Xenopus Cytogenetics and Chromosomal Evolution

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Chromosomal evolution · Cytogenetics · FISH · Genetic map · *Xenopus laevis* · *Xenopus tropicalis*

Abstract

The genus *Xenopus* represents important model organisms in the field of developmental biology and chromosomal evolution. Developmental processes are tightly coupled with the analysis of gene function via genetic linkage and mapping. Cytogenetic techniques such as chromosome banding or FISH are essential tools for the determination of gene position and subsequently for the construction of linkage and physical maps. Here, we present a summary of key achievements in *X. tropicalis* and *X. laevis* cytogenetics with emphasis on the gene localization to chromosomes. The second part of this review is focused on the chromosomal evolution regarding both above-mentioned species. With respect to methodology, hybridization techniques such as FISH and chromosome-specific painting FISH are highlighted.

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Xenopus tropicalis and *Xenopus laevis* belong to the group of African clawed frogs. Both represent valuable model organisms in the fields of molecular, cellular and developmental biology. The existence of polyploid spe-

cies in the genus *Xenopus* with multiples of 18 chromosomes (36 in *X. laevis* and other tetraploid species, 72 in *X. amieti* and other octoploid species, and 108 in *X. ruwenzoriensis* and other dodecaploid species) and with the diploid chromosome number of 20 in *X. tropicalis* and 40 in the tetraploid *X. epitropicalis* makes these amphibians suitable for the study of chromosomal evolution after a genome duplication event. The reason for such variable chromosomal counts in this group is the combination of bifurcating and reticulating speciation during evolution. Bifurcation refers to scenarios in which one ancestral species splits into 2 descendants. Reticulation speciation in African clawed frogs is characterized by the emergence of allopolyploids through the merging of 2 ancestral species [Evans, 2008].

Xenopus Genetic Maps

X. laevis and later *X. tropicalis* play a pivotal role in the study of gene function. External fecundation and embryonic development of hundreds of embryos in water allow the application of forward genetic methods. This approach comprises treatment of fertilized eggs or embryos with a mutagen such as ethylnitrosourea [Goda et al., 2006] or UV light [Noramly et al., 2005] followed by the selection and analysis of mutants with interesting pheno-

types. The identification of the gene(s) responsible for the observed abnormalities has been facilitated by the development of a genetic map.

The first attempt to arrange polymorphic loci into linkage groups (LGs) via segregation analysis in *Xenopus* was performed by Graf [1989a]. The author studied the allelic inheritance of 29 electrophoretically detected isoenzymes and 1 pigment locus in *X. laevis*. As a result, 8 LGs were established including 1 relating to sex determination (LG7) [Graf, 1989b]. A concern with this linkage map was the enzymatic nature of the polymorphic markers. Since *X. laevis* is an allotetraploid, the isoenzyme pairs may represent protein products of homeologous genes, and, if this is the case, both members of a particular pair can react with the same enzyme substrate. As a result, only isoenzymes differing in their electrophoretic mobility can be distinguished. We tried to reanalyze the findings of Graf [1989b] concerning the linkage of the *mME2* gene to sex (LG7). Sequencing of the *X. laevis mME2* gene revealed Xstir tandem repeats [Hikosaka et al., 2000] in one of the introns. Since repeat numbers differed among individuals, we used Xstir as a polymorphic marker for the subsequent linkage analysis. Unfortunately, the distribution of Xstir polymorphisms between parents and offspring revealed no linkage to sex. There are 2 possible reasons for this discrepancy: (1) Graf had worked with another enzyme reacting with the same substrate, for example with the mitochondrial malate dehydrogenase 2 (*Mdh2*), or (2) there is another *ME2* gene linked to sex [Mácha et al., 2003].

X. laevis has an allotetraploid genome, which complicates genetic and gene function studies because of the existence of homeologous chromosomes [Graf and Kobel, 1991]. *X. tropicalis* is the only known diploid clawed frog; thus, it is more suitable for gene function studies using linkage analysis of a mutated genome. The first *X. tropicalis* genetic map based on the segregation of 51 amplified fragment length polymorphism [Vos et al., 1995] and 2 isoenzyme markers was constructed by Kochan et al. [2003]. Thirteen multipoint linkage groups were defined reflecting the maternal strain and 3 separate LGs from the paternal counterpart.

Another approach to establish LGs in *X. tropicalis* is using lampbrush chromosomes (LBC) from germinal vesicles of growing oocytes [Penrad-Mobayed et al., 2009]. Since amphibian LBCs are large and relatively easy to isolate, they have been used for the study of transcriptional processes for several decades. Each homologous pair of LBCs forms a bivalent in the diplotene stage, and the thousands of transcriptionally active lateral loops give

the chromosomes a feather-like character [Callan, 1986; Morgan, 2002; Gall et al., 2004].

Interestingly, the arrangement of the lateral loops is not uniform along the chromosome axis, but reveals distinctive visible granular structures. In some cases, their position and shape are typical for the particular chromosomal bivalent, and thus, they can be used as landmarks for LBC determination. In addition, sites of active RNA polymerase III transcription are also valuable markers after their visualization using specific antibodies. Penrad-Mobayed et al. [2009] analyzed 100 LBC spreads from oocytes in stages V–VI and found a unique combination of lateral loops and active RNA polIII sites for each of the 10 *X. tropicalis* chromosomes. Since LBCs are transcriptionally active, the authors proposed that future work could visualize mRNA transcripts using the FISH coupled with tyramide signal amplification (FISH-TSA) method [Krylov et al., 2007] and could construct a physical map based on protein-coding genes. For detailed information concerning the FISH-TSA technique, see Development of FISH Techniques in *Xenopus* below.

A disadvantage of isoenzymes and amplified fragment length polymorphism-based linkage maps is the lack of information about the DNA sequence of the polymorphic markers. This means that further marker addition using genome sequencing data is impossible. In 2002, the US Department of Energy Joint Genome Institute (DOE JGI) initiated the establishment of a committee for sequencing the 1.7-Gbp-long *X. tropicalis* genome. A seventh-generation inbred Nigerian strain female served as the source of genomic DNA for 7.6-fold redundant random shotgun sampling. Eight years later, its reading and assembling was finished [Hellsten et al., 2010]. This article describes the genome assembly version 7.1 derived from version 4.1 and supplemented with synteny data from a comparison of *X. tropicalis*, human and chicken genomes. Presently, the assembly is still being improved, and the latest version is 8.0.

The *X. laevis* genome is also being sequenced using the ABI SOLiD platform. Both genome assembly versions, 6.0 and the latest 7.1, were produced by the *Xenopus* Genome Project Consortium including laboratories at the University of California, Berkeley, DOE JGI, University of Tokyo, and University of Texas, Austin, and they are currently available on the Xenbase webpage prior to publication (<http://www.xenbase.org/entry/>).

Data from the *X. tropicalis* sequencing project allowed the initiation of the construction of a *X. tropicalis* genetic map based on linkage markers with known DNA sequence [Wells et al., 2011]. There are at least 2 sequence

Fig. 1. Physical map of *X. tropicalis*. In total, 70 cDNA markers were localized to *X. tropicalis* chromosomes by FISH-TSA [Krylov et al., 2007]. Genes were selected from scaffolds (genome assembly 4.1) assigned to particular LGs (1–10). LGs 2, 3, 4, 7, and 8 show p-q orientation, while LGs 1, 5, 6, 9, and 10 are q-p oriented [adapted from Wells et al., 2011].

types present in the *X. tropicalis* genome that are well suited for high-throughput identification of polymorphic markers: (1) short tandem repeats also known as simple sequence repeats (SSRs) and (2) single nucleotide polymorphisms (SNPs). SSRs represent short 2–4-bp repeat clusters occurring with a frequency of 1/20–1/40 kbp, flanked by unique DNA sequences [Strachan and Read, 2004]. The number of repeat units is variable among individuals. On the other hand, the surrounding sequences are conserved in the 2 well-established *X. tropicalis* lines (Ivory Coast and Nigerian strain) and, thus, suitable as primer sites for PCR amplification. SNPs are more frequent than SSRs, but to utilize them, it is necessary to know the sequences surrounding the polymorphic nucleotide from both parental strains.

The construction of the *X. tropicalis* genetic map was performed using single sequence length polymorphism (SSLP) markers derived from SSR clusters. On the basis of sequencing data (Genome Assembly 2.0) comprising more than 27,000 scaffolds, around 14,000 3–4 nucleotide SSRs were identified. Those passing a PCR amplification test were subsequently analyzed for polymorphism between the 2 strains (Ivory Coast and Nigerian strain) involved in the linkage map construction and between pa-

rental animals and the F2 generation. As a result 2,886 SSLP markers were established. Linkage analysis divided these SSLPs into 10 LGs and 4 smaller linkage clusters. Assignment of all LGs and clusters to particular chromosomes was performed by FISH-TSA technique [Krylov et al., 2007] (fig. 1). In total, 70 cDNA probes were hybridized to metaphase chromosomes, and their positions were in agreement with the scaffold order within LGs [Wells et al., 2011].

However, large gaps without SSLP markers remained, especially in the short arms of XTR2 and XTR7. The reasons for this could be a lower incidence of SSR clusters or suppressed meiotic recombination due to the presence of the sex-determining region. To further explore this issue, Seifertova et al. [2013] used laser microdissection for the isolation of ~15 copies of the short arms of chromosome 7 in *X. tropicalis*. Subsequent whole genome amplification (WGA) using the WGA4 and WGA3 kits [Höckner et al., 2009] and Illumina high-throughput sequencing identified more than 200 previously unmapped scaffolds to the analyzed area based on the v. 4.1 and 7.1 genome assemblies. Laser microdissection and WGA were previously used for the preparation of *X. tropicalis* whole chromosome painting (WCP) probes [Krylov et al., 2010] (for

detailed information, see Study of *Xenopus* Chromosomal Evolution Using Cytogenetic Techniques).

The *X. tropicalis* genetic map and gynogenesis were successfully employed in the gross mapping of 9 candidate genes responsible for selected mutant phenotypes coming from pilot forward genetic screens [Grammer et al., 2005; Noramly et al., 2005; Goda et al., 2006; Khokha et al., 2009]. Jiang et al. [2011] published the first *X. tropicalis* map based on the HAPPY mapping approach (haploid DNA breakage samples using the polymerase chain reaction). This method is based on mechanical DNA breakage by gamma irradiation or shearing. Individual fragments are isolated via pulsed-field gel electrophoresis and diluted to the concentration corresponding to a haploid genome equivalent. After whole genome amplification of all isolated fragments, specific PCRs are performed in order to find the physical linkage of 2 or more markers within particular DNA fragments. The existence of a dense genetic map is the key prerequisite for the construction of a high-resolution genome map based on the HAPPY mapping.

In *X. laevis*, a comprehensive physical map based on the chromosome mapping of 60 expressed sequence tag (EST) clones was constructed [Uno et al., 2013]. The markers were derived from a previous study focused on the chromosomal evolution among *X. tropicalis*, selected nonavian reptilian species, chicken and human [Uno et al., 2012]. Their localizations were determined by the indirect FISH technique [Matsuda and Chapman, 1995] described in Development of FISH Techniques in *Xenopus*. This study resulted in the determination of all 9 chromosome groups (quartets). Moreover, strong conservation of genetic linkage between homeologous chromosome pairs and also between *X. tropicalis* and *X. laevis* orthologs was observed.

Construction of linkage and physical maps in *X. tropicalis* and *X. laevis* raised concerns over how chromosomes should best be numbered and adhered, for example, to the cytogenetic rules applied to the human karyotype. There are 2 basic principles. The first is that the order of chromosomes should follow their size, i.e. chromosome 1 is the largest and the last one is the smallest. The second principle addresses the problem of 2 chromosomes having approximately the same length. In this case, the more metacentric chromosome has a lower number. The first karyotypes of both *Xenopus* species were described in the 1970s. In *X. tropicalis*, Tymowska [1973] suggested an unconventional chromosome nomenclature based on the relative sizes of p and q arms. This numbering was used until 2009 when a new system reflecting

the above-mentioned cytogenetic rules was proposed [Khokha et al., 2009].

Chromosome nomenclature in *X. laevis* was first published in 1972 [Tymowska and Kobel, 1972]. As in *X. tropicalis*, this system took into account the p/q arms relative sizes rather than total chromosome lengths. Uno et al. [2013] suggested a new arrangement based on ordering *X. laevis* homeologous chromosome pairs with respect to their *X. tropicalis* orthologs. The determination of triplets (1 *X. tropicalis* chromosome and 2 *X. laevis* orthologs) was done by indirect FISH [Matsuda and Chapman, 1995]. However, the numbering of *X. laevis* chromosomes still corresponded to the system proposed by Tymowska and Kobel [1972]. Masanori Taira and co-workers from the *Xenopus laevis* Genome Project suggested renumbering *X. laevis* homologous pairs with regard to the new *X. tropicalis* nomenclature [Khokha et al., 2009]. For example, the *X. laevis* pair XLA1 and XLA2, which correspond to the *X. tropicalis* orthologous chromosome 1 (XTR1), would be numbered as XLA1L and XLA1S. The letters L and S mean large and small and reflect the different chromosome lengths in the particular quartet [pers. commun.]. However, unlike the new *X. tropicalis* chromosome numbering, both above-mentioned nomenclature systems reflect chromosome evolution between the 2 species rather than standard cytogenetic rules. Recently, a discussion between advocates and opponents of the proposed chromosome numbering was initiated. A comparison of the old and new numbering systems in both species is presented in table 1.

Development of FISH Techniques in *Xenopus*

Construction of physical (cytogenetic) maps via assignment of LGs to particular chromosomes or studies of chromosomal evolution would not be possible without the establishment of cytogenetic techniques such as karyotyping and localization of DNA sequences on metaphase chromosomes or LBCs. The first attempt to visualize protein-coding repetitive clusters in *X. laevis* was presented by Courtet et al. [2001]. The authors localized tandemly arrayed genes of MHC complexes II α and II β and immunoglobulin heavy-chain loci on metaphase chromosomes. For that purpose, they employed a relatively new technique called FISH-TSA [van de Corput et al., 1998]. In principle, this approach is based on horseradish peroxidase-mediated production of tyramide free radicals that covalently bind to protein tyrosine residues in the vicinity of the enzyme. A DNA probe is usually la-

Table 1. Chromosome numbering systems in *X. tropicalis* and *X. laevis*

<i>X. tropicalis</i>		<i>X. laevis</i>	
old ^a	new ^b	new ^c	old ^d
Xtr1	Xtr1	Xla1L Xla1S	Xla1 Xla2
Xtr2	Xtr2	Xla2L Xla2S	Xla3 Xla8
Xtr9	Xtr3	Xla3L Xla3S	Xla12 Xla16
Xtr3	Xtr4	Xla4L Xla4S	Xla13 Xla17
Xtr4	Xtr5	Xla5L Xla5S	Xla4 Xla5
Xtr8	Xtr6	Xla6L Xla6S	Xla9 Xla6
Xtr5	Xtr7	Xla7L Xla7S	Xla7 Xla10
Xtr10	Xtr8	Xla8L Xla8S	Xla14 Xla11
Xtr6	Xtr9	Xla9_10L ~Xla9L	Xla15
Xtr7	Xtr10	Xla9_10S ~Xla9S	Xla18

Chromosome numbering according to ^a Tymowska [1973], ^b Khokha et al. [2009], ^c personal communication, and ^d Tymowska and Kobel [1972] and Uno et al. [2013].

beled with digoxigenin or biotin. Horseradish peroxidase is introduced into the system by an anti-digoxigenin antibody or streptavidin coupled with this protein. To visualize the DNA probe, tyramide is linked to a fluorophore. The advantage of the method is high sensitivity, up to 100–1,000× higher compared with standard FISH. However, the high sensitivity is also a disadvantage, especially when the sequence of interest contains repetitive regions. In this case, a strong background is visible, and precise identification of the probe position is almost impossible. For this reason, the localization of cDNA instead of whole gene probes can be more effective (pers. observ.).

As noted above, Courtet et al. [2001] succeeded in the localization of tandemly arrayed genes where at least 3 copies were present. Our group adopted the TSA system and simplified the original protocol. Since a unique cDNA probe hybridizing to a defined locus was used, we tried to

remove the competitor salmon sperm DNA from the hybridization mixture. In addition, the RNase step was also omitted. These protocol modifications made it possible to perform simultaneous denaturation of metaphase spreads with the DNA probe. The first publication describing the localization of a single copy gene on metaphase chromosomes in *X. laevis*, and in amphibians in general, was focused on the visualization of the *c-src1* locus encoding the cellular form of the Src kinase [Krylov et al., 2003]. To validate our simplified protocol, we used a probe that interrogated the *MHC1b* locus, whose genomic position had been identified earlier by Courtet et al. [2001]. We observed the same fluorescence area on the identical pair of chromosomes. The subsequent localization of an ~1-kb cDNA probe for the *c-src1* gene revealed specific signals in the subcentromeric region of another but morphologically similar acrocentric chromosome pair. Since we were not able to get satisfactory results with the original protocol from Courtet et al. [2001], we suppose that its general simplification was the key to the successful visualization of a short single copy gene. In our second study [Tlapakova et al., 2005], we tested the FISH-TSA sensitivity in a situation where 2 target sequences with ~95% sequence identity were present, as is the case for many *X. laevis* homeologous gene pairs. We selected the 2 homeologs of malate dehydrogenase 2 (*Mdh2a* and *Mdh2b*) as model genes. Following Graf [1989b], we had been studying this enzyme as a potential sex linkage candidate. When we hybridized a cDNA probe complementary to the *Mdh2a* gene, in 85% of the cases we found tyramide deposits on chromosome 3. The remaining 15% of signals were present on chromosome 8. We obtained reciprocal results after hybridization of the *Mdh2b* probe (85% on XLA8 and 15% on XLA3).

After successful visualization of single copy genes in *X. laevis*, we tried to adopt the FISH-TSA technique in *X. tropicalis* as well. With some minor optimization, we were able to localize the *Mdh2* gene to its chromosomes. We found the gene position in a subcentromeric region of XTR4, indicating that in *X. laevis* XLA3 and XLA8 form a homeologous pair [Krylov et al., 2007].

Another approach for the localization of single copy genes was presented by Uno et al. [2008]. In that paper, the authors described the identification and characterization of an *X. laevis* sex-determining gene, *DM-W*, which evolved from *DMRT1* by gene duplication. Unlike in *Xenopus*, *DMRT* plays the same role as amphibian *DM-W* in Medaka (*Oryzias latipes*). Both genes were localized to *X. laevis* chromosomes using FISH technique adapted from the mouse model [Matsuda and Chapman,

1995]. In principle, this method represents standard indirect FISH employing short, ~1.5-kbp-biotinylated EST probes. Their visualization was done using a goat anti-biotin antibody followed by the incubation with an anti-goat antibody coupled to a fluorophore. The hybridization mixture contained a competitor prepared from sonicated *X. laevis* genomic DNA. The *DM-W* gene was localized only in female karyotypes to a single XLA3 chromosome. Its homolog in females and both XLA3 in males bore no signal. These results confirmed the ZW configuration of sex chromosomes in *X. laevis*. Fluorescence signals from genes paralogous to *DMRT1* were observed on XLA1 and XLA2 indicating their common origin.

Study of *Xenopus* Chromosomal Evolution Using Cytogenetic Techniques

The genus *Xenopus* undoubtedly represents important model organisms in the study of chromosomal evolution after a genome duplication event [Evans, 2008]. Moreover, it comprises species with the greatest variability in ploidy level in vertebrates [Schmid and Steinlein, 1991]. For a long time, karyotyping coupled with various banding techniques was a key tool for the elucidation of interchromosomal rearrangements. The karyotype was reported for *X. tropicalis* [Tymowska, 1973] and several other polyploid species [Tymowska and Fischberg, 1973, 1980a, b, 1982; Tymowska and Kobel, 1972; Tymowska, 1977; Tymowska et al., 1977].

The most studied polyploid representatives of clawed frogs are *X. laevis* and *X. epitropicalis*. *X. laevis* is of an allotetraploid origin, i.e. the successful pairing of 2 previously independent diploid genomes. Since *X. tropicalis* contains 20 chromosomes in the 2n set and *X. laevis* contains 36 chromosomes, it is clear that differences in the chromosome number relative to *X. tropicalis* were present in the most recent diploid ancestor(s) of *X. laevis*. Standard karyotyping [Morescalchi, 1977], G-banding [Sekiya and Nakagawa, 1983] and especially high-resolution replication banding [Schmid and Steinlein, 1991] revealed 9 chromosome quartets determined on the basis of the chromosome size and banding pattern. The most informative replication banding employing the incorporation of BrdU during the S phase of the cell cycle [Schmid and Steinlein, 1991] grouped *X. laevis* chromosomes into the following quartets: (1 + 2), (3 + 8), (4 + 5), (6 + 9), (7 + 17), (10 + 11), (12 + 13), (14 + 15), and (16 + 18), where the pairs (3 + 8), (4 + 5), (10 + 11), and (12 + 13)

revealed almost complete correspondence regarding the size and relative positions of replicative bands.

However, this chromosome arrangement was not in agreement with the study of Krylov et al. [2010]. In that publication, we examined chromosomal evolution between *X. tropicalis* and *X. laevis* karyotypes using Paint- and Zoo-FISH techniques. Both methods are based on the preparation of directly or indirectly labeled DNA probes derived from isolated chromosomes or chromosome fragments. The difference between these methods concerns the target karyotype. Paint-FISH involves chromosome spreads and a probe from the same species, whereas Zoo-FISH involves hybridization of a probe to the karyotype of a different species.

There are 2 major ways to obtain many copies of a particular chromosome. The first one is based on the flow-sorting apparatus and can be used in model organisms with clearly distinguishable chromosomes. The second approach is laser microdissection where the required chromosomes are directly cut from metaphase spreads dropped onto a special foil. The next step toward the preparation of a painting probe is the amplification of the chromosomal material. The most prevalent technique is the degenerate oligonucleotide-primed (DOP) PCR [Telenius et al., 1992]. This method has been successfully applied to humans [Blennow, 2004] and other higher vertebrates [Frönicke et al., 2003; Rubtsov et al., 2004; Rubes et al., 2005; Stanyon et al., 2005]. Unfortunately, in amphibians, DOP-PCR amplification resulted in the enrichment of isolated repetitive clusters such as the NOR or other tandemly arrayed regions [Krylov et al., 2010].

In our laboratory, isolation of ~15 copies of a particular *X. tropicalis* chromosome was done by laser microdissection. Because DOP-PCR amplification did not give satisfactory results, we tried a different WGA approach based on the heat fragmentation of chromosomal DNA, subsequent ligation of sequence-specific adaptors and adaptor-specific amplification employing the WGA4 kit from the Sigma-Aldrich company [Höckner et al., 2009]. Simultaneous reamplification and labeling of the WGA4 product was achieved using the complementary WGA3 kit from the same company. As a result, 8 of the 10 *X. tropicalis* WCP probes were prepared and hybridized with *X. laevis* metaphase spreads. All WCPs, except one derived from XTR10, stained a certain chromosome quartet. Schmid and Steinlein [1991] discussed an evolutionary scenario concerning the loss of the XTR10 ortholog from the *X. laevis* karyotype, where the authors proposed its nonreciprocal translocation in the ancestor. Our results strongly supported that hypothesis. XTR10

Fig. 2. **A** *X. laevis* chromosome evolution based on the hybridization of *X. tropicalis* WCPs (Zoo-FISH) and EST mapping. **B** Data from Zoo-FISH experiments [Krylov et al., 2010] revealed 2 ancestral translocations of XTR10 on XTR8 and XTR9. **C** Uno et al. [2013] confirmed only the fusion of XTR10 with XTR9 by localization of EST clones and indirect FISH. Apart from the translocation event, there are 2 other discrepancies between the 2 studies concerning the proper determination of the morphologically similar *X. tropicalis* chromosomes XTR2, 4 and 5. According to Uno et al. [2013], the paralogous chromosome of the *X. laevis* quartet XLA3 and 8 is XTR2. Based on the FISH-TSA studies of Tlapakova et al. [2005] and Krylov et al. [2007], the *X. tropicalis* ortholog of this quartet is XTR4. XTR5 WCP hybridized to XLA13 and 17 (**B**). The same *X. laevis* quartet was assigned to XTR4 using EST clones (**C**) [adapted from Krylov et al., 2010].

WCP stained a pericentric area of 2 chromosome pairs XLA14 and XLA18. However, these chromosomes revealed quartets with other counterparts, XTR8 WCP labeling XLA11 + 14 and XTR9 labeling XLA15 + 18. This result indicates that the nonreciprocal translocation of XTR10 occurred in both ancestors forming the allotetraploid *X. laevis*. In the first ancestor, the smallest chromosome fused with XTR8; in the second ancestor, it fused with XTR9 (fig. 2).

As mentioned above, chromosome quartets revealed by Zoo-FISH were in disagreement with those determined by the replication banding technique [Schmid and Steinlein, 1991] so that we were able to confirm only 2 of them (XLA1 + 2 and XLA6 + 9). On the other hand, the Zoo-FISH data were supported by Uno et al. [2013] reporting the construction of an *X. laevis* cytogenetic map. However, there are some discrepancies between these studies concerning the translocation events of ancestral XTR10 and proper determination of the similar *X. tropicalis* chromosomes XTR2, 4 and 5. Uno et al. [2013] confirmed only the fusion of XTR10 with XTR9, but not with XTR8. Since XLA14 and XLA15 are similar, the explanation probably lies in their incorrect determination after the hybridization of an XTR10 painting probe. If that was the case, then the quartet formed by XLA15 and XLA18 would be orthologous for both ancestral chromosomes XTR9 and XTR10. In Uno et al. [2013], the authors coupled the XTR2 chromosome with XLA3 and 8 orthologs. One of the mapped genes supporting this conclusion was *Mdh2*. However, our previous studies [Tlapakova et al., 2005; Krylov et al., 2007] describing *Mdh2* localization showed its positions on XTR4, XLA3 and 8. Another discrepancy concerns the distinguishing of XTR4 and XTR5. In Krylov et al. [2010], we showed the hybridization signal of XTR5 WCP on XLA13 and 17. However, based on the EST mapping, the same *X. laevis* quartet was assigned to XTR4 [Uno et al., 2013]. Since there is concordance in the determination of appropriate *X. laevis* quartets, it is clear that the problem lies in the improper identification of morphologically similar XTR2, 4 and 5 in one of the mentioned studies.

The significance of *X. tropicalis* as a model organism for the study of chromosomal evolution was also demonstrated in studies comparing gene content of particular *X. tropicalis* chromosomes with the karyotypes of nonavian reptiles, birds, marsupials, and human employing FISH techniques [Mácha et al., 2012; Uno et al., 2012].

Concluding Remarks

Cytogenetic techniques such as FISH or chromosome banding play a pivotal role in the establishment of new model organisms for research. The knowledge of genetic linkage and the identification of polymorphic nucleotide positions are important for the construction of linkage and physical maps. These, in turn, are indispensable for the identification of genes involved in, for example, early embryonic development. In addition, the introduction of

the Paint-and Zoo-FISH methods allows the study of chromosomal evolution among related species. The genus *Xenopus* represents relatively new model organisms with the advantage of forward genetics and easily distinguishable chromosomes suitable for the study of chromosomal evolution after a genome duplication event. In this review, we summarized the key achievements in the above-mentioned fields, where this genus is now well established.

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