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Sister chromatid exchange test detection of toxin-induced damage in cultured fish

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INTRODUCTION

In recent years, industrial and agricultural toxic wastes in waters from the Guadalquivir Basin, Gulf of Cádiz and the Strait of Gibraltar have increased. This growing contamination has motivated our team to develop the cytogenetic sister chromatid exchange (SCE) test, with the aim of detecting the effects of these wastes on the autochthonous cultured and wild fish species of the Southern Atlantic off the coast of Spain.

The SCE test consists of the detection of increases in the normal mean number of sister chromatid exchanges in metaphase M2 cells. These cells are those which have undergone two rounds of replication in the presence of 5-bromodeoxyuridine (BrdU). The chromatic chromatid differentiation is easily detected by simple acridine orange staining or with the fluorescence plus Giemsa (FPG) technique.

MATERIALS AND METHODS

Fishes belonging to *Rutilus alburnoides* (Teleosteens Cyprinidae) and to *Liza aurata* (Teleosteens Mugilidae) species were used in this study because of their representativeness of the Southern Atlantic Spanish ichthyological fauna.

Four groups of individuals of these species were maintained in well-aerated aquaria at 25–27°C. Forty-eight hours before sacrifice, they were injected ip with 0.5 mg of phytohemagglutinin (PHA-M)/ 50 g of fish body weight. One dose (0.8 mg of BrdU/g of fish) was injected directly into the cephalic kidney 24, 22, 20, 16 or 12 h before sacrifice, in order to obtain the maximum M2 cell (cells with sister chromatid differentiation) yield. Finally 0.3 μg of colchicine per gram of fish was injected ip 2 h before sacrifice.

After sacrifice, the kidney was removed and macerated in 0.075 M KCl for 90 min. The cell suspension was fixed in 5 changes of 1:3 acetic acid–methanol.

Air-dried preparations were obtained and stained with acridine orange (Dutrillaux and Lejeune, 1973).

RESULTS

The BrdU treatment time which offered the maximum yield of M2 cells was 16 h for *Rutilus alburnoides* and 22 h for *Liza aurata.*

Figure 1 shows an M2 metaphase cell with 4 SCE. The image belongs to the first species of triploid nature.

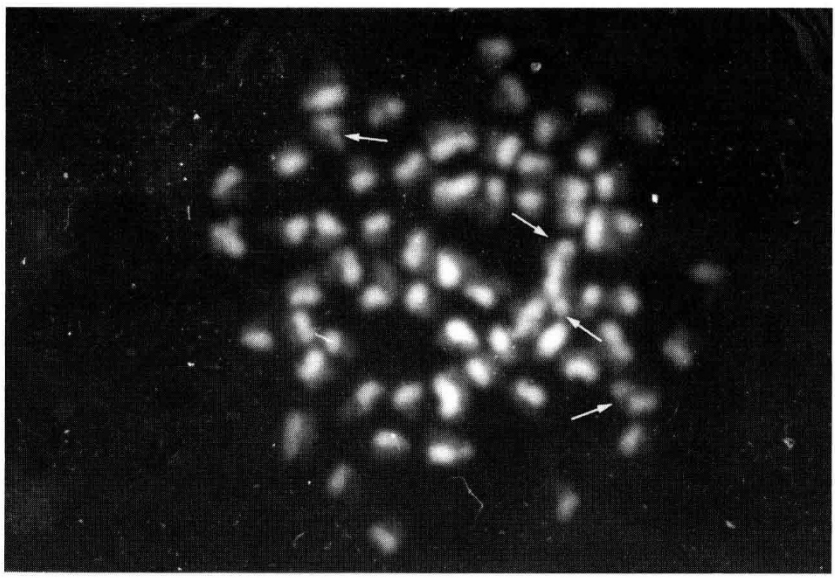

Fig 1. Triploid M2 metaphase cell of *Rutilus alburnoides* showing 4 SCE.

Mean SCE values for M2 metaphase cells were 3.57 ± 0.59 in *Rutilus alburnoides* and 2.91 ± 0.43 in *Liza aurata.* Table I summarizes the results of the SCE assay for M2 metaphase cells in both species.

Table I. Summarized results of SCE for metaphase cells in both species studied.

Species	*Tissue*	*Tested metaphases*	*SCE/metaphase*					
			0	1	2	3	4	mean
R alburnoides	cephalic kidney	480	12	21	56	271	120	3.57
L aurata	cephalic kidney	160	8	17	44	69	22	2.81

DISCUSSION

Although the meaning and mechanism of SCE is still unknown, it has been demonstrated that most of the genetically active chemical toxins (mutagens and/or carcinogens) induce significant increases in SCE when they are tested in *in vivo* animal systems or *in vitro* systems with activation (Stetka and Wolff, 1976; Carrano *et al*, 1978; Latt, 1982). At present, the SCE test is a very sensitive and rapid method for detecting chromosome mutagenicity and provides a powerful means of detecting environmental mutagens, as a complementary test to other tests of the same nature.

The application of BrdU incorporation to the detection of SCE in fish was used for the first time by Klingerman and Bloom (1976).

Since their report, other authors have used the detection of SCE as a sensitive cytogenetic test for determining chemically induced genetic damage, in *in vivo* (Alink *et al*, 1980; van der Hoeven *et al*, 1982; van der Kerkhoff and van der Gaag, 1985) and *in vitro* (Barker and Rackhan, 1979) systems.

In this study we established the mean number of SCE in fish maintained in high quality water, in the absence of toxins. This value constitutes the infrastructure of the SCE genotoxicity test, because any increase of this mean value in fish exposed to contaminated water indicates the presence in these waters of substances producing genetic damage. We are now testing the genotoxic activity of several agricultural pesticides whose results will be presented in the future.

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