

# Characterization of a Novel Tc1-Like Transposon From Bream (Cyprinidae, *Megalobrama*) and Its Genetic Variation in the Polyploidy Progeny of Bream–Red Crucian Carp Crosses

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**Abstract** Crossing the cyprinids diploid blunt snout bream *Megalobrama amblycephala* (BSB) and *Carassius auratus* red var. (RCC) generated sterile triploid (3nRB) and fertile tetraploid (4nRB) hybrid offspring. Utilizing inverted terminal repeats (ITRs) of transposon *Tdr1* from *Danio rerio* as PCR primer, the results showed that evident change in the number of Tc1-like transposons in 4nRB relative to BSB occurred, whereas such change did not arise in 3nRB compared to BSB. No Tc1-like transposon was found in RCC. A novel transposon was isolated from both BSB and 3nRB and designated as *Tma1*, which consisted of multiple copies after dot-blot hybridization. Based on the analysis of PCR amplified flanking sequence, characterization of *Tma1* indicated that this element flanked by a duplicated TA dinucleotide and harbored an ITR of about 224 bp. *Tma1* also harbored an incomplete transposase gene. Another novel transposon designated as *Tte1* was detected in 4nRB, which harbored an ITR of roughly 130 bp and consisted of multiple copies, but had no transposase gene. The analysis of PCR amplification and Southern blot hybridization showed that DNAs of 4nRB, which were hybridized to DIG-labeled pTma1, did not give band by PCR with Tma1 primer, on the other hand, 7 of 15 DNA samples from BSB, which were

hybridized to DIG-labeled pTte1, did not produce band by PCR with Tte1 primer. These results suggest that *Tte1* may be a recent invasion in BSB population and burst in 4nRB offspring. Our data provide clues as to the possible role of transposons as a driving mechanism for genomic evolution.

**Keywords** Hybrid fish · Polyploidy · Transposon · Transposase · Tc1 · Evolution

## Introduction

The crossbreeding of distantly related species should generate quite a shock to the genome of either parent species. This has long been considered an important mechanism for the generation of polymorphism and speciation (Evgen'ev 2007). This shock is indicated by genome-level alterations for the hybrid offspring, including the occurrence of triploid and tetraploid hybrids (Liu et al. 2007). It has been postulated that at least three major incidents involving genome duplication are supported by the studies of ancestral teleosts, with another more recent occurrence documented in salmonids (Hoegg et al. 2004). The duplicated chromosomes reorganized and mutated to enable stable inheritance, eventually leading to extant species whose inherent polyploidy is masked and only evident by comparative studies between species and lineages.

Large-scale movements of transposable elements have been postulated to play an important role in the speciation events (de Boer et al. 2007). It is thought that transposable element invasion and subsequent amplification act as a main morphogenetic factor ensuring adaptation of populations to environmental changes (Evgen'ev 2007). Transposable elements can be bifurcated based upon their modes of transposition into retrotransposons and DNA transposons

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(Finnegan 1985). The latter has an inverted terminal repeats (ITRs) and an encoded transposase gene required for transposition. The Tc1-like family of DNA transposons was initially discovered within the genome of the nematode *Caenorhabditis elegans* (Emmons et al. 1983), and members have since been found in numerous diverse teleosts (Radice et al. 1994). Active elements in the family have been isolated in invertebrates including *C. elegans* and *Drosophila* species (Lampe et al. 1996). However, extensive searching for vertebrate transposons of this family has thus far failed to uncover any active elements (Miskey et al. 2005): transposition inactivation has been the universally observed state for all vertebrate representatives of this family. This inactivity is the result of accumulated mutations during transmission from one generation of host to the next, a process known as “vertical inactivation” (Lohe et al. 1995). However, an active Tc1-like element named *Sleeping Beauty* (*SB*) was reconstructed/reactivated by deducing a consensus sequence of an active transposase based on molecular phylogenetic sequence data collected from the different salmonid species (Ivics et al. 1997). Naturally active transposons of other DNA-based families have been found in vertebrates, for instance the *Tol2* transposon of hAT family was isolated from genome of medaka fish (*Oryzias latipes*) (Koga et al. 1996).

Bursts of transposition occur during species hybrids, and this element activation was been involved in chromosomal remodeling, contributing to produce morphological novelties of hybrid speciation, thus transposable elements play a decisive role in hybrid genome evolution (Fontdevila 2005). For instance, hybridization of *Drosophila buzzatii* and *Drosophila koepferae* was shown to induce transposition of retrotransposon *Osvaldo* at a much higher level ( $10^{-2}$ ) than that of parental *D. buzzatii* ( $10^{-3}$ ) (Labrador et al. 1999). The results of transpositions displayed a hybrid dysgenesis syndrome, including nucleotide mutation, chromosome aberration, nondisjunction, sterility, etc. (Bingham et al. 1982). In rice hybrids, the inactive retrotransposons of *copla* and *gypsy* were activated by introgression of DNA from the wild species *Zizania latifolia*, but the elements’ activity was ephemeral and followed by rapid repression (Liu and Wendel 2000). Hybrids between two kangaroo species *Macropus eugenii* and *Wallabia bicolor* gave rise to transposition bursts associated with an intensive repatterning (O’Neill et al. 1998). These well-documented cases suggest a causal link between species hybrids and transposition activity.

In this article, we study the transmission variation of Tc1-like transposons among the diploid blunt snout bream *Megalobrama amblycephala* (BSB), red crucian carp *Carrasius auratus* red var. (RCC), and their hybrid offspring: sterile triploid hybrids (3nRB) and fertile tetraploid hybrids (4nRB). Results include isolation of a novel inactive

transposon given the appellation *Tmal* in the BSB genome and burst of another novel transposable element termed *Tte1* in the 4nRB genome. Our work perhaps provides some clues as to how transposons may serve as drivers for genomic evolution.

## Materials and Methods

### Animals and Crosses

BSB and RCC were obtained from the Protection Station of Polyploid Fish at Hunan Normal University. During the reproductive seasons (from April to June) in 2004–2007, 15 mature females and 15 mature males of each RCC and BSB were chosen as maternal and paternal gene sources, respectively. Our reproduction performing procedure is that we made the crosses step-by-step, in which one female was only crossed with one male. However, because the ponds were limited for raising each pair’s offspring, all the offspring from different pair’s crossing were mixed.

### Genomic DNA Extraction and PCR Amplification

A total of 1–2 ml of red blood cells was collected from the caudal vein of each fish using syringes containing ~200–400 units of sodium heparin. These blood mixtures were expelled into a 2-ml EP tube, from which a 100- $\mu$ l mixture was taken, mixed with lysis enzyme, and incubated at 55°C overnight. Genomic DNA was extracted following the procedure outlined in the DNA extractor kit user manual (Sangon, Shanghai, China). Analyzing the terminal repeated sequences of the transposon *Tdr1*, which is found in zebrafish (*Danio rerio*) (Izsvák et al. 1995), led to the design of an IR primer (Table 1), using to amplify Tc1-like transposable elements from the fish, including all the parents of 15 BSB and 15 RCC, and progeny of 10 4nRB and 10 3nRB in this study. PCR reaction of 25  $\mu$ l total volume composed of 0.3 units Ex Taq polymerase (Takara, Dalian, China), 2.5  $\mu$ l 10 $\times$  buffer, 0.5  $\mu$ l dNTP mixture (2.5 mM), 1  $\mu$ l primer (10  $\mu$ M), and ~100 ng genomic DNA. PCR amplifications were carried out in a Bio AB thermal cyler. The temperature profile was an initial denaturing for 5 min at 94°C followed by 35 cycles of 94°C for 30 s, 55°C for 1 min, 72°C for 2 min, and lastly a final extension at 72°C for 5 min.

### Transposable Elements Cloning and DNA Sequencing

The products of PCR amplification were separated from 1% agarose gels and visualized by ethidium bromide staining. After the bands were excised from the agarose gels, these fragments of PCR products were purified using a gel extraction kit (Sangon, Shanghai, China) and inserted

**Table 1** Primers were used in PCR amplification and Dig-labeled probe

Primer		Sequence (5' → 3')	AT <sup>a</sup> (°C)	
IR primer		TACAGTTGAAGTCGGAAGTTTACATAC	55	
Tma1 primer	Sense	TGGAGGAATTTGCTCTGGTCT	58	
	Antisense	TGAAGTCAGGGCTTTGTGGTG		
Tte1 primer	Sense	CACAATGAGGATAACTGAGG	49	
	Antisense	AAAAGAAGGAAAGGAAAAAC		
Adaptor primer	AP1	GTAATACGACTCACTATAGGGC		
	AP2	ACTATAGGGCAGCGTGGT		
Tma1 flanking sequence	5'	GSP1	TTGTTTGAACAGATTAACGTGGCACCT	67
		GSP2	GTTCTGTTTCTGATGTCTTGGCTGATT	65
	3'	GSP1	TTGAAACAGCATCTGAAGACATAAGCC	65
		GSP2	CAGAAGTAAAAAGCGTGTACGAGCA	67
Tte1 flanking sequence	5'	GSP1	CAGAAGTTCAAATACTCAATGATGGG	67
		GSP2	GCGTTTTTCTTCTTCTTTTATCTG	65
	3'	GSP1	AAATCATACAGTCGTTGTTGGAAAGGG	66
		GSP2	ATTTTTCTGAAGAACAGCAGGCAGTT	64

<sup>a</sup> Anneal temperature in PCR amplification

directly into the pMD18-T plasmid vector (Takara, Dalian, China). Plasmids were propagated in *DH5a*. Clones with expected sizes were chosen for sequencing analysis. The vector-specific primers M13 and SP6 were employed in the sequencing, which utilized an automated DNA sequencer (ABI PRISM 3730).

#### Sequence Analysis and Genomic Size Measurements

DNA sequences were analyzed using Jellyfish 2.0 and Primer Premier 5.0 software. Homology searches for DNA sequences and putative proteins were performed via BLAST at NCBI databases ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). A putative nucleus localization signal (NLS) motif was analyzed using the PSORT II Prediction program available at the PSORT website (<http://psort.ims.utokyo.ac.jp>). Alignments of transposon sequences were done using CLUSTALX software.

Chicken blood was used for controls (Tiersch et al. 1989). Genome size, which reflects the ploidy level of a specimen, was determined with a flow cytometer (Partec, Dortmund, Germany). Red blood cells of each fish were collected and stained with DAPI solution for genome size measure as described (Liu et al. 2007).

#### The Flanking Sequence Analysis of Transposons

The flanking sequences of transposons were accessed using the adaptor ligation in Genome Walker Universal Kit (GWUK, Clontech, USA). According to the manufacturer's instruction, firstly genomic DNA was digested with *Dra*I, *Eco*RV, *Pvu*II, and *Stu*I, respectively, then Adaptors (included in GWUK) were ligation with the ends of digested DNA under T4 DNA ligase reaction. According to the

known transposon sequence, two gene-specific primers (GSP) were designed: one for primary PCR (GSP1) and the other for secondary PCR (GSP2) (Table 1). The flanking sequences of transposons were obtained by PCR with two primer pairs of GSP1-AP1/GSP2-AP2 (Table 1, AP1 and AP2 included in GWUK). Using LA Taq DNA polymerase (Takara, Dalian, China), PCR amplification conditions were 30 cycles at 95°C for 30 s, and 64–67°C for 1 min (for each annealing temperature see Table 1), and 72°C for 4 min, and a final extension at 72°C for 10 min, and then PCR products were cloned and sequenced.

#### DNA Dot-Blot Hybridization

DNA dot-blot hybridization was done essentially as described (Izsvák et al. 1995). First, known amounts of genomic DNA and the plasmid DNA fragment containing the cloned transposons were serially diluted. Then DNA samples were prepared for dot-blotting by addition of an equal volume of 0.6 M NaOH, incubation at room temperature for 10 min, and then addition of an equal volume of ice-cold 2 M ammonium acetate. Next 1.5 µl DNA samples were hand-blotted onto positively charged nylon membranes (Amersco, New Jersey, USA), followed by irradiation with ultraviolet light for 30 s. Both prehybridization for 2 h and hybridization for 12 h were at 65°C in a Dig-Easy hybridization buffer (Roche Biochemical, Mannheim, Germany), followed by washing at low stringency (twice in 2× SSC/0.1% SDS for 15 min at room temperature), then washing at high stringency (twice in 0.1× SSC/0.1% SDS for 15 min at 55°C). A Dig-labeled probe was prepared using the PCR method (Roche Biochemical, Mannheim, Germany). In dot-blot hybridizations, the probes containing a 340-bp internal sequence of *Tma*1 were used for BSB and 3nRB samples,

which were obtained using *Tma1* primer (Table 1). The probes containing the full-length sequences of *Tte1* were used for 4nRB samples, which was produced by IR primer. The hybrid signal-determination was performed according to the protocols of the chemiluminescent method (Roche Biochemical, Mannheim, Germany). Auto-radiograms were scanned with an EC3 Imaging System (UVP, California, USA). The intensity of the hybrid signal was quantified using a Gel-Pro analyzer (UVP, California, USA).

#### Southern Blot Analysis and Detection of Transposons

The procedure of Southern blot hybridization was performed as described (Zhang 2002). 10 µg of genomic DNA isolated from BSB, RCC, and 4nRB was electrophoresed on a 0.8% agarose gel for 12 h after *HindIII* digestion, and then transferred to positively charged nylon membranes. Prehybridization and hybridization were performed as mentioned in previous section. The *Tma1* and *Tte1* primers (Table 1), which were used to synthesize DIG-labeled probes, were designed based on the internal sequence of transposon (deleted the ITRs). The DNAs isolated from BSB, RCC, and 4nRB were hybridized to both the 340-bp *Tma1* probe (p*Tma1*) and the 250-bp *Tte1* probe (p*Tte1*). The hybridization signal pattern was detected as discussed in previous section.

Both *Tma1* and *Tte1* primers were also used to detect the numbers of each transposon in fish. PCR amplification was performed in a 25-µl reaction mixture composed of 0.3 µl Ex Taq polymerase (Takara, Dalian, China), 2.5 µl 10× buffer, 0.5 µl dNTP mixture (2.5 mM), 1 µl each primer (10 µM), and ~100 ng of genomic DNA. PCR cycling conditions were 94°C for 5 min, 30 cycles at 94°C for 30 s, 58°C/49°C (*Tma1/Tte1*) for 30 min, 72°C for 2 min, and a final extension at 72°C for 5 min.

## Results

#### Hybrid Progeny and PCR Amplification of Tc1-Like Transposons

The crossing of RCC♀ × BSB♂ produced sterile 3nRB and fertile 4nRB. However, RCC♂ – BSB♀ crosses failed to produce any living progeny. Chromosomal number counts were performed using kidney tissue from both 3nRB and 4nRB specimens, and their DNA content was measured using a flow cytometer as described (Liu et al. 2007). The offspring between 3nRB and 4nRB were set apart from each other based on some morphological traits, e.g., 4nRB had a pair of evident barbells, whereas 3nRB did not present any barbell on the mouth.

Genomic DNAs of RCC, BSB, 3nRB, and 4nRB were amplified by PCR with IR primer. Saving that in RCC the

PCR reactions yielded no products. Both 1.5 and 1.3 kb bands were visible in BSB and 3nRB. In 4nRB, a 0.8-kb band was seen. Results were checked for all the parents of 15 BSB specimens and 15 RCC specimens, and 10 randomly selected samples for each of the two offspring specimen types.

#### Characterization of Transposon *Tma1* in BSB

The 1.3 and 1.5 kb bands from BSB genome were excised from the gel for the cloning of DNA fragments. A total of four recombinant clones were chosen for sequence analysis (three clones of the 1.5 kb, one of the 1.3 kb). Nucleotide sequence analysis revealed that all three clones of the 1.5 kb bands had an exact size of 1526 bp. The 1.3 kb band clone had an exact size of 1329 bp. Following the standard nomenclature for the Tc1-like family, these sequences will herein be referred as belonging to the *Tma1* subfamily (for Tc1-like elements from *M. amblycephala*); specifically, the three 1526 bp clones will be called *Tma1-2n1–Tma1-2n3* and the 1329 bp clone will be referred to as *Tma1-2n4*. Their complete sequences have been deposited into GenBank (EU585769-70, EU910267-68). One nucleotide at site 1486 was variable (T → C) between *Tma1-2n1* and *Tma1-2n2*, whereas *Tma1-2n2* and *Tma1-2n3* were identical. Alignment of *Tma1-2n3* with *Tma1-2n4* revealed that the latter had been truncated relative to the former by loss of a fragment of about 193 nucleotides at the 5' end. Excluding *Tma1-2n4*'s 5' 27-bp primer sequence, the remaining portion of *Tma1-2n4* was perfectly included in *Tma1-2n3*. Therefore, *Tma1-2n3* may be regarded as a *Tma1* consensus sequence by the majority rule.

The flanking sequence of *Tma1* was difference of two nucleotides from IR primer (12 and 13 G → T, see Fig. 1). Both 5' and 3' flanking sequence harbored conservatively two nucleotides “TA” (data not shown), which is a target site duplication sequence. *Tma1* consensus sequence harbored imperfectly ITRs. The 5' ITR was 224 bp in length and the 3' ITR was 212 bp in length (Fig. 1). Alignment of these two ITRs indicated a similarity of 58.1%. Each of these ITRs is a 29 bp block consisting of a perfectly inverted repeat. Per ITR contained two transposase binding sites repeating in a direct orientation (direct repeat sequences, DRs). DRs of the 5' ITR were 14 bp in length and 100% identity. DRs of the 3' ITR were 17 bp in length and 88.2% similarity.

Transposase's open reading frame (ORF) of *Tma1* started with an ATG codon at positions 283–285. The full length of ORF was 295 amino acids excluding 6 internal stop codons. The secondary structure of *Tma1* transposase was deduced based upon these 295 amino acids. Compared to the reconstructed *SB* transposon (Ivics et al. 1997), the *Tma1* transposase possessed of something as *SB*, including

**Fig. 1** Characterization of the ITRs in the ends of *Tma1* (5' → 3'). The perfectly matched nucleotides between 5' and 3' ITRs were shown with *single underlines*. The direct repeats of per ITR were shown in *gray boxes*; the internal sequences of *Tma1* were shown with *suspension points*

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CAGTTGAAGTCTTAAAGTTTACATACACTTAGATTGAAGTCATTA AAACTAATTTTTTAAA
CCACCCTAGAGATTTTCATACTAAAATAATGTAGGTTTAGCAAGTTGTTTAGGACATGA
ACTTTGTGCATAACACATCATT TTTTCCAAAAACAATGTTTACAGATGGATTGTTTCACT
TATATTTCAATTATATCACAATTGTCATGGGTCAGAAGTTTACATACAC.....AAGTGTAT
GCGAACTTATGACCTACTTTGTGAATGTGGTGAAAAC TTTTAACTGGAATAAATCAGT
TTGACCAGTATTATTCTGATATTTACATATATACAATAAAAAC TGTGATTTGAGGTAAC
ATTACCAGGATTTGTGAAAGGAGTTGTGAAAGAATTGTGTTTAAATGCATTTGGGTA
AAGTGTATGTAAACTTAAGACTTCAACTG

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a paired-like domain with a Leucine-zipper (LLLLV; residues 11, 18, 25, 32, and 39) (11 L → I) and a GRRH-like (GRRH) putative AT-hook motif. Analysis by PSORT II Prediction program identified a bipartite NLS motif at site 103 (KRVLYHHNLKGC SARKK) and a pat4-type NLS at site 117 (RKKP). The DD34E motif (aspartate, aspartate, 34 amino acid residues, then, a glutamate) presented in *SB* was not found in *Tma1*. Alignment of the 152 amino acids in N-terminal (DNA-binding domain) in *Tma1* transposase with the counterpart of *SB* found a similarity of 76.3%, whereas alignment of C-terminal (catalysis domain) amino acids between them showed only a 32.2% similarity—the latter low value being due to insertion/deletion mutations destroying the protein-coding function in *Tma1*. This implies that DNA-binding domain sequences are more conservative than catalysis domain sequences for *Tma1* and presumably other members of the Tc1-like family.

Homology searches in the NCBI database based upon the putative *Tma1* transposase netted a maximum sequence similarity of 39.8% with a Tc1-like transposase (BAF37936) in the rainbow trout *Oncorhynchus mykiss*. Using *Tma1* DNA sequences to search the NCBI database unearthed maximal sequence similarities of 80% with Atlantic salmon *Salmo salar* growth hormone 2 gene (EU621899,  $E = 3e-178$ ), and 75.2% with *Tsn1* transposon (AF017232,  $E = 1e-177$ ) in lake trout *Salvelinus namaycush*.

#### Bursts of *Tte1* in the Tetraploid Hybrids

The 0.8 kb band of 4nRB genome was excised from the gel for the cloning of DNA fragments. The results revealed that this element consists of exactly 829 bp, and the name *Tte1* is adopted to refer to such Tc1-like transposable elements in the tetraploid hybrid genome. *Tte1* is considered to be a

Tc1-like transposon due to the presence of imperfectly ITRs at opposite ends of this element (as in other Tc1-like transposons). A total of four recombinant clones were chosen for sequence analysis. The complete sequences of *Tte1-1* to *Tte1-4* clones have been deposited into GenBank (EU585775, EU910269-71). *Tte1-1* and *Tte1-2* were identical (they were consensus sequences), whereas *Tte1-3* and *Tte1-4* (*Tte1-2* and *Tte1-3*) diverged at only one (three) nucleotide site(s). The percent divergences between pairs among these four sequences were in the range 0–0.4%. The flanking sequence of *Tte1* was showed that two nucleotides differ from IR primer, and a conservatively nucleotides “TA” sequence located at the outer of each ITR’s end (data not shown). 5' ITR was 137 bp in size and 3' ITR was 127 bp in size, and they shared sequence similarity of 80.7%. A 5'-AAGTTTACATACA-3' block in *Tte1* was similar to the outer direct repeats of *Tma1*, but the inner direct repeats of *Tma1*'s ITRs were not found in *Tte1*.

BLASTN searches using the *Tte1* DNA sequence did reveal matches with several very short fragments, including ITR of other Tc1-like transposons in some species such as *Tsn1* and *Tdrl* (data not shown). The top blast hit was a 178-bp long C-8 *Tc1* transposon from *Labeo rohita* (AY769636,  $E = 3e-37$ ). Alignment of the entire C-8 *Tc1* only with 5' end sequence of *Tte1* was a 125/142 (gap = 4/142) match, whereas the entire C-8 *Tc1* only with 3' end sequence of *Tte1* was a 134/158 (gap = 13/158) match. This suggests that C-8 *Tc1* maybe represent an ITR of *Tte1*. In addition, homology searches by BLASTX against GenBank yielded no matches to the conceptive *Tte1* transposase, highlighting the evolutionary novelty of this newly found transposon. Using *Tte1* primer to detect genome of BSB, RCC and 4nRB aimed for the elimination of the effect resulted from ITR mutation of *Tte1*. The results were shown that a 250-bp band was given in 4nRB

genome while no band was obtained from BSB or RCC genome. The data imply that whether *Tte1* is absent in parent genome or this element is evidently diversity in comparison with parental element.

#### Transmission of *Tmal* From BSB to Hybrid Progeny

Two DNA fragments from 3nRB genome were isolated and cloned. Sequence analysis of these clones showed that these 3nRB transposons consisted of 1528 and 1329 bp elements. High sequence similarities with *Tmal* in BSB indicate these 3nRB transposons were copies of *Tmal*. The 1528-bp length (1329-bp length) sequence was therefore named *Tmal*-3n1 (*Tmal*-3n2). Their complete sequences have been deposited into GenBank (EU585771-72). Comparison of *Tmal*-2n3 (used as consensus sequence) in BSB with *Tmal*-3n1/2 in 3nRB (excluding the 5' primer sequence of *Tmal*-3n2) indicates that nucleotide insertions were 683 (T) and 771 (A) in *Tmal*-3n1, and transitions were 918 (G → A) in *Tmal*-3n1 and 969 (G → A) and 1276 (T → C) in *Tmal*-3n2.

*Tmal* does not be amplified in 4nRB genome by IR primer. In attempt to assess the transmission of *Tmal* from BSB to 4nRB, *Tmal* primer was used for the detection of number of transposons in 4nRB genome. A 340-bp fragment was given in BSB genome by PCR with said primer, yet no fragment was observed in 4nRB genome. These results indicate that *Tmal* may be excised from 4nRB or this element gave rise to distinctly diversity in the progeny genome.

#### The Number of Tc1-Like Elements in Fish with Different Ploidy Levels

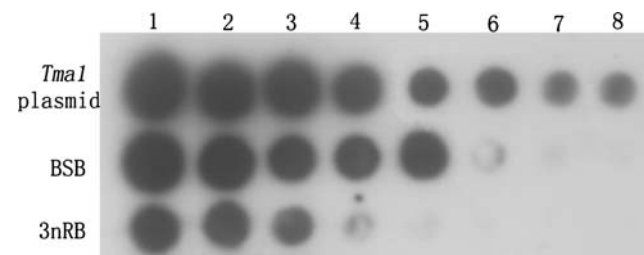
DNA contents of the samples were measured using a flow cytometer. Chicken red blood cells were used as controls. Mean values for the haploid genome sizes (C-values) of the fish specimens and chicken controls along with fish–chicken ratios are shown in Table 2.

To measure the number of Tc1-like transposon copies in a genome via a dot-blot analysis, varying amount of genomic DNA (1200, 600, 200, 100, 50, 25, 0.5, and

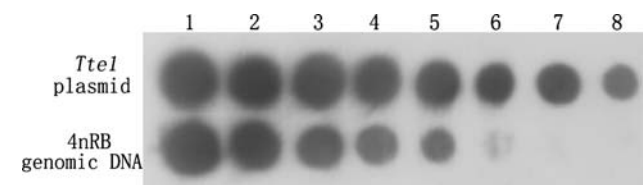
0.05 ng) were dotted onto a charged nylon membrane alongside known amounts of a plasmid bearing complete transposon sequences (100, 50, 25, 12.5, 1, 0.4, 0.1, and 0.01 ng). The number of element copies could be calculated based upon three facts: the similarity of hybrid signal intensity between genomic DNA and plasmid DNA (Figs. 2 and 3), the genome's C-values (Table 2), and the known amounts of genomic DNA and plasmid DNA. This yielded an estimate of *Tmal* 480–720 copies per haploid genome in 15 BSB samples, and 120–250 copies per haploid genome in 10 3nRB samples; 840–950 *Tte1*'s copies per haploid genome were estimated in 10 4nRB samples.

Genomic DNAs of fish were hybridized to p*Tmal*, and the results of Southern blot hybridization showed that molecular weight of genomic DNA was above 0.8 kb after *Hind*III digestion, and hybrid signal patterns exhibited a smear in all of 15 BSB samples and 10 4nRB samples, yet no such result was given in 15 RCC samples (Fig. 4). These results demonstrated one fact: multiple copies of *Tmal* presented both in BSB genome and 4nRB genome.

Genomic DNAs of fish were hybridized to p*Tte1*, and the results indicated that DNAs isolated from 4nRB can be hybridized to the probe, while seven of 15 BSB samples were also found to display the same hybrid signal patterns as 4nRB (Fig. 5). But the other 8 of 15 BSB samples and all RCC samples did not present such hybrid signal patterns. The fragments of the hybrid patterns were smear and their molecular weight were above 0.8 kb. These results



**Fig. 2** Dot-blot hybridizations were done to estimate copy number of *Tmal*. BSB (second row) and 3nRB (bottom row) genomic DNAs were hybridized to p*Tmal* using recombinant plasmid as controls (top row)



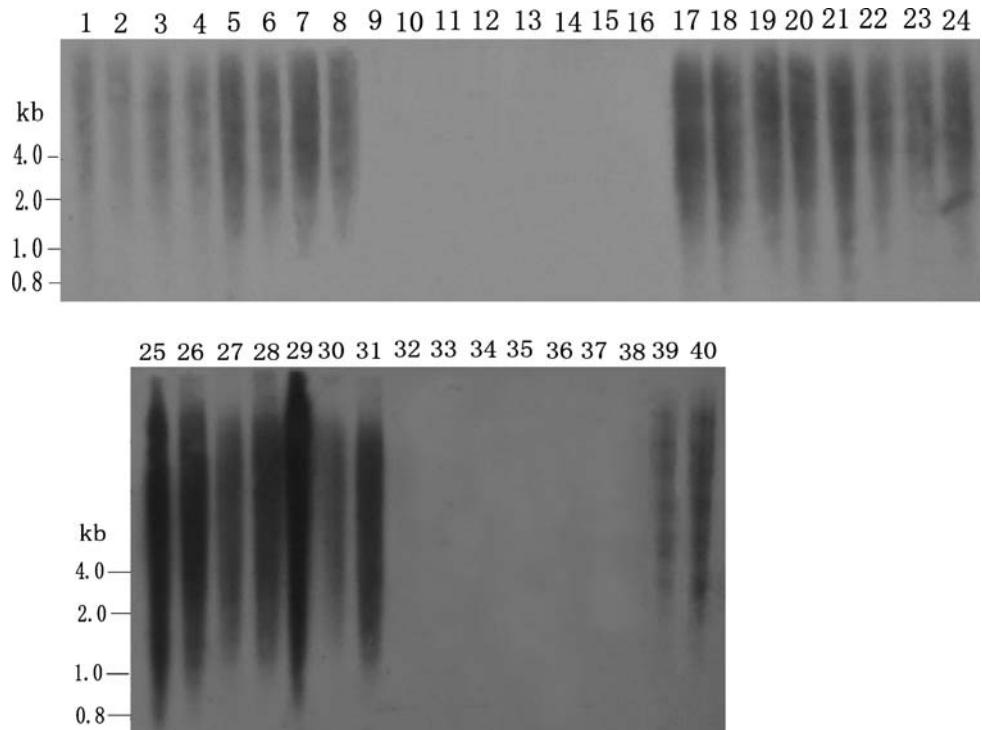
**Fig. 3** Dot-blot hybridizations were done to estimate copy number of *Tte1*. 4nRB genomic DNAs (lower row) were hybridized to p*Tte1* using recombinant plasmid as controls (upper row)

**Table 2** Haploid genome sizes of the fish with different ploidy levels

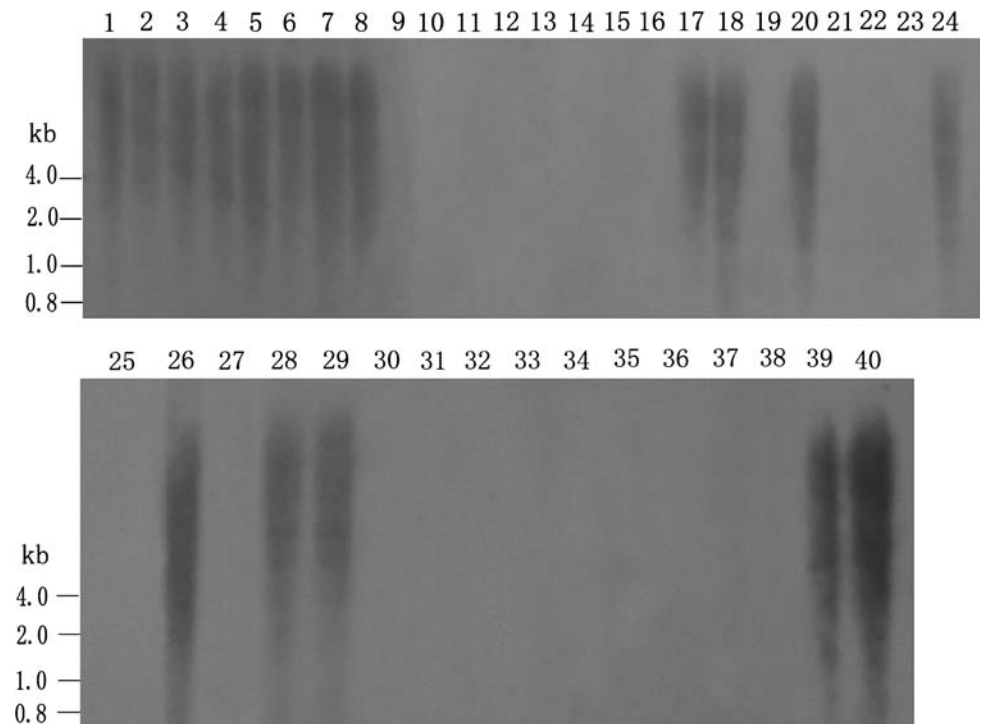
Sample	Mean DNA content	Fish–chicken ratios	Haploid genome size (pg/nucleus)
Chicken	68.23		1.30 <sup>a</sup>
BSB	81.33	1.19	1.55
3nRB	137.62	2.02	1.75
4nRB	183.90	2.70	1.76

<sup>a</sup> From Tiersch et al. (1989)

**Fig. 4** Southern blot hybridizations were done to estimate genome copy number of *TmaI* in fish. Genomic DNAs of fish were digested with *HindIII*. Lines 1–8 and 39–40 indicated ten 4nRB samples; lines 9–16 and 32–38 indicated 15 RCC samples; lines 17–31 indicated 15 BSB samples. Genomic DNAs were hybridized to p*TmaI*



**Fig. 5** Southern blot hybridizations were done to estimate genomic copy number of *TteI* in fish. Genomic DNAs of fish were digested with *HindIII*. Lines 1–8 and 39–40 indicated ten 4nRB samples; lines 9–16 and 32–38 indicated 15 RCC samples; lines 17–31 indicated 15 BSB samples. Genomic DNAs were hybridized to p*TteI*



indicated that the 4nRB genome has multiple copies of *TteI*, and the heterogeneity of *TteI* copy number presented in a few BSB samples, as well as indicated that the interspecific hybridization between distantly related fish can result to the bursts of the heterogeneous transposable element.

**Discussion**

Characterization analyses of the novel transposon *TmaI* and *TteI* showed that there is an ITR in both ends of them, and the internal sequences of these elements are lowly variability (0–0.4%) between individual copies in

comparison with other Tc1-like transposons, e.g., *Tdr1* in zebrafish is divergent of approximately 4–6% in four clones (Izsvák et al. 1995); *Tsn1* in lake trout is 7.8–10% divergence in three clones (Reed 1999). Tc1-like family is marked by the variability in its members' ITRs and three groupings (at least) are recognized in previous study (Benjamin et al. 2007). Each ITR in 224 bp of *Tma1* contains two DRs with 14 bp, which clearly point to this element being a member of the third IR-DR group, along with the reconstructed *SB* (Ivics et al. 1997)—which is the best-characterized member of this group found in fish. The ITR traits of *Tte1* are roughly 130 bp in length and possess no DRs, which place *Tte1* in the first group of the family. According to whether transposable elements harbor transposase gene, *Tma1* may represent an autonomous DNA transposable element, whereas *Tte1* represents a nonautonomous transposable element (Liu et al. 1999).

*Tma1* is found in 3nRB genome by PCR with IR primer. Nucleotide differences of this element in BSB from its copies in 3nRB only include several nucleotide substitutions and insertions. These phenomena may be resulted from orthologous forms of *Tma1* clones in parent, in other words, the diversity between these clones can be the diversified isoforms existed in BSB genome. Although *Tma1* cannot be detected in 4nRB genome by PCR with IR or *Tma1* primers, the results of Southern blot hybridization showed that genomic DNAs isolated from ten 4nRB samples can be hybridized to p*Tma1* (Fig. 4). It might be likely that parent individuals had the *Tma1*'s isoforms amplified by the IR/*Tma1* primers while the 4nRB offspring has lost those isoforms. It might be more likely that molecular mutation occurred in *Tma1*, especially in IR/*Tma1* primers sequence blocks. The past data suggest that sequence similarities between Tc1-like transposons are restricted to the ITR, in which there are transposase-binding sites triggering excision of transposable elements (Benjamin et al. 2007). Our data reveal that *Tma1* was induced to rapid diversity probably either by DNA lost or by molecular mutation during the vertical transmission. One explanation is rapidly diversification would limit the chances for cross interactions between related copies (Abrusan and Krambeck 2006), and the direct contribution of the inactivated transposons to host genome evolution is to act as a source of raw material, which can be used for the assembly of new gene and functions (Feschotte and Pritham 2007).

A novel transposon *Tte1* is detected in the fertile 4nRB by PCR with IR primer. Utilizing the internal sequence of this element to design *Tte1* primer, a 250-bp band is given in 4nRB genome, whereas no band can be obtained from BSB or RCC genome. Interestingly, Southern blot hybridization analysis shows that 7 of 15 DNAs samples from BSB were hybridized to p*Tte1*. This may be unique among studies of transposable element. The results indicate that,

after two cyprinid species crossbreeding of BSB belonging to Cultrinae and RCC belonging to Cyprininae (Yu 1989), this element emerged to burst, and hence increased the apparent number of *Tte1* copies in 4nRB genome. During *S. salar* genomic evolution history, bursts of activity of 14 families of DNA transposons have been identified by phylogenetic reconstruction analysis of transposons copies within salmonid species and other closely and distantly related species (de Boer et al. 2007). In invertebrate, the interspecific crosses of *Drosophila virilis* caused transposition burst of *Penelope* (Evgen'ev et al. 1997). In an interspecific mammalian hybrid of kangaroo, the activity of mobile element resulted from the occurrence of genome-wide under methylation, and then the activated mobile element was involved in chromosome remodeling (O'Neill et al. 1998), but the activity of transposons was ephemeral and rapidly repression during species hybrids (Liu and Wendel 2000). Here, we have shown that, after *Tte1* burst, this element may be involved in chromosomal replicate within the fertile 4nRB genome. This could potentially enhance the organism's ability to tolerate/absorb the selected stress from the crossbreeding, overcoming the barrier of hybrid dysgenesis.

We note that the heterogeneity of *Tte1* copy number was only detected in a few BSB samples but not detected in the others. Interesting, this element can also be detected in all the randomly selected 4nRB samples (Fig. 5). The results indicated that this element possibly invaded a few BSB individuals in recently, and burst in the hybrid offspring when BSB with the heterogeneity of *Tte1* copies was used as the paternal materials of two fish crossbreeding. The mechanism underlying the transposable element burst is worthy to further study. In summary, this is the first report to show that the bream–carp crossing can lead to transposon diversification and burst in host genome.

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