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Transmission electron microscope observations of C-banded skipjack tuna chromosomes

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C-banded mitotic chromosomes of *Katsuwonus pelamis* (Linnaeus) were examined by transmission electron microscope (TEM) and results compared with light microscopy images. Advantages of the TEM technique are noted.

I. INTRODUCTION

Electron microscopy is a powerful technique used in chromosome studies on humans (Christenhuss *et al.*, 1967) and other mammals (Kingsley-Smith, 1970). However, there have been very few studies of fish or other lower vertebrate chromosomes using electron microscopy. Webb (1974) was the first to report using scanning electron microscopy on fish chromosomes in an investigation of the karyology of the gobiid teleost *Pomatoschistus minutus*. In the present study we examined C-banded mitotic chromosomes of the skipjack tuna, *Katsuwonus pelamis* (Linnaeus), using transmission electron microscopy (TEM) and have compared the results with light microscopy (LM) images. Our work was patterned after that of Xu & Wu (1983) who conducted comparative studies of LM and TEM images of G-banded human mitotic chromosomes and successfully karyotyped them using the technique developed by Wu & Waddell (1982).

II. MATERIALS AND METHODS

The blood samples from skipjack tuna were collected from freshly caught specimens, the lymphocytes were isolated on a density gradient of ficoll and sodium diatrizoate (Boyum, 1968) and the samples were cultured at 26° C in marine teleost medium (Michael & Beasley, 1973). After 3–5 days cells were harvested, arrested in metaphase with colcemid and placed on slides for C-banding. A detailed description of the methods used is given in Ratty *et al.* (in press).

After the C-banding was completed each field of chromosomes was examined under LM and TEM, using procedures similar to those developed by Xu & Wu (1983). Chromosome preparations were coated with a thin layer of Parlodion by dipping the slide into a stock solution of 0·7% Parlodion in amyl acetate. The edges of the coated slides were scored to free the Parlodion films and 75-count copper grids were placed over selected chromosome complements with the aid of a phase contrast microscope. The Parlodion films with the superimposed grids were then carefully floated off the slide onto the surface of an aqueous solution of 0·01 M hydrofluoric acid. The grids were reexamined

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with phase contrast microscopy to select suitable chromosome complements. The preparations selected were air-dried, stained with saturated uranyl acetate in 50% ethanol for 8 min, and air-dried again. Chromosomes were examined at 50 kV with an RCA EMU-3H electron microscope and the TEM images were recorded on 4489 film. Direct comparisons were then made of the chromosome complements in the LM and TEM images.

III. RESULTS

Light microscopy and TEM images of C-band patterns are presented in Fig. 1: they show that the skipjack tuna has 48 telocentric chromosomes, which is in agreement with the observations reported by Ratty *et al.* (in press). Figure 2 is a karyotype of C-banded chromosomes constructed from the LM and TEM images given in Fig. 1: it shows that the distributions of C-banding on corresponding chromosomes are identical in the TEM and LM images, e.g., chromosome pairs 1, 2, 4, 6, 15, and 21 have the same prominent centromeric C-bands in both images. While the C-banding patterns are the same in the LM and TEM images, considerably greater detail is apparent in the TEM images. For example, chromosome fibres are visible in the TEM images, but not in the LM images. Figure 3 shows the fibres for chromosome pairs 2, 15, and 24 at 1800X magnification. The fibres are discernible in the centromeric regions and in the arms of the chromosomes where they form diffuse boundaries. The size of the fibres is uniform among chromosomes of varying size and measures about 300 ångströms, which is consistent with the observations of Harrison *et al.* (1981) and Harrison *et al.* (1983) for human chromosomes observed under SEM.

The TEM images also show a multiple banding pattern in addition to the C-bands on some chromosome pairs, e.g., 1, 2, 4, 6, 15, and 21 (Fig. 2). The bands in pairs 2 and 15, shown in Fig. 3, correspond to ridges while the interband regions appear as depressed grooves. The multiple banding pattern was not apparent in the LM images.

In the TEM images the centromeric C-bands all appear as darkly stained bands of uniform width, while in the LM images the centromeric C-bands of the corresponding chromosomes appear irregular in width with prominently stained swollen knobs. In addition, some chromosome regions appear as lightly stained gaps in the LM images, whereas the same chromosome region observed in the TEM images is actually morphologically continuous.

IV. DISCUSSION

Our results show that the TEM technique developed by Wu & Waddell (1982) for investigating human chromosomes has significant advantages over LM in studying fish chromosomes. The high number and the extremely small size of chromosomes of many fish species make it difficult to investigate the karyology of fishes. Using TEM images it is possible to study the complete set of diploid chromosomes and to analyze chromosome complements of cells with a high degree of resolution not possible with LM. For example, the greater resolution of TEM made it possible to distinguish chromosome fibres, which allowed us to readily discriminate small chromosomes and chromosome satellites from stain particle artifacts, which under LM sometimes resembled small chromosomes.

FIG. 1. Comparison between light microscopic images and transmission electron microscopic images of the same C-banded metaphase chromosomes in skipjack. $2n = 48$. (a) Light micrograph. (b) Electron micrograph. Scale indicates 2μm.

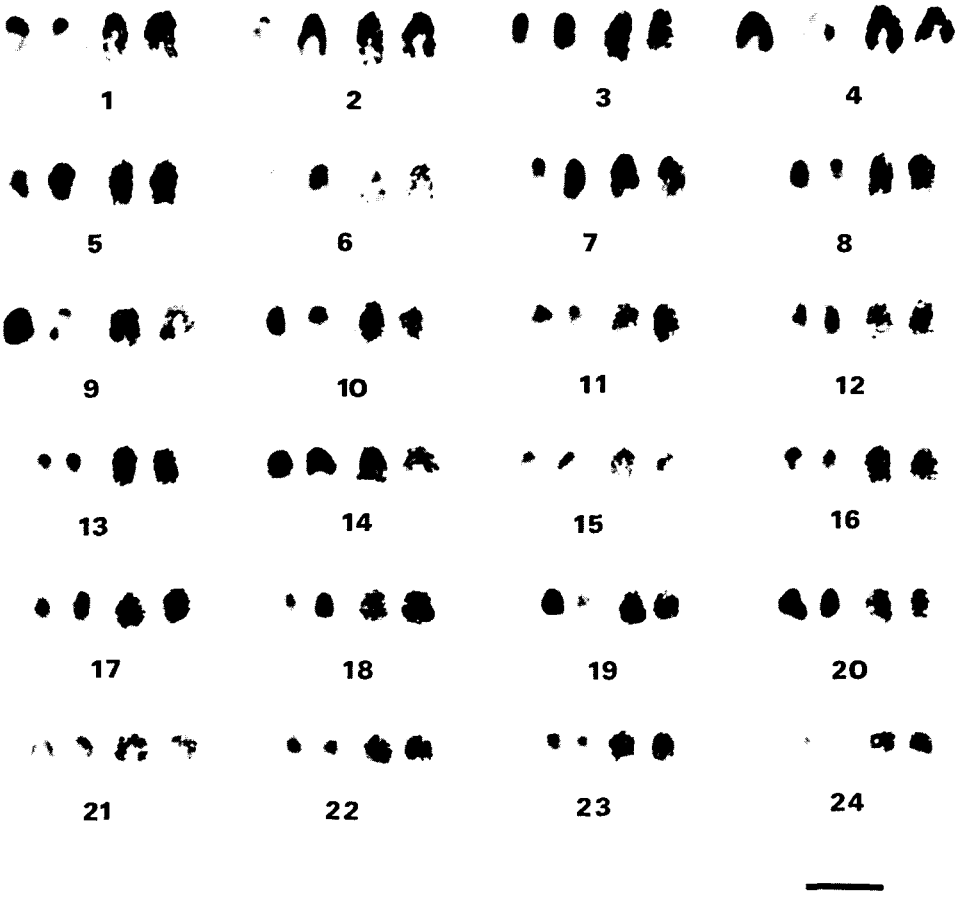

FIG. 2. C-banding karyotype of skipjack, using transmission electron microscope micrographs. 2n = 48. For each pair of chromosomes, light micrographs (left) are included for comparison. Scale indicates 2μm.

According to Dupraw (1970), Golomb (1976) and Bahr (1977), fibres are present in chromosomes but are not associated with stain particles. The use of TEM has confirmed that all of the skipjack chromosomes are telocentric. Ratty *et al.* (in press) reported that skipjack tuna chromosomes were probably all telocentric, although some of the chromosomes observed under LM resembled subtelocentrics. The discrepancy between LM and TEM observed in some chromosomes probably is due to differences in staining properties. Giemsa staining, which is used in LM, results from side stacking of thiazine dyes on DNA and the accumulation of dye at the bands (Blakey & Filion, 1967; Cervenka *et al.*, 1973; Ross & Gormley, 1973). Excessive accumulation of dye could be the reason for the subtelocentric appearance of chromosomes observed under LM. This is not a problem when chromosomes are stained with uranyl acetate for TEM, since only the density changes and the morphology of the chromosome is accurately preserved. While Giemsa was used in our C-banded preparations, it was inactivated following treatment with uranyl acetate (Xu & Wu, 1983).

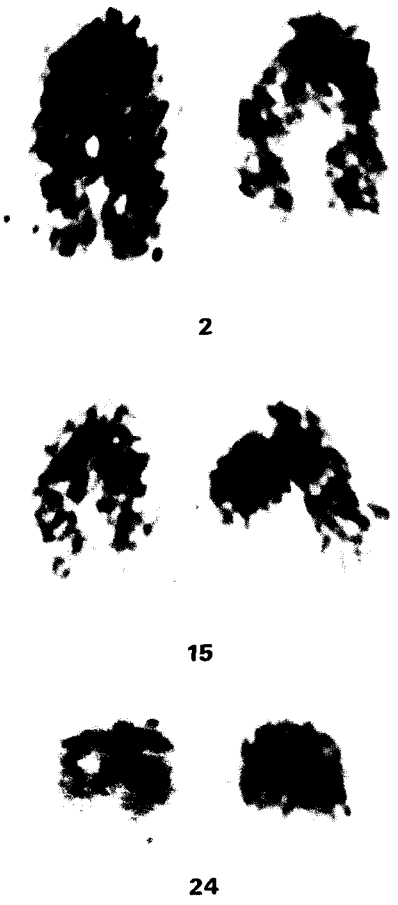

FIG. 3. Electron micrographs of chromosome pairs 2, 15 and 24 of skipjack. Scale indicates 1μm.

The banding we observed under TEM in addition to C-bands may possibly be G-bands. The multiple band and interband regions that we observed on some of the chromosomes is similar to the description of G-banding observed in SEM images by Utsami (1982). It also corresponds to G-bands observed in human chromosomes treated with trypsin and examined under TEM by Xu & Wu (1983). If the ridge and groove patterns we observed are actually G-bands, the TEM techniques of Xu & Wu (1983) may provide a mechanism for studying G-banding in fish for which ideal methods have yet to be established.

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