Genome Response of Model Insect Group (Chironomidae Diptera) to Trace Metal Contaminants in the Environment

PARASKEVA MICHAILOVA, GABRIELLA SELLA*, NINEL PETROVA§,
Institute of Zoology, Bulgarian Academy of Sciences, 1Tzar Osvoboditel, 1000 -Sofia, BULGARIA e-mail: michailova@zoology.bas.bg
*Department of Animal and Human Biology, University of Turin, Via Accademia Albertina 13, 10123, Turin, ITALYe-mail: gabriella.sella@unito.it
§ Institute of Zoology, Russian Academy of Sciences, ul. Universitetska 1, 199034,St.Petersburg, RUSSIA e-mail: chironom@zin.ru

Abstract: Insects of the family Chironomidae (Diptera) have different advantages that make them particularly suitable for evaluating the action on the genome of heavy metal contaminants in freshwater ecosystems. We describe different types of genome response to heavy metal contamination of some Chironomid model species which could be used as biomarkers of genotoxicity of heavy metal polluted environments. Functional alterations of the activity of Balbiani Rings and NOR as well as somatic aberrations of polytene chromosomes of salivary gland cells are described. Their frequency significantly increased in larvae of *Chironomus riparius* growing on heavy metal polluted sediments. Therefore they are proposed as a tool providing early warning of adverse long term effects of toxic agents on the genome.

Key-Words: *Chironomus riparius, Chironomus piger*, salivary gland polytene chromosomes, chromosome aberrations, functional alterations, heavy metals, somatic inversions.

1 Introduction

Contamination of the environment is a worldwide serious problem. Biodiversity destruction is increasing, many species run the risk of becoming extinct, structures and functions of the ecosystems are changed. The main task of our society is to maintain and possibly to improve the quality of environment and life. To this aim, the biological approaches for detecting and assessing the impact of contaminants on ecosystems have a number of advantages. The biological communities provide direct means of studying the impact of contaminants in the environment.

1.1 Invertebrates as early indicators of environmental impact of genotoxic agents

In some test protocols invertebrates can be used to replace vertebrates, because invertebrates allow improving evaluation of ecological contaminants at individual, population and community level [1]. In acute and chronic tests for hazard identification, invertebrates can be maintained in controlled conditions and therefore their response to specific toxic agents has a high level of repeatability.

Insects of the family Chironomidae (Diptera) possess different advantages that make them particularly suitable for evaluating the action of contaminants in freshwater ecosystems. Currently, benthic larval stages of Chironomids, being exposed to contaminants, are considered a typical bio-monitoring model for ecotoxicological tests [2, 3, 4] and are included in both the Extended Biotic Index [5] and the Annex V.1.2.6 of the E. C. Water Framework Directive. Chironomids inhabit every type of aquatic habitat, their larvae account for at least 50 % of the macro-invertebrate species of any given freshwater ecosystem, and they display an exceptionally wide sensitivity to environmental stresses [1]. Chironomids are not noticeably mobile and therefore they may reveal long term effects of intermittent or chronic pollution of sediments. They provide a direct means of observing the impact of contaminants because they are exposed and directly involved in the transformation that contaminants undergo in freshwater ecosystems. They can be used as bio-indicators for determination and classification of lake eutrophications [6]. Moreover some model species can be reared in lab conditions and used in dose-response experiments to validate and calibrate responses observed in natural situations. As a fish food Chironomids larvae are a primary link in the transmission of contaminants from sediments to the higher levels of the food chain. Evidence from usage of Chironomid larvae as biomarkers shows a narrow linkage between some morphological deformities and concentration of contaminants in their bodies [3]. Since Chironomids...
represents the most basic unit of biological freshwater communities, they contribute to integrate into a single whole the lower levels of biological organization (molecular, cellular and organismic) and form the building blocks for higher levels of organization (populations, communities and ecosystems). Chironomid larvae possess excellent salivary gland chromosomes with a distinct and well-characterized band structure that allows precise cytogenetic analysis [7, 8] and the follow up of the effects of contaminants like heavy metals on the structure and function of the polytene chromosomes. Moreover, the standard karyological characteristics of some species [7, 8, 9] can be employed as a basis to reveal the genotoxic effects of environmental mutagens by studying chromosome aberrations, changes in heterochromatic regions and in functional activity of the polytene chromosomes. Many recent data indicate that the Chironomid genome is much more sensitive to contaminations of aquatic ecosystems that the external larval morphology [10, 11, 12, 13, 14, 15, 16, 17, 18].

1.2 Heavy metals as important contaminants
Certain heavy metals are required as trace elements for normal cellular functions. Their presence in trace amounts is considered important for living organisms, because they represent active sites for a number of enzymes which are involved in oxidation-reduction reactions. However, when their quantity exceeds the physiological concentration, heavy metals are toxic to cells. Effects of heavy metals can be deleterious when they are released in water either in high concentrations for short periods, causing acute toxicity, or in low concentrations but for long periods, causing chronic toxicity and leading to disorders in development, growth, maturation, reproduction, hatching, which not necessarily result in early death. Therefore, we used some model Chironomid species for tracing the effects of some heavy metals on the genome’s structure and functional organization. Moreover we established biomarkers for detecting and assessing the biological significance of contaminants in aquatic ecosystems.

In the present study we reviewed the different types of genome responses of some model species of the genus Chironomus, and present circumstantial evidence of genome instability processes ongoing in contaminated areas.

2 Results
2.1 Genome response to trace metal contaminants of two closely related Chironomus species

As a model species we used Chironomus riparius Meigen, a phylogenetically young, benthic and widely distributed species. It can be easily reared in the lab and has a small set of well banded salivary gland chromosomes which allow to perform detailed analyses of the effect of different stress agents on the genome structure. Its closely related and phylogenetically older species C. piger Strenzke very often occurs sympatrically with C. riparius. This allows comparing the genome responses according to the phylogeny of the species. Being homosequential and sibling species, they are morphologically nearly indistinguishable, with identical chromosome banding patterns. Both species are differentiated at the cytological level by their amount of DNA and appearance of the constitutive heterochromatin [19, 20]. The phylogenetically youngest species, C. riparius is rich in heterochromatin than the older species C. piger. In C. piger, the pericentromeric regions contain far lower numbers of satellite DNA clusters and copies of repetitive sequences than in C. riparius [21]. The Alu and Hinf DNA clusters also showed species-specific localization. For instance, the Alu cluster has only one site in C. piger, while 22 sites were observed in C. riparius. Hinf clusters have 34 sites in C. riparius and 38 in C. piger. Therefore both species can be identified by the location of specific and easily established molecular markers, like Alu and Hinf clusters[22, 23]. Both species have the diploid chromosome set 2n = 8, with chromosomes AB CD EF G. Chromosomes AB and CD are metacentric, EF submetacentric and G acrocentric. Chromosome G comprises three Balbiani rings (BRa, BRb, and BRc) and a Nucleolar Organiser (NOR). BRa is active only in four cells of the G chromosome of a special lobe of salivary glands. The standard chromosome maps done by Hägele [9] and Kiknadze et al. [8] were used for comparative detailed cytogenetical analysis.

Samples of C. riparius larvae (total N = 535) were collected from 13 Palaearctic populations living in sediments with different concentrations of heavy metals [18]. All sediments of the 13 Palaearctic populations apart from one were being considered polluted by trace metals. To establish the degree of heavy metal pollution of sediment, the concentration of heavy metals in fossil sediments was used as a reference [24]. We considered as heavy metal polluted those sediments that had at least one heavy metal concentration twice that of the same heavy metal in fossil sediments [18]. For instance concentrations of Pb ions in two Bulgarian stations were 27 and 12 times higher respectively than those of fossil sediments. All other ions of trace metals occurred in concentrations from two to six times higher than those of fossil sediments.
2.1 Structural and functional chromosomal alterations

In the studied Palaearctic populations alterations in salivary gland chromosomes were either inherited (i.e., affecting all cells of both salivary glands of the same individual) or somatic (i.e., when nuclei with and without alterations were detected in both salivary glands). Overall, 77 different types of inherited inversions were observed in the 13 populations. All the inherited inversions were in heterozygous state and paracentric apart from three pericentric inversions in chromosome EF. Inversion variants were found in all chromosome arms. Arm F was the most strongly polymorphic with 16 different types of inversions. It was followed by arms B and C with 15, arm E with 10, arm A with four and arm G with one inversion only. Most of the inversions were present in only one out of the 13 populations. Only six inherited heterozygous inversions were shared by two populations [18]. All the observed inherited inversions occurred in very low frequency. The median percent frequency of inherited inversions was 1.4. Similar low frequencies have been reported also in two Russian populations [25] and other European populations [26]. Therefore, on account of their < 1% frequency, the majority of inversions were considered endemic and rare [27]. The degree of hereditary variability of each population was estimated by dividing the number of different hereditary inversions in a population by the number of sampled larvae (H index). No relationship could be established between the H index values and the overall level of heavy metal pollution of the sediments from which larvae were collected [18]. Indeed, in three populations there were almost no inherited inversions, though concentrations of some heavy metals in their sediments were among the highest. Conversely, the highest H index value was observed in a Russian population with a relatively low level of pollution [18].

As for somatic aberrations, 184 different paracentric and five different pericentric inversions were established [18]. Generally one somatic inversion per larva was observed. Values of the S index (i.e. the ratio of the number of different somatic inversions in a population to the number of sampled larvae) strongly differed from those of the H index of the same populations [18]. The lowest values of the S index were found in populations from far less polluted sediments. Populations from the most polluted sediments had S values from six to eight times higher than those of larvae from the unpolluted basins [18]. Somatic aberrations were found also in the G chromosome of C. riparius, mainly somatic deletions affect either BRb or BRc or both. Their frequencies were highly variable. For example, the population frequency of deletion of Brb+BrC varied from 63.16% to 0.34% of the examined cells [18]. This aberration modifies the shape of the chromosome G in a so called "pompon" chromosome. The appearance of "pompon" chromosomes has been proposed as a biomarker of heavy metal contamination in aquatic ecosystems [10]. On the whole, in the examined populations the frequencies of different somatic inversions and chromosome G deletions show an increasing trend paralleling that of the overall heavy metal pollution. Therefore, these findings give support to the hypothesis that the increased frequency of somatic rearrangements in C. riparius from long term heavy metal contaminated areas may be a consequence of genomic stress induced by heavy metal pollution.

In C. riparius chromosomes rearrangements are not randomly distributed along the chromosomes [23, 26]. A significant correlation between locations of inversion breakpoints, satellite DNA clusters (Alu and Hinf elements) and transposable elements (NLRCth1) were observed [23]. Breaks seem to occur most frequently in chromosome sections containing blocks of repetitive DNA, be they composed by satellite DNA or transposable elements. The same tendency has been observed in Drosophila species where ectopic recombination, occurring between two inverted TE copies, was associated with inversion formation [28].

No structural chromosome alterations were observed in C. piger larvae occurred together with C. riparius from polluted sediments.

In C. riparius genomic stress is indicated not only by somatic structural rearrangements but also by some functional alterations in the Nucleolar Organizer (NOR) and Balbiani Rings (BRs) regions. The Balbiani Rings are key sites of intensive transcription of genes encoding for silk proteins [29]. These proteins are very important for Chironomids due to their participation in the construction of the tubes where larvae develop. The function of Nucleolar Organizer is essential for cellular maintenance and ribosomal production machinery, which is highly conserved through evolution [30].

The response of Chironomid genomes to different stress agents is characterized by changes in puff activity of Balbiani rings (BRs) and Nucleolar Organizer (NOR). The level of puffing activity of these two very important genomic structures significantly decreased compared to the normal activity [10, 23, 31]. Normally in C. riparius the puffing activity of BRc is relatively stable in fourth instars larvae and shows a fully expanded state in the prepupal stage [32, 33, 34] and BRb is very active in young larvae and pre-pupa stage, while in the middle larval stages BRb is collapsed [32, 33]. For instance, in some larvae from polluted stations there was a clear reversal of BRb and BRc activity in the polytene chromosomes compared to their activity in larvae from unpolluted basins. Indeed, a drastic regression of BRc was observed and a parallel expansion of BRb [10, 35]. In other larvae both BRs could appear in slight activity or in a heterozygous state or completely repressed. Trace
metals seem to induce changes in the puffing activity of *C. riparius* Balbiani rings which are similar to those induced by heat shock or sugar feeding [34, 36, 37]. The same tendency has been observed also in other Chironomus species [15, 16, and 17]. These findings suggest that the BR transcription mechanism can react in a similar way to different stress situations. Furthermore, it is noteworthy that the Nucleolar Organizer also showed various changes in its activity, from a very high activity of both homologues to a heterozygous state, or a slight activity or a complete collapse [10, 13, 14, 15, 16, 17, and 35]. Ribosomal genes are direct target for cadmium toxicity [30]. Therefore not only the increase in frequency of somatic inversions and deletions of G chromosome but also the drastic disruption of activity of BRs and NOR can be considered as very early and sensitive signals of genome toxicity of trace metals.

In contrast to the genome of *C. riparius* larvae, the genome of the *C. piger* larvae reacted to heavy metal contamination mainly by functional alterations (i.e. by decreasing the activity of the BR systems and NOR as well as by the appearance of new unknown puffs at the telomeres [20]). Such interspecific different reactions could depend on the well established different organization of repetitive DNA fractions of their genome, as already suggested by Ross et al. [38]. In addition to the high level of somatic chromosomal aberrations and functional alterations in heavy metal polluted environments some cases of disruption of the interspecific isolating reproductive barrier have been observed. For instance, in the heavy metal polluted basins (Pancharevo, Bulgaria) and river Latka in the Barok region (Jroslavskay, Russia), where *C. riparius* and *C. piger* as well as *C. riparius C. annularius* are sympatric, and few hybrids have been found recently between *Chironomus riparius* and *Chironomus piger* and between *Chironomus riparius* and *Chironomus annularius* Meigen [20]. The chromosomes of the hybrid between *C. riparius* and *C. piger* show many sections, which are conjugated and sections where the pairing of both homologues does not occur. Hybrids between *Chironomus riparius* and *Chironomus piger* are very rare in nature and in laboratory they have very low or nil fertility. The hybrids between *Chironomus riparius* and *Chironomus annularius* show almost completely lack of synapses and are obviously sterile.

Tentatively the hypothesis can be advanced that isolating mechanisms involved in pre-mating inter-specific barriers have been damaged in some individuals.

### 2.1.2 Endemisms in trace metal polluted regions?

Recent circumstantial findings in heavy metal polluted environments of new species resulting from deep re-organisation of the basic karyotype are suggestive of a possible indirect role of heavy metal pollution in this process. The Chironomus species *Kiefferulus tendipediformes* Goetghebuer occurs in Bulgaria and Hungary lakes with a specific cytotype (2n = 8). These basins are mainly unpolluted. Recently in a very heavy metal polluted region near Krakow, Poland (Boleslaw Pb mining ponds dating back from the 12th century,) a new cytotype has been found with 2n = 6 [39]. The two cytotypes were called cytotype 1 (2n = 8) and cytotype 2 (2n = 6) [39]. Chromosomes AB and CD are similar in both cytotypes, while the third chromosome of the cytotype 2 results from fixed specific chromosome rearrangements - tandem fusion of two acrocentric chromosomes (EF, G) of cytotype 1[39].

In the Boleslaw pool a still undescribed new *Chironomus* species has been collected. The new species belongs to the cytocomplex “*psedothummi*” AE BF CD G [40] but can be distinguished from the other species of this cytocomplex by fixed homozygous inversions in arm A and specific band patterns in arms B, C, D and G. These permanent chromosome rearrangements (tandem fusions and fixed homozygous inversions) created new gene linkage groups.

Further studies are necessary to ascertain whether the new *Chironomus* sp. and Cytotype 2 of *Kiefferulus tendipediformes* are endemic species which are adapted to this peculiar environment or whether they are present also in other regions, independently of the level of heavy metal of pollution of sediments.

### 3 Conclusions

- Polytene chromosomes of Chironomids can be used as indicators of genotoxic concentration of pollutants in aquatic ecosystems.
- Functional alterations of the two key structures Balbiani Rings and Nucleolar Organizing Regions and the somatic chromosomal damages (heterozygous inversions, deletions, deficiencies, formation of “pompon” G chromosomes) are particularly suitable as biomarkers because they can be easily identified.
- Most somatic chromosome rearrangements are not randomly distributed along the chromosomes they occur more frequently in specific sections of the chromosomes. These sections are composed either by satellite DNA or by transposable elements. Type and frequency of rearrangements depend on the DNA structure and organization.
- Closely related *Chironomus* species show
different genome response to stress induced by toxic agents.
- The analysis of the cytogenetic response is therefore a potentially powerful tool in providing early warning signals of adverse long term effects of toxic agents at the individual, population and community level.

References


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