REARRANGEMENTS IN CHIRONOMIDAE (DIPTERA) GENOMES INDUCED BY VARIOUS ENVIRONMENTAL STRESS FACTORS

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Invertebrates are known to be an appropriate tool for assessment of the state of aquatic ecosystems. Some insect groups are frequently used in alternative test methods due to their specific characteristics such as: higher sensitivity than in vertebrates, short live cycles, large population size, availabity at a very low cost, etc. Species of family Chironomidae are very suitable invertebrates for risk assessment within the scope of programs of aquatic ecosystem and human health protection. They are widely distributed, sensitive to many pollutants; some of them can be cultivated; they have a short life cycle; and their larvae are of great importance in aquatic food chains. They have excellent salivary gland chromosomes, which are promising tools to assess genotoxicity in the environment. Due to their good resolution they allow detection of a broad range of cytogenetic aberrations at the structural and functional levels. Somatic structural aberrations (inversions, deletions, deficiencies, amplification, and others) can be used as biomarkers at the cytogenetical level for measurement of the toxic effects of different stress agents in the environment. Due to deletions in chromosome G in some chironomid species, it was transformed to so called pompon-like chromosome G, which can be used to test the genotoxicity of various agents in an aquatic ecosystem. It is emphasized that the BR system is a very interesting model for studying the response of the genome to pollutants in the environment. Together with the standard activity of this system, a clear reversed level of activity was observed. Changes of the activity of the Nucleolar Organizer were also detected. It is shown that these important key structures are affected by trace metal toxicity. The protective role of heterochromatin is discussed. It has been shown that in the model ecotoxicological species *C. piger* and *C. riparius* the distribution of somatic rearrangements in their genome is nonrandom. They concentrate mainly at or close to sites rich in repetitive DNA loci. Structural and functional aberrations in polytene chromosomes can serve as early-warning indicators of environmental pollutions. Changes at the population and community levels can be analyzed by using these biomarkers of individual response.

Key words: Chironomidae, genotoxicity, polytene chromosomes, structural aberrations.

To the famous scientist, colleague and friend Prof. Dr. Iya Ivanovna Kiknadze, who is still full of energy and keeps working in the field of Genetics: Cytogenetics and Evolution, especially of family Chironomidae, Diptera

Invertebrates as early indicators of environmental impact

Environmental pollution is one of the serious problems in the world. Nowadays we witness degradation of the surroundings. Many species have disappeared, ecosystem structures and functions have changed, and biodiversity is being destroyed. The main task of our society is to protect the environment and improve life quality. Methods applied to detection and assessment of the impact of pollutants on ecosystems are of great importance. Biological approaches have a number of advantages that make them particularly suitable for accessing the dynamic process in ecosystems (Walker *et al*., 1998).

Biological communities provide direct means for studying the impact of pollutants in the environment. They are exposed to and directly involved in the transformation of pollutants in ecosystems.

Different vertebrate species are in use as test system to monitor the environment (Walker *et al.*, 1998).

Nowadays, though, invertebrates can replace vertebrates in some testing protocols. With invertebrates, pollutants can be better evaluated at the individual, population and community levels (Lagadic, Caquet, 1998). They provide evidence for the existence of causal links between individual responses and changes at the population and community levels. Changes of individual responses assessed through biomarker measurements at the population and community levels are probably among the most important initial steps in the development of early indicators of the environmental impact of agents. The toxicological and ecotoxicological responses of invertebrates have a key role in the environmental agent impact assessment. The development of bioassays with invertebrates is stimulated by both biological and toxicological features of these organisms. Biological aspects are related to their maintenance under controlled conditions. Their specific responses to various stress factors determine their toxicological significance. They have been successfully used in acute and chronic tests for hazard identification (Lagadic, Caquet, 1998). These authors note that invertebrates occupy key positions in the food webs of aquatic ecosystems and some of them are present in a wide range of habitats. The invertebrates have been used largely for the monitoring of chemicals in the environment. Biological, ecological and toxicological characteristics render invertebrates useful in detecting pollution in specific habitats, mainly through their bioaccumulation potential, and in evaluation of individual effects of exposure (Lagadic, Caquet, 1998). Predictions can be made about how future contamination would act at the population and community levels, and therefore, invertebrates can be used as early warning indicators of deterioration and restoration of ecosystem structure and function. With regard to their positions at different levels of food webs, invertebrates play key roles in ecosystems. Most of them occur as large populations and have intimate contact with the substrate where pollutants are accumulated. Also, they have limited ability to move freely and thus cannot escape the exposure to pollutants. Molting appear to be a critical step in the arthropod life cycle and is very important to assess toxic effects of stress factors on individual development. The duration of the intermolt period, mortality rate, and frequency of body part abnormalities can be used as individual criteria to assess the effect of pollutants.

The advantages of family Chironomidae for evaluating the effect of pollutants in freshwater ecosystems

One very important group of invertebrates is Chironomidae. They have many advantages for assessment of the state of the environment. Chironomids are a widely distributed and abundant group of species, which inhabit aquatic ecosystems and play there an important role. Chironomid larvae account for at least 50 % of the macro-invertebrate species in any freshwater ecosystem. Individual larvae represent basic units of biological communities. They integrate into a single whole at lower levels of biological organization (molecular, cellular and organ) and form building blocks for higher organization levels: populations, communities, and ecosystems (Warwick, 1990). Their larva stage is the most critical, the most responsive to environmental stress, and the most metabolically active stage of their life.

From the viewpoint of pollution assessment in biomarker studies, benthic larval stages of Chironomidae (Diptera) are regarded as a typical biomonitoring model for ecotoxicological tests (Warwick, 1988; Jannssen de Bisthoven *et al.*, 1992). They are included in both the Extended Biotic Index (De Pauw *et al.*, 1992) and the Annex V.1.2.6 of the E. C. Water Framework Directive. The classification of lakes as oligotrophy, mesotrophy and eutrophy can be performed on the basis of chironomid communities. In this way they can be used for defining the trophic state of a lake (Saether, 1979). Also, they play a large role in lake purification. Chironomid larvae are directly exposed to pollutants, and their tissues can accumulate various pollutants, which later are transferred to fish and ducks and then to humans. Chironomid larvae are the primary link of the food chain, from the sediment to the higher levels of the chain. At present, chironomid larvae are used in environmental tests owing to their wide range of sensitivity to different environmental factors: temperature, pH, depth, food, freezing, salinity, oxygen, pollutants and etc. The external morphology of the larvae (mandibles, antenna, submentum, and epipharynx) is very sensitive to these parameters (Warwick, 1990). Warwick also indicated that morphological abnormalities possessed a number of advantages for assessment and detection of the impact of pollution on aquatic ecosystems. However, later it was shown that the chironomid genome was more sensitive and responsive to pollutants in aquatic ecosystems than external morphology (Michailova *et al*., 1996, 1998, 2000, 2001a, b, 2003, 2006, 2008, 2009a, b, c; Petrova, Michailova, 2002; Petrova *et al*., 2004; Sella *et al*., 2004; Planello *et al*., 2007). Chironomid larvae (stage exposed to pollutants) possess salivary gland chromosomes, which make chironomids appropriate subjects for cytogenetic monitoring. Due to chromosome aberration analysis, chironomids can be used for tracing mutagen effects of a number of stress agents. The following features of polytene chromosomes are applicable for this purpose: size and polyteny with a very good band pattern along the chromosomes (Michailova, 1989). Also, standard karyological characteristics of some species (Hägele, 1970; Wülker, Klötzli, 1973; Kiknadze, 1987; Michailova, 1987, 1989; Michailova, Contreras-Lichtenberg, 1995; Kiknadze *et al*., 1991; Michailova, Todorova, 1998) can be employed as a basis to reveal environmental mutagens by studying chromosome aberrations; appearance of heterochromatin; and changes in the functional activity of the polytene chromosomes and the activity of key structures, such as Balbiani rings (BRs) and Nucleolar Organizer (NOR).

Having in mind the advantages of chironomids, we used this knowledge for tracing the effect of several factors in the environment, especially the consequences of trace heavy metal pollutants on the structure and functional organization of polytene chromosomes in the species. We also tried to establish biomarkers for detection of pollutants in aquatic ecosystems and assessment of their significance and their toxic effects.

The effect of heavy metals and important species appropriate for characterization of their genotoxicity

Heavy metals are very important as trace element for normal cellular functions. Most of them form active sites in a number of enzymes involved in oxidation-reduction reactions. However, they can be toxic to cells once their levels exceed physiological values. The effect of heavy metals can manifest itself at high concentrations for short periods, causing acute toxicity, or at low concentrations but for long periods of time, when it can cause chronic toxicity, leading to disorders in vital functions, such as abnormalities in morphology, growth, maturation, reproduction, hatching, etc.

In order to study the effect of various trace metals on the genome structure, we can use species of genera *Chironomus* Meigen and *Glyptotendipes* Kieffer, subfamily Chironominae. It is important that the species can be reproduced in the laboratory (Fischer, 1969; Michailova, 1985) and used in dose-response experiments in order to validate and calibrate responses observed in the field. Such species are *Chironomus riparius* Meigen 1804, *C. piger* Strenzke, 1959, *Glyptotendipes barbipes* (Staeger, 1839), *G. salinus* Michailova, 1987, *G. glaucus* Meigen, 1818 and *G. pallens* Meigen 1804 (Michailova, Belcheva, 1990; Michailova *et al*., 1996, 1998, 2000; Ilkova, 2004). The response of the genome to various stress agents was also detected in *C. bernensis* Wülker, Klötzli, 1973 (Petrova, Michailova, 2002; Michailova *et al*., 2009b) and *C. plumosus* L. (Michailova, Mettinen, 2000; Ilkova, 2004; Michailova *et al*., 2009b). Also, the reaction of *C. acidophilus* Keyl, Keyl, 1959 genome from an acidic lake was analyzed (Michailova *et al*., 2009a). The polytene chromosomes of the above species have clear band structures, and good standard chromosome maps are available for them (Hägele, 1970; Michailova, 1987, 1989; Kiknadze *et al*., 1991; Petrova, Michailova, 2002). This allows tracing the cytogenetic effects of some metals on the structure and function of polytene chromosomes. Cytogenetic effects can also be studied under laboratory conditions, and typical chromosome rearrangements are known to be signals for certain pollutants.

Knowledge of the biology of the species, their phylogeny, and DNA organization of their genome is very important in consideration of genomic responses.

Chironomus riparius and *C. piger* are homosequential species, distinguished by the amount of heterochromatin (Hägele, 1977; Michailova *et al*., 2009c) and by the amount and location of repetitive DNA (Schaefer, Schmidt, 1981; Michailova *et al*., 2009c). The former is a phylogenetically younger benthic chironomid species, most widely distributed in freshwater ecosystems. *Glyptotendipes barbipes* and *G. salinus* are sibling species, also benthic. They differ in specific homozygous inversions (Michailova, 1987; Andreeva *et al*., 1998) as well as in phylogeny: *G. barbipes* is younger (Michailova, 1987).

Glyptotendipes glaucus and *G. pallens* are miner species. They have species-specific band patterns of the polytene chromosomes. *Glyptotendipes pallens* is phylogenetically older (Hoffrichter, 1977). In this way, it is possible to trace the effects of biology and phylogeny on the genome response of Chironomids. Timmermans *et al.* (1989) established different external morphology reactions of *Chironomus* species depending on their biology. In order to understand the genome response to anthropogenic factors, especially to trace metal pollution, it was necessary to perform two types of studies: field and laboratory experiments.

Field studies of various *Chironomus* and *Glyptotendipes* species were performed in lakes or ponds characterized by higher concentrations of different trace metals in comparison with fossil sediments (Forstner, Salomons, 1980; Michailova *et al*., 1996, 1998, 2000, 2009a, b; Ilkova, 2004; Petrova *et al*., 2004; Sella *et al*., 2004).

Laboratory experiments were performed with fertilized egg masses from unpolluted ponds: *C. riparius*, *C. piger*, *G. salinus*, *G. barbipes* and *G. pallens* (Michailova, Belcheva, 1990; Michailova *et al*., 2001a, b, 2003, 2006, 2009c). Acute and chronic experiments involved treatment with Al ions, $CuCl_2$, $Cr(NO_3)_3$ and $Pb(NO_3)_2$. All experiments were carried out under standard conditions at 20 °C and with a photoperiod of 16h light, constantly aerated, and pH 7, which was kept by adding NaOH or HNO_3. Parallel control experiments were done.

Cytogenetic characteristics of the genomes of species under study

The species of genus *Chironomus* belong either to the cytocomplex thummi (Keyl, 1962) (*C. riparius*, *C. piger*, *C. plumosus*), with chromosome arm combinations AB CD EF G or to cytocomplexes *lacunarius* (Wülker, Klötzli, 1973) (*C. bernensis*) and *pseudothummi* (Keyl, 1962) (*C. acidophilus*), with chromosome arm combinations: AD BC EF G and AE, BF, CD G respectively. In all complexes the chromosome set is $2n = 8$, chromosomes AB, AD, BF, BC, CD are metacentric, chromosomes AE and EF – submetacentric and chromosome G – is either acrocentric (*C. riparius*, *C. piger*, *C. plumosus*, *C. acidophilus*) or telocentric (*C. bernensis*). In most cases chromosome G bears the key structures: Balbiani rings (BRs) and a Nucleolar Organizer (NOR). In *C. bernensis*, NORs occur in chromosomes AD and EF. The centromere regions of the polytene chromosomes of these species are distinct. They look like dark heterochromatin bands. The species of genus *Glyptotendipes* also have $2n = 8$, with chromosome arm combinations tentatively designated as AB CD EF G, Chromosome G of *G. glaucus* and *G. pallens* carries BRs and NORs, while in *G. barbipes* and *G. salinus* chromosome G has BRs only, each of the other chromosomes having a NOR. Chromosomes AB CD EF of the studied species are metacentric, in *G. pallens*, *G. salinus*, *G. barbipes* chromosome G is telocentric, and in *G. glaucus* it is acrocentric. (Michailova, Contreras-Lichtenberg, 1995). The centromere regions in *G. barbipes* and *G. salinus* are characterized by large dark knobs, whereas in *G. glaucus* and *G. pallens* they look like dark heterochromatin bands.

Well done standard chromosome maps are available for all aforementioned species (Hägele, 1970; Michailova, 1987, 1989; Kiknadze *et al*., 1991; Michailova, Contreras – Lichtenberg, 1995; Petrova, Michailova, 2002), which allow detailed analysis of chromosome rearrangements under different stress factors.

Structure chromosome rearrangements

In all species studied, belonging to geographically isolated populations (Bulgaria, Italy, Russia, Poland, and Finland) and collected from lakes and ponds with elevated concentrations of trace metals (Michailova *et al*., 1996, 1998, 2009a, b; Petrova *et al*., 2004; Sella *et al*., 2004), we found both inherited (Chromosomes are affected in all salivary gland cells.) and somatic rearrangements (Only few cells of one individual are affected.) (Sella *et al*., 2004). The **inherited aberrations** were dominated by paracentric heterozygous inversions, appearing as complex heterozygous inversions affecting large regions of polytene chromosomes, with a frequency exceeding 1 % (Petrova, Michailova, 2002; Michailova *et al*., 2009a, b). An exception was *C. riparius*, whose inherited aberrations occurred at a frequency below 1 % (Kiknadze *et al*., 1988; Sella *et al*., 2004). According to Sella *et al*. (2004), the observed inversions in this species can be considered endemic, which have occurred recently or have been randomly floating in populations for a long time and should disappear sooner or later (Sella *et al*., 2004). The observed heterozygous frequency did not differ significantly from those expected from the Hardy – Weinberg equilibrium (Sella *et al*., 2004). Only few pericentric inversions were detected in a separate population of *C. riparius* (Petrova *et al*., 2004, Sella *et al*., 2004). No correlation or relationships were detected between the H index (obtained by dividing the number of different hereditary aberrations by the number of studied larvae) and pollutant levels in the sediments (Sella *et al*., 2004). Inherited heterozygous inversions in *C. riparius* were observed also in larvae exposed to different concentrations of Cr, Pb, Al, or Cu (Michailova *et al*., 2001a, b, 2003, 2006), although at a higher frequency.

Somatic rearrangements. Somatic chromosome aberrations of different types were detected in the *Chironomus* and *Glyptotendipes* species studied, both by field and laboratory exposure to different concentrations of trace metals (Al, Cr, Pb, Cu): para-pericentric heterozygous inversions, deficiencies, amplifications, and deletions (Michailova *et al*., 2000, 2001a, b, 2003, 2006, 2008, 2009a, b, c). The inversions, amplifications and deficiencies affected small regions of the chromosomes and only few cells of a particular individual. They occurred at very low frequencies (Michailova *et al*., 2001a, b, 2003, 2006, 2009c; Petrova *et al*., 2004; Sella *et al*., 2004). With regard to the phylogeny of the species, it is important to emphasize that the genome of the phylogenetically younger species is more sensitive than the genome of an older species, *C. piger*. For instance, the frequency of somatic inversions observed in *C. riparius* sampled in the field (51,9 %) was significantly higher than that observed in *C. piger* (21,3 %) (G = 61,923, df 1, P < 0,001) (Ilkova *et al*., 2007). Having in mind King's (1993) idea that the chromosome rearrangements are not randomly distributed in the chromosome but depend on DNA organization, note that the genome of *C. riparius* showed the tendency for more variable aberrations compared to other species. It can be a result of the more interspersed repetitive DNA distribution in its genome. However, some breakpoints of inversions were shared by both sibling species (Michailova *et al*., 2002; Ilkova *et al*., 2007). Benthic and miner species showed different reactions. Benthic species are also more sensitive than miner ones owing to their direct contact with pollutants in the sediments (Ilkova, 2004).

In addition to inversions, we observed homozygous deletions and heterozygous deficiencies in some of the polluted sites and exposed larvae of *C. riparius* and *C. piger* (Michailova *et al*., 1996, 1998, 2000, 2001a, b, 2003, 2006; Sella *et al*., 2004; Michailova *et al*., 2009c). Deletions affected only chromosome G of sibling, homosequential species, *C. piger* and *C.riparius*, where in fact very important structures are located: BRs and NOR. They affected either BRb or BRc, or both BRc + BRb, again with a higher frequency in the phylogenetically younger species *C. riparius* – 16,8 % compared to *C. piger* – 7,9 % (Ilkova *et al*., 2007). Some of these deletions can induce the formation of a so-called «pompon»-like chromosome (Fig. 1, a, b). This is a specific phenomenon of the reaction of a small chromosome: it has lost much of its genetic material, and other parts become very active. Sometimes, chromosome G assumed a very compact state in few cells of studied individuals (Fig. 1, a, c) (Michailova *et al*., 2003).

Somatic deficiencies appear very seldom. For instance, in few cells of *C. riparius* from Moncalieari, Italy, a deficiency was detected in arm E (Michailova *et al*., 1998), and in individuals from Santena, Italy, in arms A, B, D, and G (Michailova *et al*., 1998, 2000). Of special interest is the appearance of heavily stained bands in some sections of *C. riparius* chromosomes. Arm D: D2, D2f, D3b, D3de, C4e; arm E: 4f, 5ef; arm F: B3h (Michailova *et al*., 1996). Some of these bands appeared in different forms: as an amplified band, as a band in the heterozygous state, and as a band in the normal homozygous state. Very interesting events were observed in chromosome EF in Italian sites studied (Moncalieari and Santena). Two sections in arm E (A5d-g and A4f-g) and two sections in arm F (B3h and B3o) were often larger than the same sections in the standard chromosome map. They could appear in the homozygous, heterozygous and

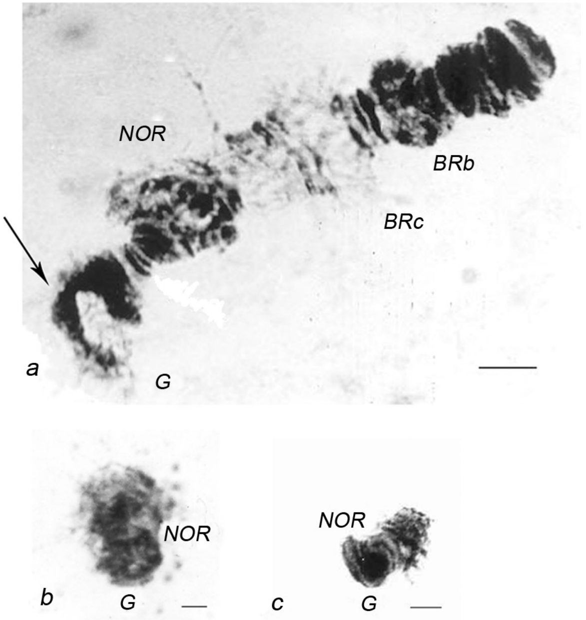

Fig. 1. Chromosome G of *Chironomus riparius*.

a – chromosome G: BRb, Balbiani Ring b; BRc, Balbiani Ring c; NOR, Nucleolar Organizer; b – chromosome G with deletions, looking as a «pompon»; c – chromosome G with deletions, in the compact state.
BR – Balbiani Ring; NOR – Nucleolar Organizer. The arrow indicates the centromere region.
Scale bar = 10 µm.

amplified homozygous states in one and the same individual. Densitometrical analysis was performed in sections A5d-g and A4f of arm E and section B3h of arm F (Sella *et al*., 2001). The mean value of DNA content in section EA4f was 18,8 pg., in section EA5d-g, 18,2 pg, and in section FB3h of arm F, 9,6 pg. Sections A4f and A5d-g of arm E and section FB3h of arm F had 7, 13 and 15 times higher DNA contents than the lowest class in the control (Sella *et al*., 2001). The authors suggested that this somatic size variation was due to somatic recombination events provoked by pollutants in the sediments of water basins.

Another interesting event, a somatic heterozygous translocation, was observed in the St. Petersburg population of *C. riparius* (Petrova *et al*., 2004).

One very important event should be mentioned: the tendency for correlation between the number of somatic aberrations and pollution in the regions under study. The maximum numbers of somatic aberrations were detected in samples of *C. riparius* and some other chironomid species from very highly polluted sites (Sella *et al*., 2004; Michailova *et al*., 2009b). Also, the frequency of somatic aberrations in chironomid larvae treated with different concentrations of trace metals was significantly higher than in control larvae (Michailova *et al*., 2001a, b, 2003, 2006). These results confirm Lagadic and Caquet's (1998) idea that somatic aberrations can be used to assess the genotoxic effect of environmental factors.

It is important to consider the observed changes of the appearance of euchromatin and heterochromatin in the polytene chromosomes of *C. riparius* from polluted areas. The field and laboratory-treated materials of *C. riparius* provide evidence for a process involving the transformation of euchromatin into heterochromatin. Chromosomes AB, CD and EF have fine interstitial dark C heterochromatin bands, which are euchromatic in unpolluted and control populations (Michailova *et al*., 1997). If we assume that heterochromatin plays a protective role in absorbing damages and structure aberrations, the transformation of euchromatin to heterochromatin might provide an adaptive advantage for cells or individuals as a whole.

Another very interesting event observed in the field populations under study is position effect variegation (PEV). Some of the somatic pericentric inversions were associated with PEV: in the homologue where the inversion is located, a pseudopuff was found, and in the other homologue the same region was in the standard condensed state (Michailova *et al*., 1997).

In this case the activity of one and the same gene can depend on its position in the genome (Henikoff, 1990). Euchromatin, near or within a heterochromatin region, usually becomes inactivated or shows variegated expression. In some studied populations in Italy and treated material, PEV was observed in arms A, D, E and F of *C. riparius* polytene chromosomes. In all these cases PEV varied from cell to cell, producing mosaic gene expression. It is known that a variety of environmental conditions can either enhance or suppress the variegating effect. In *Dr. melanogaster* PEV has been observed after ionizing radiation (Henikoff, 1990), inducing chromosome rearrangements. In populations studied by us and the treated material of *C. riparius* PEV could be caused by a heterozygous inversion, produced by the high concentrations of some heavy metals or complexes of various trace metals in the field. In this way the euchromatin adjacent to the centromere heterochromatin becomes inactive owing to a heterozygous inversion, and turns into heterochromatin (Michailova *et al*., 1997). Assuming the hypothesis that PEV results from inactivation of normal genes by histone-mediated condensation of chromatin within some cells (Mottus *et al*., 1980), one may suggest that it is accumulation of specific proteins that is involved in gene inactivation.

There are different hypotheses concerning the effect of trace metals on the species and its genome. Naturally, the chemical speciation of trace metals exists, and it can therefore increase their bioavailability. Kleimann *et al*. (1981) found that chironomids from an acidic lake polluted with trace metals also could regulate the accumulation of copper, zinc and, to some extent, manganese. Karlik *et al*. (1980) noted that, as aluminum forms different complexes depending on pH, at high pH the presence of Al has a stabilizing effect upon DNA as the metal binds to its phosphate groups. At low pH, aluminum can destabilize the DNA double helix, for it preferentially binds to denaturized DNA and forms cross links between strands, forming the so-called Complex II (Karlik *et al*., 1980).

Copper is known to destabilize DNA, possibly through localized production of hydroxyl radicals (Bremner, 1998). Zinc generally has a beneficial effect on the genome by, for example, reducing the toxicity of cadmium (Nocentini, 1987; Coogan *et al*., 1992). Excess zinc can, however, leads to an increase in reactive iron, which can damage DNA through reaction with hydrogen peroxide to produce hydroxy radicals (Elgohary *et al*., 1998). Copper and zinc also influence gene expression by, for example, regulating the activity of detoxication-associated metallothionein genes. Sanderson *et al*. (1980), who studied the black fly *Simulium vitatum*, found that aluminum toxicity may be related to alterations in gene expression. However, special molecular analysis of the genome is required to confirm that such mechanisms operate in the chironomid species studied by us.

Functional changes in chromosomes

Many functional alterations were observed both in field and lab materials of chironomids exposed to different concentrations of trace metals (Michailova *et al*., 2001a, b, 2003, 2006, 2008, 2009a–c). The response of chironomid genomes to different stress agents in the nature, as well as to trace metals in the lab, is characterized by changes in gene expression, clearly manifesting themselves in the puff activity of BRs and NOR. Balbiani rings are key structures, as they are sites of intense transcription of genes encoding silk proteins (Wieslander, 1994). These proteins are very important for chironomids due to their participation in the construction of the tubes where larvae develop. The Nucleolar Organizer function is essential for cell maintenance and ribosomal production, which is highly conserved through evolution (Planello *et al*., 2007).

In most cases, the activity of BRs in *C. riparius* is below the normal value (Michailova *et al*., 1996, 1998, 2001a, b, 2003, 2006). For instance, in *C. riparius* the puffing activity of BRs is normally relatively stable in forth instar larvae but occurs in the fully expanded state up to the prepupa stage (Santa Cruz et al., 1978; Kiknadze et al., 1976, 1985; Diez et al., 1990). BRb is very active in young larvae and at the prepupa stage, while in the middle larval stage BRb is slightly active or collapsed (Santa Cruz *et al*., 1978; Kiknadze *et al*., 1985). Pollution by trace metals or complexes of different trace metals affect the BRs transcription mechanism dramatically. For instance, in some polluted sites of Italian rivers, BRc and BRb of *C. riparius* (IV larva stage, 6–7th phase) are very sensitive, whereas the BRa activity does not change. Both BRs (BRb and BRc) can be in collapse or become very active, and a drastic regression of BRc, paralleled by an expansion of BRb was observed (Michailova *et al*., 1998; Petrova *et al*., 2004). Changes of the activity of these key structures were also recorded after exposure of *C. riparius* larvae to different concentration of Cr, Pb, Al, Cu ions (Michailova, 2001a, b, 2003, 2006). Both the BRs could show slight activity or be in the heterozygous state, or be completely repressed. So, a clear reversal of BRb and BRc activity was observed in polytene chromosomes of *C. riparius* from polluted or exposed lab materials in comparison with an unpolluted population or control. It is important to note that trace metals can induce changes in the puffing of *C. riparius* Balbiani rings relatively similar to those induced by heat shock or sugar feeding (Beermann, 1973; Yagi, 1984; Diez *et al*., 1990). The same tendency was also observed in other chironomid species (Michailova *et al*., 2009a, b, c). It is possible that the BR transcription machinery can respond in a similar way to different stress situations in the nature.

A very interesting mechanism was observed in *C. acidophilus* (Michailova *et al*., 2009a). In cases when one of BRs was not expressed, a puff at the telomere of chromosome was observed. It is considered to be a compensatory mechanism. Many specific puffs in other chromosomes were also detected in species collected from polluted sites or exposed to trace metals (Michailova *et al*., 1996, 2000, 2001a, b, c). In *C. riparius* an induction of a puff in chromosome G (Dc, E2de) was detected, in either a homozygous or heterozygous state (Kiknadze, Panova, 1972; Michailova *et al*., 1998).

Furthermore, it is noteworthy that the Nucleolar Organizer also shows a change in its activity: from a very high activity of both the homologues in a heterozygous state to a slight activity or complete collapse (Michailova *et al*., 1998, 2003, 2006, 2009c; Petrova *et al*., 2004). Intermediate activity was recorded very often (Michailova *et al*., 2009b, c). Planello *et al*. (2007) to show that ribosomal genes and the Nucleolar function are direct targets for cadmium toxicity.

Thus, the results of drastic disruption of activity of BRs and NOR appear to be the earliest and sensitive signal of toxicity of trace metals. These key cell structures may play the main role in monitoring and responding to stress in the environment.

Some other functional alterations were also observed in both populations and lab experiments. Few specimens of *C. riparius* had two or three chromosomes with telomeres having a loose, granular structure or the centromere looking like a pseudopuff (Michailova *et al*., 2001a, b, 2003). Very often, larvae treated with various concentrations of Cr ions had polytene chromosomes with granular structures in the mosaic state (Michailova *et al*., 2001a).

In few cells of the polytene chromosomes of *G. glaucus* (Ilkova, 2004), of *C. acidophilus* (Michailova *et al*., 2009a), of *C. riparius* (Michailova *et al*., 1996, 2000), an ectopic pairing between different chromosomes was detected. For instance, in *C. riprius*, an ectopic conjugation was observed between chromosome G, section Ee and telomeres A, C, D, E and F (Michailova *et al*., 1996). The lowest degree of contacts was noticed between centromeres AB, EF and G chromosomes. Normally in *C. riparius* the homologues of chromosome G are paired. However, material cases of disturbed pairing were observed in some populations studied or exposed: either both homologues were completely asynapted or they were asynapted in a specific site of the chromosome. The frequency of asynapsis increased from section A to E (Michailova *et al*., 1996, 1998, 2003).

Other very interesting events were observed in centromere regions of chromosome G of *C. riparius* (Michailova *et al*., 1996, 1998, 2001a, b) and *C. bernensis* (Petrova, Michailova, 2002; Michailova *et al*., 2009b). The species had been either collected from polluted basins or treated with ions of trace metals. Centromere heterochromatin condensation, which led to «dark-knob» formation in *C. bernensis*, was detected. The formation of a «dark knob» had been observed in *C. nuditarsis* from a Swiss population (Fischer, Tichy, 1980). The authors' hypothesis is that the mutation resulted from the absence of a Balbiani ring, replaced by a compact heterochromatin mass. The observed condensation of heterochromatinin in species living in polluted sediments can be formed by additional DNA replication in these chromosome segment under the influence of heavy metal pollution. However, additional molecular studies are required to understand the causes of this phenomenon.

Association between localization of repetitive DNA clusters (Alu and Hinf) and the transposable elements (NRCth1, CRTR) and break points of rearrangements in Chironomid genome

Michailova *et al*. (1996) put forward the hypothesis that a correlation between the chromosomal distribution of some mobile and repetitive DNA elements and sites of chromosomal breakpoints exists in *C. riparius*. In the sibling homosequential species *C. riparius* and *C. piger* the repeats of DNA elements (Alu and Hinf) are very stable in different geographically isolated populations from Germany, Bulgaria, and Italy (Hankeln, 1990; Bovero *et al*., 2002; Ilkova *et al*., 2007; Michailova *et al*., 2007). The Alu and Hinf elements seem to be structural components of *C. piger* and *C. riparius* heterochromatin-like Cla elements (Schmidt, 1984).

The genome of the phylogenetically younger species, *C. riparius*, is rich in these elements in comparison with the older *C. piger* species. On the one hand, as these cluster have constant positions in both the homosequemtal species, they can be used as molecular markers for their identification. On the other hand, they may cause the higher sensitivity of the *C. riparius* genome to different stress agent. Also, both species are distinguished in the locations of retrotransposons NLRCth1, and larger numbers of signals were detected in the *C. riparius* genome than in *C. piger* (Ilkova *et al*., 2007; Michailova *et al*., 2007, 2009c). Zampicinini *et al.* (2004) performed PCR-based Transposon Insertion Display (TID) and found a high level of insertion variability in *C. riaprus*. The greater number of copies of dispersed repetitive sequences in the *C. riparius* genome than in *C. piger* determines the higher sensitivity of the *C. riparius* genome, where somatic aberrations affected the whole chromosome, whereas in *C. piger* they are concentrated mainly in pericentromeric regions and in few sites of arms D and F (Michailova *et al*., 2009c). Thus, both species, which differ in DNA organization (*Chironomus riparius* has a greater content of DNA elements rich in repetitive DNA elements, dispersed among all chromosomes.) respond to stress agents in different ways. None or significantly less chromosome rearrangements (inversions) were found in *C. piger* (Michailova *et al*., 2009c). In some localities *C. piger* responded mainly by changing the functional activity of its polytene chromosomes, whereas the response of the *C. riparius* genome involved numerous structural and functional alterations. So, these different responses to environmental agents may be associated with the structural organization of their genomes.

In both species the distribution of break points was nonrandom, and proximal regions of the chromosome had significantly more breakpoints than distal ones (Bovero *et al*., 2002). These are common breakpoints, which, according to Bovero *et al*. (2002), are cytological points where a breakage was observed in at least two larvae, either from the same or different populations.

Like breakpoints, repetitive DNA clusters appear to be significantly more abundant in regions of constitutive heterochromatin, such as pericentromeric, proximal regions, but they are rare in or absent from distal sections of chromosomal arms. Also, Gunderina and Aimanova (1998) observed that γ-induced breakpoints concentrated in the pericentromeric regions of *C. riparius* chromosomes and that in some cases their locations coincided with those of repetitive DNA sequences. However, it was proven that the significant association formed between breakpoints and sites of distributions of NLRCth1, Alu, and Hinf clusters in the genomes of both species (Ilkova *et al*., 2007). For instance, twenty of the total number of weak points (58,8 %) occurred in the vicinity of bands hybridizing to probes of Alu and Hinf satellite DNA (Bovero *et al*., 2002). Such correlations were found also for the distribution of CRTR clusters and breakpoints of rearrangements in *C. piger* (Michailova *et al*., 2009 c). Location of the pCthC12HR transposable element (TE) in the genome of *C. riparius* was studied by Kiknadze *et al*. (1987) in larvae of the Novosibirsk population, and coincidences between the location of this element and sites of chromosomal breaks were detected. The data about the sensitivity of the genome to stress agents confirm the idea put forward by Bovero et al. (2002) that repetitive DNA and TE-rich regions are more sensitive to genotoxic agents and that they are potentially prone to rearrangements.

According to Golubovsky (2000), the genome consists of obligate and facultative components, and there is an interaction between them. The obligate component consists of unique sequences of structural genes. The facultative components include repetitive DNA and transposable elements. Our data support Golubovsky's (2000) suggestion of interaction between the facultative and obligate components of the genome. Different stress agents can activate transposable elements and induce a burst of mutational activity.

Conclusion

Chironomids can be used as an indicator of genotoxic concentrations of pollutants in aquatic ecosystems.

Polytene chromosomes of chironomids, due to their great resolution, are very useful in applied investigations on environment quality. Important structures of polytene chromosomes, such as the BR system and NOR, may be regarded as a model for studying the response of the genome to heavy metal pollution.

The functional aberrations plus somatic cytogenetic damages (heterozygous inversions, deletions, deficiencies, formation of «pompon» chromosome G) are particularly suitable as biomarkers, as these cytogenetic changes are easily identifiable and provide early alarm signals of adverse long-term effects in organisms.

Most somatic chromosome rearrangements are nonrandomly distributed: they occur more frequently in specific sections of the chromosomes composed either by satellite DNA or by transposable elements.

Closely related *Chironomus* species show different genome responses to stress agents. The type and frequency of the rearrangements depend on DNA structure and organization.

Analysis of cytogenetic responses is therefore a potentially powerful tool in preventing long-term effects of anthropogenic stress at the population and community level.

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