

サケ科魚類の2異種間交雑による染色体の切断と消失

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Chromosome Fragmentation and Loss in Two Salmonid Hybrids

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Abstract

Chromosomal aberrations, including fragments, chromatid breaks and uncondensed chromatin were found in metaphase preparations from masu salmon-pink salmon and Japanese charr-chum salmon hybrid embryos. Light microscopic examination of charr-chum embryo tissue sections showed an apparent loss of chromatid matter into the cytoplasm at the time of mitotic division.

Introduction

Arai (1984) noted the presence of chromosome fragments in metaphase cells of Japanese charr (*Salvelinus leucomaenis*) and chum salmon (*Oncorhynchus keta*) hybrids and a mean chromosome number significantly reduced from the expected 79 (mode of 58 in embryo samples taken between 10 and 17 days, and 52 from a single cross sampled at 41 days). To further investigate these phenomena, the present study reexamined samples taken during two previous hybridization experiments for chromosome abnormalities. One experiment involved a female masu salmon (*Oncorhynchus masou*; n=33) and male pink salmon (*Oncorhynchus gorbuscha*; n=26) cross (Ma and Yamazaki, 1986) and the other a female Japanese charr (n=42) and male chum salmon (n=37) cross. In the first case, some hybrids show normal development. The charr-chum cross is inviable, the embryo severely deformed and dying prior to hatching.

Methods

All eggs were fertilized by the dry method and maintained in circulating water of 8 to 12°C. Chromosome preparations were made according to the chopping method of Yamazaki et al. (1981), air-dried in the case of the masu-pink salmon cross and flame-dried in the charr-chum experiment. Hybrid samples utilized from the former experiment (n=4) were taken at 14 days (no control was available); those of the latter at 44 to 58 days for the hybrid group (n=10) and 46 days and larval stage for the control group. Bouin-preserved embryos from the latter experiment were cut in 5- μ m sections and stained with hematoxylin-eosin. These included 1 3-day, 1 4-day, 3 7-day and 1 14-day old embryos from the hybrid group, and 1 3-day, 1 4-day, and 1 14-day old embryos from the control group. In addition, selected metaphases from a reciprocal chum-charr cross and its control (32 to 45 days) were examined for abnormalities.

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Results

The present study confirms chromosome loss during early development of charr-chum hybrids, but at a rate significantly less than that reported by Arai (Table 1). Also noted was the occurrence of not one but 3 modal peaks: near 79 (as anticipated), the largest at 69 and another near 58. Individual embryos tended to be mosaics, having a range of chromosome numbers.

Table 1. Distribution of chromosome numbers of Japanese charr (F) × chum salmon (M) hybrid embryos, experiment #2.

| Embryo | | Chromosome numbers | | | | | | | | | | | | | | | | | | | No. of cells | | |
|--------|------------|--------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|--------------|---------------|-------|
| No. | Age (days) | 56 | 57 | 58 | 59 | 62 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 75 | 77 | 78 | 79 | 80 | >80 | with fragment | Total |
| H1 | 44 | | | 1 | | | | | | 1 | 2 | 2 | | | | 1 | | | | | | 2 | 7 |
| H2 | 44 | | | | | | | | 1 | | | 2 | | | | | | 1 | 1 | 2 | | 3 | 7 |
| H3 | 44 | | | 1 | 2 | 1 | | | | | | 2 | 1 | | 1 | | | | | | | 3 | 8 |
| H4 | 52 | 1 | 2 | 2 | 1 | | | 1 | | | | | 1 | | | | | | | | 1 | 4 | 9 |
| H5 | 52 | | 1 | | | | 1 | | 1 | | 4 | 1 | | | 1 | | | | | 1 | | 2 | 10 |
| H6 | 52 | | | | | | | | | | 1 | | | | | | | | | 1 | | 0 | 2 |
| H7 | 52 | | | | | | | | 1 | | | | | | | | | | | | | 0 | 1 |
| H8 | 58 | | | | | | | | | | | | | | 1 | | 1 | | 2 | | 1 | 3 | 5 |
| H9 | 58 | | | | 1 | 1 | 1 | | | 1 | | 3 | | | | | | | | | | 3 | 7 |
| H10 | 58 | 1 | 1 | | | | | | | 1 | | 2 | | | | | | 1 | | | 2 | 4 | 8 |
| Total | | 2 | 5 | 4 | 4 | 1 | 2 | 1 | 3 | 3 | 7 | 12 | 2 | 1 | 2 | 2 | 2 | 3 | 2 | 3 | 3 | 24 | 64 |

The masu-pink salmon cross displayed two modes at 59 (the expected) and 52 chromosomes (Ma and Yamazaki, 1986). In both the masu-pink and charr-chum crosses (and to much lesser degree the chum-charr embryos) chromosomal fragments and the occasional chromatid break were evident (PLATE I and II). The fragments in some cases remained attached to the chromosome arms by chromatid fibres. Trailing fibres were also noted among the three hybrid groups. In certain charr-chum embryos, frequency of fragment occurrence may have been as high as 50 percent of the cells.

PLATE III displays selected charr-chum hybrid cells in which chromatid matter has failed to migrate to the poles at metaphase and so is not incorporated into the nuclei at the time of cytokinesis. This apparent chromatid loss within the cytoplasm was not evident within the control samples. In the hybrid embryos it was detected for all of the above noted sampling times, but was least evident at 3 days and most visible at 7 days after fertilization (however, no 7-day control was available).

Discussion

Chromosome fragmentation, reminiscent of the present hybrid phenomena, has

also been observed in intraspecies fertilization experiments using gamma-irradiated (Yamazaki, 1981) and UV-irradiated sperm (unpublished data), and artificially aged eggs (Yamazaki, 1981). Furthermore, abnormal mitotic chromosomal migration similar to that of the present study has been reported for radioactive mutagen-treated medaka eggs (Egami, 1981).

Arai (1984) noted chromosome fragments and reduced chromosome numbers in the case of the charr-chum cross and suggested that chromosome loss may arise from an incompatibility of the foreign sperm genome and the egg cytoplasm, which selectively eliminates exotic chromosomes. Present inability to adequately distinguish maternal and paternal chromosomes precluded testing this hypothesis. The abnormal mitotic migration shown in PLATE III suggests that asters may fail to attach with some chromosomes. Perhaps the problem lies not only with cytoplasm incompatibility, but also with inability of the sperm and its centriole to initiate normal aster formation in the newly fertilized egg. Obviously, detailed time sequence and karyological analyses of the developing hybrid embryos are required.

Acknowledgements

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EXPLANATION OF PLATES

PLATE I. Chromosome aberrations. Black line represents 0.01 mm.

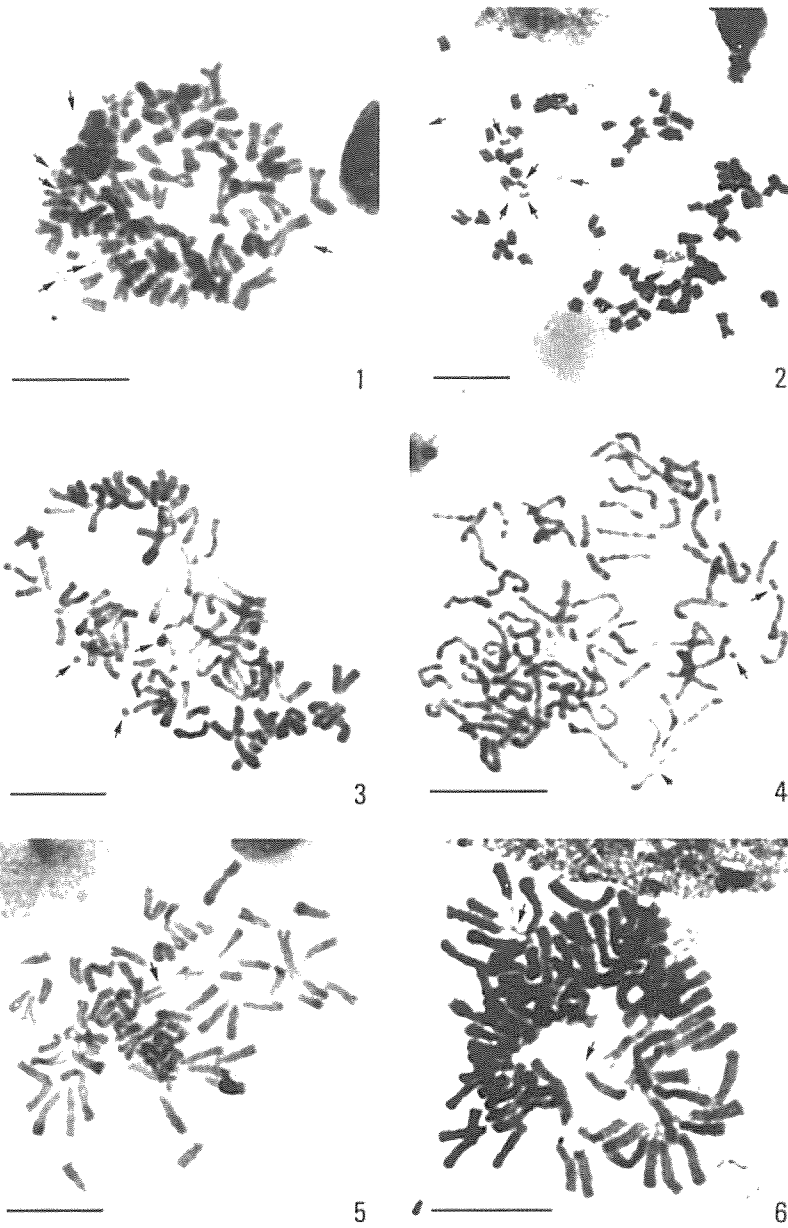
- Fig. 1. Japanese charr (F) × Chum salmon (M). Embryo number H9, CN (Chromosome no.): 58. Fragments
- Fig. 2. Charr-chum. H5, CN: 57?. Fragments
- Fig. 3. Charr-chum. H8, CN: not known. Fragments
- Fig. 4. Charr-chum. H11, CN: not known. Fragments and chromatid breaks.
- Fig. 5. Charr-chum. H5, CN: not known (not all chromosomes are included in photograph). The isochromatid break is bridged by fibres.
- Fig. 6. Chum salmon (F) × Japanese charr (M). CN: not known. Chromatid fibres.

PLATE II. Chromosome aberrations. Masu salmon (F) × Pink salmon (M). Single embryo. Black line represents 0.01 mm.

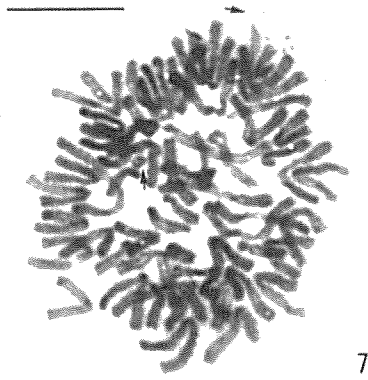
- Fig. 7. Fibre and fragment.
- Fig. 8. Fibre and possible fragment. Note similarity with Fig. 1.
- Fig. 9. Isochromatid break.
- Fig. 10. Isochromatid break and fragment.
- Fig. 11. Fragment.
- Fig. 12. Fragment connected with fibre.

PLATE III. 5-micron sections of char-chum cells.

- Fig. 13. Control (char × char), 4 days.
- Fig. 14. Control, 14 days.
- Fig. 15. Hybrid, 4 days. Telophase. Isolated chromosomes at the equator fail to migrate to the poles.
- Fig. 16, 17. Hybrid 1, 7 days.
- Fig. 18. Hybrid 2, 7 days. Cytokinesis. Extranuclear chromosomes isolated in cytoplasm.
- Fig. 19. Hybrid 3, 7 days.
- Fig. 20-22. Hybrid 1, 7 days.
- Fig. 23, 24. Hybrid, 14 days.



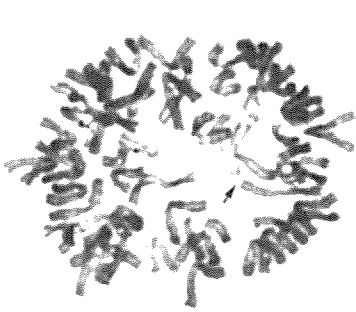
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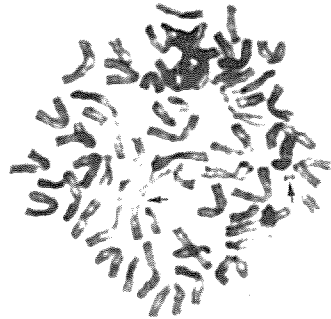
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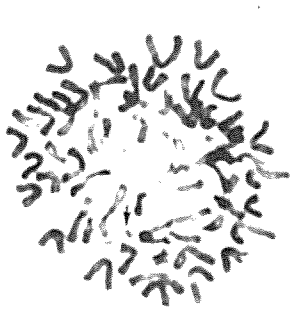
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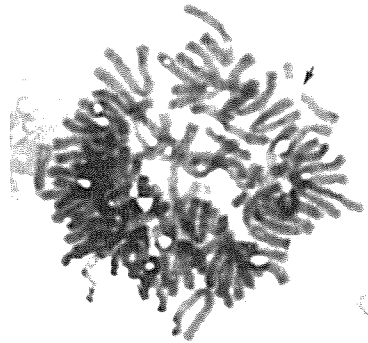
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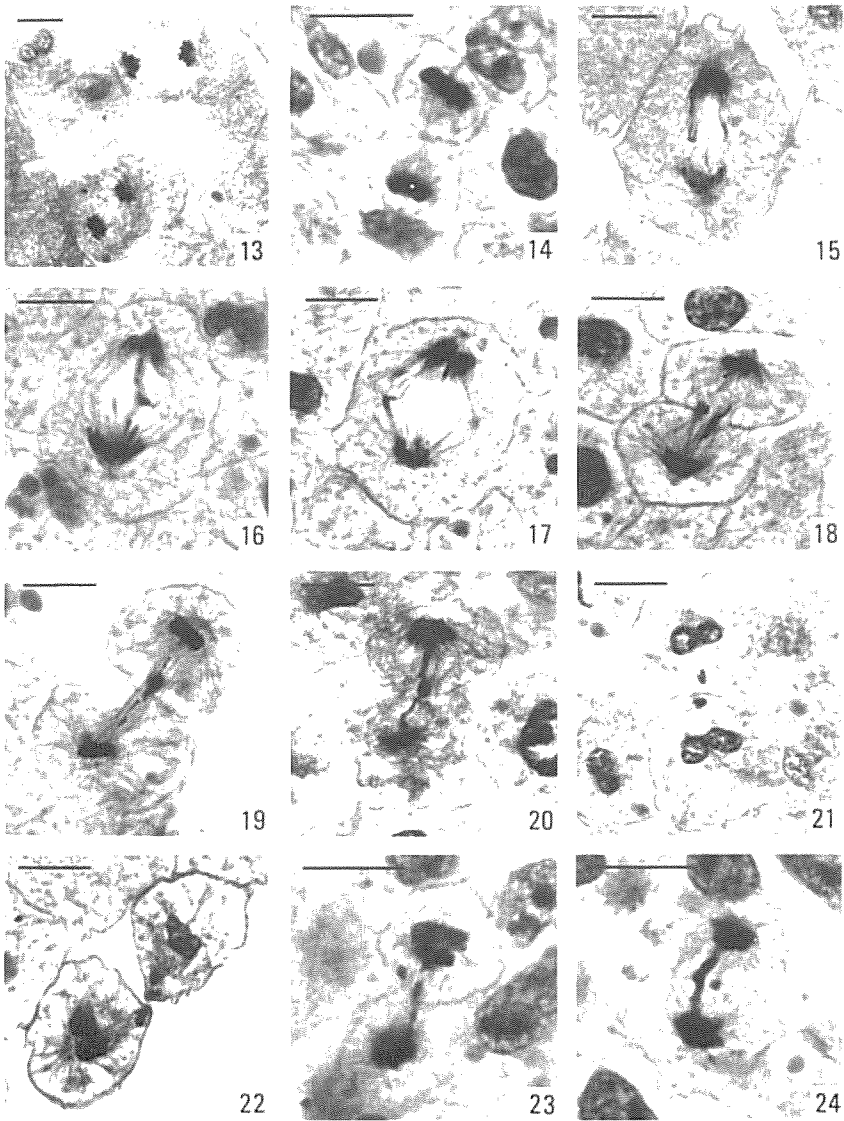
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