Exp. Anim. 59(4), 395–405, 2010

—Review—
Review Series: Animal Bioresource in Japan

Xenopus tropicalis: An Ideal Experimental Animal in Amphibia

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Abstract: Studies using amphibians have contributed to the progress of life science including developmental biology and cell biology for more than one hundred years. Since the 1950s *Xenopus laevis* in particular has been used by scientists in many fields for experiments, resulting in the development of various techniques such as microsurgery on early embryos, biosynthesis of gene-encoded protein in oocytes by mRNA injection, misexpression experiments by mRNA injection into embryos, gene knockdown studies by injection of morpholino anti-sense oligonucleotide into fertilized eggs, transgenesis by the *I-SceI* meganuclease method, and so on. In this paper we will introduce *Xenopus tropicalis* as an alternative experimental animal. It has a shorter generation time and smaller diploid genome, together with whole-genome sequence data. The procedures available for *Xenopus laevis* can work well with *Xenopus tropicalis*, and embryos of both species develop at similar rates according to the developmental staging system of Nieuwkoop and Faber. Experimental systems of *Xenopus tropicalis* will pave the way for a new era of vertebrate genomics and genetics.

Key words: *Xenopus* genetic divergence, *Xenopus laevis*, *Xenopus tropicalis* (*Silurana tropicalis*) laboratory lines

Introduction

Amphibians metamorphose from a juvenile water-breathing form to an adult air-breathing form, and have an important evolutional position as the first terrestrial tetrapods. Since the late 1800s amphibians have been employed in studies aiming to elucidate vertebrate development [16, 24]. About 100 years ago, Gudernatsch demonstrated that administration of thyroid gland induces precocious metamorphosis of tadpoles in a most dramatic way [13], leading to the discovery of thyroid hormone. Some of the superb characteristics of amphibians as experimental animals are as follows. 1) Amphibians have internal organs and skeleton similar to mammalian ones, for example, lungs, pancreas, kidneys, thymus, spleen, and clavicles. 2) Investigators can obtain many relatively large eggs by hormonally controlling the time of ovulation. 3) Embryogenesis proceeds *ex vivo*,

(Received 4 March 2010 / Accepted 21 April 2010)
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which makes its observation easy and allows us to study the influence of physical and chemical treatments on the eggs and embryos. 4) Amphibian embryos are robust and amenable to surgical manipulation including ablations and cut-and-paste transplantation. Spemann and Mangold discovered by making use of these merits that the dorsal lip of a blastopore can induce the organization of the main body axis [36]. Briggs and King succeeded in the production of clonal progeny by transplanting nuclei from early embryos (advanced blastula cells) into enucleated eggs [3], suggesting that all cells of the body contain the same genome. Since the 1950s, the African clawed frog, *Xenopus laevis* (*X. laevis*), has been the most widely used anuran amphibian research organism, which is supported by a precise, elaborate and highly reproducible description of its early development [26]. Gurdon and Uelinger obtained normal larvae of *X. laevis*, and subsequently fertile adults, by transplanting nuclei of differentiated intestinal epithelial cells into enucleated eggs [15], which demonstrated that even differentiated cells have all the genes required to produce an individual, and that the process of cell differentiation does not necessarily involve any stable inactivation of differentiation genes. Molecular biologists have also been utilizing *X. laevis* to obtain gene products by injecting mRNA into oocytes since the 1970s [14]. Furthermore, Kroll and Amaya established the transgenesis method by injecting sperm nuclei containing an exogenous DNA fragment into eggs [23]. A large body of literature based on *X. laevis* studies has made many outstanding and splendid contributions to developmental biology and cell biology.

However, *X. laevis* has some demerits as an experimental animal. First, *X. laevis* is an allotetraploid-derived species, which is thought to have arisen from a tetraploidization event 30 million years ago [19]. Electrophoretic studies of enzymes and blood proteins suggested that copies of duplicated pairs are expressed at approximately one-half of all loci [11]. This tetraploidy hampers the use of powerful technologies, gene knockdown, mutagenesis and genetic linkage mapping experiments, because two morpholino anti-sense oligonucleotides might be necessary to reduce the expression of duplicate genes, and mutation of all four loci is essential to know a recessive phenotype. Second, the female *X. laevis* is reported to become sexually mature from 10 to 24 months post metamorphosis (PM), and viable spermatozoa is observed in testes by 6 months PM, although complete sexual maturity of the males is considered to occur later [30]. Such a long generation time is a heavy burden on genetic analysis including the crossing of mutagen-treated frogs and the construction of genetic linkage maps. Third, the database for the *X. laevis* genome sequence is not yet available. The genomic sequence information is important for analyses of promoters, characterization of the gene structure, and comparison with other genomes. Another emerging frog model system, *Xenopus tropicalis* (tropical clawed frog, Western clawed frog) is expected to overcome these handicaps as we describe below. Since 2002, we have been inbreeding, maintaining, and supplying lines of *Xenopus tropicalis* (*X. tropicalis*) with the laboratory of Dr. M. Asashima (University of Tokyo, Japan) in National BioResource Project supported by the Ministry of Education, Culture, Sports, Science and Technology, Japan.

What is *Xenopus tropicalis*?

The taxonomy of *X. tropicalis* is controversial. *X. tropicalis* was classified into the genus *Silurana*, because the phylogenetic analysis of morphological features indicated that the species *tropicalis* and *epitropicalis* are more closely related to *Hymenochirus* + *Pipa* than to the remaining species of *Xenopus* [4]. However, the comparison of ribosomal DNA sequences and the re-analysis of the morphological data have revealed that *Silurana* is phylogenetically closest to *Xenopus*, and that the recognition of the genus *Silurana* is not required in order to render *Xenopus* monophyletic [6]. Therefore, we have adopted the name of *X. tropicalis*. Its English name is tropical clawed frog, according to Amphibian Species of the World 5.3, an Online Reference (http://research.amnh.org/vz/herpetology/amphibia/).

The advantages of *X. tropicalis* over *X. laevis* have been reviewed previously [18] (Table 1). First, *X. tropicalis* has a smaller diploid genome than *X. laevis*. The cytogenetic analysis of the genus *Xenopus* showed that *X. tropicalis* is the only known diploid species [10] and has 20 chromosomes (2n), while many species including *X. laevis* have chromosome number 2n=36 [39]. Correspondingly, the genome sizes of *X. tropicalis* and *X.*

Table 1. Comparison between *Xenopus tropicalis* and *Xenopus laevis*

	Xenopus tropicalis		*Xenopus laevis*	References
Rearing temperature	22–28°C		18–24°C	
Ploidy	Diploid		Allotetraploid	[10, 19]
Haploid number of chromosomes	10		18	[39]
Genome size	1.7×10^9 bp		3.1×10^9 bp	[38]
Reproductive maturation				
Male	22 weeks PM		6 months PM~	[30]
Female	30 weeks PM		10–24 months PM	
Adult size	Yasuda line	Ivory Coast line		
Male	4.5 cm	4 cm	7.5 cm	
Female	6 cm	5 cm	10 cm	
Egg size	0.7–0.8 mm		1–1.3 mm	[18]
Eggs per spawning	1,000–9,000		300–1,000	[18]

PM: post-metamorphosis.

laevis are 1.7×10^9 and 3.1×10^9 bp, respectively [38]. When using *X. tropicalis*, it is necessary to confirm the ploidy level, before starting the experiment, because the sister species of *X. tropicalis*, *X. epitropicalis*, is tetraploid like *X. laevis* [40].

Second, *X. tropicalis* has a shorter generation time. The first obvious sign of a male's sexual maturity is the development of purple nuptial pads on the ventral forelimbs. *X. tropicalis* male frogs exhibit nuptial pads at 4–5 months of age. Once they develop obvious nuptial pads, their mating is successful. Mature females develop clearly protruding cloacas within 4–6 months of age. Although 5-month-old females can lay eggs, the percentages of successful fertilization rise from 50% to more than 90% till 9 months of age [12].

A diploid genome and faster generation time of *X. tropicalis* alleviate the burden on *Xenopus* researchers who carry out morpholino anti-sense oligonucleotide loss-of-function studies, mutation analyses and multi-generational experiments. When reducing the expression of a specific gene in *X. tropicalis*, the target is only a single gene, not two genes as in *X. laevis*, and identifying recessive phenotypes requires mutagenizing two independent loci, not four. More than 40 mutants were identified in *X. laevis* by inbreeding and gynogenesis after several decades of efforts by the early 1990s [25], while 122 potential mutations of *X. tropicalis* have been reported by the similar approaches, and 42 have been confirmed as genetically heritable mutations [9, 12, 27].

Third, the *X. tropicalis* genome has been sequenced by the Joint Genome Institute of the US Department of Energy (JGI) using a frog from the sixth inbred generation of the Nigerian line (http://faculty.virginia.edu/xtropicalis/) (Table 3). The sequence data have been assembled to create a genome of approximately 1.5 Gb in the genome assembly release v. 4.1. The genome is composed of 19501 scaffolds with an average coverage of 7.65X. Approximately 95% of *X. tropicalis* full-length cDNAs are mapped to the v. 4.1 assembly that contains approximately 28,000 gene models. The average gene length is 16.5 kb and the average transcript length is 1.3 kb, with the average protein containing 409 amino acids. There are approximately 6.5 exons per gene averaging 200 bp each with intron spacing of 2.8 kb. The *X. tropicalis* genome sequence data can be accessed at the JGI genome browser (http://genome.jgi-psf.org/Xentr4/Xentr4.home.html), and the sequence is very useful for understanding the gene structure and promoter sequence. It is known from the genomic sequence comparison that *X. tropicalis* is the most distantly related vertebrate species to humans that still exhibits long-range synteny [31].

Fourth, many experimental systems developed for *X. laevis* are also applicable to *X. tropicalis* [20]. *X. tropicalis* embryos develop and grow up at similar rates to *X. laevis* according to the developmental staging system of Nieuwkoop and Faber. The *X. laevis* protocol for whole-mount *in situ* hybridization to mRNA transcripts can be applied to *X. tropicalis*, and *X. laevis* probes often hy-

bridize to *X. tropicalis* mRNAs. Antibodies against *X. laevis* proteins can effectively detect *X. tropicalis* proteins. Antisense morpholino oligonucleotides will work in *X. tropicalis* in studies of loss of a specific gene activity during development. The animal cap cells from *X. tropicalis* have the same competence to differentiate into several different cell lineages in response to activin as those from *X. laevis* [34]. The transgenesis in *X. tropicalis* is enabled by microinjection of *I-SceI*-digested DNA construct into fertilized eggs (*I-SceI* meganuclease method) [28].

Standard Lines and Their Characterization

The following lines are maintained at the Institute for Amphibian Biology and the laboratory of Dr. M. Asashima.

1) Nigerian line

The Nigerian line originated from frogs caught in the wild, which were obtained by Dr. M. Kirschner (University of California, USA). The Nigerian inbred lines were generated by Dr. R. Grainger (University of Virginia, USA) [25], and confirmed to be diploid by karyotyping (http://faculty.virginia.edu/xtropicalis/KaryotypeXtropicalis.htm). The sixth generation was used for the genome sequencing by JGI as described above. This line was obtained as a generous gift from Dr. Grainger in 2003.

2) Ivory Coast line

The Ivory Coast line originated from Adipodoume, Ivory Coast, and has been shown to be diploid [39]. Ten male Ivory Coast frogs and four females were obtained in 1998 as a kind gift from the live collection of the Institute of Zoology at the University of Geneva.

3) Yasuda line

Dr. K. Yasuda (Nara Institute of Science and Technology, Japan) obtained *X. tropicalis* frogs from the laboratory of Dr. R. Grainger in 1996, and carried out several generations of inbreeding. This line was provided to us in 1998 and 2003 (Fig. 1). The karyotype of the Yasuda line was examined and shown to be diploid by the laboratory of Dr. M. Asashima [41] and us (Fig. 2).

A skin graft transplantation assay was undertaken to

Fig. 1. A female frog of the *Xenopus tropicalis* Yasuda line. The snout-vent length is 6 cm.

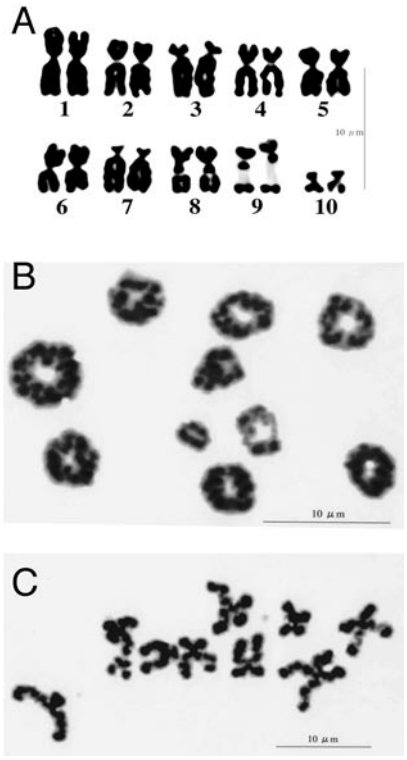


Fig. 2. Karyotype of the Yasuda line. (A) Mitotic chromosomes observed in the testis. (B) Metaphase of primary spermatocyte showing 10 bivalents. (C) Metaphase of secondary spermatocyte showing 10 chromosomes.

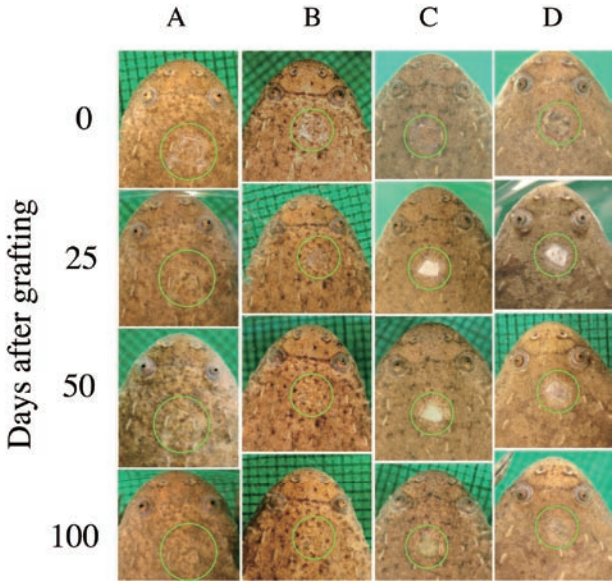
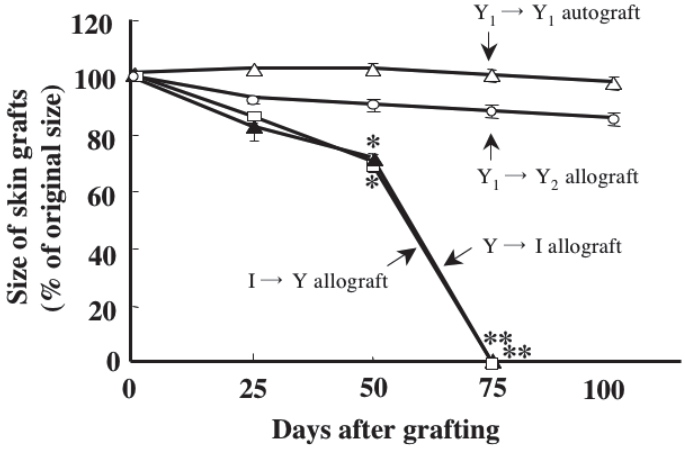


Fig. 3. Skin graft transplantation assay to assess the degree of inbreeding. Autografts of the Yasuda line (A) and allografts from the Yasuda line to a different frog of the same line (B), from the Ivory Coast to the Yasuda line (C), and from the Yasuda to the Ivory Coast line (D) were observed for more than 100 days. Representative pictures are shown here.

assess the degree of inbreeding using the fourth generation of the Yasuda line at the Institute for Amphibian Biology (Fig. 3). Adult *Xenopus* frogs can recognize and reject skin allografts from donors that differ either by major histocompatibility complex or by minor histocompatibility antigens. Generally, major histocompatibility complex-disparate allografts are rejected more promptly (18 to 22 days) than are skin grafts transplanted from donors that have the identical major histocompatibility complex haplotype, but multiple different minor histocompatibility antigens (30 to 50 days) [22, 33]. Autografts, namely, skin grafts that were excised from the dorsal side of bodies and returned to the same place, were retained for more than 100 days after transplantation (Fig. 3A), whereas allografts transplanted from the Ivory Coast line to the Yasuda line or from the Yasuda to the Ivory Coast became smaller, edematous and whitish within 25 days, and detached by day 75 (Fig. 3C and 3D). Skin grafts transplanted among the fourth generation Yasuda line frogs remained unchanged for more than 100 days, like autografts, except for a 10% reduction in size (Figs. 3 and 4). These results suggest that the Yasuda line is quite syngenic and that organ transplanta-



*Significantly less ($P < 0.05$) than corresponding values for $Y_1 \rightarrow Y_1$ autograft.
**Significantly less ($P < 0.01$) than corresponding values for $Y_1 \rightarrow Y_1$ autograft (χ^2-test).

Fig. 4. Quantitative analysis of size of skin grafts between the same or different lines. The sizes of autografts of the Yasuda line (open triangles) and allografts from the Yasuda line to a different frog of the same line (open circles), from the Ivory Coast to the Yasuda line (open squares), and from the Yasuda to the Ivory Coast line (closed triangles) were measured and quantified at days 0, 25, 50, 75, and 100. Each data point is the average skin graft size with error bars denoting standard deviations.

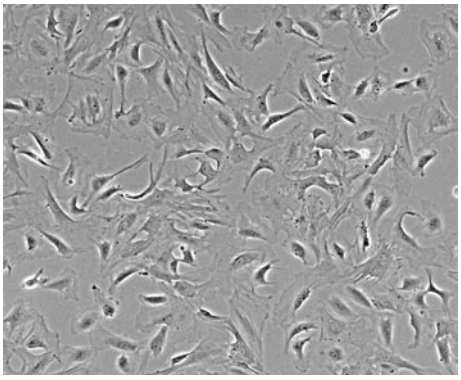


Fig. 5. A cell line derived from hind-limbs of a *Xenopus tropicalis* Yasuda line tadpole.

tion is not rejected between individuals, although some minor histocompatibility loci are not homozygous yet.

We have succeeded in the establishment of several cell lines derived from hind-limbs of stage 54, 56, and 57 Yasuda line tadpoles (Fig. 5). They respond to thyroid hormone and are induced to express a series of genes that thyroid hormone stimulates in hind-limbs (unpublished data). To our knowledge, this is the first report on the establishment of *X. tropicalis* cell lines.

4) Asashima line

The Asashima line originated from frogs caught in the wild, which were obtained by Dr. M. Asashima in 1998. It has been demonstrated to be diploid [41]. The chromosome mapping and complete karyotype by G- and Ag-banding have been carried out. The seventh generation of this inbred line is available. This frog is close to *X.* cf. *tropicalis* as mentioned below.

5) Golden line

The Golden line was established by selection of fast growth and short times to sexual maturation in the laboratory of Dr. Harland (University of California, USA). Male frogs exhibit nuptial pads, starting at 8 weeks post-metamorphosis, and testes sizes reach their peak at 22 weeks post-metamorphosis with sperm counts peaking at the same time. Vitellogenic oocytes increase in number up to 15,000 per female at 30 weeks post-metamorphosis, and account for 75% of the total number of oocytes present in the ovary. The ovary and oviducts continue to grow in mass until 30 weeks. Male and female frogs reach reproductive maturation at 22 and 30 weeks post-metamorphosis, respectively [30].

Phylogenetic Relationship of Standard Lines

To survey the genetic divergence among the *X. tropicalis* laboratory lines, an approximately 3.6 kbp mitochondrial genomic DNA fragment containing *Cytb*, *tRNA* for threonine, the control region (CR), *tRNA* for proline, *tRNA* for phenylalanine, and *12S rRNA* was amplified and sequenced (Table 2). The resulting sequences were compared with each other, the *X. tropicalis* caught in the wild and related taxa. The Nigerian, Yasuda, and Ivory Coast lines showed the same nucleotide sequence in the sequenced region, excluding the repeat numbers in the control region and very few substitutions in the Ivory Coast line. In contrast, the Asashima line possessed a somewhat divergent sequence from the other lines (from 78.9% in CR to 98.5% in *tRNA* for proline). The 5' portion (290 bp) of the Asashima line *12S rRNA* exactly matched that of a possibly different species, *X.* cf. *tropicalis* (= *Silurana* cf. *tropicalis*), from Liberia. Based on this sequence, the most-likelihood tree was reconstructed (Fig. 6). In this tree, the Nigerian, Yasuda, and Ivory Coast lines form a clade, and the Asashima line is their sister taxon. According to Evans *et al.* [7], the extant clawed frog lineages originated well after the breakup of Gondwana, about 63.7 million years ago, with a 95% confidence interval from 50.4 to 81.3 million years ago, when the split of ancestors between *X. laevis* and *X. tropicalis* occurred. The ancestor of *X. tropicalis* diverged from that of *X.* cf. *tropicalis* 13.8 million years ago. We estimate that the Ivory Coast line separated from the Nigerian and Yasuda lines about 1.8 million years ago, and the Nigerian line cannot be distinguished from the Yasuda line in the sequenced mitochondrial region.

How to Obtain and Maintain *Xenopus tropicalis*

Both Hiroshima University and the University of Tokyo exploit, proliferate, maintain, and supply inbred lines of *X. tropicalis* to researchers as the National BioResource Project. A list of maintained lines and detailed information on material transfer agreements are available

Table 2. Comparison of the *Cytb-12S rRNA* region sequenced among *X. tropicalis* laboratory lines and wild-caught samples and *X. laevis*

Regions	*X. tropicalis* laboratory lines			*X. laevis*
	Nigerian & Yasuda[a)]	Ivory coast (Similarity vs. Nigerian & Yasuda)	Asashima (Similarity vs. Nigerian & Yasuda)	(Similarity vs. Nigerian & Yasuda)
Cytb[b)]	962 bp	962 bp 99.6%	962 bp 88.6%	959 bp 80.5%
tRNA for Thr	71 bp	71 bp 100.0%	71 bp 94.4%	70 bp 93.0%
Spacer	29 bp	29 bp 100.0%	28 bp 80.1%	27 bp 66.7%
tRNA for Pro	69 bp	69 bp 100.0%	69 bp 97.1%	69 bp 91.3%
Control region	2,042 bp including 93 bp × 2 repeats	2,233 bp including 93 bp × 4 repeats (96.3% excluding additional repeats)	2,038 bp including 93 bp × 2 repeats 78.9%	2,135 bp including 93 bp × 2 repeats 60.5%
tRNA for Phe	68 bp	68 bp 100.0%	68 bp 98.5%	69 bp 94.2%
12S rRNA[c)]	398 bp	398 bp 100.0%	397 bp 93.0%	397 bp 88.7%
Comparison of *12S rRNA* sequence (290 bp) with the wild-caught *X. tropicalis*[d)] and *X.* cf. *tropicalis*[e)] from Evans *et al*. [7]				
Wild *X. tropicalis* from Nigeria	100.0%	100.0%	93.8%	–
from Ivory coast	100.0%	100.0%	93.8%	–
from Sierra Leone	99.7%	99.7%	93.4%	–
X. cf. *tropicalis* from Liberia	93.8%	93.8%	100.0%	

[a)] Nigerian and Yasuda lines show completely the same nucleotide sequence in this region. [b, c)] *Cytb* and *12S rRNA* were partially sequenced. [d, e)] The genus *Silurana* was adopted in Evans *et al*. [7] and *Silurana* cf. *tropicalis* was suggested as a different species from the real *X. tropicalis*. Note: *12S rRNA*, 12S ribosomal RNA gene; *16S rRNA*, 16S ribosomal RNA gene; CR, control region; *Cytb*, cytochrome b gene; *tRNA* for Thr, transfer RNA gene for threonine; *tRNA* for Pro, transfer RNA gene for proline; *tRNA* for Phe, transfer RNA gene for phenylalanine.

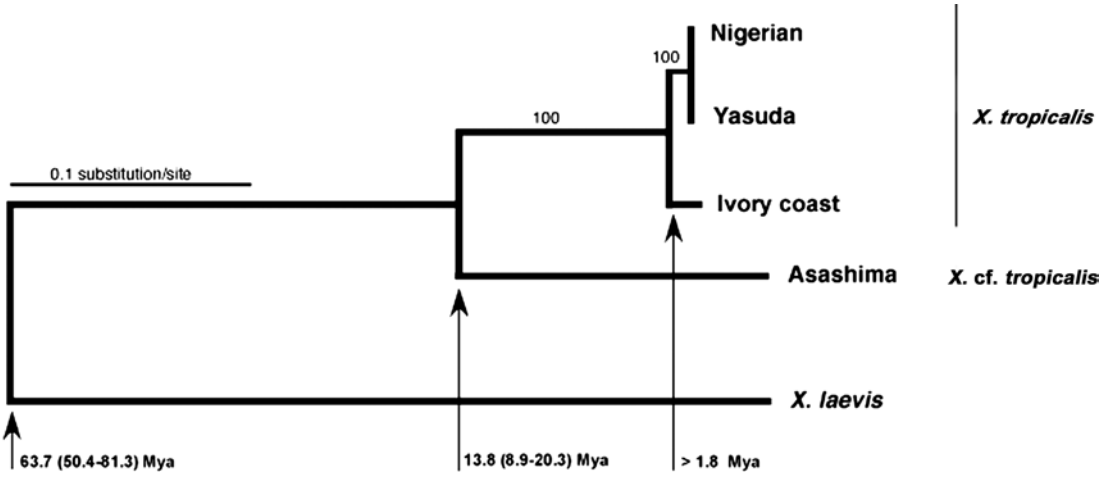


Fig. 6. Maximum likelihood tree of the *Xenopus tropicalis* laboratory lines. The –log likelihood value of this tree is 10160.79. The tree was reconstructed from 3,564 bp of mitochondrial genomic sequence with a GTR+G substitution model. Tree reconstruction was performed by PAUP ver. 4.10b [37] and the substitution model was suggested by Akaike information criteria implemented in Modeltest ver. 3.06 [32]. The numbers on the upper branches are nonparametric bootstrap probabilities calculated from 100 pseudo-replicates. The possible branching times of the *X. tropicalis* lines and related taxa, estimated by Evans *et al*. [7] based on a mitochondrial *12S rRNA-tRNAVal-16S rRNA* sequence are also shown. Mya; million years ago.

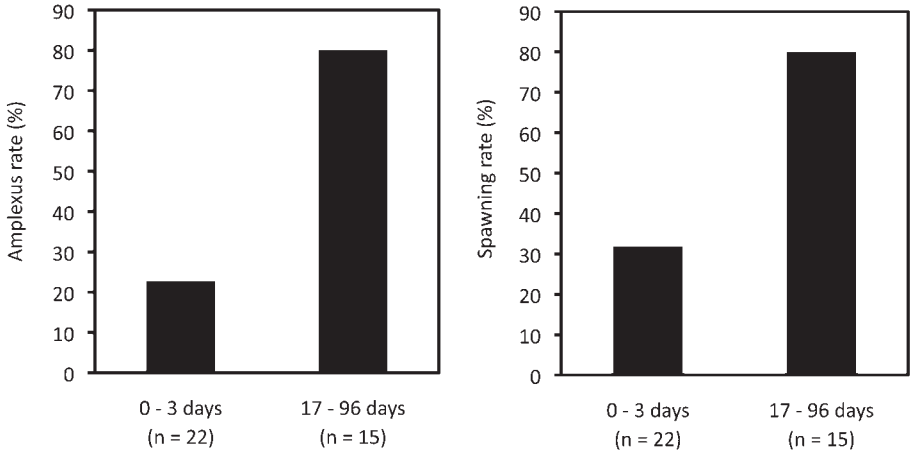


Fig. 7. The effect of maintenance at 28°C on both the amplexus and spawning rates of natural matings. The amplexus and spawning rates were examined using adult frogs of the Yasuda line that had been kept at 28°C for more than one year and then transferred into a room at 24°C for the indicated periods. The fertilization rate was 30 to 100%, when a pair of frogs had been maintained at 24°C for 17–96 days.

at the website of *Xenopus* National BioResource Project (http://home.hiroshima-u.ac.jp/~amphibia/NatBio/index.html) (Table 3).

Excellent protocols on *X. tropicalis* husbandry are published at the website of *Xenopus tropicalis* Home (http://faculty.virginia.edu/xtropicalis/) and Harland *Xenopus tropicalis* Site (http://tropicalis.berkeley.edu/home/). At our facility, embryos are raised at 25–28°C with exchange of water every day. As tadpoles grow up, the water is changed less frequently, and finally once a week. Tadpoles are fed the powdered mix, Sera Micron (Sera Partners), daily. Since the rate of development depends on temperature, froglets just after metamorphosis are maintained at 27°C to promote growth, and fed with cricket larvae, accompanied by total water change every day for half a year. Adult frogs are kept at 22–24°C, and fed with crickets or eel food pellets, followed by exchange of water and cleaning of the water tanks every other day.

Matings are effectively done at 22–25°C, but not at warmer temperatures [18]. We have estimated the effect of maintenance at 28°C on both the amplexus and spawning rates of natural matings using frogs that had been kept at 28°C for more than a year (Fig. 7). It took a few months to recover at 24°C, indicating that adult frogs should be raised at 24°C or less to obtain embryos for experiments.

Examples of Published Papers

The grafting experiments of Spemann and Mangold clarified that the transplantation of the dorsal lip of the blastpore induces the formation of a secondary embryo. Spemann's organizer, the dorsal blastopore lip, induces the host ventral cells to form dorsal embryonic cells such as a neural tube and dorsal mesodermal cells, and expresses at least five bone morphogenetic protein (BMP) antagonists, all of which can mimic the effect of an organizer in a gain-of-function assay, suggesting overlapping functions. Harland's group demonstrated that the antisense morpholino knockdown of three BMP antagonists, chordin, noggin, and follistatin, resulted in both catastrophic failure of dorsal development and expansion of the ventral and posterior fates in an *X. tropicalis* inbred line. The kockdown of two BMP antagonists had effects, but the neural plate still formed. They concluded that BMP antagonists are required for formation of the dorsal structures, and that neural specification requires the continuous activity of BMP antagonists from the blastula through to the gastrula stages [21]. Their study showed that *X. tropicalis* is an ideal frog to test for redundancy in two or more genes and overlap in gene function using antisense morpholino, considering that *X. laevis* is allotetraploid, and has four alleles for each

Table 3. Information on *X. tropicalis*

Resource name	URL (Contents)
National BioResource Project—*Xenopus tropicalis*—	http://home.hiroshima-u.ac.jp/~amphibia/NatBio/ (Strains, husbandry, distribution, cDNA database)
JGI *X. tropicalis*	http://genome.jgi-psf.org/Xentr4/Xentr4.home.html (Genome database)
Ensemble genome browser 57: *Xenopus tropicalis*	http://uswest.ensembl.org/Xenopus_tropicalis/Info/Index (Genome database)
UCS Genome Bioinformatics	http://genome.ucsc.edu/ (Genome Bioinformatics)
CHORI BAC Resouces	http://bacpac.chori.org/ISB1.htm#ref (BAC library)
Xenopus tropicalis: UniGene Build #49	http://www.ncbi.nlm.nih.gov/UniGene/UGOrg.cgi?TAXID=8364 (cDNA database)
The *Xenopus tropicalis* EST Project	http://www.sanger.ac.uk/Projects/X_tropicalis/ (cDNA database)
DFCI—*X. tropicalis* Gene Index	http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=x_tropicalis (cDNA database)
Xenopus Gene Collection	http://xgc.nci.nih.gov/ (cDNA database)
Gurdon Institute *Xenopus tropicalis* Full-Length Database	http://genomics.nimr.mrc.ac.uk/online/xt-fl-db.html (cDNA database)
Xenbase	http://www.xenbase.org/common/ (cDNA, genome, and expression database)
X. tropicalis Home	http://faculty.virginia.edu/xtropicalis/ (Husbandry, protocols)
Harland *Xenopus tropicalis* Site	http://tropicalis.berkeley.edu/home/ (Husbandry, protocols)

gene, and this inbred line is not generally available.

A pigmented animal half (animal cap) of the late blastula of *Xenopus* is a presumptive ectodermal region that is composed of undifferentiated cells. It is known that the undifferentiated cells of *X. laevis* animal cap is capable of differentiating into several different cell lineages and developing *in vitro* into such organs/tissues as muscle, notochord, heart, liver, pronephros, and pancreas by treating them with activin at various concentrations, and reacting them with retinoic acid [1, 2, 35]. Animal cap cells of *X. laevis* and *X. tropicalis* display comparable competence in differentiation into mesodermal and endodermal tissues, when induced by activin, in a dose-dependent manner. Organizer and mesoderm markers are expressed in a similar temporal and dose-dependent manner in tissues from both organisms [34].

While inbreeding a population of Nigerian frogs, three mutations were identified, and all were recessive embryonic lethal [12]. The technique of gynogenesis, the generation of embryos with only a maternal genetic contribution, is faster and easier than conventional and traditional screens. Forty-two potential mutant phenotypes were isolated by a gynogenetic screen of naturally occurring recessive mutations in wild *X. tropicalis*. Ten lines of the developmental mutants have genetically heritable recessive phenotypes [27]. The forward and reverse genetic screens were undertaken for chemically-induced mutations in *X. tropicalis* [9]. In the forward genetic screen, 77 candidate phenotypes were uncovered in diverse organogenesis and differentiation processes. Twenty-nine mutants were shown to be heritable by a gynogenetic screen design. It was demonstrated that the reverse genetic approach, TILLING, is feasible for obtaining carriers of mutations in specific genes. These reports have not only established *X. tropicalis* as a genetic system, but have also shown that it can be used to discover novel gene functions.

The genomic sequence data of *X. tropicalis* are utilized

in the bioinformatic search for transposable elements [17] and binding sites of transcriptional factors such as thyroid hormone receptor [5, 8], and in the genome-wide comparisons with mammalian sequences to predict the cis-regulatory elements, for example, conserved non-coding elements [29].

Acknowledgments

The authors thank Drs. M. Asashima and S. Takahashi for their extensive contribution to the National BioResource Project of *Xenopus tropicalis* and Asashima line frogs. We thank Junko Nishiyama, Chiori Kojou, Reiko Imamura, Satomi Kobayashi, Yasuko Kawano, and Taeko Nakajima for their technical assistance. The National BioResource Project is funded by the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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