A FAST AND EASY METHOD FOR CHROMOSOME KARYOTYPING IN ADULT TELEOSTS

JOHN R. GOLD

Fisheries Biology Research Facility
Department of Animal Science, University of California
Davis, California 95616

AS GENERALLY DISCUSSED BY ROBERTS [*8*], chromosome cytology of adult teleosts is routinely accomplished either by conventional tissue squashes [*4, 5, 6*] or by cell culture methods [*2, 3, 7*]. Although both methods are employed with varying success, each suffers from disadvantages when large numbers of well-spread metaphases are desired from a large sample of adult individuals. For example, tissue squashes often result in low numbers of poorly spread mitotic preparations and in addition require some degree of manipulatory dexterity. Cell culture techniques, on the other hand, while providing excellent spread numbers and resolution of chromosome detail, require laboratory skills and equipment not always available. Moreover, the cost of cell culture procedures necessary for karyotyping a large sample of adult fish would preclude its use in all but very specialized laboratories.

At the Davis trout genetics laboratory, the possibility of using karyotypic differences as racial and/or species markers among salmonid fish is being pursued. Since a large number of adult individuals are examined, alternate methods of chromosome karyotyping have been sought which are both inexpensive and simple in design and execution. The procedure described below has satisfied both criteria, and is currently employed as the sole method of chromosome karyotyping at the Davis laboratory.

MATERIALS AND METHODS

1. Three to five hours prior to sacrifice, inject each fish intramuscularly (dorsally, either anterior or posterior to the dorsal fin) with 0.8 ml/100 grams body weight of a 0.5% colchicine in 0.85% sterile saline solution (after Ohno et al. [*6*]).

2. Following sacrifice, remove 2-5 mm^3 tissue pieces and place in 3-4 ml of a *cold* (on ice, about 5°C) 25% balanced salt solution in 0.85% sterile saline solution.

3. After all samples are removed (usually 20-30 minutes), gently homogenize the tissue pieces in a glass homogenizer. Decant into marked centrifuge tubes.

4. Centrifuge tubes 5 minutes at 67 × *g* and then gently decant supernatant.

5. Resuspend pellet by slowly adding 4 ml of 0.75% potassium chloride hypotonic solution, gently agitating each tube. Let stand at room temperature for 8 minutes, and then centrifuge 5 minutes at 67 × *g*. Decant supernatant with a 5¾-inch Pasteur pipette.

6. Add 4 ml of freshly prepared fixative (3:1 methanol — acetic acid), pouring the fixative slowly down the side of the tube. Let stand 10 minutes at room temperature, then centrifuge for 5 minutes at 67 × *g*. Decant supernatant.

7. Repeat step 6 three times, adding 4 ml of fixative and gently resuspending pellet each time.

8. At this point, tubes may be stored overnight in 4 ml of fixative to insure complete fixation. If slides are to be made the same day, the fixed cells (pellet) should be gently agitated before the last centrifugation.

9. Prepare slides by dropping cells in fixative (4-5 drops) onto a clean microscope slide to which 2-3 drops of 55% ETOH has already been placed. Cells are dropped from a height of 10-12 inches using a 5¾-inch Pasteur pipette.

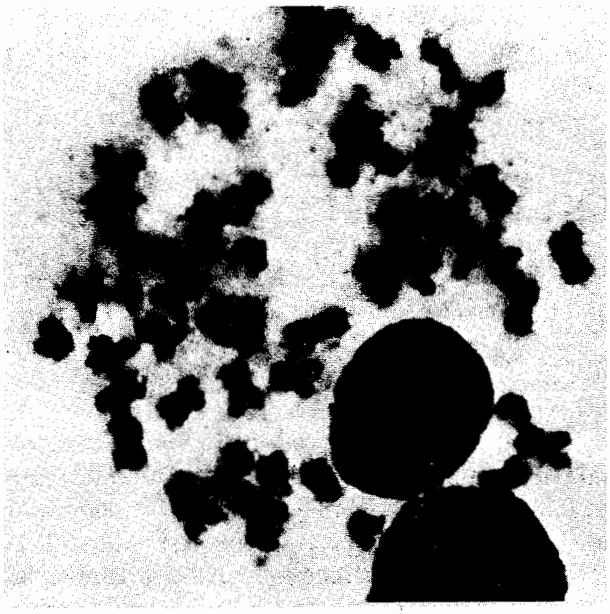

Figure 1.—A photomicrograph of head kidney metaphase chromosomes of *Salmo gairdneri*. Regular optics, oil immersion (1,000 ×). $2N = 60$.

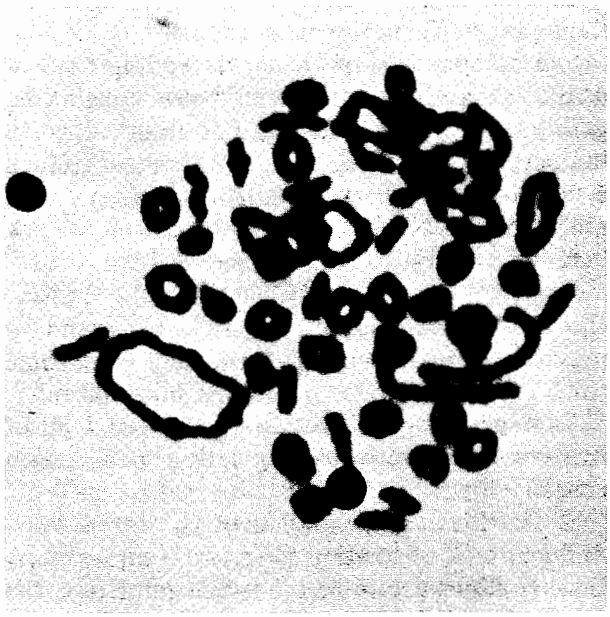

Figure 2.—A photomicrograph of a polyploid meiosis (metaphase I) of *Salmo gairdneri* from testis. Regular optics, oil immersion (1,000 ×).

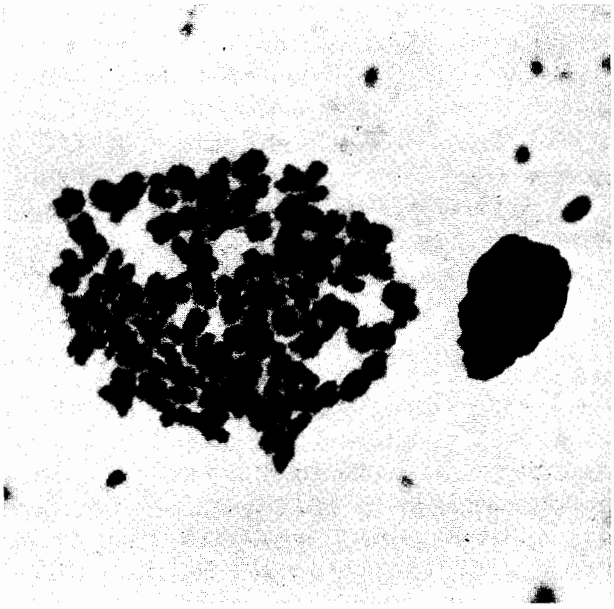

Figure 3.—A photomicrograph of head kidney metaphase chromosomes of *Salmo aguabonita*. Regular optics, oil immersion (1,000 ×). $2N = 58$.

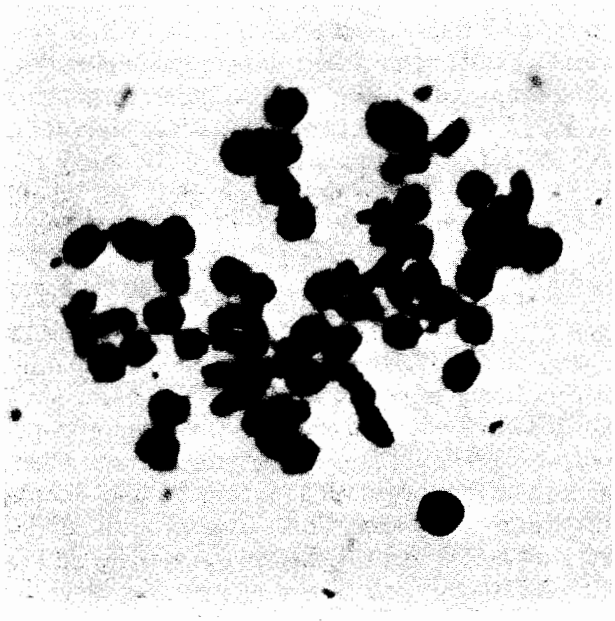

Figure 4.—A photomicrograph of a polyploid meiosis (metaphase I) of *Salmo aguabonita* from testis. Regular optics, oil immersion (1,000 ×).

Care must be taken in applying the cells as too many cells per slide will impede single spread resolution. Slides are quickly flamed with a lighted match or a low flame gas burner, vigorously shaken to remove excess liquid, and then allowed to air-dry (after Evans et al. [*1*]).

10. When dry, slides may be stained, cleared, and mounted in permount.

RESULTS AND DISCUSSION

As shown in figures 1-4, the above method results in good chromosome spreading, permitting positive identification of both centromere position and chromosome enumeration. Moreover, in the majority of spreads analyzed the cell outline is clearly visible, a prerequisite necessary to determine whether the cell is intact [*7*].

Since only 4-5 drops of cells are placed on each slide, the number of metaphase cells per slide is less than that obtained from cell culture techniques. However, since the resultant preparations are usually one cell layer thick, good contrast and definition of chromosomes is achieved; and an entire slide can be scanned rapidly, yielding approximately 75-100 good metaphase cells per slide.

To date, 90 individuals from two species of trout (*Salmo gairdneri* and *S. aguabonita*) have been examined using this procedure. Excellent metaphase spreads have been obtained from the adult tissues spleen, head kidney, and testis. Differences in fish size and age have had no apparent effect on the quality of spreads examined. Thus, the method should be applicable for chromosome cytology of other teleosts as well.

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