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A New Nomenclature of *Xenopus laevis* Chromosomes Based on the Phylogenetic Relationship to *Silurana/Xenopus tropicalis*

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BrdU replication banding pattern · Homoeologous chromosomes · Nomenclature · *Xenopus laevis* · *Xenopus tropicalis*

Abstract
Xenopus laevis (XLA) is an allotetraploid species which appears to have undergone whole-genome duplication after the interspecific hybridization of 2 diploid species closely related to *Silurana/Xenopus tropicalis* (XTR). Previous cDNA fluorescence in situ hybridization (FISH) experiments have identified 9 sets of homoeologous chromosomes in *X. laevis*, in which 8 sets correspond to chromosomes 1–8 of *X. tropicalis* (XTR1–XTR8), and the last set corresponds to a fusion of XTR9 and XTR10. In addition, recent *X. laevis* genome sequencing and BAC-FISH experiments support this physiological relationship and show no gross chromosome translocation in the *X. laevis* karyotype. Therefore, for the benefit of both comparative cytogenetics and genome research, we here propose a new chromosome nomenclature for *X. laevis* based on the phylogenetic relationship and chromosome length, i.e. XLA1L, XLA1S, XLA2L, XLA2S, and so on, in which the numbering of XLA chromosomes corresponds to that in *X. tropicalis* and the postfixes 'L' and 'S' stand for 'long' and 'short' chromosomes in the homoeologous pairs, which can be distinguished cytologically by their relative size. The last chromosome set is named XLA9L and XLA9S, in which XLA9 corresponds to both XTR9 and XTR10, and hence, to emphasize the phylogenetic relationship to *X. tropicalis*, XLA9_10L and XLA9_10S are also used as synonyms.

Xenopus laevis (XLA) is allotetraploid and has 36 chromosomes (4x = 2n = 36), consisting of 2 sets of 18 chromosomes, which are cytologically distinguished [Wickbom, 1945; Weiler and Ohno, 1962; Morescalchi, 1963; Tymowska and Kobel, 1972]. Its relative *X. tropicalis*

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(XTR) is diploid and has 20 chromosomes (2x = 2n = 20). The conventionally stained karyotypes of the genus *Xenopus*, including those of *X. laevis* and various other subspecies, were presented and reviewed in detail by Tymowska [1977, 1991]. A high-resolution replication-banding karyotype of *X. laevis* was first published by Schmid and Steinlein [1991]. In that study, the chromosomes were arranged according to the karyotype system established by Tymowska and Kobel [1972] and Tymowska [1991], and the identification of homoeologous chromosomes (quartets; each quartet consists of a homoeologous set of 2 homologous chromosomes) was attempted on the basis of similarities in morphology and banding patterns.

In recent years, detailed analyses of the homoeologous chromosomes of *X. laevis* have been conducted at the molecular level. To investigate the process of genomic and chromosomal reorganization in *X. laevis* after allotetraploidization, chromosome painting of *X. laevis* was carried out using DNA probes made from microdissected *X. tropicalis* chromosomes [Krylov et al., 2010]. Subsequently, comparative cytogenetic mapping of functional genes between *X. tropicalis* and *X. laevis* was performed by chromosome fluorescence in situ hybridization (FISH) using 60 cDNA clones derived from *X. laevis*, which covered all *X. tropicalis* chromosomes [Uno et al., 2013]. By these molecular cytogenetic analyses of chromosome homoeologies between the 2 species, all 9 pairs of homoeologous chromosomes were precisely identified at the molecular level. In parallel, the *X. laevis* genome project was progressed by the US-Japan *X. laevis* Genome Project Consortium, further confirming the data of Uno et al. [2013] on the homoeologous *X. laevis* chromosomes (quartets). Therefore, this is the time to propose a new nomenclature of *X. laevis* chromosomes based on both molecular and cytogenetic data, which easily allows identifying the phylogenetic relationship of the homoeologous chromosome pairs of *X. laevis* and *X. tropicalis*. In this new nomenclature, the numbering of *X. laevis* chromosomes corresponds to that in *X. tropicalis*, and the postfixes 'L' and 'S' stand for 'long' and 'short' chromosomes in the homoeologous pairs of *X. laevis*. We also propose a standard idiogram of BrdU-banded *X. laevis* chromosomes to indicate the position of the genes mapped by in situ hybridization.

Materials and Methods

Late-replication banding with 5-bromodeoxyuridine (BrdU) and Hoechst 33258 staining of *X. laevis* chromosomes was performed according to Uno et al. [2008, 2013]. For this chromosome banding, fibroblast cells derived from heart or kidney tissues were cultured at 26°C and treated in the late S-phase with 25 μg/ml BrdU for 6 h before harvesting. Chromosome preparations were made following a standard air-drying method. After staining with Hoechst 33258 (1 μg/ml) for 5 min, the slides were heated to 65°C for 3 min on a hotplate and then exposed to UV light for an additional 5–6 min at 65°C.

Chromosome lengths were measured using 5 metaphase chromosome spreads from 3 females, which were prepared as described above. Each chromosome or arm length was normalized by the total chromosome length of the respective metaphase.

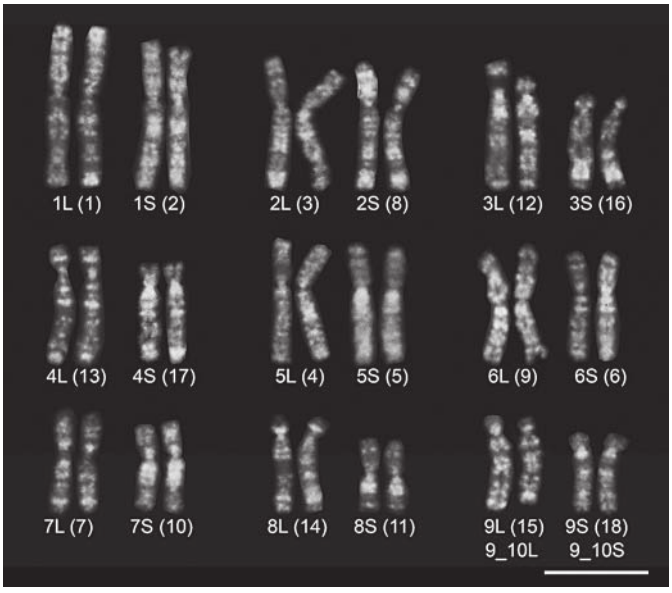

Fig. 1. Hoechst 33258-stained late-replication-banded karyotype of *X. laevis* chromosomes from cultured fibroblasts. The lightly stained bands correspond to G-positive bands produced by GTG-banding (G-bands by trypsin using Giemsa) in mammalian and avian chromosomes. The homoeologous chromosomes are arranged in quartets and numbered according to the new nomenclature system. The numbers in parentheses correspond to the chromosome numbers used by Tymowska and Kobel [1972], Tymowska [1991], Schmid and Steinlein [1991], and Uno et al. [2013]. Scale bar = 10 μm. Image modified from Uno et al. [2013].

Results and Discussion

Recent *X. laevis* genome analyses and chromosome FISH experiments using about 800 BAC clones confirmed all 9 quartets of chromosomes, which were previously identified by cDNA FISH [Uno et al., 2013], and also demonstrated that there are no gross interchromosomal rearrangements [unpublished data of the US-Japan *X. laevis* Genome Project Consortium co-led by D.S.R. and M.T.]. Figure 1 shows the BrdU/Hoechst late-replication

Table 1. Numbering system of *X. tropicalis* and *X. laevis* chromosomes based on their homoeologies, chromosome lengths, centromere indices, and average length ratios of short to long chromosomes in each of the 9 chromosome quartets of *X. laevis*

Chromosome numbering system			Chromosome length (p + q arm), %[c]	Centromere index, %[d]	S/L length ratio ± SD[e]
X. tropicalis[a]	*X. laevis* new	*X. laevis* old[b]			
XTR1	XLA1L	1	7.64 (3.38+4.26)	44.2	0.91±0.01
	XLA1S	2	6.96 (2.80+4.16)	40.2	
XTR2	XLA2L	3	6.68 (2.61+4.07)	39.1	0.94±0.03
	XLA2S	8	6.28 (2.15+4.13)	34.2	
XTR3	XLA3L	12	6.69 (1.70+4.99)	25.4	0.75±0.01
	XLA3S	16	5.05 (1.16+3.89)	23.0	
XTR4	XLA4L	13	5.60 (1.36+4.24)	24.3	0.86±0.03
	XLA4S	17	4.79 (1.20+3.59)	25.1	
XTR5	XLA5L	4	5.92 (2.28+3.64)	38.5	0.92±0.04
	XLA5S	5	5.46 (1.89+3.57)	34.6	
XTR6	XLA6L	9	5.91 (2.74+3.17)	46.4	0.90±0.03
	XLA6S	6	5.34 (2.12+3.22)	39.7	
XTR7	XLA7L	7	4.88 (2.08+2.80)	42.6	0.90±0.04
	XLA7S	10	4.37 (1.92+2.45)	43.9	
XTR8	XLA8L	14	5.17 (1.28+3.89)	24.8	0.75±0.05
	XLA8S	11	3.87 (1.79+2.08)	46.3	
XTR9 + XTR10	XLA9L (9_10L)	15	5.00 (1.10+3.90)	22.0	0.88±0.03
	XLA9S (9_10S)	18	4.41 (1.11+3.30)	25.2	

[a] Numbering used by Khohka et al. [2009]. [b] Numbering used by Tymowska and Kobel [1972], Tymowska [1991], Schmid and Steinlein [1991], and Uno et al. [2013]. [c] Ratio of individual chromosome length to the total length of all chromosomes. [d] Ratio of the short arm length to the total chromosome length. [e] Average ratio calculated for each of the 4 chromosomes in the quartets.

banding patterns that are unique to each *X. laevis* chromosome and have been used for identification of chromosomes and physical assignment of loci on the chromosomes. Based on this chromosome identification, we measured the lengths of short and long chromosome arms and compared the total lengths of the 2 homoeologous chromosome sets in each quartet. Table 1 shows that the length ratio of shorter versus longer chromosome of each homoeologous pair ranges from 0.75 ± 0.01 to 0.94 ± 0.03. Therefore, in each of the quartets, homoeologous chromosome sets can be distinguished based on their overall lengths in addition to their specific banding patterns, with one chromosome pair being long (L) and the other being short (S) (fig. 1; table 1).

Based on the highly conserved synteny of *X. laevis* and *X. tropicalis* chromosomes [Uno et al., 2013], their unique chromosome banding patterns and length differences between homoeologous chromosomes, we propose a new nomenclature of *X. laevis* chromosomes that reflects the phylogenetic relationship with *X. tropicalis* chromosomes as shown in figures 1 and 2. In this nomenclature system, we used *X. tropicalis* as the reference, because it is the extant diploid species closest to *X. laevis*, in which its 10 pairs of chromosomes are traditionally arranged by decreasing lengths and numbered consecutively from 1 through 10 (XTR1–XTR10) [Khokha et al., 2009]. We designated *X. laevis* chromosomes as XLA1, XLA2, and so on for each homoeologous pair, where the XLA1 pair, for example, phylogenetically corresponds to XTR1. The designations L or S were further added to the chromosome numbers as postfixes (XLA1L, XLA1S, XLA2L, XLA2S, and so on) to distinguish the long and short chromosomes of a homoeologous set in each quartet (fig. 2; table 1).

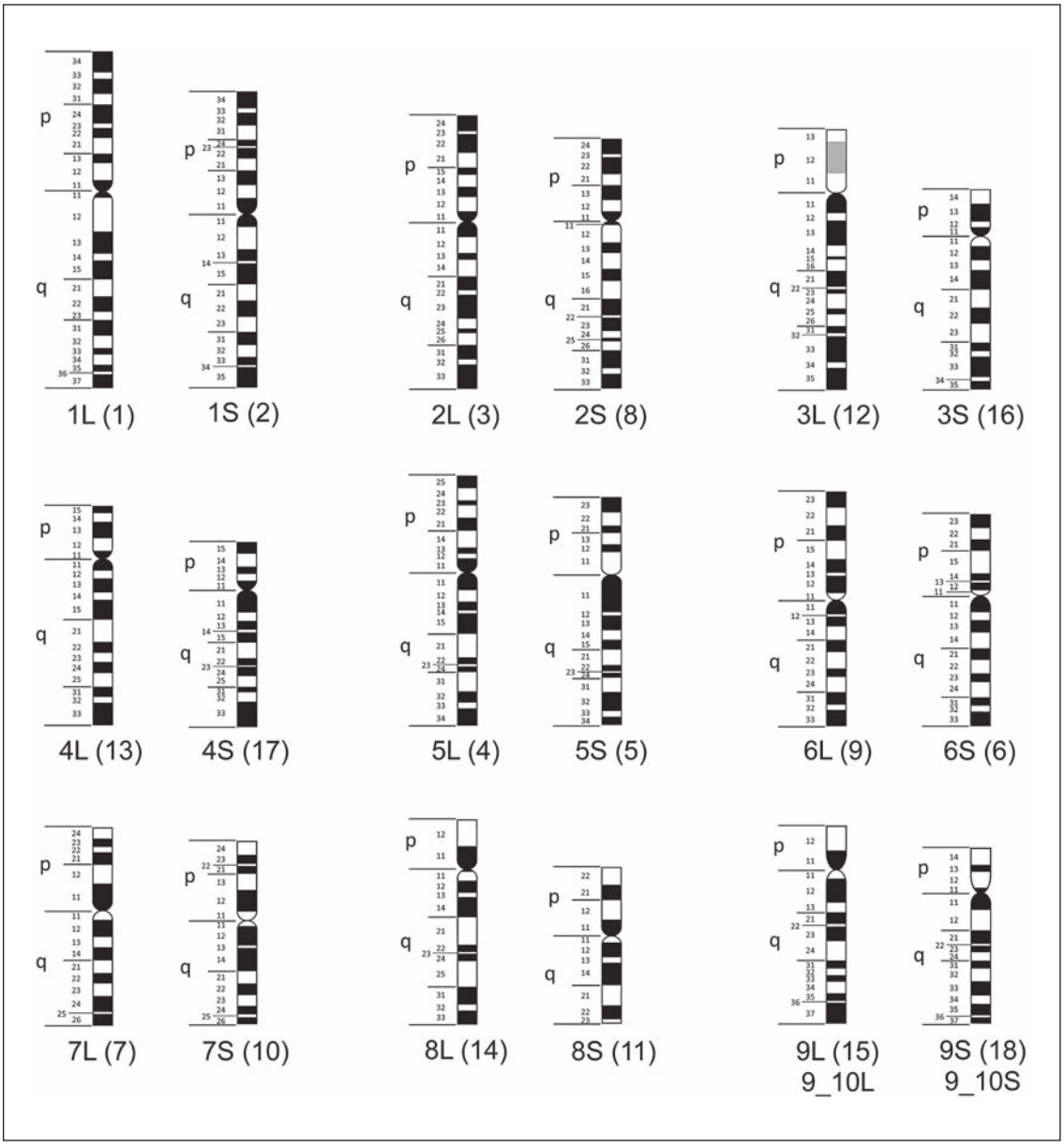

Fig. 2. Idiogram of the late-replication banding patterns of *X. laevis* chromosomes stained with Hoechst 33258. The chromosomes are numbered according to the new nomenclature system. The numbers in parentheses correspond to the chromosome numbers used by Uno et al. [2013]. The grey band (p12) in chromosome 3L is the nucleolus organizer region.

XTR1 to XTR8 phylogenetically correspond to pairs XLA1 to XLA8, respectively, with the same consecutive numbering. However, the last 2 *X. tropicalis* chromosomes (XTR9 and XTR10) are homoeologous to the single 9th chromosome pair of *X. laevis*. It is likely that these chromosomes derived from a fusion event of 2 chromosomes that correspond to XTR9 and XTR10 in the ancestral lineage of *X. laevis* [unpublished data of the US-Japan *X. laevis* Genome Project Consortium]. Although each of these chromosomes corresponds to XTR9 and XTR10, we designated them as XLA9L and XLA9S. Alternatively, the 9th *X. laevis* chromosomes can also be referred to as XLA9_10L and XLA9_10S as synonyms of XLA9L and XLA9S, respectively, because this designation is easily un-

derstandable and takes into account the chromosome rearrangement that occurred in karyotype evolution (tandem fusion of 2 chromosomes followed by centromere repositioning). Cytogeneticists might prefer XLA9L and XLA9S to prevent the confusion due to yet unidentified and undetectable chromosomal rearrangements that may have occurred in the ancestral genomes. However, the designation XLA9_10L and XLA9_10S is convenient for comparative genomics of *X. tropicalis* and *X. laevis,* which are used extensively as model organisms for biomedical research where easy direct genome/chromosome comparisons between the 2 species are extremely valuable. However, this system should be applied only to *Xenopus* and its closely related species but not to any other far-related species beyond Xenopodinae whose chromosomes might have been rearranged extensively [Morescalchi, 1968; Tymowska, 1991]. We note that this system is not suited to cope with complex chromosome reshufflings, especially reciprocal translocations, in karyotypes of even closely related species.

The designation of chromosomes in an allopolyploid karyotype should reflect the ancestral diploid genomes prior to hybridization, but need not indicate every chromosome rearrangement that may have occurred before and after the hybridization event. However, in the present case of *X. laevis*, there is no evidence of complex karyotype repatterning because the synteny of each chromosome is highly conserved in *X. tropicalis* and *X. laevis* [Uno et al., 2013; the *X. laevis* genome sequence ver. 7.1 at http://gbrowse.xenbase.org/fgb2/gbrowse/xl7_1/; unpublished BAC-FISH data of the US-Japan *X. laevis* Genome Project Consortium].

The *X. laevis* chromosome nomenclature system proposed here has been approved on September 9, 2014 by the *Xenopus* Gene Nomenclature Committee chaired by A.M.Z. (http://www.xenbase.org/gene/static/geneNomenclature.jsp). The complete *Xenopus* chromosome nomenclature guidelines can be found on Xenbase (http://www.xenbase.org/), the *Xenopus* model organism database [Karpinka et al., 2014].

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References

Karpinka JB, Fortriede JD, Burns KA, James-Zorn C, Ponferrada VG, et al: Xenbase, the *Xenopus* model organism database; new virtualized system, data types and genomes. Nucleic Acids Res 43(database issue):D756–D763 (2014).

Khokha MK, Krylov V, Reilly MJ, Gall JG, Bhattacharya D, et al: Rapid gynogenetic mapping of *Xenopus tropicalis* mutations to chromosomes. Dev Dyn 238:1398–1406 (2009).

Krylov V, Kubickova S, Rubes J, Macha J, Tlapakova T, et al: 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 (2010).

Morescalchi A: Conferma della presenza di eterocromosomi in *Xenopus laevis* Daudin. Rend Acc Sci (Napoli), Ser 4 30:310–314 (1963).

Morescalchi A: I cromosomi di alcuni *Pipidae* (Amphibia Salientia). Experientia 24:81–82 (1968).

Schmid M, Steinlein C: Chromosome banding in Amphibia. XVI. High-resolution replication banding patterns in *Xenopus laevis*. Chromosoma 101:123–132 (1991).

Tymowska J: A comparative study of the karyotypes of eight *Xenopus* species and subspecies possessing a 36-chromosome complement. Cytogenet Cell Genet 18:165–181 (1977).

Tymowska J: Polyploidy and cytogenetic variation in frogs of the genus *Xenopus*; in Green DS, Sessions SK (eds): Amphibian Cytogenetics and Evolution, pp 259–297 (Academic Press, San Diego 1991).

Tymowska J, Kobel HR: Karyotype analysis of *Xenopus muelleri* (Peters) and *Xenopus laevis* (Daudin), Pipidae. Cytogenetics 11:270–278 (1972).

Uno Y, Nishida C, Yoshimoto S, Ito M, Oshima Y, et al: 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 (2008).

Uno Y, Nishida C, Takagi C, Ueno N, Matsuda Y: Homoeologous chromosomes of *Xenopus laevis* are highly conserved after whole-genome duplication. Heredity 111:430–436 (2013).

Weiler C, Ohno S: Cytological confirmation of female heterogamety in the African water frog *(Xenopus laevis)*. Cytogenetics 1:217–223 (1962).

Wickbom T: Cytological studies on Dipnoi, Urodela, Anura, and *Emys*. Hereditas 31:241–346 (1945).