


# Analysis of Frog Chromosomes by Karyogram

By HAROLD L. HOUSER

In some biology courses much research and classroom attention is focused on the cell's nuclear composition. A simplified technique has been developed that allows the novice to prepare microscope slides containing analyzable chromosome spreads of one of our most common laboratory animals, the frog. This is a flame-dry technique refined from Tjio and Whang (1962), Kiossoglou (1964), and DIFCO (1965). It has been used successfully by high school students for science fairs and in the school laboratory. The technique has aided taxonomic differentiation in the family Ranidae (Houser and Sutton, 1969; Reynhout and Kimmel, 1969). With proper alterations this simplified process might help to indicate possible chromosome morphologies as they relate to *in vivo* chemical cultures.

## Procedure for Obtaining Karyograms

Karyograms showing chromosome spreads from frog testis and femoral-bone marrow have been obtained in the following manner:

1. Expose prerefrigerated frogs (it is not necessary to refrigerate frogs) to warm room temperatures (about 25 C) for at least 48 hours.
2. Inject 1.5 cc of 0.05% aqua colchicine per 100 g of body weight into the thoracic intraperitoneal cavity.
3. Incubate frogs for 22 to 24 hours at 30 to 35 C in individual containers containing 50 to 100 ml of water.
4. Pith the frogs and remove testis and femoral-bone marrow. (The epiphysis marrow is mechanically exposed and readily removed with small, sharp forceps. The red, bloodlike tissue seems to contain the greater number of mitotic cells.)

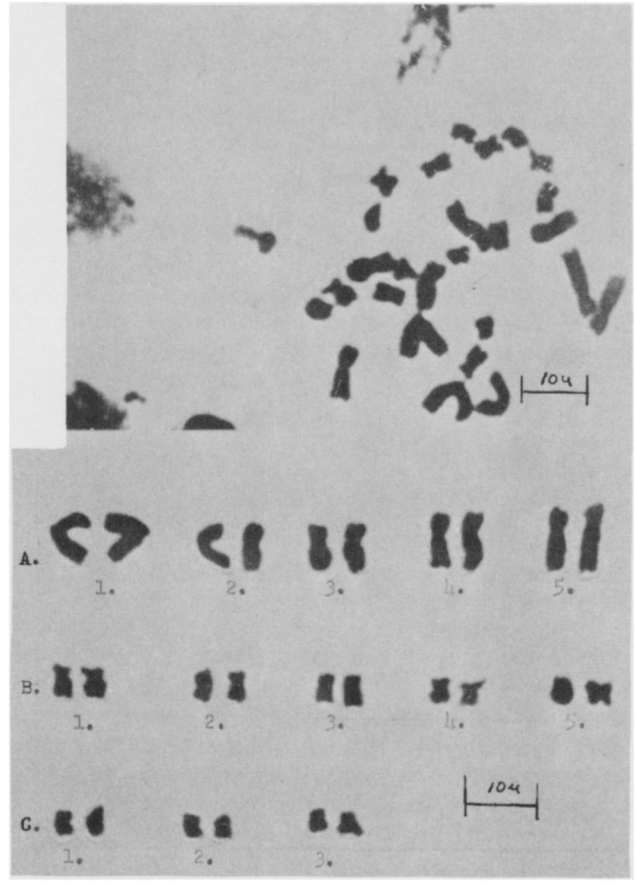


**Fig. 1.** *Above*: photomicrograph from a chromosome spread of female *Rana aurora*, femoral marrow cell. *Below*: corresponding (2N) karyogram prepared from the upper photomicrograph. (Chromosome preparations by K. Hemmelrick, student, Colfax High School, Colfax, Calif.; photographs by author.)

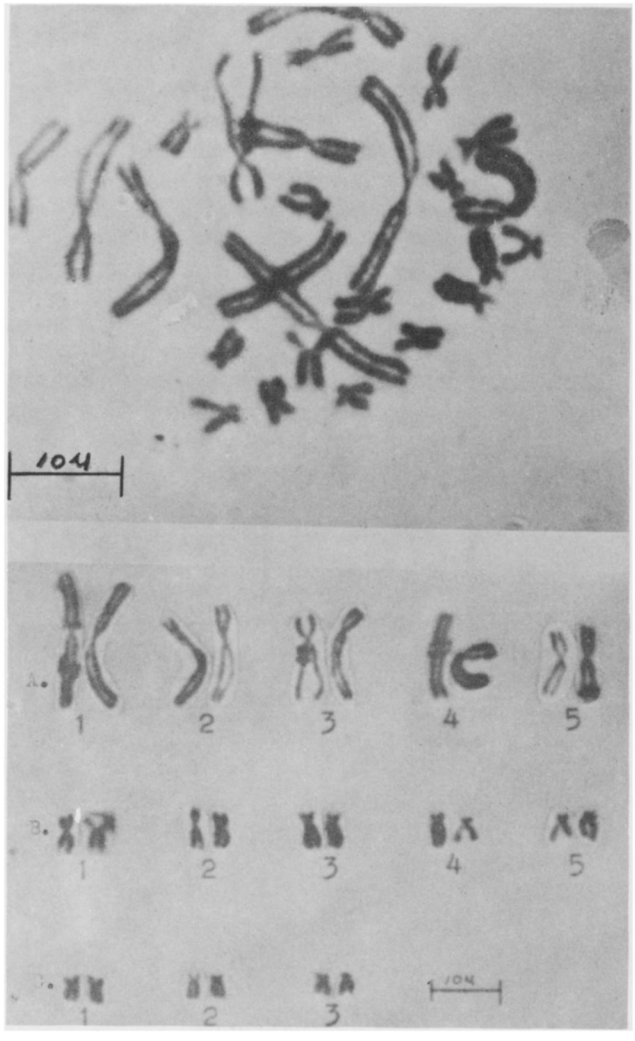


**Fig. 2.** *Above*: photomicrograph from a chromosome spread of female *R. boylii*, femoral-marrow cell. *Below*: corresponding (2N) karyogram prepared from the upper photomicrograph. (Preparations and photographs by author, in this and the following figures.)

5. Place potential mitotic tissue in centrifuge tubes containing 2 ml of 1% sodium citrate solution and incubate at warm room temperature for 30 to 45 minutes. Tease and disperse the tissue gently with a stirring rod or carefully aspirate the cell suspension with a pasteur pipette.

6. Centrifuge at approximately 1,000 rpm for five minutes and decant the supernatant. (Fatty materials may sometimes be at the surface. Eliminating these substances does not seem to alter the results.)

7. Fix the remaining tissue with 2 ml of 2:1 methanol–glacial acetic acid and incubate at warm room temperature for 10 to 20 minutes.

8. Resuspend cells by aspirating with a pasteur pipette and recentrifuge for 3 to 5 minutes.

9. Repeat steps 7 and 8 two more times.

10. Use a pasteur pipette to resuspend cells in approximately 1 ml of fixative. Transfer 3 or 4 drops of the suspension to the top of a well-cleaned slide wetted with 60% ethyl alcohol. After a momentary pause ignite the alcohol with direct flame. When the flame has gone out allow the slide to dry. Stain for 30 minutes in a concentrated aqueous-Giemsa solution. Rinse in two baths of distilled water.

11. Allow slide to air-dry and examine under a microscope. If it is well stained, mount cover slips with balsam or Permount. If necessary, the slides may be cleared by putting them through an alcohol–xylene bath before the mounting of the cover slips.

12. Select fields containing properly spread and isolated sets of chromosomes. This may be the most difficult step. The slide should be scanned at 100× and 400×. Final chromosome analysis should be made under oil immersion. The greater number of cells will not possess analyzable chromosome spreads. Most cells will contain a large nuclear ball of nondifferentiated chromosomes. Each slide may possess up

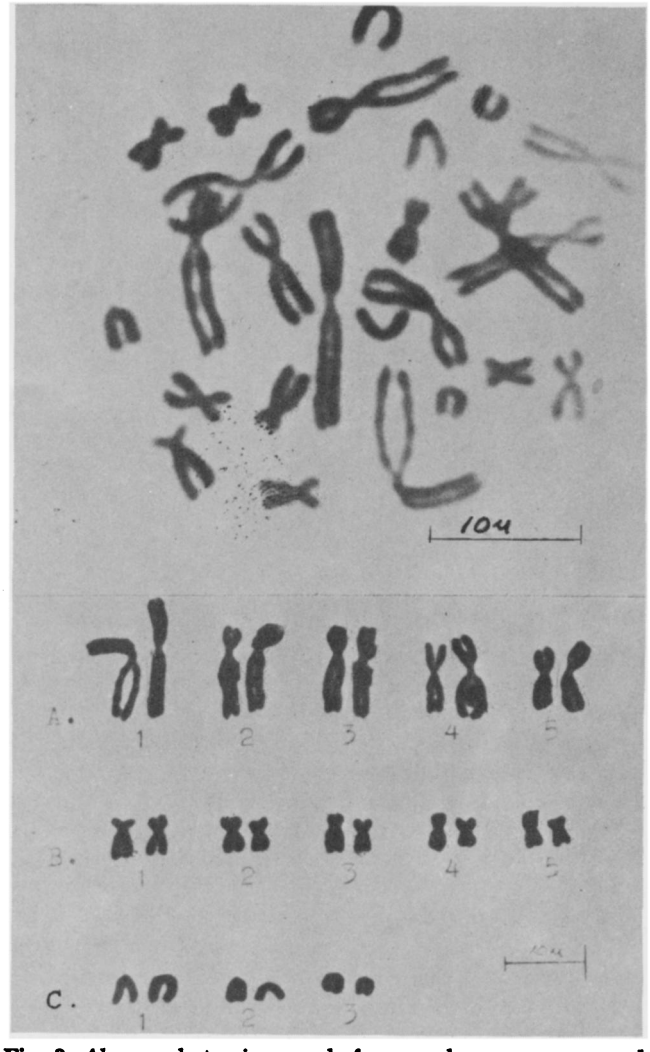


**Fig. 3.** *Above*: photomicrograph from a chromosome spread of female *R. muscosa*, femoral-marrow cell. *Below*: corresponding (2N) karyogram prepared from the upper photomicrograph.

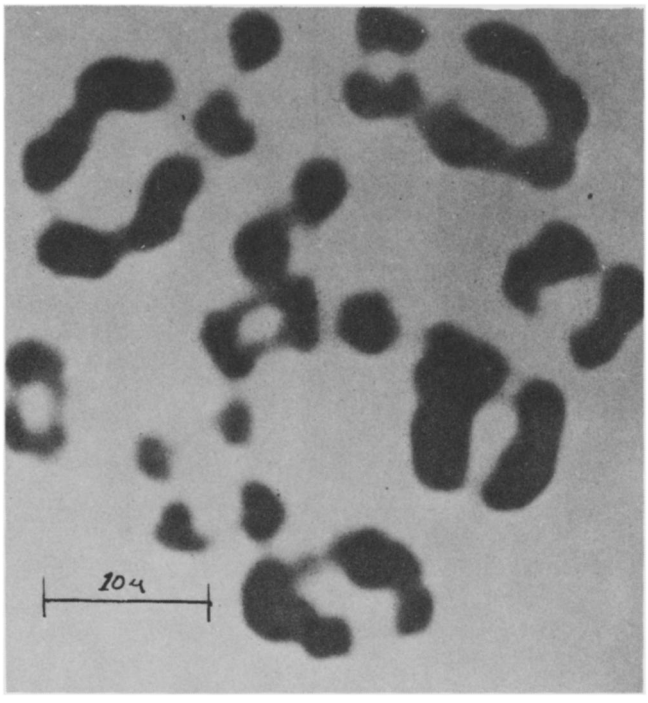


**Fig. 4.** Photomicrograph of diakenesis in meiosis I. Prepared from the testis of male *R. muscosa*.

to 20 cells with analyzable chromosome spreads. (I tell my students that it takes one class period per slide to methodically examine each slide's chromosome contents.)

13. Obtain enlarged photomicrographs.

14. Construct karyograms by using scissors to cut the chromosomes from micrographs of the chromosome spreads. When excising the chromosomes from the photomicrographs make sure to leave a background margin around each chromosome. If two chromosomes overlap on the photomicrograph, use two prints, excising one chromosome from each print.

15. Prepare the karyogram by arranging the excised photograph of each chromosome with the approximate comparative homologous pair.

## Use of Frog Karyograms

This technique has produced spreads of chromosomes that demonstrate many phenomena of cytogenetics.

Differences and similarities in karyographs help establish phylogenetic relationships, as is exemplified in a comparison of karyograms of *Rana aurora* (fig. 1) and *R. boylii* (fig. 2) with the group C set of chromosomes of *R. muscosa* (fig. 3).

The testis tissues reveal examples of meiotic diakenesis (fig. 4) and chromosome spreads of haploid sperm cells (fig. 5). These karyograms may be compared with similarities in the species diploid karyotype from female marrow-tissue (fig. 3).

In some cases enlarged photographs of chromosomes exemplify the lamp-brush effects (fig. 6) of chromosomes with activated gene loci.

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KIOSSOGLOW, A., W. J. MITUS, and W. DAMASHEK. 1964. A direct method for chromosome studies of human bone

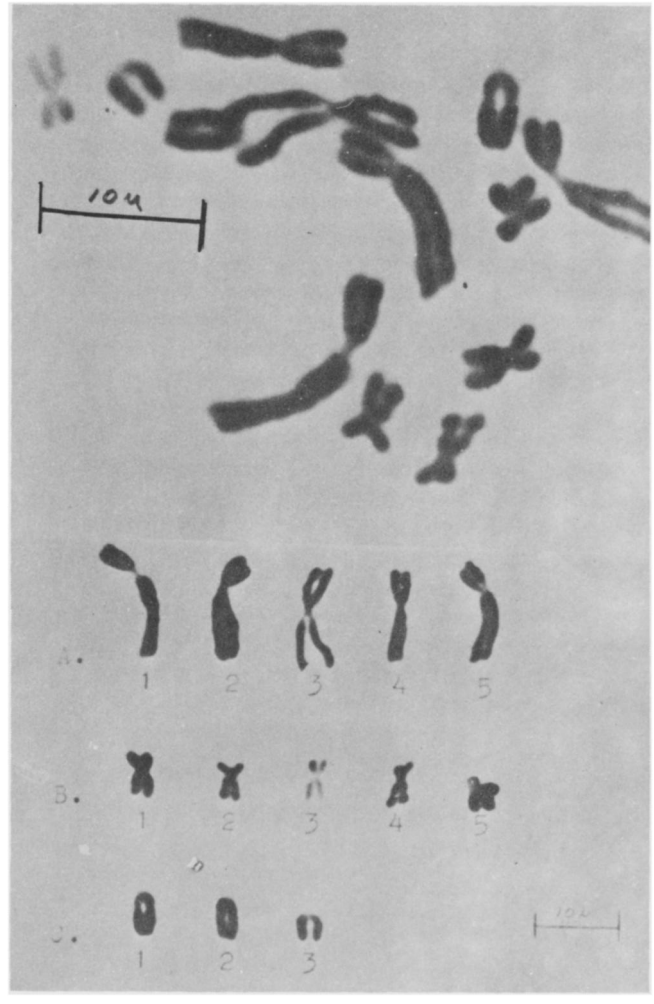


**Fig. 5.** *Above*: photomicrograph of a chromosome spread from male *R. muscosa*, testis cell. *Below*: corresponding (N) karyogram prepared from the upper photomicrograph.

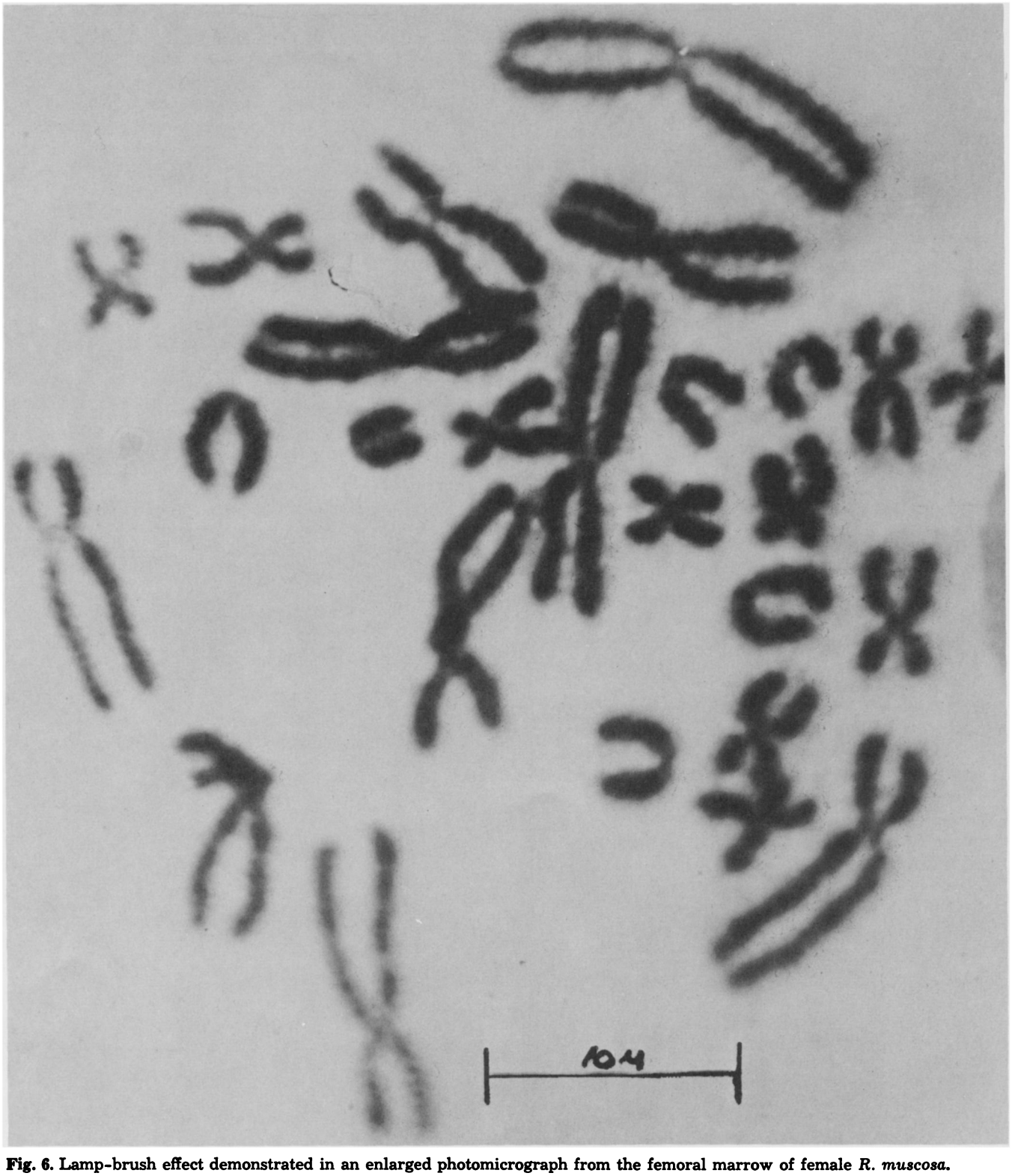


**Fig. 6.** Lamp-brush effect demonstrated in an enlarged photomicrograph from the femoral marrow of female *R. muscosa*.

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