non-exposed larvae into metal-exposed field populations to estimate the

BOX 1.2: fluctuating asymmetry

Fluctuating asymmetry (FA) is defined as the occurrence of random differences between the phenotypic values of characters on the left and right sides of a normally bilaterally symmetrical individual organism (Van Valen 1962). When fitness depends on morphology, individuals which can develop the phenotype reliably or show greater developmental stability, should be more fit (cf Brakefield 1997; Clarke 1997). Departure from the law of symmetry as measured by FA has frequently been suggested as a reliable index of genetic or environmental health and of the effects of different types of stress (Leary & Allendorf 1989; Parsons 1990; 1992; Zakharov & Graham 1992). Experimental work has shown that females of the chironomid *C. plumosus* prefer to mate with more symmetric males (McLachlan & Cant 1995; McLachlan 1997) and that higher levels of asymmetry coincide with different types of serious environmental stressors, such as increased toxicant concentrations (Zakharov & Yablokov 1990; Pankakoski et al 1992), larval crowding (Clarke & McKenzie 1992), or a less optimal temperature regime (Bradley 1980).

An ideal demonstration of FA should exclude other forms of asymmetry normally occurring in certain animal populations (Palmer & Strobeck 1986; Palmer 1994; Swaddle et al 1994). The first of these is antisymmetry, a form of asymmetry due to negative interaction between sides. This type of asymmetry is found for example in fiddler crabs (*Uca* spp), where the large signalling claw of the males occurs at an equal frequency on the left and right sides in a population. Directional asymmetry, a systematic bias in one direction, is another form of normal development, but can seriously interfere with FA-analysis. Directional asymmetry is for example occurring in humans where the right gonads are normally larger than those on the left (cf Leary & Allendorf 1989).

In larval chironomids FA is studied in relation to environmental stress on rare occasions only. Clarke (1993) showed a decreased developmental stability in *C. salinarius* populations under the influence of a fertiliser manufacturing facility. In addition, in another *Chironomus* species tentative evidence was put forward for an increased FA-level caused by chlorpyrifos contamination (Clarke

rate of inbreeding between metal exposed and non-exposed chironomids

- 2) to examine the developmental stability of metal-exposed chironomids as compared with their non-exposed conspecifics, by developing and investigating morphological markers for development
- 3) to determine whether a higher net metal handling capacity is present in metal adapted populations as compared with non-exposed chironomids
- 4) to describe temporal variability in the actual level of metal adaptation that might occur as a result of immigration of non-exposed midge larvae
- 5) to develop a method for crossbreeding chironomids allowing to mimic gene flow under experimental conditions and to examine the heritability of metal adaptation after crossbreeding.

BOX 1.2: fluctuating asymmetry (continued)

et al 1995). However, Bleeker et al (1999) did not detect significant deviations from bilaterally symmetrical *C. riparius* larvae exposed to different nitrogen substituted polycyclic aromatic hydrocarbons. In the present thesis, FA was used to estimate the developmental stability of metal-exposed *C. riparius* populations in the River Dommel. As in the mentioned studies on FA in chironomids, the character chosen was the number of teeth on the comb-like pecten epifaryngis (figure 1.3), which is situated at the anterior part of the larval head capsule. The difference in the number of teeth between left and right sides served as a measure for asymmetry. The centre of the pecten epifaryngis was determined by an indentation on the anterior side of the pecten. The tooth or pair of teeth in the middle of this indentation was used as the division point between right and left. It is argued that this character is not influenced by antisymmetry or directional asymmetry and, in addition, the remaining statistical criteria allowing FA-analysis were also met (cf chapter 3; Clarke 1993; Clarke et al 1995; Bleeker et al 1999).

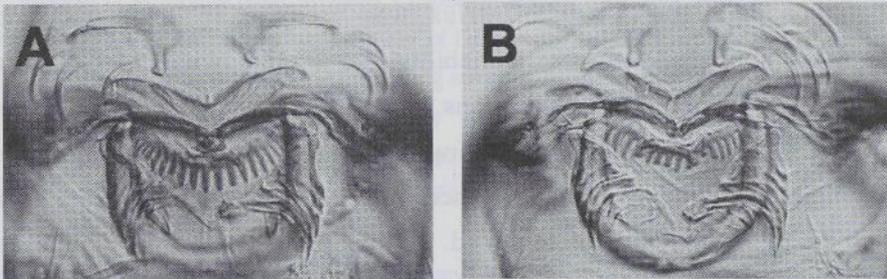


FIGURE 1.3: Pecten epifaryngis of *Chironomus riparius* situated at the anterior part of the larval head capsule. A method was developed to incorporate high frequencies of asymmetrical individuals (cf picture B) in midge populations in a measure describing fluctuating asymmetry.

At first therefore, a detailed year round field study was carried out to analyse seasonal dynamics of *C. riparius* at both metal exposed and non-exposed field sites in the River Dommel. Three different sampling sites in the River Dommel were visited every fortnight. Life cycles and density patterns were examined by analysing instar composition. An estimation of the immigration rate was made by measuring the amount of non-tolerant larvae drifting into the metal-exposed area (chapter 2). In the third chapter the developmental stability of eight different chironomid Dommel populations and their progeny in clean cultures was measured. This was carried out by studying the level of fluctuating asymmetry and the occurrence of mentum deformities, which were both analysed by light microscopy. First a suitable technique for the application of the theory of fluctuating asymmetry was developed and improved for use on *C. riparius* fourth instar larvae (cf box 1.2). In the fourth chapter, metal accumulation and the fate of metals during metamorphosis were investigated because it

was assumed that the presence of metal-adapted specimens would influence the metal handling capacity of the chironomids. For this purpose, zinc and cadmium content was measured in simultaneously sampled larvae and imagoes of *C. riparius* from both reference and metal-polluted sites. Large samples were obtained to estimate shedding rates in four different chironomid populations from the River Dommel. To examine the possible consequences of gene flow in metal-adapted field populations of *C. riparius*, certain life-history characteristics were followed over time to detect temporal fluctuations in the level of metal adaptation (chapter 5). Three different field populations were visited seven times in a five-month period. In addition, a laboratory population of *C. riparius* was used as an extra reference population to exclude temporal variability due to unknown environmental influences or experimental conditions in the laboratory. Populations were tested in chronic experiments using first generation laboratory-reared animals and interpopulation differences can, therefore, be assumed to be at least partly genetically determined. To allow crossbreeding under experimentally controlled conditions, an emergence trap was designed and metal adapted and non-adapted chironomid strains were crossbred successfully (chapter 6). This crossbreeding technique was used in the seventh chapter to simulate gene flow from non-adapted strains in metal-adapted chironomids and to examine the heritability of metal tolerance. This unit focused on two chironomid field populations flanking the point source of metal pollution and it is, therefore, hypothesised that these populations interbreed under natural conditions. Short-term toxicity experiments were carried out on eight occasions during a period spanning more than a year to measure control mortality rates, control growth, and EC_{50} values in the parental populations and in both reciprocal crosses.

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