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Natural Mating and Tadpole Husbandry in the Western Clawed Frog *Xenopus tropicalis*

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INTRODUCTION

The routine generation and successful husbandry of tadpoles is essential for investigators using the Western clawed frog *Xenopus tropicalis* for developmental and genetic studies. We describe a method to induce natural mating by injecting a hormone into sexually mature *X. tropicalis*, and to raise the offspring through the embryonic, tadpole, and metamorphosis stages. *X. tropicalis* develop at a faster rate than *Xenopus laevis*, reaching metamorphosis in as little as 4 wk. Moreover, natural mating produces large numbers of offspring and is therefore particularly useful for mutation screening and genetic mapping. The method includes a technique for the anesthesia of frogs, which makes them easier to handle during injection of the hormone, reducing the likelihood of injury to the animals.

RELATED INFORMATION

A protocol for **Egg Collection and In Vitro Fertilization of the Western Clawed Frog *Xenopus tropicalis*** (Showell and Conlon 2009a) is also available. Embryos should be staged according to criteria described by Nieuwkoop and Faber (1967). See **The Western Clawed Frog *(Xenopus tropicalis):* An Emerging Vertebrate Model for Developmental Genetics and Environmental Toxicology** (Showell and Conlon 2009b) for an introduction to *X. tropicalis* as a model organism.

MATERIALS

CAUTIONS AND RECIPES: Please see Appendices for appropriate handling of materials marked with **<!>**, and recipes for reagents marked with **<R>**.

Reagents

Aquatic system water, chlorine-free (pH 6.5–7.0; conductivity 600–800 μS)

This water must be sterile for Steps 14, 17, and 18.

<!>Cysteine hydrochloride dejellying solution (2%, w/v) (for chemical-induced dejellying treatment; see Steps 16 and 17)

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Prepare a 2% (w/v) cysteine hydrochloride dejellying solution by dissolving L-cysteine hydrochloride monohydrate (reagent grade, ≥98%) in distilled H_2O and adjusting the pH to 8.0 with 10 M NaOH.

Frog and Tadpole Bites (HBH) or similar finely pelleted solid food (for post-metamorphic frogs; see Step 20)

Human chorionic gonadotropin (hCG)

Dissolve the contents of the vial (Sigma CG10; 10,000 U) in 10 mL of sterile H_2O to produce a 1000 U/mL stock solution. Store at 4°C. (A further dilution is required to produce the 100 U/mL solution used in Step 4.)

Leibovitz L-15 medium supplemented with 0.3 g/L L-glutamine (Sigma)

Add bovine calf serum (defined, iron-supplemented, sterile-filtered; e.g., Hyclone) to the medium to a final concentration of 10% (v/v) before use.

Nasco Frog Brittle for Post-metamorphic *Xenopus* or similar pelleted food (for larger juveniles and adults; see Step 20)

Sera Micron (for tadpoles; see Step 19)

For stock, suspend 2 g of Sera Micron in 40 mL of distilled H_2O. Store at 4°C.

<!>Tricaine (ethyl 3-aminobenzoate methanesulfonate salt)

Store as a 2.5% (w/v) stock solution in sterile H_2O at 4°C.

X. tropicalis frogs (male and female)

Equipment

Aquarium (glass; 10-gal, 38-L, or similar) (for the tadpole stage and older; see Step 19)

The use of an aquarium air pump (e.g., Tetra/Second Nature) with an airstone (e.g., Top Fin) connected using vinyl airline tubing (e.g., Top Fin) is recommended. In addition, a 50-W aquarium heater (e.g., Visi-Therm Deluxe, Marineland) should be used where necessary to maintain water temperature at 25°C–28°C (77.0°F–82.4°F).

Dissection microscope

Housing tanks (plastic; 9 1/8 in. × 6 in. × 6 5/8 in.) (e.g., Kritter Keeper, Lee's Aquarium and Pet Products)

Needles (25 gauge, 5/8 in.)

Petri dishes (10 cm)

Sheet to cover tank (optional; see Step 10)

Syringe (1 mL)

Transfer pipettes (plastic)

METHOD

In Steps 1–13, do not expose frogs to temperatures below 23°C (73.4°F), because this can lead to death. Ideally, water temperatures should be maintained between 25°C (77.0°F) and 28°C (82.4°F).

Anesthesia

1 Place male and female *X. tropicalis* frogs (Fig. 1) into plastic housing tanks that contain chlorine-free aquatic system water at a depth of ~2 in.

2 Add the tricaine solution to a final concentration of 0.025% (w/v).

3 Allow ~25–30 min for the anesthetic to take effect. Do not leave the frogs in the anesthetic for longer than is necessary.

It is not necessary or desirable to fully anesthetize the frog during this procedure. Anesthetization is only intended to minimize the risk of distress and injury to the frogs while administering the hormone injection. When properly anesthetized, the frogs should put up minimal resistance to handling.

Prepriming

4 Inject each frog with 0.1 mL of a 100 U/mL solution of hCG. Make the injection into a dorsal lymph sac using a 1-mL syringe and a 25-gauge needle (Fig. 2).

5 Once each injection is complete, allow the frog to recover from anesthesia for 30–60 min. in shallow water (not >2 in. deep).

6 Return active frogs to normal housing conditions for 20–24 h.

Priming and Mating

7 Anesthetize the frogs as described in Steps 1–3.

8 Inject each frog with 0.2 mL of a 1000 U/mL stock solution of hCG. Make the injection into a dorsal lymph sac (Fig. 2).

9 Allow the frogs to recover as described in Step 5.

10 Transfer each mating pair into a tank of shallow water (with a depth of 2–3 in.), so that there is one pair per tank.

Tanks should be situated in a quiet place if possible, and covered with a sheet to prevent any disturbance to the frogs during mating.

11 Monitor for the occurrence of amplexus and egg-laying (~5 h).

See Troubleshooting.

12 Take samples of eggs from each mating pair using a plastic transfer pipette that has been precoated with Leibovitz L-15 medium containing 10% calf serum to prevent sticking. Examine the eggs for fertilization using a dissection microscope.

The occurrence of the first embryonic cleavages indicates that fertilization has occurred.

See Troubleshooting.

13 After mating and egg collection, separate the mating pairs and transfer the frogs to holding tanks (i.e., plastic housing tanks containing clean aquatic system water). Keep male and female frogs isolated overnight to monitor for possible adverse health effects and to allow females to finish laying eggs.

Female frogs should not be returned to recirculating aquatic systems immediately, because they may continue to lay eggs for several hours after mating.

Dejellying Embryos

Two alternative approaches may be taken. The first of these (Steps 14 and 15) allows embryos to hatch naturally from their jelly coats and is suitable for clutches in which >80% of the eggs are fertilized. The second approach (Steps 16 and 17) dissolves the jelly coat using a chemical treatment and is suitable for clutches in which <80% of eggs are fertilized, or where early-stage embryos are required for analysis.

Natural Dejellying

14 Replace the water in the tanks containing clutches of embryos with at least 2 L of sterilized aquatic system water. Culture overnight at 25°C–28°C (77°F–82.4°F).

Survival may be improved by teasing apart any large clumps of eggs and embryos at this stage.

15 Replace the water twice daily, as in Step 14, until the embryos hatch from their jelly coats. As the embryos hatch, proceed with Step 18.

*Embryos reach the hatching stage (*Nieuwkoop and Faber [1967] *stage 26 and 27) at ~24–26 h post-fertilization at 25°C.*

Chemical-Induced Dejellying

16 Replace the water from the tanks containing clutches of embryos with 100 mL of 2% (w/v) cysteine hydrochloride dejellying solution. Gently agitate the embryos using a plastic transfer pipette to ensure even exposure to the dejellying solution.

Dejellying should take between 3 and 5 min. The clear separation between abutting embryos, which results from the presence of the outer jelly coat, will disappear when the dejellying process is complete. Embryos in dejellying solution must be monitored continuously by eye or by dissection microscope to avoid lethal overexposure.

See Troubleshooting.

17 Remove the dejellying solution and rinse the embryos thoroughly with sterilized aquatic system water (three to five rinses, ≥200 mL per rinse). Proceed to Step 18.

See Troubleshooting.

Husbandry of Embryos, Tadpoles, and Froglets

18 Culture the early embryos in Petri dishes or plastic housing tanks containing sterilized aquatic system water at 25°C–28°C (77°F–82.4°F). Replace the water on a daily basis. Make sure that the water used for water changes is at the same temperature as the water it replaces, to avoid potentially lethal temperature fluctuations. If the embryos are cultured in Petri dishes, leave them there until the feeding tadpole stage (~4 d post-fertilization), and then transfer them to small plastic housing tanks.

See Troubleshooting.

At any of these stages, the embryos may be harvested for analysis. Embryos should be staged according to criteria described by Nieuwkoop and Faber (1967). *If developmental stages beyond the*

feeding tadpole are required, or to raise animals to adulthood, continue with Step 19.

19 Raise the tadpoles in small plastic housing tanks for 1–2 wk before transferring them to larger (e.g., 10-gal) aquariums. Once the tadpoles reach the feeding stage, feed them once or twice daily by adding Sera Micron to the water (feed 0.6 mL of Sera Micron per liter of tank H_2O). For small tanks, replace one-third of the water daily, and clean the tanks every 2–3 d to remove waste and uneaten food. For larger aquariums, replace one-quarter of the water daily.

The use of an air pump and airstone is recommended when housing tadpoles in aquariums, as this helps to aerate and circulate the water. When raising large numbers of tadpoles, survival may be improved by housing in darkened conditions.

See Troubleshooting.

20 After the tadpoles go through metamorphosis, move the froglets to a separate tank and feed them daily with Frog and Tadpole Bites or a similar finely pelleted solid food. Feed larger juveniles and adults Nasco Frog Brittle for Post-metamorphic *Xenopus* or similar pelleted food in place of Frog and Tadpole Bites.

To increase their chances of survival, house newly metamorphosed froglets in tanks that are separate from larger juvenile frogs, which tend to be aggressive, particularly while feeding.

TROUBLESHOOTING

Problem: Amplexus does not occur.

[Step 11]

Solution: Try to select males that have dark nuptial pads on their inner forelimbs, because this is a sign of sexual maturity.

Problem: Females do not lay eggs.

[Step 11]

Solution: Consider the following:

- Make sure that the concentration of the hormone solution is 1000 U/mL.
- Make sure that the hormone solution has not been repeatedly frozen and thawed, or stored for >2 wk at 4°C.
- Make sure that the hormone solution does not leak from the injection site at Steps 4 and 8.

Problem: The eggs are not fertilized.

[Step 12]

Solution: Try to select males that have dark nuptial pads on their inner forelimbs, because this is a sign of sexual maturity.

Problem: Embryos die during the chemical dejellying treatment. (This is indicated by embryos turning white and/or lysing soon after treatment.)

[Steps 16 or 17]

Solution: Consider the following:

- Check the concentration and pH of the 2% (w/v) cysteine hydrochloride solution.
- Minimize the treatment time in Step 16.
- Increase the number of rinses to ensure removal of all dejellying solution in Step 17.

Problem: Embryos or tadpoles die during culturing.

[Steps 18 and 19]

Solution: Consider the following:

- Reduce the number of embryos or tadpoles per dish (or per tank).
- Make sure that the water and the dishes/tanks are kept free of excess food and waste.
- Make sure that the water is properly aerated in Step 19.

DISCUSSION

Natural mating has two main advantages over in vitro fertilization for the production of *X. tropicalis* embryos. First, it typically results in larger numbers of embryos (usually in excess of 1000 per clutch, and often many more), which is an advantage for applications such as genetic screens. Second, it does not involve sacrificing male frogs to obtain testes and spermatozoa. This is particularly important when breeding valuable animals, such as those carrying transgenes or mutations of interest. However, the method also has limitations. Not all pairs will mate on a given day and, of those that do, not all will produce embryos. In our experience, it is necessary to set up a minimum of six mating pairs to ensure a reasonable chance of obtaining one or more successful matings. Investigators should also bear in mind that the eggs are not fertilized simultaneously and so the embryos produced will not undergo development in synchrony. For synchronous development through early embryogenesis, embryos must be produced by in vitro fertilization; see **Egg Collection and In Vitro Fertilization of the Western Clawed Frog *Xenopus tropicalis*** (Showell and Conlon 2009a).

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FIGURE 1.
Adult *X. tropicalis*. Female frogs (*left*) are typically larger than males (*right*) and have noticeably pear-shaped bodies and a prominent cloaca. Males often have dark nuptial pads on the inner surfaces of their forelimbs (not shown).

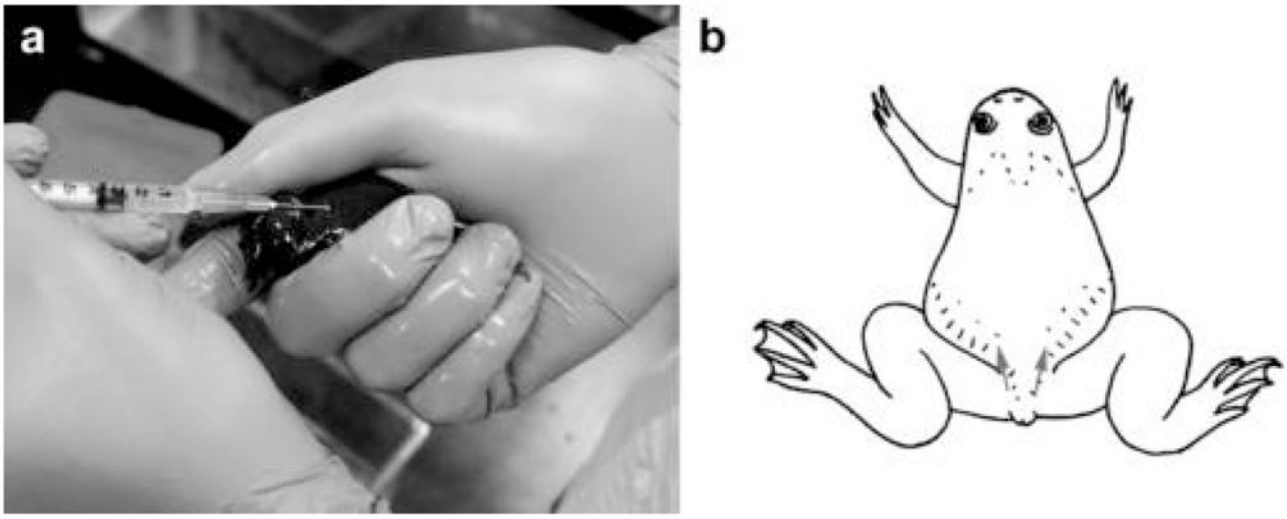

FIGURE 2.
Priming frogs. (*a*) The technique for restraining *X. tropicalis* for hormone injection. (*b*) The sites at which frogs may be injected to administer the priming dose of hCG into either dorsal lymph sac are indicated (arrows).