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FREQUENCY OF GAP AND BREAK TYPE CHANGES ON THE CHROMOSOMES OF THE FISH Perca fluviatilis CAUGHT AT SOME LOCALITIES ON THE DANUBE

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In this work the karyotype figure of the fish species Perca fluviatilis (Percidae) is presented. In addition, the frequency of gaps and breaks on the chromosomes of fish specimens caught at four different localities (by Beška, Zemun, Višnjica and Grocka) during three consecutive years (1986, 1987 and 1988) was determined. The highest frequency of these changes was recorded in fish caught at the localities situated downstream from Belgrade, i. e. by Višnjica and Grocka. Some of the values found in fishes caught at these localities ranged within the values of the presumed critical zone (3.0-3.5%) or were even higher. In that connecteion, these localities can be taken as periodically or permanently hazardous from genotoxical point of view.

Key words: Kariotype, Perca fluviatilis, genotoxicity, chromosomal aberrations

INTRODUCTION

An increased frequency of changes in chromosomes such as breaks and gaps (Brogger, 1982; Fišter et al., 1986; Zimonjić et al., 1990) points to the effects of different genotoxic agents. This phenomenon has often been applied in laboratory tests (Preston et al., 1981) for the possible mutagenic action of different chemical compounds (e. g. medical agents, pesticides, food additives etc.).

However, all these tests were performed using either cell cultures or laboratory animals as model systems, while data on the effects of compounds possibly expressing mutagenic action in animals from natural habitats are extremely rare in the available literature. This prompted us to estimate the frequency of chromosomal gaps and breaks in the fish species Perca fluviatilis caught at four localities of the Danube differing very much with regard to the level of contamination.

MATERIAL AND METHODS

Specimens of the fish species *Perca fluviatilis* were caugh at four different localities of the Danube (Beška, Zemun, Višnjica and Grocka) during three consecutive years (1986, 1987 and 1988). They were injected with colchicine (1.0 ug per gram b. w.) and sacrificed. Mitotic figures of metaphase

chromosomes were obtained after the preparation of kidney tissue by applying the method of Fontana et al. (1977) somewhat modified.

Metaphase chromosomes were analyzed and the number of breaks and gaps was determined. The results were statistically analyzed.

RESULTS

In order to estimate the frequency of changes of the break and gap types on the chromosomes, a number of individuals of the fish species Perca fluviatilis was analyzed. It was found that the karyotype of this species has a diploid chromosome number (2n = 48) consisting of 4 metacentric (M), 12 submetacentric (SM), 16 subacrocentric (SA) 16 acrocentric (A) chromosomes. The value of the arm number was found to be 80 (Figure 1). It should be emphasized that the presence of 4 M and 12 SM, which were classified into the same group, was observed.

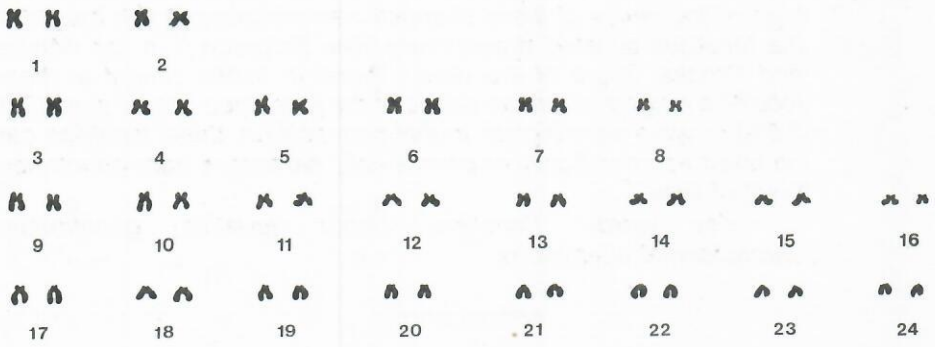

Figure 1. Karyotype of the fish species Perca fluviatilis (Percidae)

As early as in 1939, Svardson et al. reported the chromosome number of this fish species to be 2n = 28 which was corrected two decades later by Lieder (1959). The latter author claimed that the chromosome number of *P. fluviatilis* amounted to 2n = 44. The data of Nygren et al. (1968) who found 2n = 48 can be considered the first correct data on the chromosome number of this fish species. Earlier erroneous results could be ascribed to insufficiently reliable and precise cytogenetic procedures applied at the beginning of cytogenetics.

Thirty mitotic figures of each individual fish caught at four different localities of the Danube were examined with the aim of through analysis of the chromosomes and precise observation of chromosomal breaks and gaps which could result from river water contamination.

The results of the cytogenetic analysis of *Perca fluviatilis* caught in the Danube near Beška village are given in Table 1. In 1986 eight individuals and 260 mitoses were analyzed and six changes of the gap and break type were

observed making a total of 2.30%. Examinations performed on the fish caught in 1987 at the same locality included seven individuals and 220 mitoses and four chromosome breaks and gaps (1.81%) were recorded. In 1988, four specimens were caught at this locality and 190 mitoses were examined to reveal four chromosomal changes of the gap and break type, making a total of 2.10%.

Table 1. Frequency of chromosomal gaps and breaks in Perca fluviatilis from the Danube caught at Beška

Year No.	1986. Mitoses examined	1986. Breaks and gaps	No.	1987. Mitoses examined	1987. Breaks and gaps	No.	1988. Mitoses examined	1988. Breaks and gaps
1.	30	—	1.	30	1	1.	30	—
2.	30	1	2.	30	—	2.	30	1
3.	35	1	3.	35	—	3.	30	1
4.	35	—	4.	30	2	4.	35	1
5.	35	1	5.	35	—	5.	30	—
6.	35	2	6.	30	1	6.	35	1
7.	30	1	7.	30	—			
8.	30	—						
Sum:	260	6		220	4		190	4
Percent:		2.30%			1.81%			2.10%

Table 2. summarizes the data obtained by cytogenetic analysis of *P. fluviatilis* individuals caught at Zemun. In 1986. five fish and 150 mitotic figures were analyzed. Four chromosomal changes seen as gaps and breaks (2.66%) were recorded.

Table 2. Frequency of chromosomal changes of the break and gap type in Perca fluviatilis individuals caught at the Zemun locality of the Danube.

Year No.	1986. Mitoses examined	1986. Breaks and gaps	No.	1987. Mitoses examined	1987. Breaks and gaps	No.	1988. Mitoses examined	1988. Breaks and gaps
1.	30	1	1.	30	1	1.	35	1
2.	30	—	2.	30	2	2.	35	—
3.	30	1	3.	30	—	3.	35	—
4.	30	2	4.	35	—	4.	30	3
5.	30	—	5.	30	—	5.	30	—
			6.	30	1			
Sum:	150	4		185	4		165	4
Percent		2.66%			2.16%			2.42%

In 1987 at the same locality six individuals were caught and cytogenetically analyzed. Examinations of 185 mitoses revealed four alterations of the gap and

break type making a total of 2.16%. During the subsequent year (1988) five *P. fluviatilis* were caught. Analyses of 165 mitoses showed four chromosomal changes of the break and gap type (2.42%).

Table 3. Frežuency of chromosomal breaks and gaps in Perca fluviatilis specimens from the Višnjica locality of the Danube.

Year No.	1986. Mitoses examined	1986. Breaks and gaps	No.	1987. Mitoses examined	1987. Breaks and gaps	No.	1988. Mitoses examined	1988. Breaks and gaps
1.	30	1	1.	30	—	1.	35	—
2.	30	2	2.	30	1	2.	35	2
3.	30	—	3.	30	—	3.	35	—
4.	30	3	4.	30	3	4.	30	2
5.	30	3	5.	30	—	5.	30	—
6.	35	1	6.	35	—	6.	30	2
7.	35	—	7.	30	2	7.	30	1
8.	35	—	8.	30	3	8.	30	1
9.	30	1	9.	30	—			
10.	30	—	10.	30	2			
Sum:	315	12		310	11		225	8
Percent		3.80%			3.54%			3.55%

Table 4. Frequency of chromosomal changes (gaps and breaks) in Perca fluviatilis from the Danube caught at the Grocka locality

Year No.	1986. Mitoses examined	1986. Breaks and gaps	No.	1987. Mitoses examined	1987. Breaks and gaps	No.	1988. Mitoses examined	1988. Breaks and gaps
1.	30	1	1.	30	—	1.	30	2
2.	30	—	2.	30	2	2.	30	—
3.	30	2	3.	35	1	3.	35	2
4.	30	—	4.	30	—	4.	30	—
5.	30	3	5.	30	2	5.	30	2
6.	35	—	6.	35	—	6.	30	—
7.	35	2	7.	35	2			
			8.	30	3			
			9.	30	—			
Sum:	220	8		285	10		185	6
Percent		3.63%			3.50%			3.24%

The data obtained by cytogenetical analyses of *P. fluviatilis* specimens caught at Višnjica, downstream from Belgrade are presented in Table 3. In 1986, the analyses included ten fish specimens and 315 mitotic figures of chromosomes. These examinations revealed twelve changes of the gap and

break type, making a total of 3.80%. During the next year (1987), another ten fish were caught and 310 mitoses were analysed. Eleven chromosomal changes (3.54%) seen as gaps and breaks were recorded. In 1988 at the same locality eight individuals of *P. fluviatilis* were caught and 225 mitoses were analyzed to reveal eight (3.55%) chromosomal gaps and breaks.

Table 3. summarizes the results obtained by cytogenetic examinations of *P. fluviatilis* individuals caught in the Danube at Grocka. In seven specimens caught in 1986, 220 mitoses were examined. In this case eight breaks and gaps were found on chromosomes. During 1987 nine individuals were caught and 285 mitotic chromosome figures were analyzed. These analyses revealed ten chromosomal changes of the gap and break types making a total of 3.50%. During the next year (1988) six iindividuals of this fish species were caught and subjected to cytogenetic analyses which involved the examination of 185 mitotic figures of chromosomes. In this case six gaps and breaks (3.24%) on chromosomes were seen.

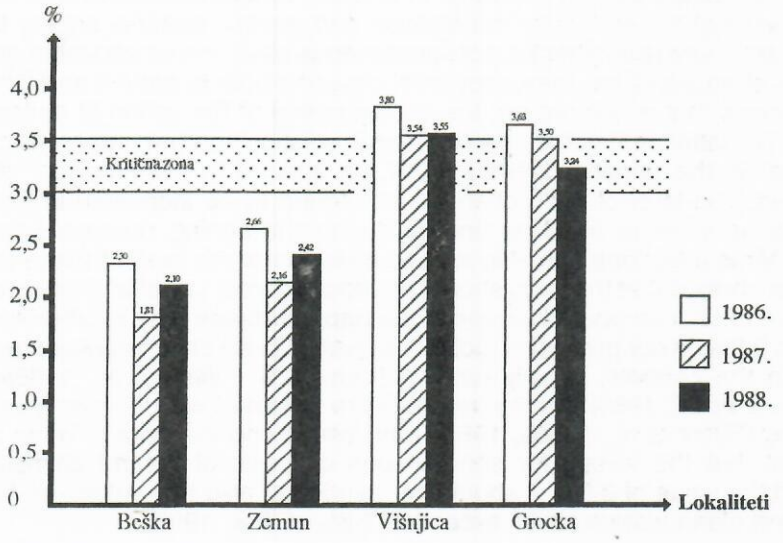

Figure 2. Frequencies of chromosomal gaps and breaks in *Perca fluviatilis* specimens caught at four different localities of the Danube during 1986, 1987 and 1988.

The results of our studies are graphically presented in Figure 2. where mean values for the changes of the gap and break type are given for each year of investigation and for each locality.

DISCUSSION

Mean values for the frequency of gaps and breaks on chromosomes of the fish species, *P. fluviatilis*, varied from the lowest value of 1.81% recorded in individuals caught during 1987 at Beška to the highest value of 3.80 observed in the fish caught during 1986 near Višnjica (Tables 1 and 3, respectively).

In Figure 2 it can be seen that the fish specimens from the localities situated upstream from Belgrade had a lower level of chromosomal changes in comparison with those caught at the localities downstream from Belgrade, where the frequency of these changes was rather high. In Figure 2 the range of values from 3.0% to 3.5% can be seen. This range was previously termed a critical zone (Fišter, 1992).

One of the drawbacks of the present study was the fact that we did not have the real controls usually applied during genotoxicity determinations under standardized laboratory conditions. This made the interpretation of the results obtained throughout the present work rather difficult. Namely, at the moment it is impossible to disciminate "spontaneous" changes on chromosomes from those that occurred as a consequence of the genotoxic action of different river water contaminants.

One should bear in mind that in addition to spontaneous errors occuring at the level of the molecular constitution and genetic material activity that are immanent, there is another kind of spontaneous alteration which would generally include changes of low frequency level occuring both in natural and laboratory populations, but which are not the consequence of the action of mutagenetic, agent. The latter changes may result from irradiation but their extent will certainly depend on the genetic constitution of the species in the broadest sense of meaning. The level of such changes was found to be increased in laboratory animals as a result of inbreeding or during the ageing process (Lilp et al., 1981). Virus infections can also lead to elevation of the level of these changes and it is obvious that this term should be reconsidered. Lilp et al. (1981) reported the level of spontaneous changes of the gap and break type in laboratory mice of even 2.8%. In our previous studies the level of these changes never exceeded 3.0% in the controls, usually ranging from 1-2% (Fišter et al., 1986a, 1987; Vujošević et al., 1986). Similar results were obtained with human lymphocyte cultures (Fišter et al., 1986a, 1987). Our results and the data of other authors suggest that the values for spontaneous changes of control animals never exceed the value of 3.0%. It should be mentioned also that values for fish from so-called clean waters never exceeded 2.0% (Fišter, 1992).

Although it is difficult at present to define the concept of clean water, or clean nature, one should accept that there are regions, or river sections significantly different with regard to the degree of contamination wich depends on the presence of industrial plant, the vicinity of big cities, the extent of application of pesticides in agriculture, etc. In the present work the value of 3% for chromosomal changes of the gap and break type was taken as a limit value, while the range between 3.0 and 3.5 was considered a zone where "spontaneous" changes are overlapping the alterations resulting from direct action of genotoxic agents (Fišter, 1992). The data presented in this work were evaluated according to these criteria.

Statistical analyses of the results (analysis of variance and Student's t test) graphically presented in Fig. 2 revealed statistically significant and very significant differences in the frequencies of chromosomal changes between the localities where the fish were caught during the three-year period. Thus, statis-

tically significant differences were recorded when the frequency of chromosomal changes in *P. fluviatilis* caught at the following Danube sections were compared: Beška versus Zemun, Višnjica and Grocka, Zemun versus Višnjica and Grocka, namely, significant differences were observed when any of the localities was compared to the others.

The lowest frequency of chromosomal changes seen as the gaps and breaks was registered in fish specimens caught at Beška, tobe increased in individuals from the locality of Zemun, while the highest frequency of these chromosomal alterations was found in the fish caught at Višnjica, the locality situated immediately downstream the city of Belgrade, to be somewhat decreased in the fish from Grocka, although the values were still within the proposed critical zone.

Frequencies of the chromosomal changes analyzed throughout this work were very high in *P. fluviatilis* specimens caught at Višnjica, exceeding the value of 3.5%, i. e. the critical zone. Since these values were so high in all three consecutive years examined, this section of the Danube can be considered as periodically or even permanently genotoxic. At this locality aberrant individuals with a large metacentric chromosome in the karyotype were caught. This occurred very probably as a consequence of Robertson's fusion and supports this view (Živković et al., 1987; Fišter, 1992). Namely, it is well documented that the presence of genotoxic agents can increase the probability of the appearance of such changes.

The chromosomal gaps and breaks recorded here do not mean that such effects occur at the level of species and populations, since the incorporation of such - structural, and more complex changes / inversions, translocations, fusions etc./ depends on numerous other factors, but primarily on selection.

Neverthelase, it is uncertain whether the chromosomal changes will remain only at the level of somatic cells. There is the possibility that some extensive changes could occur also in reproductive cells and tissues which would affect the progeny and finally lead to population polymorphism and/or changes of the fish species. However, the results presented in this paper suggest that genotoxic agents present in the river water can trigger the above mentioned events and support the suggestions of numerous authors on the indispensability of applying carefully elaborated programmes for the protection of water resources.

REFERENCES

1. *Bozhko, S. I., Meszaros, B. and Toth, E.* 1978. The karyological studies on some species of Percidae. *Ichtyologia, 10,* 17—28.
2. *Brogger, A.* 1982. The chromatid gap — a useful parameter in genotoxicology? *Cytogenetics and Cell Genetics, 33,* 14—19.
3. *Fišter, S.* 1992. Genetičko-populaciona analiza kariotipa nekih vrsta riba familije Cyprinidae. *Doktorska disertacija, Veterinarski fakultet Univerziteta u Beogradu.*
4. *Fišter, S., Živković, S. and Soldatović, B.* 1986. Effects of mebendazole on bone marrow cell chromosomes of mice. *Acta Veterinaria, 36,* 3—12.
5. *Fišter, S., Živković, S. i Veljović, P.* 1986a. Efekti nekih jedinjenja piperazina na hromozomima sisara. *Genetika, 18,* 175—180.

6. *Fišter, S., Soldatović, B. and Živković, S.* 1987. The effects of tetramisole chloride on murine bone marrow cells and human lymphocyte chromosomes. *Acta Veterinaria, 37,* 41—46.
7. *Fontana, F., Janković, D. and Živković, S.* 1977. Somatic chromosomes of Acipenser ruthenus L. *Arhiv bioloških nauka, 27,* 1—2.
8. *Lieder, U.* 1959. Die Chromosomenverhaltnisse von Esox lucius L., Perca fluviatilis L. und Lucioperca lucioperca L. (Vertebrata Pisces). *Arch. Zool., 12,* 123—126.
9. *Lilp, J. G. and Korogodina, Yu, V.* 1981. Spontaneous and induced chromosome aberrations in bone marrow cells of mice of different strain and age. *Cytologia (USSR) XXIII, No 10,* 1174—1179.
10. *Nygren, A., Edlund, P., Huisch, V., Ashgren, L.* 1968. Cytological studies in perch (Perca fluviatilis L), pike (Esox lucius L.) and ruff (Acerina cernua L.). *Hereditas, 59, 2—3,* 518—524.
11. *Preston, J. R., William, A., Bender, A. M., Brewen, J. G., Carrano, A. V., Heddle, J. A., McFee, A. F., Wolff, S., Wassom, J. S.* 1981. Mammalian in vivo and in vitro cytogenetic assays: A report of the U. S. EPA's Gene-Tox Program. *Mutation Research, 87,* 143—188.
12. *Svardson, G. and Wickbom,* 1939. Notes on the chromosomes of some Teleosts, Esox lucius L., Lucioperca lucioperca L. and Perca fluviatilis L. *Hereditas, 25,* 472.
13. *Vujošević, M., Živković, S. and Fišter, S.* 1986. In vivo mammalian bone marrow cytogenetic test (chromosomal analysis) — results of testing Urotovet. *Acta Veterinaria, 36,* 91—94.
14. *Zimonjić, D., Savković, N., Anđelković, M.* 1990. Genotoksični agensi. *Naučna Knjiga 1990. Beograd.*
15. *Živković, S., Fišter, S. and Vujošević, M.* 1987. Cytogenetic analysis of fishes from the Percidae (Pisces) family. *Genetika, 19,* 227—233.

UČESTALOST PROMENA TIPA GAPA I PREKIDA NA HROMOZOMIMA RIBA VRSTE PERCA FLUVIATILIS UHVAĆENIH NA NEKIM LOKALITETIMA DUNAVA

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SADRŽAJ

Izvršena su citogenetička ispitivanja vrste Perca fluviatilis, na primercima riba sakupljenih sa četiri lokaliteta Dunava, kod Beške, Zemuna, Višnjice i Grocke, u toku 1986., 1987. i 1988. godine.

Analizom kariotipa je ustanovljeno da vrsta poseduje diploidan broj 2n=48 hromozoma i to: 4 metacentrična (M), 12 submetacentričnih (SM) 16 subakrocentričnih (SA) i 16 akrocentričnih (A) hromozoma.

Kod riba uhvaćenih na različitim lokalitetima Dunava, analizirane su strukturne promene na hromozomima — tipa gapa i prekida, da bi se ustanovilo da li postoji povećana učestalost ovih promena, koja bi mogla da ukaže na eventualno prisustvo genotoksičnih agenasa u rečnoj vodi na nekim od ispitivanih lokaliteta.

Prema postavljenom kriterijumu, ustanovljeno je da je učestalost ovih promena povišena na lokalitetima Dunava nizvodno od ušća Save i Beograda, kod Višnjice i Grocke, te da na ovim lokalitetima postoji izvestan genetički rizik od povremenog ili stalnog prisustva genotoksičnih agenasa u rečnoj vodi.