

ORIGINAL ARTICLE

Homoeologous chromosomes of *Xenopus laevis* are highly conserved after whole-genome duplication

Y Uno¹, C Nishida², C Takagi³, N Ueno^{3,4} and Y Matsuda¹

It has been suggested that whole-genome duplication (WGD) occurred twice during the evolutionary process of vertebrates around 450 and 500 million years ago, which contributed to an increase in the genomic and phenotypic complexities of vertebrates. However, little is still known about the evolutionary process of homoeologous chromosomes after WGD because many duplicate genes have been lost. Therefore, *Xenopus laevis* ($2n=36$) and *Xenopus (Silurana) tropicalis* ($2n=20$) are good animal models for studying the process of genomic and chromosomal reorganization after WGD because *X. laevis* is an allotetraploid species that resulted from WGD after the interspecific hybridization of diploid species closely related to *X. tropicalis*. We constructed a comparative cytogenetic map of *X. laevis* using 60 complimentary DNA clones that covered the entire chromosomal regions of 10 pairs of *X. tropicalis* chromosomes. We consequently identified all nine homoeologous chromosome groups of *X. laevis*. Hybridization signals on two pairs of *X. laevis* homoeologous chromosomes were detected for 50 of 60 (83%) genes, and the genetic linkage is highly conserved between *X. tropicalis* and *X. laevis* chromosomes except for one fusion and one inversion and also between *X. laevis* homoeologous chromosomes except for two inversions. These results indicate that the loss of duplicated genes and inter- and/or intrachromosomal rearrangements occurred much less frequently in this lineage, suggesting that these events were not essential for diploidization of the allotetraploid genome in *X. laevis* after WGD.

Heredity (2013) **111**, 430–436; doi:10.1038/hdy.2013.65; published online 3 July 2013

Keywords: *Xenopus laevis*; *Xenopus tropicalis*; comparative gene mapping; FISH; whole-genome duplication; homoeologous chromosomes

INTRODUCTION

Whole-genome duplication (WGD: polyploidization) is an evolutionary event that played an important role in the diversification of most eukaryotic lineages (Ohno, 1970; Kellis *et al.*, 2004; Kasahara, 2007; Otto, 2007). It is generally accepted that each duplicated gene evolved independently after WGD and that the polyploid genome quickly turned into a diploid state, referred to as diploidization, through repeated genomic and chromosomal reorganization including the loss of homoeologs, with some genes being consistently maintained as duplicates. Diploidization through genomic and chromosomal reorganization after ancient WGDs has been identified in yeast and most plant genomes (Song *et al.*, 1995; Seoighe and Wolfe, 1998; Pontes *et al.*, 2004; Simillion *et al.*, 2004; Yu *et al.*, 2005; Fischer *et al.*, 2006; Tuskan *et al.*, 2006; Woodhouse *et al.*, 2010). A total of 80% of the duplicated genes in yeast were lost after WGD 80 million years ago (Mya) (Kellis *et al.*, 2004), 70% in *Arabidopsis* after WGD 86 Mya (Bowers *et al.*, 2003) and 47% in maize after WGD 5–12 Mya (Woodhouse *et al.*, 2010).

Polyploidization is less prevalent in animals than in plants. Comparative analyses of genome sequences in vertebrates and chordates revealed that WGD occurred twice (2R-WGD) in the early evolutionary history of vertebrates around 450 and 500 Mya, and

3R-WGD occurred in the teleost fish lineage >350 Mya after 2R-WGD (Ohno, 1970; International Human Genome Sequencing Consortium, 2001; Jaillon *et al.*, 2004; Kasahara *et al.*, 2007; Putnam *et al.*, 2008). Comparative genome analyses of four teleost fish species (medaka fish, *Takifugu*, *Tetraodon* and zebrafish) suggested that genomic and chromosomal reorganization frequently occurred in the teleost fish lineage after 3R-WGD (Kasahara *et al.*, 2007; Sémon and Wolfe, 2007). It has been suggested that the common ancestor of salmonid fishes underwent one additional WGD 50–100 Mya after 3R-WGD (Allendorf and Thorgaard, 1984). Comparisons of genetic linkage between salmonid fishes (Atlantic salmon and rainbow trout) and other teleost fishes (medaka, stickleback and zebrafish) revealed that the genetic linkage of teleost fishes is conserved in salmonid fishes, although several chromosomal rearrangements occurred after WGD in salmonid genomes (Danzmann *et al.*, 2008; Guyomard *et al.*, 2012). However, little is known about the process of genomic and chromosomal reorganization after WGD in vertebrates because many duplicate genes derived from WGD have been lost in vertebrate genomes. For example, genome sequence analyses revealed that 76–80% of duplicated genes derived from 3R-WGD have been lost in extant teleost fish lineages, and it is speculated that approximately 60% of these duplicated genes were rapidly lost

¹Laboratory of Animal Genetics, Department of Applied Molecular Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan; ²Department of Natural History Sciences, Faculty of Science, Hokkaido University, Sapporo, Japan; ³Division of Morphogenesis, National Institute for Basic Biology, Okazaki, Japan and ⁴Department of Basic Biology, School of Life Science, The Graduate University of Advanced Studies (SOKENDAI), Okazaki, Japan

Correspondence: Dr Y Matsuda, Laboratory of Animal Genetics, Department of Applied Molecular Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan.

E-mail: yoimatsu@agr.nagoya-u.ac.jp

Received 17 January 2013; revised 6 May 2013; accepted 9 May 2013; published online 3 July 2013

within about 75 million years after 3R-WGD (Jaillon *et al.*, 2004; Sato *et al.*, 2009).

The African clawed frog (*Xenopus laevis*, Pipidae, Anura) ($2n = 36$) and the western clawed frog (*Xenopus (Silurana) tropicalis*, Pipidae, Anura) ($2n = 20$) are widely used as experimental animals in a wide range of scientific fields such as developmental, cellular, immunological and molecular biological research. More than 20 species of extant clawed frogs classified into two genera (*Silurana* and *Xenopus*) have been reported, which exhibit a variable number of chromosomes ($2n = 20$ –108). It is suggested that these species except for *X. tropicalis* resulted from polyploidization, which occurred after the hybridization of two different species (Tymowska and Fischberg, 1973; Bisbee *et al.*, 1977; Tymowska, 1991; Hughes and Hughes, 1993; Kobel, 1996; Evans *et al.*, 2004, 2008). The allotetraploidization events occurred at least twice in clawed frogs after the divergence of the ancestor of the diploid species *X. tropicalis*, which has 20 chromosomes and the ancestor of diploid species now thought to be extinct, which had 18 chromosomes (Tymowska, 1991; Evans, 2008), and this divergence probably occurred 50–65 Mya (Evans *et al.*, 2004; Hellsten *et al.*, 2007). In *Silurana*, *X. tropicalis* and another diploid species underwent allopolyploidization to give rise to three tetraploid species with 40 chromosomes, including *X. epitropicalis* and two undescribed species. Allopolyploidization between two 18-chromosome species occurred at least once 21–40 Mya, giving rise to the ancestor of all *Xenopus* species with 36, 72 or 108 chromosomes (Evans *et al.*, 2004; Chain and Evans, 2006; Hellsten *et al.*, 2007). Therefore, *X. laevis* and *X. tropicalis* are good animal models for understanding the process of genomic and chromosomal reorganization after WGD. We recently constructed a high-resolution chromosome map consisting of 140 genes for *X. tropicalis* (Uno *et al.*, 2012). The draft genome assemblies of *X. tropicalis* were reported previously (Hellsten *et al.*, 2010), and several comparative studies of complementary DNA (cDNA) sequences have been performed between *X. tropicalis* and *X. laevis* (Morin *et al.*, 2006; Hellsten *et al.*, 2007; Sémon and Wolfe, 2008). However, the information is still insufficient to know genetic linkage of *X. laevis*, and no comprehensive comparative analyses using genomic sequencing or cytogenetic mapping have been conducted for *X. laevis* and *X. tropicalis*.

In this study, we constructed a comparative cytogenetic map of *X. laevis* by fluorescence *in situ* hybridization (FISH) mapping of the functional genes that had been localized to *X. tropicalis* chromosomes. We consequently identified all nine homoeologous chromosome groups of *X. laevis* and then revealed the chromosome rearrangements that occurred between the two species and also between the homoeologous chromosomes of *X. laevis*. We discussed the process of genomic and chromosomal evolution in the *X. laevis* lineage after WGD on the basis of our comparative cytogenetic map of *X. laevis*.

MATERIALS AND METHODS

Animals, cell culture and chromosome preparation

We used adult females of the J strain of *X. laevis*, which were purchased from the breeder. After pithing, heart, lung and kidney tissues were collected for cell culture. All experimental procedures using animals conformed to the guidelines established by the Animal Care Committee, Nagoya University. Tissues were minced, and cells were cultured at 26 °C in a humidified atmosphere of 5% CO₂ in air for 10–14 days (Uno *et al.*, 2008). Primary cultured cells were harvested using 0.5% trypsin and then subcultured. For replication-banded chromosome preparation, 5-bromo-2'-deoxyuridine (25 µg ml⁻¹) was added to the cell cultures at log phase, and cell culturing was continued for 6 h including 1 h colcemid treatment (0.17 µg ml⁻¹) before harvesting. Chromosome preparations were made following a standard air-drying method. After staining with Hoechst 33258 (1 µg ml⁻¹) for 5 min, slides were heated to 65 °C

for 3 min on a hot plate and then exposed to ultraviolet light for an additional 5–6 min at 65 °C (Matsuda and Chapman, 1995). Slides were kept at –80 °C until use.

Fluorescence *in situ* hybridization

For chromosome mapping, 60 *X. laevis* cDNA clones were selected from 140 clones that were used for the chromosome mapping of *X. tropicalis* in our previous study (Uno *et al.*, 2012). These clones were isolated based on a web data catalog of the NIBB/NIG/NBRP *Xenopus laevis* EST project (XDB3, <http://xenopus.nibb.ac.jp/>). FISH mapping was performed as described previously (Matsuda and Chapman, 1995). DNA probes were labeled with biotin-16-dUTP (Roche Diagnostics, Basel, Switzerland) using a nick translation kit (Roche Diagnostics) following the manufacturer's instruction and ethanol precipitated with sonicated salmon sperm DNA and *Escherichia coli* transfer RNA. After hybridization, the hybridized probes were reacted with a goat anti-biotin antibody (Vector Laboratories, Burlingame, CA, USA) and then stained with Alexa Fluor 488 rabbit anti-goat IgG (H + L) conjugate (Molecular Probes, Life Technologies, Carlsbad, CA, USA). Chromosome slides were counterstained with 0.75 µg ml⁻¹ propidium iodide. To discriminate the Z and W chromosomes, a 15-kb genomic DNA fragment of the *DM-W* gene (Yoshimoto *et al.*, 2008) was hybridized to the chromosome slide, where the hybridization signal was detected on chromosome 3. The hybridized probe was removed from the slides by redensaturation in 70% formamide/4 × standard saline citrate at 70 °C for 2 min, and then the *DM-W* probe was hybridized with a 100-time volume of the sonicated whole genomic DNA of *X. laevis* to the same slide. After hybridization, slides were incubated with fluorescein isothiocyanate-avidin (Roche Diagnostics), and hybridization signals were observed.

RESULTS

High-resolution Hoechst-stained bands of *X. laevis* chromosomes were obtained using a replication banding method (Figure 1), and their ideograms were slightly modified from our previous one (Uno *et al.*, 2008). Based on our previous mapping data of 140 genes in *X. tropicalis* (Uno *et al.*, 2012), we selected 60 genes, which covered the entire chromosomal regions of 10 pairs of *X. tropicalis* chromosomes, for their comparative mapping to *X. laevis* chromosomes (XLA) (Figure 2 and Table 1). The identification of each chromosome and subchromosomal localization of the hybridization signals was performed using the Hoechst G-banded ideogram. Hybridization signals were detected on two pairs of homoeologous chromosomes for 83% of the cDNA clones (50/60) (Figure 3). For instance, seven genes (*KDM3A*, *ACSL1*, *PCDH10*, *EEF2*, *DMRT1*, *NF2* and *DEPDC1B*), which were mapped on *X. tropicalis* chromosome (XTR) 1, were all localized to XLA1 and XLA2, indicating that XLA1 and XLA2 are homoeologous. All the other homoeologous chromosome pairs of *X. laevis* and their homologies with *X. tropicalis* chromosomes were as follows: XTR2 was homologous to XLA3 and XLA8, XTR3 to XLA12 and XLA16, XTR4 to XLA13 and XLA17, XTR5 to XLA4 and XLA5, XTR6 to XLA6 and XLA9, XTR7 to XLA7 and XLA10 and XTR8 to XLA11 and XLA14. The genes on XTR9 and XTR10 were all localized to XLA15 and XLA18, suggesting that the homoeologous chromosome pair XLA15 and XLA18 was derived from a tandem fusion of XTR9 and XTR10 or that XTR9 and XTR10 are derivatives of a fission event that occurred in an original chromosome pair of homoeologous XLA15 and XLA18.

Genetic linkage has been highly conserved between the two species and also between homoeologous chromosome pairs of *X. laevis*. No interchromosomal rearrangements (reciprocal translocations) were detected, and gene orders were identical between homologous chromosomes of *X. laevis* and *X. tropicalis* and between *X. laevis* homoeologous chromosome pairs, except for intrachromosomal rearrangements (inversions) that were detected between XLA12 and XLA16, XLA11 and XLA14, and XTR10 and homoeologous XLA15

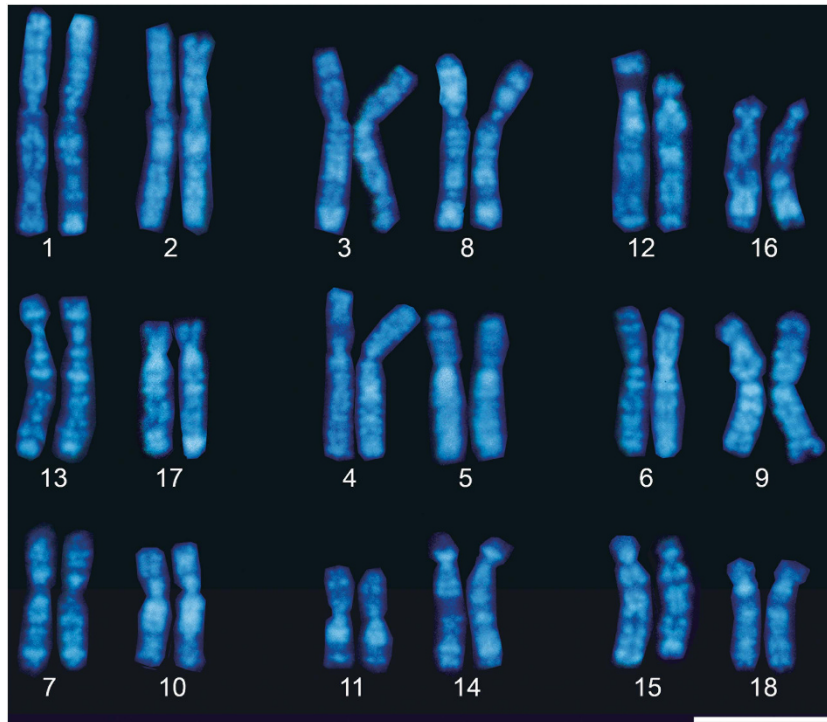


Figure 1 Hoechst-stained replication-banded karyotype of the female *X. laevis* (XLA). Each chromosome was numbered following our previous karyotypic study of *X. laevis* (Uno *et al.*, 2008). Chromosomes were grouped as homoeologous chromosome pairs (XLA1 + 2, 3 + 8, 12 + 16, 13 + 17, 4 + 5, 6 + 9, 7 + 10, 11 + 14 and 15 + 18) according to comparative gene mapping in this study (Figure 3). Small size variation of chromosomes between individuals was found for XLA12p (Uno *et al.*, 2008), which depends on the size differences of the 18S–28S ribosomal gene cluster on the short arm (data not shown). Scale bar = 10 μ m.

and XLA18 pairs. These results indicate that the genetic linkage of *X. tropicalis* chromosomes has been retained almost intact in *X. laevis* chromosomes with no interchromosomal translocations after WGD.

Five XTR2-linked genes (*POU2F1*, *EIF2S3*, *MDH2*, *LARP4* and *RAB6A*) were all localized to the homologous chromosomes of XLA3 and XLA8. XLA3 was identified as the Z and W sex chromosomes in our previous study (Yoshimoto *et al.*, 2008); however, no morphological differences have been found between the Z and W sex chromosomes (Schmid and Steinlein, 1991; Uno *et al.*, 2008). We identified the W chromosome by FISH mapping of the W-linked sex (ovary)-determining gene, *DM-W* (Yoshimoto *et al.*, 2008) (Figure 2m). None of the five genes on the Z or W chromosome differed in chromosomal locations and hybridization efficiencies (data not shown), indicating that structural differentiation hardly occurred between the Z and W chromosomes except for the W-specific region containing the *DM-W* gene.

DISCUSSION

In this study, we identified all nine quartets (the homoeologous chromosome groups) of *X. laevis* (XLA1 + 2, 3 + 8, 12 + 16, 13 + 17, 4 + 5, 6 + 9, 7 + 10, 11 + 14 and 15 + 18), whose genetic linkage has been highly conserved in *X. tropicalis*. Paleontological studies have suggested that *X. tropicalis* is a more ancient species than other extant polyploid *Xenopus* species, providing the possibility that the ancestral diploid *Xenopus* species had $2n=20$ chromosomes similar to *X. tropicalis* (Estes, 1975). Therefore, the chromosome number of *X. laevis* ($2n=36$) may have occurred as a result of allotetraploidization of the interspecific hybrid between two different species with $2n=18$ chromosomes, which was derived from the fusion of two

chromosome pairs of the ancestral diploid species with $2n=20$ (Schmid and Steinlein, 1991; Tymowska, 1991). Our results suggest that the ancestral bi-armed chromosome pair of homoeologous XLA15 and XLA18 may have been derived from the fusion of XTR9 and XTR10 in the ancestral species of *X. tropicalis*. The nine quartets identified in this study were not consistent with those determined by 5-bromo-2'-deoxyuridine/dT replication banding (Schmid and Steinlein, 1991) and cross-species chromosome hybridization with *X. tropicalis*-derived chromosome painting probes (Krylov *et al.*, 2010). Krylov *et al.* (2010) demonstrated that XLA11 + 14 and XLA15 + 18 were painted with XTR8 and XTR9 probes, respectively, and XLA14 and XLA18 were hybridized with XTR10 paint; however, XTR10 showed homology with XLA15 and XLA18 by comparative gene mapping in the present study. This discrepancy may be due to a difference in the numbering system of *X. laevis* chromosomes.

High rate of loss of duplicated genes (50–75%) after WGD has been reported in *X. laevis* using a large number of expressed sequence tags (ESTs) (20 223 ESTs reported by Hellsten *et al.* (2007) and 28 463 ESTs by Sémon and Wolfe (2008)), which is not so different from the rate of gene loss in teleost fishes after 3R-WGD (76–80%) (Jaillon *et al.*, 2004; Sémon and Wolfe, 2007), yeast after WGD (80%) (Kellis *et al.*, 2004), *Arabidopsis* (70%) (Bowers *et al.*, 2003) and maize (47%) (Woodhouse *et al.*, 2010). However, in this study, hybridization signals were detected on both homoeologous chromosomes for most clones (50 of 60 genes: 83%), which indicated that the loss of duplicated genes after WGD was much lower in *X. laevis* (17%). These results suggest that the loss of one copy of duplicated genes could not be determined accurately using only a partial collection of

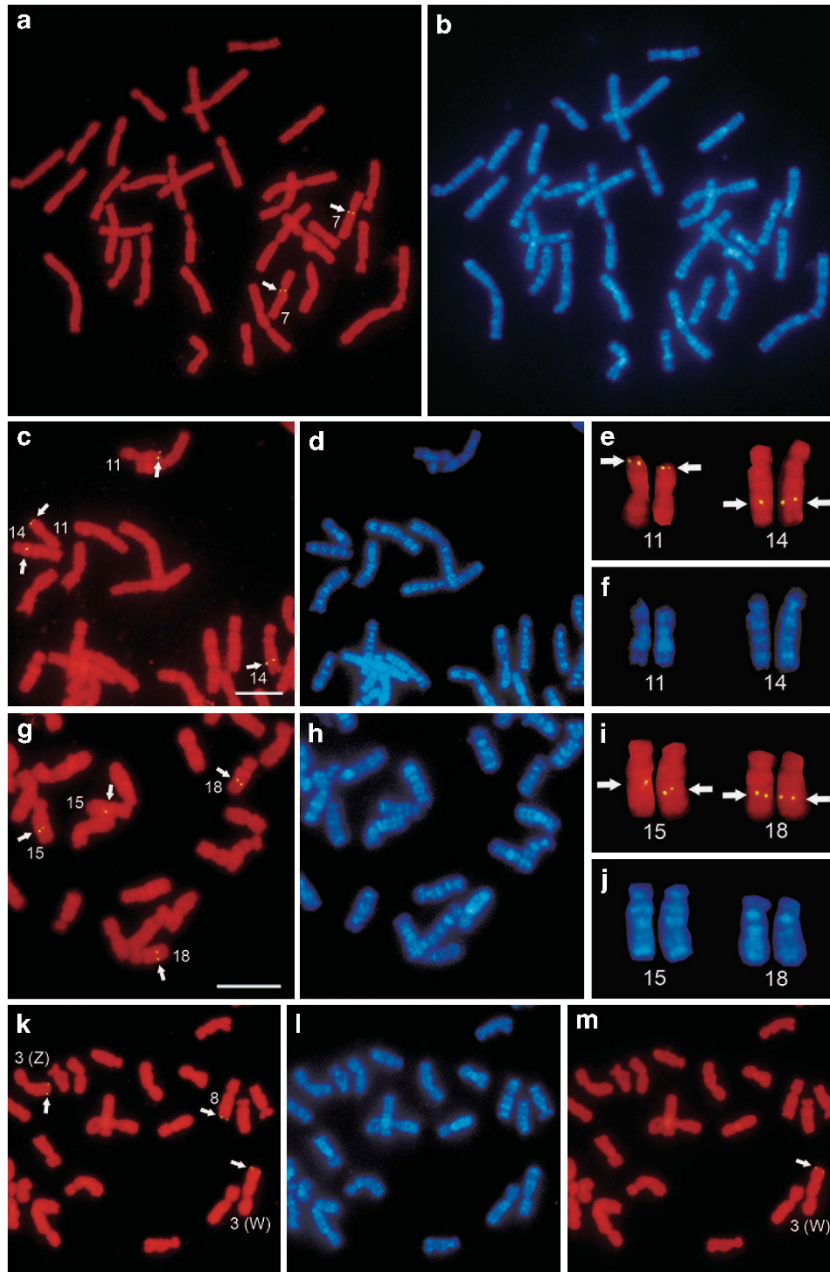


Figure 2 Chromosomal localization of DNA clones to metaphase chromosome spreads of the female *X. laevis*. FISH patterns of the cDNA clones of *TUBGCP2* on chromosome 7p (a), *ACTN1* on chromosomes 11p and 14q (c, e), *FNI* on chromosomes 15q and 18q (g, i) and *RAB6A* on chromosomes 3q and 8q (k) on propidium iodide-stained metaphase chromosome spreads. Arrows indicate hybridization signals. The FISH pattern of a genomic DNA fragment of the *DM-W* gene on chromosomes 3q (m) on the same metaphase spread that was used for mapping of the *RAB6A* gene (k). The *DM-W* probe was hybridized to the metaphase spread after the *RAB6A* probe was removed. The Z and W sex chromosomes were precisely identified by FISH with the *DM-W* probe. Hoechst-stained patterns of the propidium iodide-stained chromosomes in panels a, c, e, g, i and k are shown in panels b, d, f, h, j and l, respectively. Scale bars = 10 μ m.

X. laevis gene ESTs (Hellsten *et al.*, 2007). Analyses using both genome sequences and chromosome mapping of a large number of transcribed genes are needed to more accurately estimate the frequency of gene loss in *X. laevis* after WGD.

Our comparative maps of functional genes between *X. tropicalis* and *X. laevis* demonstrated no evidence of interchromosomal rearrangements between two species and revealed that genetic linkage has been highly conserved between *X. tropicalis* and *X. laevis* except

for inversions between XTR10 and homoeologous XLA15 and XLA18 pairs. The gene orders on *X. tropicalis* chromosomes have also been highly conserved in two homoeologous pairs of *X. laevis* chromosomes except for inversions between XLA12 and XLA16 and between XLA11 and XLA14. According to the gene orders of XTR3 and XTR8, which are considered to be the ancestral types of homoeologous XLA12 and XLA16 pairs and XLA11 and XLA14 pairs, respectively, a large paracentric inversion may have occurred in the long arm of

Table 1 List of 60 genes localized to *X. laevis* chromosomes

Gene symbol ^a	Clone no. ^b or accession no.	Chromosomal location			
		<i>X. laevis</i>	<i>X. tropicalis</i> ^c	Chicken ^d	Human ^d
<i>KDM3A</i>	XL413k20ex	1p, 2p	1p	4q	2p11.2
<i>ACSL1</i>	XL318g05ex	1p, 2p	1p	4q	4q34–q35
<i>PCDH10</i>	XL003c24	1p, 2p	1p	4q	4q28.3
<i>EEF2</i>	XL470i07ex	1q, 2q	1q	28	19pter–q12
<i>DMRT1</i> ^e	AB201112	1q, 2q	1q	Zq	9p24.3
<i>NF2</i>	XL085b04	1q, 2q	1q	15	22q12.2
<i>DEPDC1B</i>	XL220a24	1q, 2q	1q	Zp	5q12.1
<i>POU2F1</i>	XL164e23	3p, 8p	2p	1q	1q22–q23
<i>EIF2S3</i>	XL408e08ex	3p, 8p	2p	1q	Xp22.2–p22.1
<i>MDH2</i>	XL165g11	3q, 8q	2q	19	7cen–q22
<i>LARP4</i>	XL014a12	3q, 8q	2q	un	12q13.12
<i>RAB6A</i>	XL038d18	3q, 8q	2q	1q	11q13.3
<i>PPFIBP1</i>	XL062p09	12p, 16p	3p	1p	12p11.23–p11.22
<i>IK</i>	XL168o22	12q	3q	13	5q31.3
<i>CSNK1A1</i>	XL221g09ex	12q, 16q	3q	13	5q32
<i>NET1</i>	XL227m13ex	12q, 16q	3q	1p	10p15
<i>CYP19A1</i> ^e	BC079750	12q	3q	10	15q21.1
<i>XPO7</i>	XL214i11	12q, 16q	3q	22	8p21
<i>WT1</i> ^e	D82051	13p, 17p	4p	5p	11p13
<i>EDC4</i>	XL473f03ex	13p, 17p	4p	11	16q22.1
<i>KARS</i>	XL480m02ex	13q, 17q	4q	11	16q23–q24
<i>UAP1</i>	XL086p04	13q, 17q	4q	8p	1q23.3
<i>ALAS1</i>	XL051o04	13q, 17q	4q	12	3p21.1
<i>NWL</i>	XL479c13ex	4p	5p	3q	1q41–q42.2
<i>XPO1</i>	XL294p07ex	4p, 5p	5p	3p	2p16
<i>CEBPZ</i>	XL039i04	4p, 5p	5p	3q	2p22.2
<i>SLC2A12</i>	XL036o21	4q	5q	3q	6q23.2
<i>TRIP12</i>	XL204i01	4q, 5q	5q	9	2q36.3
<i>GATA4</i>	XL039m17	4q, 5q	5q	3q	8p23.1–p22
<i>ABCF2</i>	XL012i18	6p, 9p	6p	2p	7q36
<i>WAC</i>	XL075d07	6p, 9p	6p	2p	10p11.2
<i>CTNNB1</i>	XL480g03ex	6p, 9p	6p	2p	3p21
<i>APCDD1</i>	XL055h12	6q, 9q	6q	2q	18p11.22
<i>EEF1D</i>	XL013c21	6q, 9q	6q	2q	8q24.3
<i>SEC23IP</i>	XL151i10	7p, 10p	7p	6	10q25–q26
<i>ZRANB1</i>	XL027a17	7p, 10p	7p	6	10q26.13
<i>CYP17A1</i> ^e	AF325435	7p	7p	6	10q24
<i>GOT1</i>	XL151j11	10p	7p	6	10q24
<i>TUBGCP2</i>	XL008b12	7p	7p	6	10q26.3
<i>SLC37A2</i>	XL082p15	7q, 10q	7q	24	11q24.2
<i>ZW10</i>	XL046i12	7q, 10q	7q	24	11q23.2
<i>DDOST</i>	XL005p17	7q, 10q	7q	21	1p36.1
<i>GSN</i>	XL300b05ex	11p, 14p	8p	17	9q33
<i>NR5A1</i> ^e	AB273177	14p	8q	17	9q33
<i>AR</i> ^e	U67129	14q	8q	4p	Xq11.2–q12
<i>FMR1</i>	XL022n18	11q, 14q	8q	4p	Xq27.3
<i>SOX3</i> ^e	— ^f	11q	8q	4p	Xq27.1
<i>PAPOLA</i>	XL035j18	11p, 14q	8q	5q	14q32.31
<i>ACTN1</i>	XL286g19ex	11p, 14q	8q	5q	14q22–q24
<i>COPA</i>	XL263c24ex	11q, 14q	8q	25	1q23–q25
<i>STAU1</i>	XL175g01	15p, 18p	10q	20	20q13.1
<i>BMP7</i>	XL056i08	15p, 18p	10p	20	20q13
<i>EFTUD2</i>	XL300a14ex	15p, 18p	10p	27	17q21.31
<i>SOX9</i> ^e	AB439583	15p, 18p	10q	18	17q24.3–q25.1
<i>NARF</i>	XL210h06	15q, 18q	10q	18	17q25.3
<i>ZEB2</i>	XL207j23	15q, 18q	9q	7q	2q22
<i>FN1</i>	XL338p06ex	15q, 18q	9q	7p	2q34

Table 1 (Continued)

Gene symbol ^a	Clone no. ^b or accession no.	Chromosomal location			
		<i>X. laevis</i>	<i>X. tropicalis</i> ^c	Chicken ^d	Human ^d
<i>NDUFS1</i>	XL034k12	15q, 18q	9q	7q	2q33–q34
<i>NOMO3</i>	XL271k19ex	15q, 18q	9p	14	16p13
<i>UQCRC2</i>	XL016d01	15q, 18q	9p	14	16p12

Abbreviations: EST, expressed sequence tag; un, unknown chromosomal location.

^aHuman gene symbol.

^bClone numbers of *X. laevis* EST clones used for mapping, which were selected from a web data catalog of the NIBB/NIG/NBRP *Xenopus laevis* EST project (XDB3, <http://xenopus.nibb.ac.jp/>) based on the *X. tropicalis* chromosome map described by Uno et al. (2012). Fragment sizes of all *X. laevis* EST clones were > 1.5 kb.

^cChromosomal locations for *X. tropicalis* taken from previous studies (Uno et al., 2008, 2012).

^dChromosomal locations of chicken and human homologs searched with the BLATN program of the Ensembl (<http://www.ensembl.org/index.html>) and/or the blastn program of NCBI (<http://www.ncbi.nlm.nih.gov/>) (searched in September 2012).

^eGenes mapped in our previous studies (Uno et al., 2008; Yoshimoto et al., 2008).

^fThe cDNA fragment of *SOX3* was isolated by Koyano et al. (1997).

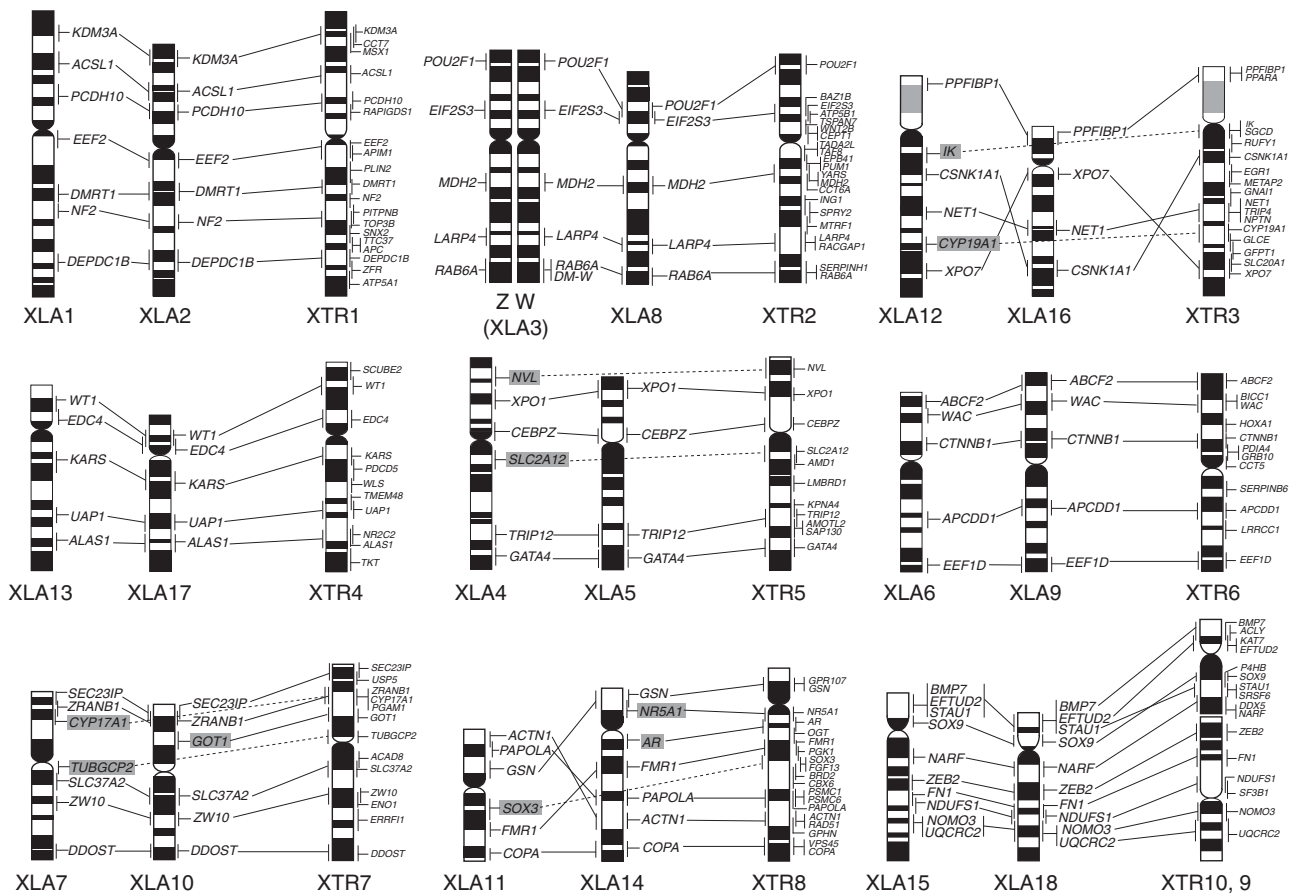


Figure 3 Comparative cytogenetic map of *X. laevis* (XLA) and *X. tropicalis* (XTR). The chromosomal locations of *AR*, *CYP17A1*, *CYP19A1*, *DMRT1*, *DM-W*, *NR5A1*, *SOX3*, *SOX9* and *WT1* in *X. laevis* and the cytogenetic map of *X. tropicalis* were taken from our previous studies (Uno et al., 2008; Yoshimoto et al., 2008). XTR9 is inverted to facilitate a comparison of the gene order with those of XLA15 and XLA18. Gene symbols enclosed in gray boxes indicate the genes that were located on only one pair of homoeologous chromosomes. The small size variations of XLA12p and XTR3p between individuals are represented as gray-colored bands in the ideogram (Figure 1; Uno et al., 2008).

XLA16, with at least two inversions containing a pericentric inversion in XLA11. The gene orders of *SOX9* and *STAU1* and their locations in XTR10q are different from those in XLA15p and XLA18p, suggesting that a pericentric inversion event occurred in the proximal region of the ancestral chromosome of homoeologous XLA15 and XLA18 pairs after the fusion between XTR9 and XTR10 of the ancestral diploid species such as *X. tropicalis*. These results provide us with the

following two possibilities: (1) gene orders were different in some chromosomes between the ancestral diploid species before WGD occurred; and (2) intrachromosomal rearrangements occurred in one of the homoeologous chromosome pairs after WGD. In either case, the present results indicate that inter- and/or intrachromosomal rearrangements occurred much less in the *X. laevis* lineage for 21–40 million years after allotetraploidization than in teleost fishes

(medaka fish, *Takifugu*, *Tetraodon* and zebrafish) (Kasahara *et al.*, 2007; Sémon and Wolfe, 2007), salmonid fishes (Danzmann *et al.*, 2008; Guyomard *et al.*, 2012), plants (*Arabidopsis*, rice and *Populus*) (Pontes *et al.*, 2004; Simillion *et al.*, 2004; Yu *et al.*, 2005; Tuskan *et al.*, 2006) and yeast (Seoighe and Wolfe, 1998; Fischer *et al.*, 2006), in which chromosome rearrangements frequently occurred after WGDs.

Neither multivalent association nor pairing between homoeologous chromosomes was observed in meiosis in *X. laevis* (Tymowska, 1991), which implies that an ancient allotetraploid genome of *X. laevis* returned to a genetically diploid state through the process of diploidization of allopolyploid genomes. The present results consequently suggest that the loss of duplicated genes and chromosomal rearrangements may not have been essential for diploidization of the allotetraploid genome after WGD in *X. laevis*. In high-polyploid *Xenopus* species with 72 and 108 chromosomes, a few multivalents have been observed at the first meiotic metaphase of some spermatocytes in *X. vestitus*, *X. wittei*, *X. amieti* ($2n=72$) and *X. ruwenzoriensis* ($2n=108$), suggesting that higher polyploidization may have been the most recent event in the *Xenopus* lineage and diploidization has yet not been fully accomplished (Tymowska and Fischberg, 1973; Tymowska, 1991).

In this study, we constructed a comparative cytogenetic map of the allotetraploid species *X. laevis*. The genetic linkage and order of genes have been highly conserved between *X. tropicalis* and *X. laevis* chromosomes and also between homoeologous chromosomes of *X. laevis*, and WGD-derived duplicated genes have been mostly retained in homoeologous chromosomes of *X. laevis*. These results collectively suggest that inter- and intrachromosomal rearrangements and loss of duplicated genes have occurred less frequently in the lineage of *X. laevis* after allotetraploidization. Whole-genome sequencing of *X. laevis* and comparative chromosome mapping of other polyploid *Xenopus* species help us to better understand the process and mechanism of genome evolution after WGD.

DATA ARCHIVING

There were no data to deposit.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This study was supported by a Grant-in-Aid for Scientific Research on Innovative Areas (No. 23113004) and a Grant-in-Aid for Scientific Research (B) (No. 22370081) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

- Allendorf FW, Thorgaard GH (1984). Tetraploidy and the evolution of salmonid fishes. In: Turner BJ (ed.) *Evolutionary Genetics of Fishes*. Plenum Press: New York, pp 1–53.
- Bisbee CA, Baker MA, Wilson AC, Haji-Azumi I, Fischberg M (1977). Albumin phylogeny for clawed frogs (*Xenopus*). *Science* **195**: 785–787.
- Bowers JE, Chapman BA, Rong J, Paterson AH (2003). Unravelling angiosperm genome evolution by phylogenetic analysis of chromosomal duplication events. *Nature* **422**: 433–438.
- Chain FJJ, Evans BJ (2006). Multiple mechanisms promote the retained expression of gene duplicates in the tetraploid frog *Xenopus laevis*. *PLoS Genet* **2**: e56.
- Danzmann RG, Davidson EA, Ferguson MM, Gharbi K, Koop BF, Hoyheim B *et al.* (2008). Distribution of ancestral proto-Actinopterygian chromosome arms within the genomes of 4R-derivative salmonid fishes (Rainbow trout and Atlantic salmon). *BMC Genomics* **9**: 557.
- Estes R (1975). *Xenopus* from the Palaeocene of Brazil and its zoogeographic importance. *Nature* **254**: 48–50.
- Evans BJ, Kelley DB, Tinsley RC, Melnick DJ, Cannatella DC (2004). A mitochondrial DNA phylogeny of African clawed frogs: phylogeography and implications for polyploid evolution. *Mol Phylogenet Evol* **33**: 197–213.

- Evans BJ (2008). Genome evolution and speciation genetics of clawed frogs (*Xenopus* and *Silurana*). *Front Biosci* **13**: 4687–4706.
- Fischer G, Rocha EP, Brunet F, Vergassola M, Dujon B (2006). Highly variable rates of genome rearrangements between hemiascomycetous yeast lineages. *PLoS Genet* **2**: e32.
- Guyomard R, Boussaha M, Krieg F, Hervet C, Quillet E (2012). A synthetic rainbow trout linkage map provides new insights into the salmonid whole genome duplication and the conservation of synteny among teleosts. *BMC Genet* **13**: 15.
- Hellsten U, Khokha MK, Grammer TC, Harland RM, Richardson P, Rokhsar DS (2007). Accelerated gene evolution and subfunctionalization in the pseudotetraploid frog *Xenopus laevis*. *BMC Biol* **5**: 31.
- Hellsten U, Harland RM, Gilchrist MJ, Hendrix D, Jurka J, Kapitonov V *et al.* (2010). The genome of the western clawed frog *Xenopus tropicalis*. *Science* **328**: 633–636.
- Hughes MK, Hughes AL (1993). Evolution of duplicate genes in a tetraploid animal, *Xenopus laevis*. *Mol Biol Evol* **10**: 1360–1369.
- International Human Genome Sequencing Consortium (2001). Initial sequencing and analysis of the human genome. *Nature* **409**: 860–921.
- Jaillon O, Aury JM, Brunet F, Petit JL, Stange-Thomann N, Mauceli E *et al.* (2004). Genome duplication in the teleost fish *Tetraodon nigroviridis* reveals the early vertebrate proto-karyotype. *Nature* **431**: 946–957.
- Kasahara M (2007). The 2R hypothesis: an update. *Curr Opin Immunol* **19**: 547–552.
- Kasahara M, Naruse K, Sasaki S, Nakatani Y, Qu W, Ahsan B *et al.* (2007). The medaka draft genome and insights into vertebrate genome evolution. *Nature* **447**: 714–719.
- Kellis M, Birren BW, Lander ES (2004). Proof and evolutionary analysis of ancient genome duplication in the yeast *Saccharomyces cerevisiae*. *Nature* **428**: 617–624.
- Kobel HR (1996). Allopolyploid speciation. In: Tinsley RC, Kobel HR (eds) *The Biology of Xenopus*. Clarendon Press: Oxford, pp 391–401.
- Koyano S, Ito M, Takamatsu N, Takiguchi S, Shiba T (1997). The *Xenopus Sox3* gene expressed in oocytes of early stages. *Gene* **188**: 101–107.
- Krylov V, Kubickova S, Rubes J, Macha J, Tlapakova T, Seifertova E *et al.* (2010). Preparation of *Xenopus tropicalis* whole chromosome painting probes using laser microdissection and reconstruction of *X. laevis* tetraploid karyotype by Zoo-FISH. *Chromosome Res* **18**: 431–439.
- Matsuda Y, Chapman VM (1995). Application of fluorescence *in situ* hybridization in genome analysis of the mouse. *Electrophoresis* **16**: 261–272.
- Morin RD, Chang E, Petrescu A, Liao N, Griffith M, Chow W *et al.* (2006). Sequencing and analysis of 10,967 full-length cDNA clones from *Xenopus laevis* and *Xenopus tropicalis* reveals post-tetraploidization transcriptome remodeling. *Genome Res* **16**: 796–803.
- Ohno S (1970). *Evolution by Gene Duplication*. Springer-Verlag: Berlin.
- Otto SP (2007). The evolutionary consequences of polyploidy. *Cell* **131**: 452–462.
- Pontes O, Neves N, Silva M, Lewis MS, Madlung A, Comai L *et al.* (2004). Chromosomal locus rearrangements are a rapid response to formation of the allotetraploid *Arabidopsis suecica* genome. *Proc Natl Acad Sci USA* **101**: 18240–18245.
- Putnam NH, Butts T, Ferrier DEK, Furlong RF, Hellsten U, Kawashima T *et al.* (2008). The amphioxus genome and the evolution of the chordate karyotype. *Nature* **453**: 1064–1071.
- Sato Y, Hashiguchi Y, Nishida M (2009). Temporal pattern of loss/persistence of duplicate genes involved in signal transduction and metabolic pathways after teleost-specific genome duplication. *BMC Evol Biol* **9**: 127.
- Schmid M, Steinlein C (1991). Chromosome banding in Amphibia. XVI. High-resolution replication banding patterns in *Xenopus laevis*. *Chromosoma* **101**: 123–132.
- Sémon M, Wolfe KH (2007). Rearrangement rate following the whole-genome duplication in teleosts. *Mol Biol Evol* **24**: 860–867.
- Sémon M, Wolfe KH (2008). Preferential subfunctionalization of slow-evolving genes after allopolyploidization in *Xenopus laevis*. *Proc Natl Acad Sci USA* **105**: 8333–8338.
- Seoighe C, Wolfe KH (1998). Extent of genomic rearrangement after genome duplication in yeast. *Proc Natl Acad Sci USA* **95**: 4447–4452.
- Simillion C, Vandepoele K, Saeys Y, Van de Peer Y (2004). Building genomic profiles for uncovering segmental homology in the twilight zone. *Genome Res* **14**: 1095–1106.
- Song K, Lu P, Tang K, Osborn TC (1995). Rapid genome change in synthetic polyploids of Brassica and its implications for polyploid evolution. *Proc Natl Acad Sci USA* **92**: 7719–7723.
- Tuskan G, Difazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U *et al.* (2006). The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science* **313**: 1596–1604.
- Tymowska J (1991). Polyploidy and cytogenetic variation in frogs of the genus *Xenopus*. In: Green DS, Sessions SK (eds) *Amphibian Cytogenetics and Evolution*. Academic Press: San Diego, pp 259–297.
- Tymowska J, Fischberg M (1973). Chromosome complements of the genus *Xenopus*. *Chromosoma* **44**: 335–342.
- Uno Y, Nishida C, Yoshimoto S, Ito M, Oshima Y, Yokoyama S *et al.* (2008). Diversity in the origins of sex chromosomes in anurans inferred from comparative mapping of sexual differentiation genes for three species of the Raninae and Xenopodinae. *Chromosome Res* **16**: 999–1011.
- Uno Y, Nishida C, Tarui H, Ishishita S, Takagi C, Nishimura O *et al.* (2012). Inference of the protokaryotypes of amniotes and tetrapods and the evolutionary processes of microchromosomes from comparative gene mapping. *PLoS One* **7**: e53027.
- Woodhouse MR, Schnable JC, Pedersen BS, Lyons E, Lisch D, Subramaniam S *et al.* (2010). Following tetraploidy in maize, a short deletion mechanism removed genes preferentially from one of the two homologs. *PLoS Biol* **8**: e1000409.
- Yoshimoto S, Okada E, Umemoto H, Tamura K, Uno Y, Nishida-Umehara C *et al.* (2008). A W-linked DM-domain gene, *DM-W*, participates in primary ovary development in *Xenopus laevis*. *Proc Natl Acad Sci USA* **105**: 2469–2474.
- Yu J, Wang J, Lin W, Li S, Li H, Zhou J *et al.* (2005). The genomes of *Oryza sativa*: a history of duplications. *PLoS Biol* **3**: e38.