Cytogenetic damage in viviparid snails inhabiting different lakes in Switzerland

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Numerical changes of chromosomes were evaluated in 252 specimens of snails *Viviparus ater, V. acerosus, V. mamillatus* and *V. contectus* inhabiting lakes Zurich, Murten, Lauerz and Constance. Cytogenetic damage in *V. acerosus, V. mamillatus* and *V. contectus* was studied in 54 specimens, which were descendants of snails collected in Hungary, Albania and Italy and cultured in cages during 1–7 years in Lake Zurich near Ruschlikon in Switzerland. Chromosome sets were studied in 5255 somatic cells of embryos and juveniles and gonadal cells of adult males. Eighteen chromosomes in diploid sets and nine bivalents in meiotic nuclei were observed in the majority of cells of *V. ater, V. mamillatus, V. acerosus.* The diploid number of chromosomes of *V. contectus* was 2n = 14. Seven bivalents were observed in the male meiosis of *V. contectus*. Moreover, changes of chromosome numbers were detected in 4% to 27.2% of the cells studied. The highest frequency of aneuploid and polyploid cells was observed in embryos of *V. ater* from the lakes Murten (27.2%) and Zurich (22.1%). The prevalence of polyploid cells in gonads and hypodiploid cells in embryos and juveniles was found in *V. ater* from all the lakes studied. A comparatively high frequency of cytogenetic changes was noted in *V. acerosus, V. mamillatus* and *V. contectus* transferred 1–6 years ago from Hungary, Albania and Italy and caged in Lake Zurich at Ruschlikon.

Key words: cytogenetic damage, *Viviparus*, snails, Switzerland

INTRODUCTION

A wide variety of industrial, domestic and agricultural wastes is discharged into aquatic systems. A large part of pollution can migrate with surface waters, and dissolved material may settle down in the bottom sediments. Molluscs, due to their filtration activity, sedentary style of life and other biological peculiarities, can accumulate harmful substances and achieve very high concentrations in tissues (Hartwig, 1995). They are recognized as very suitable organisms for the studies of aquatic genotoxicity. In cytogenetical studies of molluscs, a numerical variability of chromosomes was often observed. The aneuploid cells (with loss or gain one or more chromosomes) frequently occurred in bivalve and in some gastropod molluscs (Thiriof-Quievreux et al., 1988; Baršienė, Petkevičiūtė, 1988; Cornet, Soulard, 1989; Cornet, Soulard, 1990; Baršienė, 1994; Baršienė et al., 1994; Baršienė et al., 1996b; Baršienė et al., 1999, Baršienė et al., 2000). Moreover, different frequency of polyploid cells in mollusc tissues was shown (Thiriof-Quievreux et al., 1988; Thiriof-Quievreux et al., 1989; Baršienė et al., 1988; Baršienė, Petkevičiūtė, 1988; Baršienė, 1994; Baršienė et al., 1994; Yaseen, 1994; 1996).

Karyological studies of five gastropod mollusc species of the genus *Viviparus* (Baršienė et al., 2000) impelled us to consider the genotoxic impacts in the lakes Murten and Zurich in Switzerland. The Zurich City and the surrounding setlements, as well as motorboat activity in Lake Zurich are the major sources of pollutant intake into the lake. Viviparid snails from the lake Murten was were collected in the same harbour as motorboat port. Two nuclear power plants are located in the environs of Lake Murten. It is a well established fact that ionising radiation, PAH, heavy metals and other chemical pollutants can induce cytogenetic damage in mollusc cells (Dixon, 1982; Brunetti et al., 1992; Baršienė, 1994; Baršienė et al., 1999; Dopp et al., 1996; Bolognesi et al., 1996; Bolognesi et al., 1999; Baršienė, Baršytė, 1997; 2000; Baršienė, Baršytė-Lovejoy, 2000).

The main aim of this study was to evaluate the cytogenetic injuries in viviparid molluscs inhabiting various lakes in Switzerland, differing by ecotoxic exposure. These snails were introduced into water bodies of Switzerland. Thus, environmental genotoxicity was evaluated in tissues of the species *V. ater* introduced about one hundred years ago and in *V. acerosus, V. mamillatus* and *V. contectus*, which were transferred from Hungary, Albania and Italy 1–6 years before and caged in Lake Zurich by Ruschlikon.

MATERIAL AND METHODS

Peculiarities of environmental genotoxicity were assessed in four lakes of Switzerland. Lake Murten is located close to Bern, Lake Lauerz is close to Zug and Zurich, and Lake Constance is by Arbon near the border with Germany. The location of Lake Zurich is well known. Snails *V. ater* were sampled in the summer of 1996 from Lake Zurich by Feldbach and Goldbach and from the lakes Lauerz, Murten and Constance. Snails *V. acerosus, V. mamillatus* and *V. contectus* were collected from cages submerged at a depth of 3 m in Lake Zurich by Ruschlikon (Table). Aneuploidy, polyploidy and meiotic injuries were evaluated in 5255 cells of the embryos, juveniles and gonads of adult males.

Pieces of somatic and gonadal tissues were dissected from molluscs and prepared according to modified methods (Baršienė et al., 1996a). The blocking of cell division at metaphase was obtained by injection of 0.1–0.2% aqueous colchicine solution into adult specimens, using *ca.* 1 ml per 100 g of mollusc weight, 4–6 h before dissection. Juveniles were placed directly in 0.01–0.02% colchicine solution. Hypotonization of mollusc tissues was performed in distilled water at room temperature for 40–90 min. The material was fixed with 3:1 ethanol acetic acid solution, which was changed three times: after 30 min, 1 h and 24 h. Tissues were dissociated in 45% acetic acid and cells were smeared on slides and slightly heated up to human body temperature on a flame. The slides were stained with 4% Giemsa for 30–50 min using phosphate buffer solution, pH 6.8. The mitotic metaphase and meiotic stages were examined with a Jena Med cytology microscope.

Table. **Material for karyological studies of molluscs genus *Viviparus***

Species	Lakes	No of snails studied
Viviparus ater	Zurich by the Feldbach	48
	Zurich by the Goldbach	57
	Lauerz	51
	Constance	42
V. acerosus	Zurich by the Ruschlikon	13
V. mamillatus	Zurich by the Ruschlikon	20
V. contectus	Zurich by the Ruschlikon	21

Chromosome number variability p was counted as a percentage according to the formula:

$$p = (\Sigma a/\Sigma x)100\%,$$

where a is the abnormal / normal cell number and x stands for all examined cells.

To evaluate the standard deviation (SD) of data the following formula was used (Rokickij, 1974):

$$SD = (p(100-p)/\Sigma x)^{1/2}.$$

Statistical analysis was done employing the PRISM statistical package using the Chi-square test.

RESULTS

The diploid chromosome set of *V. ater, V. acerosus* and *V. mamillatus* consists of 18 chromosomes. Fourteen chromosomes were prevalent in the sets of *V. contectus.* The frequencies of diploid sets in the species of viviparid snails inhabiting different lakes varied. In tissues of *V. ater* inhabiting Lake Murten only 72.8% of diploid cells were observed, which is a significantly lower level ($p < 0.001$) than in those from the Lake Lauerz. In males of *V. acerosus* caged in Lake Zurich by Ruschlikon, the most stable chromosome numbers were determined. Eighteen chromosomes were counted in 96% of the gonadal cells studied, whilst in the somatic tissues of their embryos the diploid chromosome number was significantly lower ($p < 0.001$). A significantly higher level of cytogenetic damage in embryos and juveniles was observed in *V. ater* from Lake Murten, in *V. mamillatus* and *V. contectus* from the Lake Zurich by Ruschlikon. The cytogenetic damage was higher in adult

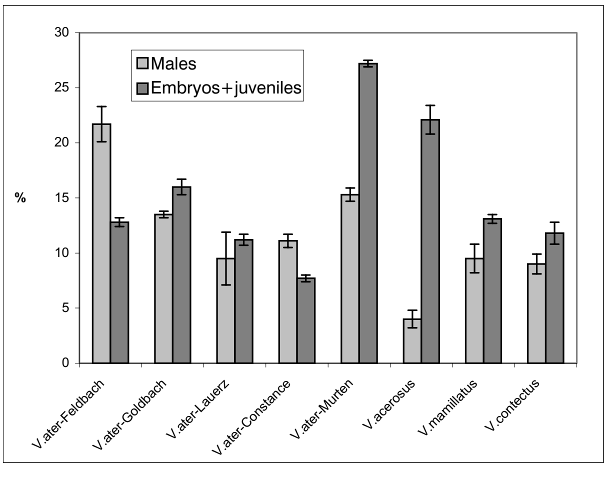

Fig. 1. Cytogenetic damage in the cells of the genus *Viviparus* snails

males of *V. ater* from Lake Zurich by Feldbach and from Lake Constance than in tissues of their embryos and juveniles (Fig. 1).

Cytogenetical disturbancies in somatic and gonadal cells were studied in *V. ater* inhabiting four lakes located at different parts of Switzerland. The most frequent hypodiploid cells occurred in the tissues of embryos and juveniles from all populations of *V. ater* (Fig. 2). Molluscs collected from Lake Murten had significantly higher levels of hypodiploid cells (26.6%) than ones sampled in the lakes Constance and Lauerz ($p < < 0.01$). It is worth stressing that in somatic tissues of embryos and juveniles polyploid cells (0.9–2.4% of the cells studied) were rare (Fig. 2).

Analysis of numerical changes in the chromosome sets of *V. ater, V. acerosus,* *V. contectus* and *V. mamillatus* showed the predominance of hypodiploid cells in somatic tissues of all species from all localities. The highest level of such cells was detected in embryos of *V. ater* from Lake Murten and in juveniles of *V. acerosus* caged for one year in Lake Zurich by Ruschlikon (Fig. 3).

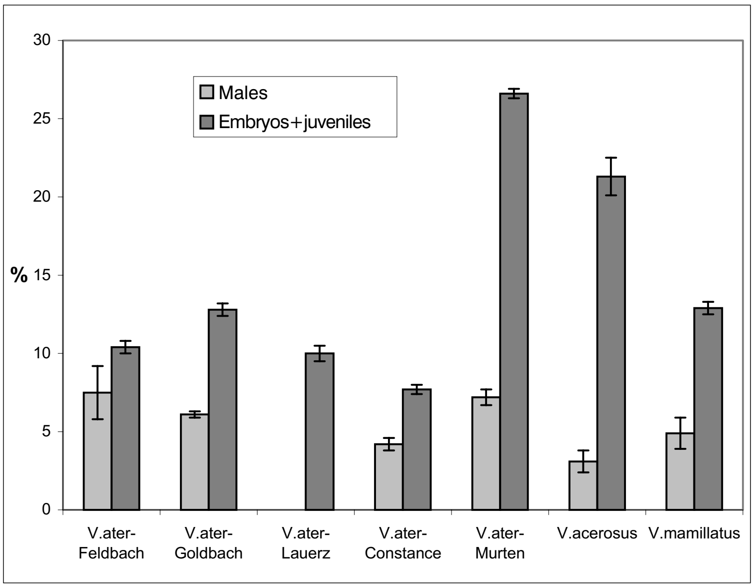

Fig. 4. Content of hypodiploid cells in the tissues of the genus *Viviparus* snails

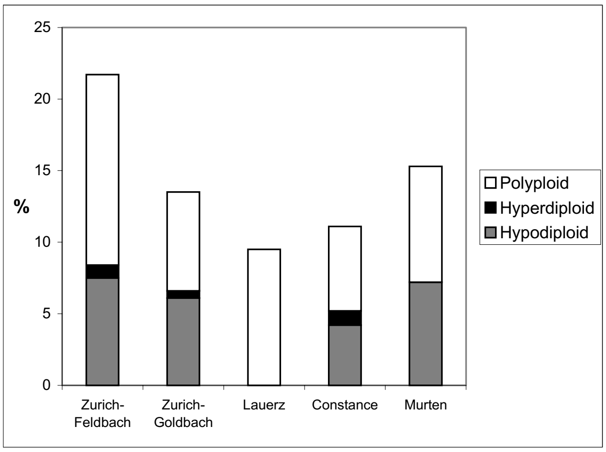

Fig. 2. Cytogenetic damage in males of *Viviparus ater* inhabiting different lakes

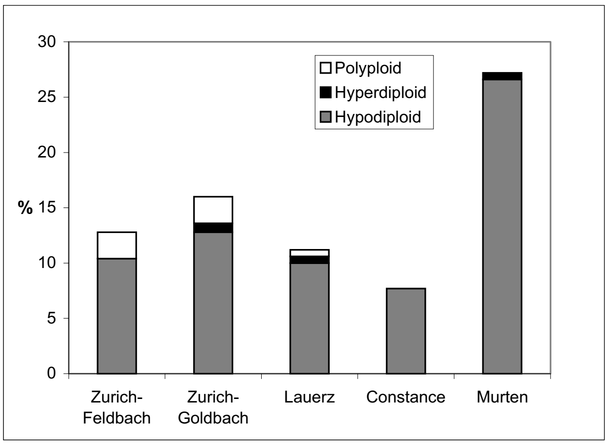

Fig. 3. Cytogenetic damage in the cells of *Viviparus ater* embryos and juveniles inhabiting different lakes

Remarkable and extensive chromosome set irregularities were observed in gonads of snails *V. ater* collected from Lake Zurich by Feldbach at the motorboat port. These molluscs possessed high amounts of cells with the hypodiploid and polyploid chromosome number (Fig. 4). Tetraploid and octoploid cells were prevalent among polyploid cells. Also, comparatively high amounts of cells with sixteen chromosomes in the sets were noted, whilst in Lauerz population of *V. ater* only cell polyploidy in gonads of males was found (Fig. 4). The hyperdiploid chromosome sets were rare occurrence in the cells of viviparid molluscs (Figs. 2, 4).

DISCUSSION

Aquatic ecosystems are known as collectors of various contaminants, and in the complex mixtures of pollutants a wide variety of interactions (additive, antagonistic or synergistic) are possible. During such processes the hazardous properties of pollutants can be changed and activation or detoxification of the derivatives could occur. Besides, in hydrosystems could be naturally synthesised so–called "natural pesticides", which are more mutagenic and cancerogenic than man-made compounds (De Flora et al., 1994).

Hazardous compounds are distributed widely in various ecosystems, and clear understanding of all dangers posed by environmental pollution to natural systems is needed. A conceptual model of genotoxic exposure effects across the levels of biological organisation was proposed by Anderson et al. (1994) and Bickham & Smolen (1994). The initial event is a contact of a pollutant with a biological molecule – DNA. This event results in DNA adducts, dimers, and DNA–protein crosslinks. As a consequence of DNA structural changes, various chromosomal aberrations can occur. Besides, the clastogenic influence of environmental pollutants, aneugenic effects (numerical changes in chromosome sets of organisms) can appear (Dixon, Clarke, 1982; Brunetti et al., 1992; Majone et al., 1990; De Flora et al., 1994; Bolognesi et al., 1996). Thus, the effects induced by the environmental contaminants are most frequently displayed first on the molecular level, and as a consequence of such disturbances various cytological, morphological, physiological and other changes can occur. Agents that cause genetic alterations may be present at very low, sublethal concentrations (Anderson et al., 1994).

During the last decades there is a growing evidence on the increased ecological risk to aquatic organisms that inhabit contaminated sites. These organisms may accumulate a variety of chemicals, radionuclides, including those with mutagenic and cancerogenic potention. A significant increment of cytogenetic damage in polluted *versus* unpolluted areas has been determined in aquatic organisms The high frequency of chromosome aberrations in polluted sites of the Po River was found in *Chironomus riparius.* In the sediments of contaminated sites higher concentrations of Cr, Pb, Cd and Cu were presented (Michailova et al., 1996). Increment of chromosome aberrations was observed in *Ch. balatonicus* from the Chernobyl region (Michailova et al., 1994). Frequent structural rearrangements of chromosomes and aneuploidy were observed in turtles inhabiting a radioactive reservoir (Lamb et al., 1991). Clams *Mya arenaria* inhabiting areas heavily polluted with PCBs showed a significant increase in micronuclei formation. Aneugenic effects were directly related to the level of carcinogenic compounds (Dopp et al., 1996). In Siberian river Tom, which during 30 years is dumped by the radioactive and chemical wastes, the micronuclei were occurred in 79% of studied cells from 12346 specimens of gastropod molluscs *Bithynia leachi.* More than 68% of snails possessed morphological abnormalities (Ilyinskikh, et al., 1996). In different aquatic habitats of Lithuania polluted by heavy metals, PAHs, PCBs and in those located in Chernobyl fallout spots, the cytogenetic damage in molluscs was significantly higher than in snails from unpolluted areas. It was noted that the frequency of polyploid cells, chromosome set instability and neoplastic lesions was higher in molluscs inhabiting areas polluted by both chemicals and radionuclides (Baršienė, 1994; Baršienė, 1997; Baršienė et al., 1994; Baršienė et al., 1999; Baršienė, Baršytė-Lovejoy, 2000).

In this study, the highest cytogenetic damage was shown in viviparids inhabiting lakes Murten and Zurich. Hypodiploid cells were most abundant in the tissues of *V. ater* from Lake Zurich by Ruschlikon and from Lake Murten. The tendency of cell polyploidization was most remarkable in viviparids from Lake Zurich by Feldbach. Radionuclides, oil, heavy metals might be considered as genotoxic agents in these lakes. Viviparid snails due to their detritus feeding and sedentary style of life are directly exposed to the harmful substances accumulated in sediments, and cytogenetic injuries are observed in their tissues (Baršienė, 1994; Baršienė et al., 1996b; Baršienė et al., 1999; Baršienė, Baršytė-Lovejoy, 2000). In the environs of Lake Murten two nuclear power plants, Lucens and Muehleberg, are located. Moreover, snails were collected from the harbour where a motorboat port was located. Ionising radiation is well known for inducing mainly structural aberrations of chromosomes (Hermine et al., 1996). Besides its clastogenecity, even at a low dose radiation showed aneugenic activity (Natarajan et al., 1996). Petroleum fuels are complex mixtures of aliphatic and aromatic hydrocarbons and some of them show a mutagenic and cancerogenic potential. The genotoxicity largely depends on the aromatic content of the fuel (Crebelli, 1995). Polycyclic aromatic hydrocarbons (PAH) are well-known genotoxins and may cause genetic damage in molluscs (Venier et al., 1996). An intense traffic of motorboats contributes to the pollution load of the water bodies (De Flora et al., 1991).

A comparatively high frequency of cytogenetic damage was noted in *V. acerosus*, which were transferred in 1995 from Hungary, and in *V. mamillatus*, which were introduced from Albania in 1989 and until 1996 were caged in Lake Zurich at Ruschlikon. Deployment in new ecological conditions as well as the action of environmental genotoxic compounds from the municipal, industrial, or motorboat activities may induce cytogenetic impairements. Heavy metals can cause an oxidative DNA damage, interfere with DNA repair and replication process (Hartwig, 1995). The formation of reactive oxygen leads to oxidative DNA damage and induction of DNA strand breaks, DNA–protein cross-links, and chromosomal aberrations could appear. It was stressed that Cd (II), Ni (II), Co (II), Pb (II) and As (III) can disturb DNA repair at low non-cytotoxic concentrations (Hartwig, 1995). Polychlorinated biphenyls (PCBs) are stable pollutants that can be found in most aquatic ecosystems. In molluscs from sites heavily polluted by PCBs the frequency of micronuclei was 3 times higher than in those from unpolluted sites (Dopp et al., 1996).

Extensive studies are carried out on the mechanisms of action of chemical compounds that affect chromosome distribution and induce aneuploidy. It was shown that the interaction of genotoxic chemicals with spindle microtubules leads to chromosome malsegregation (Crebelli et al., 1995). As a result, aneuploidy of cells occurs, *i.e.* in their nuclei fewer or more chromosomes than the exact multiple of the haploid set occur. Two different mechanisms of aneuploidy origination are known. One is non-disjunction of chromosomes at the anaphase when one daughter cell becomes trisomic and the other monosomic. The second mechanism of aneuploidy development is chromosome loss, when the lost chromosome may form a micronucleus. Studies of the genotoxic (aneugenic) properties of some chemicals or environmental genotoxicity *in situ* are mainly based on evaluation of micronuclei. Consequently, the aneuploidy which a rises as a result of chromosome non-disjunction remains unscored. Conventional cytogenetic studies are unable to show what kind of micronuclei exist in the cells, as they can be formed of whole chromosome or its acentric fragments (Scarpato et al., 1996). The scoring of cytogenetic damage at metaphase stage and meiotic rearrangements allow to evaluate more correctly the genotoxic influence of the environment.

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CITOGENETINĖS PAŽAIDOS SKIRTINGŲ ŠVEICARIJOS EŽERŲ VIVIPARIDŲ AUDINIUOSE

S a n t r a u k a

Chromosomų rinkinių pažeidimai tirti 252 pilvakojų moliuskų *Viviparus ater, V. acerosus, V. mamillatus* ir *V. contectus* somatinėse ir lytinėse ląstelėse. *V. ater* individai surinkti iš Ciuricho, Murten, Lauerz ir Constance ežerų. *V. ater* rūšis į Ciuricho ežerą buvo introdukuota praėjusio šimtmečio pradžioje, iš kur paplito ir į kitus Šveicarijos vandens telkinius. Moliuskai *V. acerosus, V. mamillatus* ir *V. contectus* atvežti iš Vengrijos, Albanijos ir Italijos ir 1–7 metus buvo veisiami varžose Ciuricho ežere prie Ruschlikon. Citogenetinė analizė atlikta embrionų, jauniklių ir suaugusių individų 5255 ląstelėse. *V. ater, V. acerosus* ir *V. mamillatus* somatinėse ląstelėse vyravo18 chromosomų rinkiniai, mejozėje – 9 bivalentai. *V. contectus* mejozėje dažniausiai rasti septyni bivalentai ir 14 chromosomų normaliuose diploidiniuose rinkiniuose. Chromosomų skaičiaus pokyčiai aprašyti 4–27,2% tirtų ląstelių.

Mažiausiai citogenetinių pažaidų rasta vivipariduose, kurie gyveno Lauerz ežere. Aneuploidinės ir poliploidinės ląstelės dažniausiai buvo aptiktos embrionuose, surinktuose Murten (27,2%) ir Ciuricho ežeruose (22,1%). Aplinkos genotoksinis poveikis Murten ežere matyt susiformavo dėl taršos motorinių valčių prieplaukose bei dėl šio ežero apylinkėse veikiančių dviejų atominių elektrinių įtakos. Ciuricho ežero tarša genotoksiniais junginiais matyt susijusi su vandens transportu, taip pat pramonės ir buitinių vandenų nuotekomis. Visose *V. ater* tirtose populiacijose ląstelių poliploidija dažnesnė suaugusių patinų gonadose, o hipodiploidinės ląstelės dažnesnės šios rūšies embrionų ir jauniklių audiniuose. Santykinai daug citogenetinių pažaidų buvo nustatyta iš kitų šalių atvežtų ir varžose veisiamų *V. acerosus, V. mamillatus* ir *V. contectus* audiniuose. Galima teigti, kad tai buvo sąlygota dirbtinio veisimo, naujų ekologinių sąlygų bei teršalų, pasižyminčių genotoksinėmis savybėmis, poveikio.

Raktažodžiai: citogenetinės pažaidos, *Viviparus*, moliuskai, Šveicarija