Dynamics of metal adaptation in riverine chironomids.

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Publication date
1999

Citation for published version (APA):
CHAPTER VI

A method for crossbreeding strains of chironomid midges (Diptera; Chironomidae) and its application to ecotoxicological studies

ABSTRACT
In this paper a newly developed technique for crossbreeding populations of Chironomus riparius is presented. It was shown that this technique is suitable in genetic and ecotoxicological studies, when crossbreeding of different strains of a midge species is necessary. In addition, no indications of parthenogenesis in C. riparius could be detected, allowing users to apply this technique for the numerous conceivable possibilities. In this study, we tested the applicability of the crossbreeding technique in analysing differences in metal sensitivity in two field populations of C. riparius from the metal polluted River Dommel, and in the resulting reciprocal crosses. It was shown that the metal adaptation present in the metal-exposed parent population, based on short term EC50 values for cadmium, vanished almost completely within one generation after crossbreeding with a non-adapted reference population. This observation will elucidate the expected wax and wane of the actual level of metal adaptation present in C. riparius populations in the River Dommel.
CHAPTER VI

Introduction

Occurrence of mating in chironomid midges is predominantly restricted to monospecific aerial swarms during calm weather. Males gradually leave the resting areas to form a swarm in which they fly in a zigzag manner over a fixed marker. Soon after the onset of swarming, females enter the swarm and copulation takes place in the air (Kon 1984). This swarming tactic may secure high mating rates of females when population densities are low (Armitage et al 1995). However, in laboratory cultures this swarming behaviour may hamper the possibilities of successful reproduction of different chironomid species, because every chironomid species needs specific circumstances to form swarms (LeSage & Harrison 1980). Even more difficulties are to be expected in genetic and ecotoxicological studies when crossbreeding of different strains of a midge species is necessary to examine the heritability of characters in both the parent populations and the reciprocal products.

In this paper we describe a newly developed method to successfully crossbreed different populations of the midge Chironomus riparius (Meigen, 1804). The possibility of parthenogenetic reproduction interfering with the crossbreeding results and the relevance of the crossbreeding system in general, are discussed in an ecotoxicological case study in which a metal adapted and a non-adapted population of C. riparius from the heavily metal polluted River Dommel were crossbred.

Materials and Methods

field sampling

Larvae of C. riparius were sampled during six occasions between November 1996 and October 1997, on two closely situated locations in the River Dommel. Metal contamination in the Dommel originates from a zinc factory which creates a distinct point source of pollution in the river. On both sides of this point source chironomid larvae were collected. The 1995
average of dissolved Cd for the reference site (R) just upstream from the zinc factory, was 5 nM Cd, whereas at the polluted site (P) just downstream from the zinc factory, 640 nM Cd was measured. Although these two sites differ more than a factor 100 in cadmium pollution, the distance between both locations is only a few hundred metres. Detailed information about metal pollution and related Dommel characteristics is available in Postma (1995) & Groenendijk et al (1999). All C. riparius larvae were transported to the laboratory on day of collection and reared to adulthood in a controlled climate room.

**culturing and crossbreeding conditions**

Field larvae were cultured using plastic aquaria (12.5 l) with a flight cage (38*21*30 cm) on top. All larvae were kept in clean sediment (sand < 300 µm) and circa 5 l of Dutch Standard Water (Maas et al 1993). Midge larvae were fed ad libitum with a solution of ground Trouvit and Tetraphyll®. After circa one week midges started to emerge. Crossbreeding of midge populations was carried out with a newly designed emergence trap. In this trap, adult, freshly emerged midges were caught separately in small plastic tubes (25 ml; 34 per aquarium) which were placed just above the water surface (figure 6.1). These plastic tubes were checked at least twice a day and tubes in which both male midges 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 (R) together with newly emerged females (f) from the polluted population (P) and conversely (figure 6.1). This crossbreeding setup produced egg masses from four different C. riparius strains. Both parental populations were cultured without emergence trap and produced pure reference (R) originated or polluted (P) originated egg masses. In both aquaria equipped with emergence traps, egg masses from the two reciprocal crosses were produced (figure 6.1).
ecotoxicity experiments

Short term experiments for testing metal tolerance and possible effects of crossbreeding on the level of metal tolerance, were performed by determining the growth rate of first instar larvae. First generation laboratory-reared animals (F1) were used in the experiments to examine the presence of a genetic component for population differentiation in metal tolerance. All experiments were started with first instar larvae less than 24 hours old from these first generation egg masses. Twenty-five larvae were exposed during four days to six concentrations of cadmium in 400 ml Dutch Standard Water. The average actual cadmium concentrations during the experiments were 0.2; 59.0; 108; 220; 600 and circa 1200 nM Cd. The food (1 ml of a solution of ground Trouvit and Tetraphyll®) was added at the start of the experiments and provided a suitable substrate for tube building. Therefore, no additional sediment was added. The initial length of larvae from each strain was measured in a sample of 25 larvae, using an ocular micrometer. After 96 hours, the surviving larvae were counted and their
length was measured. Population differences in the growth rates of larvae in the unexposed control situation were observed. Therefore, growth in each of the four strains exposed to cadmium, was expressed by calculating the percentage reduction of the growth relative to the corresponding control. In addition, short term EC$_{50}$ values were estimated with 95% confidence limits, by fitting a model for logistic response. When subtoxic stimulus was present an extended model for logistic response (van Ewijk & Hoekstra 1993) was used to calculate the EC$_{50}$ value and its accessory 95% confidence limits.

**parthenogenesis**

Two experiments (A and B) were performed to examine the possibility of parthenogenesis in *C. riparius*, using the above mentioned culturing conditions. For this purpose circa 300 larvae from the laboratory culture of *C. riparius* present at our department, were placed in plastic aquaria with a flight cage on top. All larvae were kept in clean sand (< 300 µm) and circa 5 l of Dutch Standard Water (Maas et al. 1993). All aquaria were equipped with an emergence trap to catch female midges, which had definitely not been in contact with male chironomids. These females were allowed to deposit their egg masses in a separate flight cage. All egg masses deposited were collected and were examined after seven days if hatching of the eggs had taken place.

**Results and Discussion**

**parthenogenesis**

Parthenogenesis is a widespread phenomenon among insects, although only a few chironomid species are reported to show parthenogenetic reproduction. Most of these examples are found in the Tanytarsini and Orthocladiinae families (Armitage et al. 1995). However, three *Chironomus* species have also been found to be partly parthenogenetic (Grodhaus 1971). In our experiments more than 180 unfertilised females of *C. riparius* were caught, using the emergence traps from the crossbreeding system (figure 6.1),
producing over 100 egg masses in experiments A and B together (table 6.1). None of these egg masses hatched, indicating that under the culturing conditions used C. riparius is a non-parthenogenetic species. In addition, a pilot study analysing allozyme polymorphisms using field captured C. riparius larvae also showed no indications of genetically identical individuals (Raijmann & van Grootveld 1997). It is therefore highly unlikely that parthenogenesis interferes with results obtained in crossbreeding experiments with different C. riparius strains.

**TABLE 6.1:** Number of used unmated females, the number of produced egg masses and the number of fertilised egg masses in two experiments examining the possibility of parthenogenesis in *Chironomus riparius*.

<table>
<thead>
<tr>
<th>experiment</th>
<th>number of females</th>
<th>number of egg masses</th>
<th>fertilised egg masses</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>42</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>139</td>
<td>95</td>
<td>0</td>
</tr>
</tbody>
</table>

crossbreeding of midge populations

Table 6.2 shows the number of egg masses produced in each of the four strains of C. riparius in the six different experiments. The production in both parental populations was on average higher than 40 egg masses, indicating a high genetic diversity in the mixture of first instar F1 larvae. Due to a minor, but systematic loss of midges during the use of the emergence traps, the number of egg masses produced in the reciprocal crosses is lower compared to both parental populations. However, the produced totals were still appropriate for further use, because each egg mass of C. riparius contains at least 300-400 eggs and a vast majority of the egg masses were found to be fertilised. Thus, the average number of more than 15 egg masses in the crossbred strains guaranteed enough genetic diversity in the following generation. Consequently, the emergence traps proved to be a suitable technique for crossbreeding C. riparius midge populations, especially because parthenogenesis in this chironomid species was absent. Therefore, this technique is suitable in genetic studies when crossbreeding of different strains of a midge species is necessary to study characters present in one or both the parent populations and its heritability in the resulting

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reciprocal products. Out of the numerous conceivable possibilities, we tested the applicability of the crossbreeding technique in studying differences in metal sensitivity in both parental field populations of *C. riparius* from the metal polluted River Dommel, and in the reciprocal crosses.

**TABLE 6.2:** Number of egg masses produced in both parental populations and reciprocal crossings of *Chironomus riparius* in six different experiments. At the bottom the average value for each strain is shown.

<table>
<thead>
<tr>
<th>experiment</th>
<th>R</th>
<th>P</th>
<th>mR*fP</th>
<th>fR*mP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>64</td>
<td>53</td>
<td>31</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>57</td>
<td>17</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>43</td>
<td>41</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>8</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>22</td>
<td>45</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>76</td>
<td>46</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td><strong>average:</strong></td>
<td><strong>44</strong></td>
<td><strong>42</strong></td>
<td><strong>16</strong></td>
<td><strong>18</strong></td>
</tr>
</tbody>
</table>

**ecotoxicological relevance**

As an example, larval growth of first instar larvae (F1) of *C. riparius* exposed to cadmium, in experiment 1 is shown in figure 6.2. Results clearly demonstrate that cadmium reduced larval growth of the reference population. Larvae from the polluted location, on the other hand, showed maximum growth at somewhat increased cadmium concentrations and were less affected by cadmium than larvae from the reference site, even at the highest cadmium concentration (figure 6.2). This resulted in a significantly different 96 h EC$_{50}$ value for both parental populations. The estimated EC$_{50}$ value for the reference population was 245 nM Cd (95% confidence limits (CL): 211-289) and for the polluted population 564 nM Cd (95% CL: 469-680). The EC$_{50}$ values for the mR*fP reciprocal crossing turned out to be almost equal to the reference population (243 nM Cd; 95% CL: 201-294). The fR*mP strain however, was significantly more affected by cadmium than the reference population, resulting in an EC$_{50}$ value of 121 nM Cd (95% CL: 102-143). All other experiments showed similar indications for an increased metal tolerance in the polluted population and a reduction of metal adaptation in the reciprocal F1 products.
The significant difference in cadmium sensitivity between *C. riparius* larvae sampled at the reference and polluted site is in agreement with the findings of Postma (1995) and Postma & Groenendijk (1999), who showed that metal adaptation in *C. riparius* in the River Dommel should genuinely be judged genetic according the standards set in Brandon (1990). However, based on the presented EC\textsubscript{50} values, the cadmium adaptation vanished almost completely within one generation after crossbreeding with a non-adapted reference population. This observation has important implications for the actual level of metal adaptation present in *C. riparius* in the River Dommel. Fluctuations in the actual level of metal adaptation are to be expected, because it was shown that mass migration of non-tolerant larvae in the Dommel regularly takes place (Groenendijk et al 1996). Because seasonal dynamics of *C. riparius* populations on both sides of the point source of metal pollution were identical, gene flow between non-adapted and adapted populations seems likely to occur in this river. This gene flow can easily reduce the speed of adaptation to pollutants (Comins 1977; Taylor & Georghiou 1979), but on the other hand it can reduce the effects of
inbreeding by introducing new genes, essential to further increase metal
tolerance (Slatkin 1987).

Most likely, C. riparius populations mix genetically in the River Dommel
on a regular base. Based on the results mentioned above, the effects of
crossbreeding in metal-adapted midge populations can now be studied easily
using the crossbreeding technique described above. The expected wax and
wane in metal adaptation and the rate at which metal tolerance can be re-
established will be examined in detail. In future research therefore, we
would like to focus on the crossbreeding effects in metal adapted C. riparius
populations in the River Dommel.

acknowledgements

The practical assistance of Miguel Dionisio Pires, Marc Plans and Suzanne Swenne during
the sampling period was greatly appreciated. Furthermore, we would like to thank Dr
Michiel Kraak for his useful comments on our manuscript and Fiona Curran for correcting the
English text.

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