Karyotyping of *X. tropicalis*

We have been karyotyping animals in the Grainger lab to confirm that the stocks maintained in the lab are indeed *X. tropicalis* and to establish that wild-caught animals being used for gynogenesis are also *X. tropicalis*.

We recently received a group of animals that looked somewhat unusual and the results of our karyotyping indicate that these are not *X. tropicalis*. Because we believe it is important for investigators to be careful to evaluate the animals they receive, we report the details of this particular case and our karyotyping results here. The animals in question were about the same size as our *X. tropicalis* but looked different from our *X. tropicalis* stocks (compare Figures 2 and 4). After karyotyping them and comparing that karyotype to one from an inbred strain raised in our laboratory for five generations (compare figures 1 and 3), we determined that these animals we not *X. tropicalis*, but an unidentified *Xenopus* species with 36 chromosomes.

Figure 1: Karyotype of *X. tropicalis* inbred (Nigerian) line

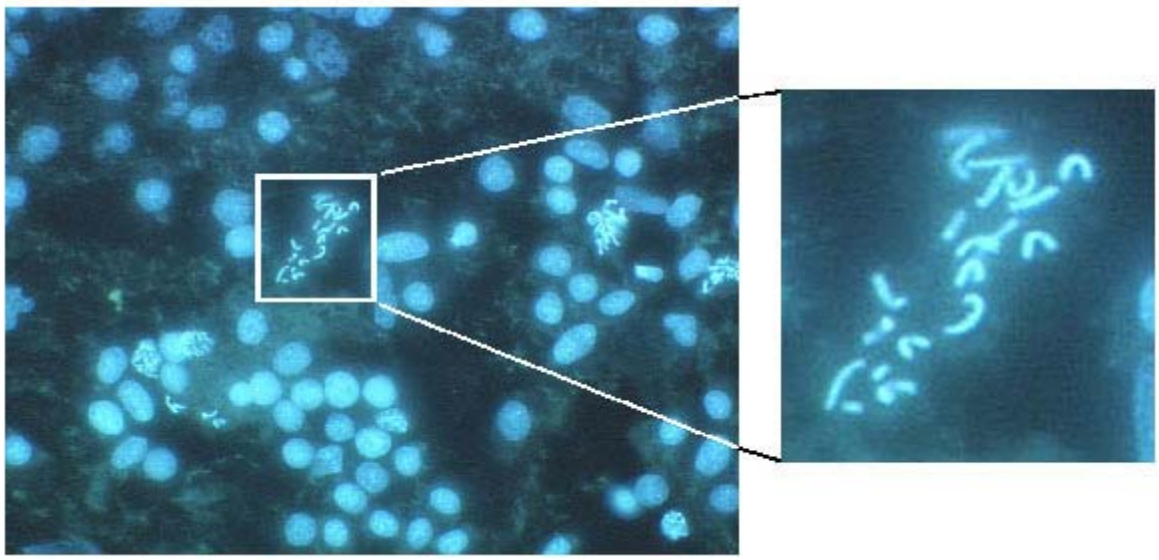


Image is a 630X magnification view of a field of Hoechst-stained nuclei obtained from the karyotyping procedure detailed below. Several chromosome spreads are visible, but in only one are the chromosomes separated sufficiently for counting. That spread is magnified in the right-hand image showing 20 chromosomes, the proper diploid karyotype of *X. tropicalis*.

Figure 2: Appearance of *X. tropicalis* inbred (Nigerian) line

Adult *X. tropicalis* in our stock lines are generally a uniform brown color, with lighter patches appearing often near the nose (see inset). The female (bottom, main panel) is larger than the male (upper, main panel). The animals have eyes that are smaller and closer together, in proportion to body size, than eyes in *X. laevis*.

Figure 3: Karyotype of Unknown *Xenopus* sp.

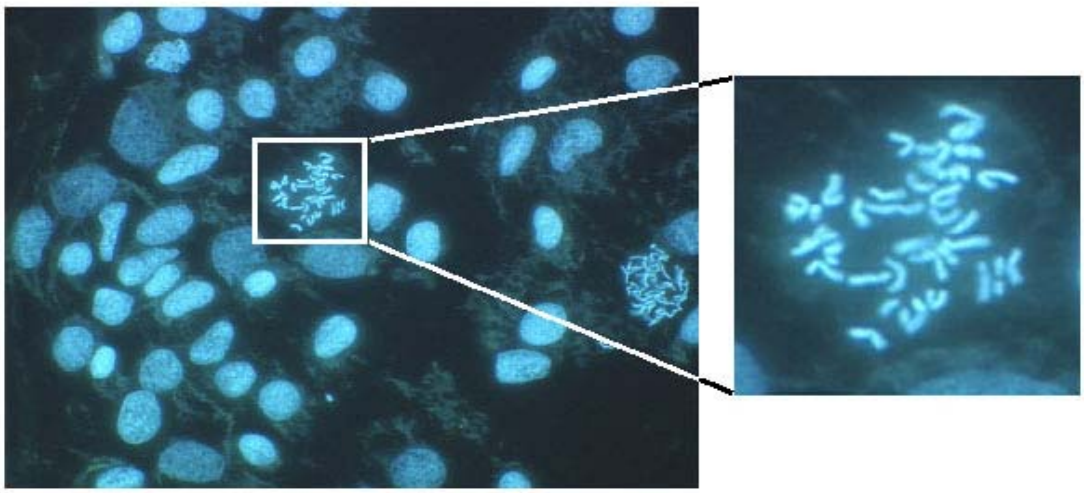


Image is a 630X magnification view of a field of Hoechst-stained nuclei obtained from the karyotyping procedure detailed below. Two chromosome spreads are visible, but in only one are the chromosomes separated sufficiently for counting. That spread is magnified in the right-hand image showing 36 chromosomes.

Figure 4: Appearance of Unknown *Xenopus* sp.

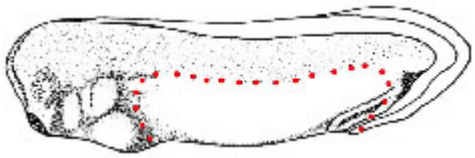
These unknown *Xenopus* are light green/brown in color and have a variegated pattern on their dorsal surface. They have lighter colored eyes then *X. tropicalis*, almost white in appearance. Their eyes are also larger and protrude more than those of our *X. tropicalis* lines. The male and female in this image are about the same size, but on the whole females are larger then males.

Protocol for Karyotyping *Xenopus* tadpoles

1. Prior to collecting blood for genomic DNA preparation, a pair of frogs were mated (see *Grainger Lab X. tropicalis Mating Protocol* for details) and the resultant tadpoles were used in the following karyotyping protocol.

2. Place 10 Nieuwkoop & Faber Stage 26-34 tadpoles into a dish of deionized, distilled water.

3. Remove the yolky **ventral** portion of the tadpole and discard. Below is a drawing of a stage 30 tadpole with the "yolky ventral portion" outlined in red.



4. Place all **dorsal** portions together in the DI water and allow to stand for 20 minutes.

5. Pipette the dorsal halves, carrying as little water with them as possible, into an Eppendorf tube containing 0.2 ml of 60% acetic acid in water.

6. Allow to stand for 5 minutes.

7. Pipette **all** of the tissue, carrying as little acetic acid with it as possible from the tube and place on a positively charged slide (ex. Superfrost Plus from Fisher).

8. Blot away excess acetic acid if necessary.

9. Place a large coverslip on the slide and press down firmly, being careful not to let the coverslip slide around.

10. Place slide and coverslip on dry ice for 5 minutes.

11. Remove slide and coverslip from dry ice and use a razor blade to gently pry up the edge of the coverslip from the still frozen slide.

12. Remove coverslip.

13. Place slide on a paper towel and stain the nuclei/chromosomes by covering the slide with Hoechst 33342 [1 microliter Hoechst 33342 (stock: 0.1mg/ml in water) in 1 ml distilled water] for 5 minutes. ***Wear gloves when working with Hoechst!***

14. Tip the slide up and allow stain to run off the slide and onto the paper towel.

15. Mount by placing a drop of PBS/glycerol on slide, add large coverslip and seal edges with clear nail polish.

16. Examine slide for presence of stained chromosomes using UV fluorescence using a high power (63X or higher) objective.

17. If done properly, this technique should result in hundreds, possibly thousands, of spread chromosomes. Observing *countable* spreads requires patience, but most slides yield several countable spreads.