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The Western Clawed Frog *(Xenopus tropicalis):* An Emerging Vertebrate Model for Developmental Genetics and Environmental Toxicology

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INTRODUCTION

Xenopus tropicalis, also known as *Silurana tropicalis* or the Western clawed frog, is a small, wholly aquatic frog that is found in the countries that lie along the west coast of equatorial Africa from Gabon to Sierra Leone. It is a diploid relative of *Xenopus laevis*, a frog that has found many uses in laboratories throughout the world. *X. tropicalis* closely resembles its relative in anatomy throughout its life cycle. It shares its advantages as a model organism for studying many aspects of vertebrate biology, particularly the genetic, biochemical, and environmental factors that influence vertebrate development from embryonic stages through adulthood. It also shares much of its biology with more distantly related species, including mammals, but unlike mammalian model organisms, routine manipulation of *X. tropicalis* in the laboratory can produce thousands of embryos for experimental analysis. *X. tropicalis* is also finding uses as an important test species for assessing the impact of environmental toxins and disease on amphibians, which are in decline in many areas of the world due to waterborne pollutants and infectious agents such as the chytrid fungus.

SOURCES AND HUSBANDRY

X. tropicalis are commercially available as partially inbred (F_6) lines. These lines were derived from stocks produced by Robert Grainger and colleagues at the University of Virginia, and originated from wild frogs caught in Nigeria. Efforts are under way to generate fully inbred lines ($>F_{20}$) that will be of enormous benefit for future genetic studies. Unlike the widely used pseudotetraploid *X. laevis*, which takes ~18 mo to reach sexual maturity, *X. tropicalis* has a short generation time of ~6 mo. Raising animals is straightforward, and they can be housed in either standing water tanks or in recirculating aquatic systems. Advice for raising *X. tropicalis* from embryo to adult is provided in Natural Mating and Tadpole Husbandry in the Western Clawed Frog *Xenopus tropicalis* (Showell and Conlon 2009a).

GENETICS

Laboratory strains of *X. tropicalis* carry a significant degree of sequence polymorphism within their breeding populations. This naturally occurring polymorphism can be a source of functionally informative recessive alleles that can be uncovered using a variety of

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approaches, including traditional forward genetic screening, amplicon resequencing (reverse genetic screening), and gynogenesis (Noramly et al. 2005; Goda et al. 2006). Efforts are also under way to carry out mutagenesis screens to produce novel mutant lines for study; these efforts are aided greatly by the large numbers of offspring that can be generated routinely and raised for screening in the laboratory. The short generation time makes multigeneration screening and genetic analysis strategies feasible. Mutations, isolated using these genetic screening approaches, can be mapped to defined regions of the genome using a simple sequence length polymorphism (SSLP) map developed by Amy Sater, Dan Wells, and colleagues at the University of Houston and Baylor College of Medicine (http://tropmap.biology.uh.edu/index.html). Candidate genes can be tested by attempting to phenocopy a mutant by microinjecting gene-specific morpholino oligonucleotides into an embryo (Khokha et al. 2002).

GENOMICS AND ASSOCIATED RESOURCES

X. tropicalis is thought to have diverged from other *Xenopus* species prior to the genome duplication event (likely a hybridization of ancestral species) that resulted in their polyploidization (M. Gilchrist, pers. comm.). Its diploid genome, confirmed by genome sequencing and expressed sequence tag (EST) analyses, contains ~1.7 billion base pairs on 10 chromosome pairs (Tymowska 1973; Thiebaud and Fischberg 1977). A variety of genomic resources exist for investigators using *X. tropicalis*, not least of which is a genome sequence produced by the Joint Genome Institute of the U.S. Department of Energy (http://genome.jgi-psf.org/Xentr4/Xentr4.home.html). In addition, large EST libraries, representing thousands of unique messenger RNA transcripts, allow the function of transcribed genes to be tested by straightforward techniques that involve microinjecting specific messenger RNAs into the embryo. Many of the resources developed for studying gene expression in *X. laevis*, such as in situ hybridization probes, antibodies, and microarrays, can also be used for studies of *X. tropicalis*.

The *X. tropicalis* genome sequence is invaluable for comparative genomics studies, because the frog occupies a unique evolutionary position. Its genome is sufficiently distant from that of mammals to have allowed the divergence of functionally unconstrained sequences, while maintaining many syntenic relationships between genes and associated regulatory elements found in both amphibians and mammals (Showell and Conlon 2007). Comparative analyses of genomes can highlight elements outside of transcribed regions, whose sequences have been conserved due to a requirement to maintain important regulatory functions. *Xenopus* provides a model system in which the function of these regulatory sequences can be rapidly tested, due to the ease and speed with which transgenic embryos expressing fluorescent proteins under the control of these sequences can be generated using the restriction enzyme-mediated integration (REMI) method developed by Kroll and Amaya (Kroll and Amaya 1996; Amaya and Kroll 1999).

TECHNICAL APPROACHES

Methods are available for Natural Mating and Tadpole Husbandry in the Western Clawed Frog *Xenopus tropicalis* (Showell and Conlon 2009a), Egg Collection and In Vitro Fertilization of the Western Clawed Frog *Xenopus tropicalis* (Showell and Conlon 2009b), and Tissue Sampling and Genomic DNA Purification from the Western Clawed Frog *Xenopus tropicalis* (Showell and Conlon 2009c).

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