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Evidence for Recent Invasion of the Medaka Fish Genome by the *Tol2* Transposable Element

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ABSTRACT

Tol2 is a transposable element of the terminal-inverted-repeat class, residing in the genome of the medaka fish *Oryzias latipes.* The genus Oryzias contains more than 10 species for which phylogenetic relationships have previously been estimated. To infer the history of *Tol2* in this genus we performed genomic Southern blots and PCR analyses of 10 of the species. It was revealed that *Tol2* occurs in 2 of the 10 species (*O. curvinotus* and *O. latipes*) and that the length and the restriction map structure of *Tol2* are identical in the two cases. Further, sequencing analysis revealed an extremely low level of divergence compared with that in a nuclear gene. These results suggest recent incorporation of *Tol2* into one or both of the two species, implying horizontal transfer of *Tol2* from one species to the other or into them both from a common source.

TRANSPOSABLE elements are repetitive sequences capable of moving from one chromosomal location to another. Several transposable elements, or transposable element families, are known to be distributed across species, genera, or even higher taxa. When the phylogeny of a transposable element is incongruent with the phylogeny of its host species, horizontal transfer of the element between species is often suggested as the explanation (for reviews, see Kidwell 1992; Capy *et al.* 1994). It is not known whether horizontal transfer is a frequent event under natural circumstances or just a rare accident. If the former is the case, it might play a significant role in the evolution of transposable elements (see Maruyama and Hartl 1991a; Kidwell 1992; Lohe *et al.* 1995), allowing them to survive the pressures of vertical inactivation and stochastic loss that would lead to their ultimate extinction from genomes.

Well-known examples of horizontal transfer are represented by the *P* element (Daniels *et al.* 1990) and the *mariner* element (Maruyama and Hartl 1991a) of Drosophila and the IS*1* element of bacteria (Lawrence *et al.* 1992). With these, high sequence similarities were found among copies isolated from distantly related species. Similar observations have been reported for many elements in Drosophila, in related insects, and in bacteria. Examples from more diverse organisms should aid in inferring how frequent this phenomenon is.

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We here report high nucleotide sequence similarity of a transposable element in two fish species, the most likely explanation for which is horizontal transfer. *Tol2* is a terminal-inverted-repeat transposable element residing in the genome of the medaka fish *Oryzias latipes* (Koga *et al.* 1996). It is 4.7 kb in length and contains four open reading frames (ORFs) having amino acid sequence similarity with members of the *hAT* family (Calvi *et al.* 1991; Atkinson *et al.* 1993), a group of terminal-inverted-repeat transposable elements including *hobo* of Drosophila (McGinnis *et al.* 1983), *Ac* of maize (McClintock 1948; Fedoroff *et al.* 1983), and *Tam3* of snapdragon (Sommer *et al.* 1985). Features concerning the similarity, including amino acid sequence alignments, are described in Koga *et al.* (1999). The medaka fish genome contains 10 to 30 copies of *Tol2* that are highly homogeneous in structure: no restriction map variation was found among more than 200 copies and no sequence variation among five randomly chosen clones (Koga and Hori 1999). This situation contrasts with other *hAT* elements in which defective, shorter elements are common. To explain this anomaly, we have proposed three hypotheses: (1) lack of factors that are involved in the generation of defective copies; (2) less efficient amplification of defective copies; and (3) a short interval between the *Tol2* invasion of the medaka fish and the appearance of evolutionary change (Koga and Hori 1999). The fact that the homogeneity is seen even at the nucleotide sequence level appears to favor the last explanation. The genus Oryzias includes more than 10 species whose phylogenetic relationships have already been studied (Sakaizumi 1985; Naruse *et*

al. 1993; Naruse 1996). If the third explanation is true, and, in addition, if the time span after the *Tol2* invasion is short on the time scale of speciation in the genus, an inconsistency between the *Tol2* phylogeny and the host phylogeny might be expected. To determine if this is the case, we first performed genomic Southern blotting and PCR to study the distribution and structure of *Tol2*-hybridizing sequences in 10 species of the genus Oryzias. Having found an inconsistency, we further conducted sequencing analysis of *Tol2* copies and genes from their host species. The results suggested recent incorporation of *Tol2* into at least one of the species.

MATERIALS AND METHODS

Medaka fish: This species (*O. latipes*) inhabits East Asia, including China, Korea, and Japan, and demonstrates geographical variation. According to data on isozyme frequencies, there are four regional populations: [1] Northern Japan, [2] Southern Japan, [3] Eastern Korea, and [4] China and Western Korea (Sakaizumi 1986; Sakaizumi *et al.* 1987). A total of 12 laboratory stocks, 3 from each population, were examined. Their original collection sites and designations were the following: [1a] Yokote, Japan; [1b] Kaga, Japan; [1c] Maizuru, Japan; [2a] Nagoya, Japan; [2b] Kasumi, Japan; [2c] Kobe, Japan; [3a] Yongchon, Korea; [3b] Sachon, Korea; [3c] Shinpyon, Korea; [4a] Shanghai, China; [4b] Maegkok, Korea; [4c] Pugang, Korea.

Other species: Nine species of the genus Oryzias were obtained from the World Medaka Aquarium of the Nagoya City Higashiyama Zoological Garden. The nine species, in addition to *O. latipes*, are listed in Figure 1 with the present consensus on their phylogenetic relationships and their original collection sites. Detailed phylogenetic trees are available in Naruse *et al.* (1993) and Naruse (1996). Swordtail fish (*Xiphophorus helleri*) and zebrafish (*Danio rerio*) were purchased from a pet shop in Nagoya. Commercially available genomic DNAs were used for assessment of the chicken and human cases.

Analysis of genomic DNA: Southern blotting and subsequent hybridization experiments were performed as described by Koga *et al.* (1995). Briefly, high molecular DNAs were digested with restriction enzymes, fractionated on 1.0% agarose gels, and transferred to nylon membranes. The membranes were then hybridized with ^{32}P-labeled probes at 65° for 16 hr. The hybridization solution contained 6× SSPE, 0.5% SDS, 50 μg/ml fragmented heterologous DNA, and 5 ng/ml probe DNA. Heterologous DNA was included as a blocking agent and salmon sperm DNA was used as long as only the two *Tol2*-containing species (*O. curvinotus* and *O. latipes*) were concerned. This is because similar analyses in our previous studies were conducted under this condition. In hybridization experiments including other species, yeast genomic DNA was used because salmon DNA might absorb the probe DNA if the salmon is phylogenetically closer to the origin of the probe DNA (medaka fish) than are the genome DNAs to be examined.

For the 10 species of the genus Oryzias, a 4-μg aliquot of genomic DNA was used for each gel slot. For the species outside the genus, 20 μg was used. The implications of this difference are described in the discussion.

Hybridization probes: The *Tol2* element was first identified as a DNA fragment inserted into the *tyrosinase* gene of an albino mutant fish (Koga *et al.* 1996). This particular *Tol2* copy is denoted *Tol2-tyr* (GenBank accession no. D84375). Parts of a *Tol2-tyr* clone generated by restriction enzyme digestion or PCR were subcloned into the pBluescript II vector. A

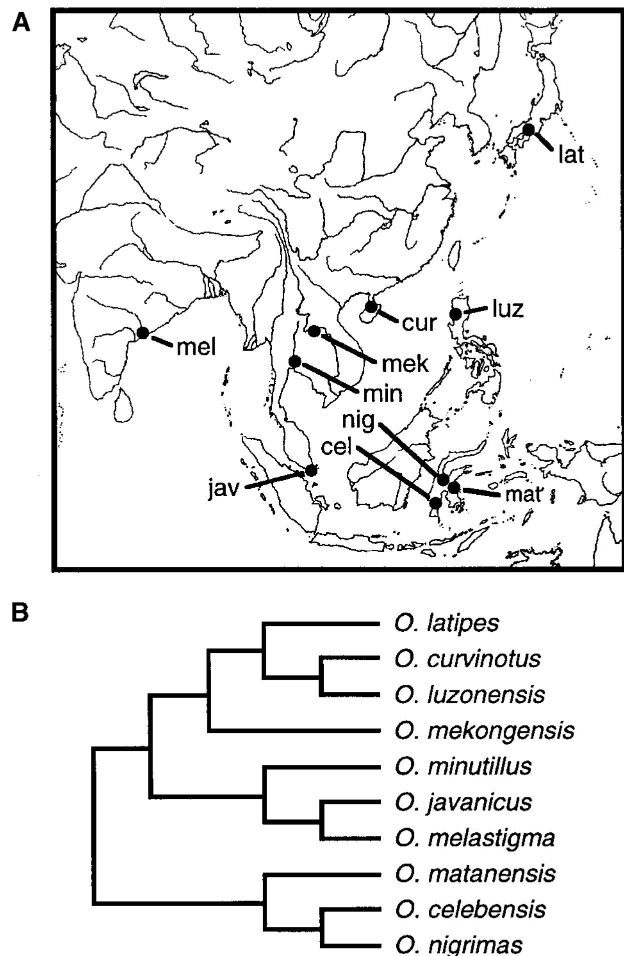

Figure 1.—The 10 species of the genus Oryzias examined in the present study. (A) The original collection sites of the 10 species are shown by dots on the map. The species names are abbreviated with their first three letters. (B) Naruse *et al.* (1993) and Naruse (1996) constructed phylogenetic trees with nucleotide sequence data for the mitochondrial cytochrome b gene and the 12S ribosomal RNA gene, respectively. The scheme was redrawn from these references for the 10 species.

fragment of the medaka fish *tyrosinase* gene was also cloned. These clones were used as probes for hybridization experiments after removal of their vector portions. The regions covered and the designations of the probes are shown in Figure 2.

Other molecular techniques: PCR, cloning, and sequencing were conducted as previously described (Koga and Hori 1999).

RESULTS

Distribution of *Tol2* among species: Figure 3 illustrates autodiagrams of Southern blots to determine the presence of *Tol2* in the 10 species of the genus Oryzias and other species outside the genus. Genomic DNAs were digested with *Bgl*II, which has a single cutting site in the *Tol2-tyr* sequence. In Figure 3A where probe Tyr-Bg (part of the medaka fish *tyrosinase* gene) was used, one or two hybridization signals were observed for all

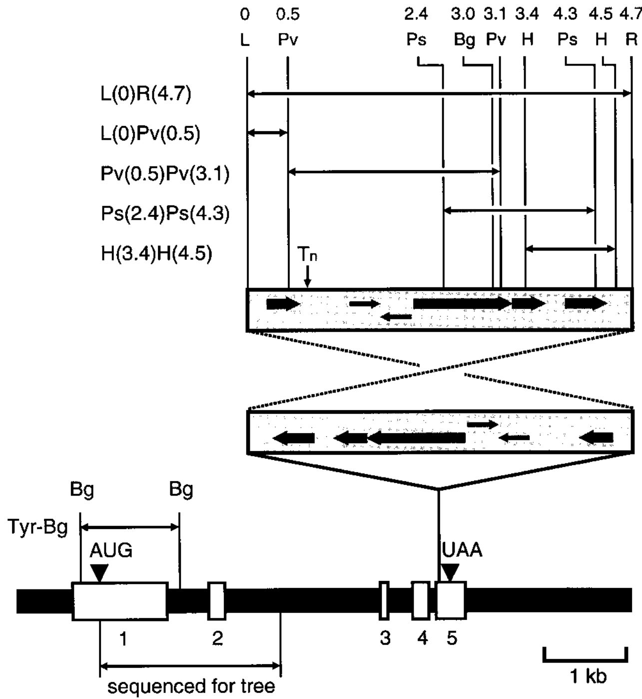

Figure 2.—Probes used for Southern blot analysis. The *tyrosinase* gene region of the medaka fish is illustrated at the bottom. The open boxes indicate exons 1 to 5. The stippled box is *Tol2-tyr*, identified as a DNA fragment inserted in exon 5 as shown. The thick arrows in the box indicate the spans and directions of the four *Tol2* ORFs. The thin arrows are internal inverted repeats of 302 bp and 303 bp. Because the direction of the *Tol2* ORFs is opposite to that of transcription on the *tyrosinase* gene, the *Tol2* portion is illustrated again by changing its direction. Parts of the *tyrosinase* gene and *Tol2*, indicated by double-headed arrows with their designations, were subcloned and used as hybridization probes. Restriction enzyme cutting sites (Bg, *Bgl*II; H, *Hin*dIII; Ps, *Pst*I; Pv, *Pvu*II) and the left and the right ends of the element (indicated by the letters L and R) are shown with their locations in kilobase pairs, 0 being assigned to the L site. T_n indicates the location of consecutive T's present in the first intron of *Tol2.* The double-headed arrow below the *tyrosinase* gene map is the region sequenced for construction of phylogenetic trees of fishes.

the fishes of the genus Oryzias, the swordtail fish, and zebrafish. In Figure 3B for which the hybridization conditions were the same as those for Figure 3A and probe L(0)R(4.7) (the entire *Tol2* element) was used, strong hybridization signals, consisting of multiple bands, were observed for *O. curvinotus* and *O. latipes.* No signals were observed with the other 8 species of the genus Oryzias. For confirmation, an additional four fish samples for each of the 8 Oryzias species were examined by the same method as for Figure 3B and no bands due to *Tol2* were observed (data not shown).

Genomic DNAs from swordtail fish and humans also lacked bands due to the *Tol2* element. However, in the lanes for zebrafish and chicken, multiple weak signals were observed.

Distribution and variation of ***Tol2*** **in** ***O. curvinotus*** **and** ***O. latipes*****:** Figure 4 shows an autodiagram of a Southern blot to determine the presence of *Tol2* in an *O. curvinotus*

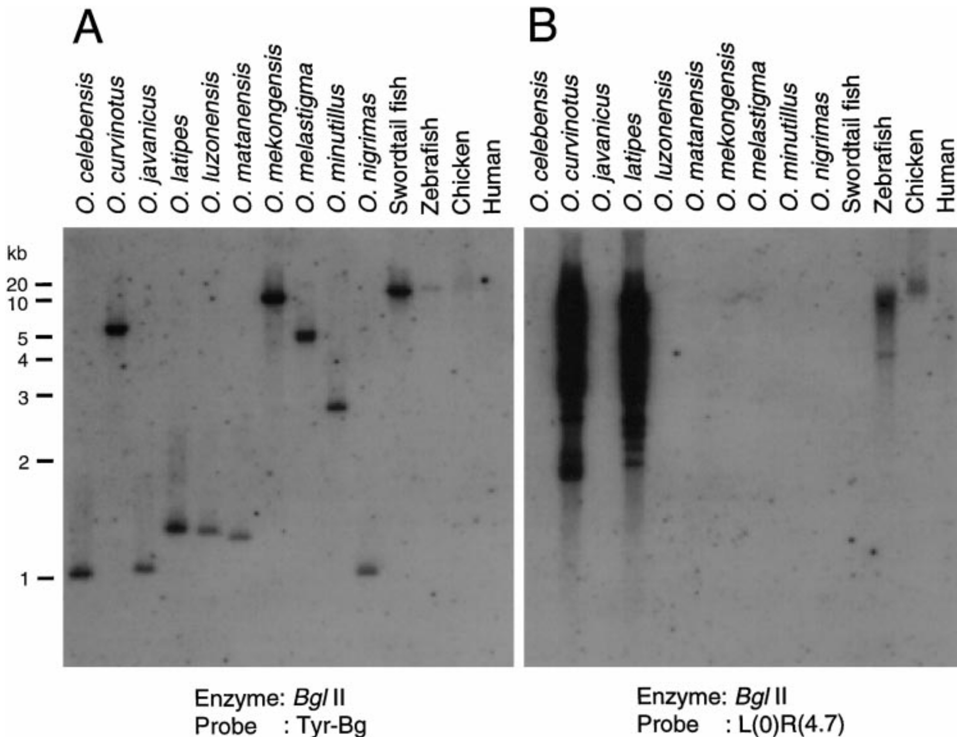

Figure 3.—Southern blot hybridization to examine the distribution of *Tol2* among species. Genomic DNAs were digested with *Bgl*II. Two sets of samples were fractionated on 1.0% agarose gels and transferred to nylon membranes. One was hybridized with probe Tyr-Bg (part of the *tyrosinase* gene) (A) and the other with probe L(0)R(4.7) (the entire *Tol2* element) (B). The fish [2c] (see materials and methods) was used as a sample of *O. latipes.* The sizes and mobilities of the size marker DNA fragments are indicated along the left margin.

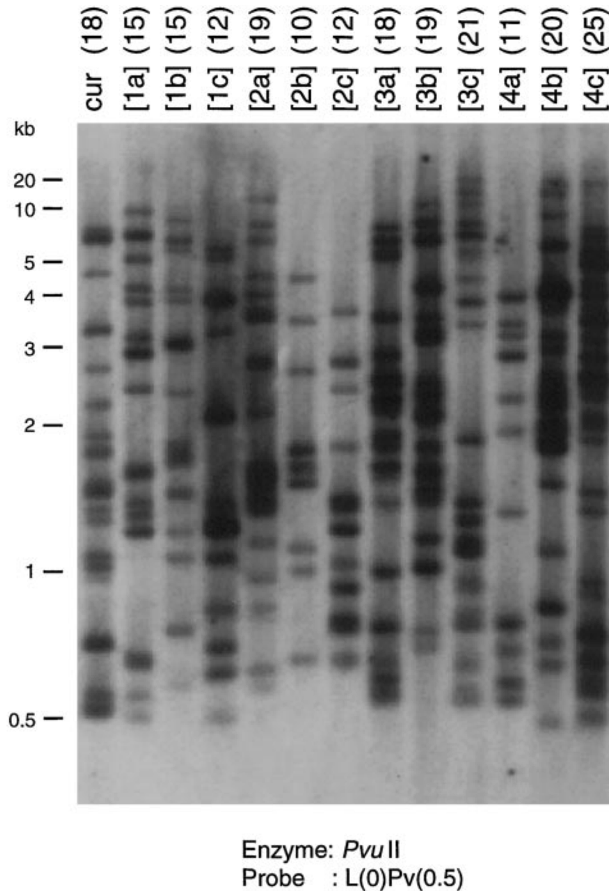

Figure 4.—Southern blot hybridization to examine the presence of *Tol2* in the samples of *O. curvinotus* and *O. latipes.* Genomic DNAs were digested with *Pvu*II. The hybridization probe was L(0)Pv(0.5). The designation "cur" stands for the *O. curvinotus* sample. [1a], [1b], etc., are the *O. latipes* samples (see materials and methods). The numbers shown in parentheses are the numbers of bands obtained by scanning the hybridization membrane along the lanes with a radioactivity scanner and by counting radioactivity peaks. The sizes and mobilities of the size marker DNA fragments are indicated along the left margin.

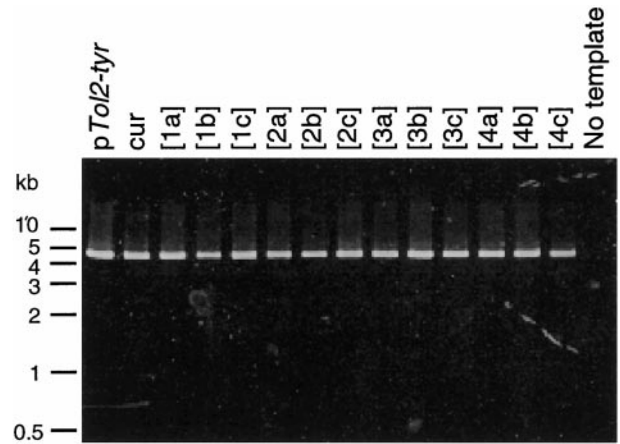

Figure 6.—PCR products from the *Tol2-tyr* clone and genomic DNAs. The template DNAs, indicated above the lanes, were 10 pg of the *Tol2-tyr* clone, 100 ng of fish genomic DNAs, and no template DNA. The PCR primers were 28 nucleotides from the ends of *Tol2-tyr.* The conditions were [2 min at 94°] and 25 cycles of [20 sec at 94°, 20 sec at 60°, 6 min at 72°]. Reaction mixtures were fractionated on a 1.0% agarose gel and stained with ethidium bromide.

sample and 12 samples of *O. latipes.* All 13 samples exhibited multiple bands hybridizing to probe L(0)Pv(0.5). Although this probe contains the left terminal inverted repeat (17 bp), it does not yield a signal for the right terminal inverted repeat (19 bp), as confirmed in a preliminary experiment using a *Tol2* clone as the target (data not shown). The numbers of bands observed are shown in Figure 4. In the *O. latipes* samples, they are distributed in the range between 10 and 25, with an average of 16. This range is not very different from that observed for a single local population (11 to 30, with an average of 19; Koga and Hori 1999).

It has previously been shown, using various parts of *Tol2* as probes, that *Tol2* copies are highly homogeneous in their restriction map structures in fish samples from a single local population (Koga and Hori 1999). In

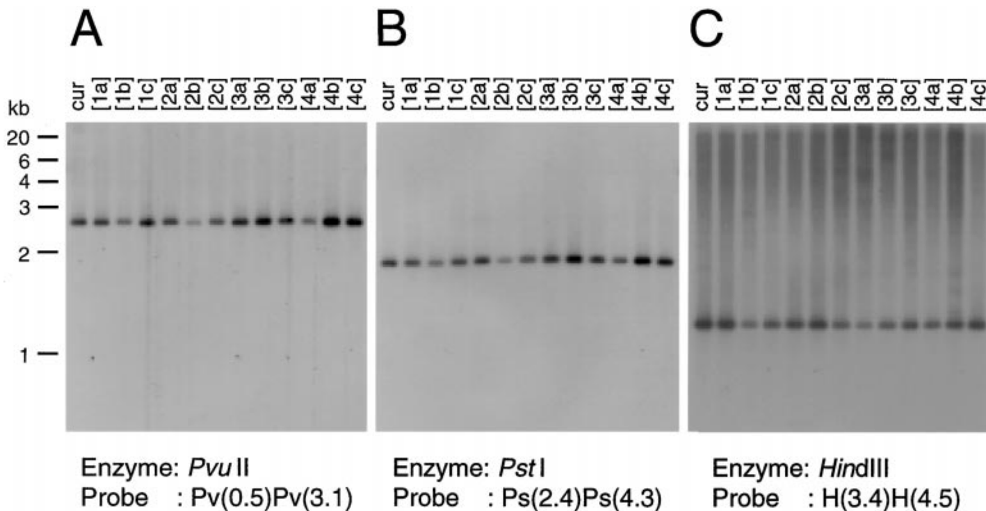

Figure 5.—Southern blot hybridization for examination of restriction patterns of *Tol2* copies. Restriction enzymes and probes are indicated under A, B, and C.

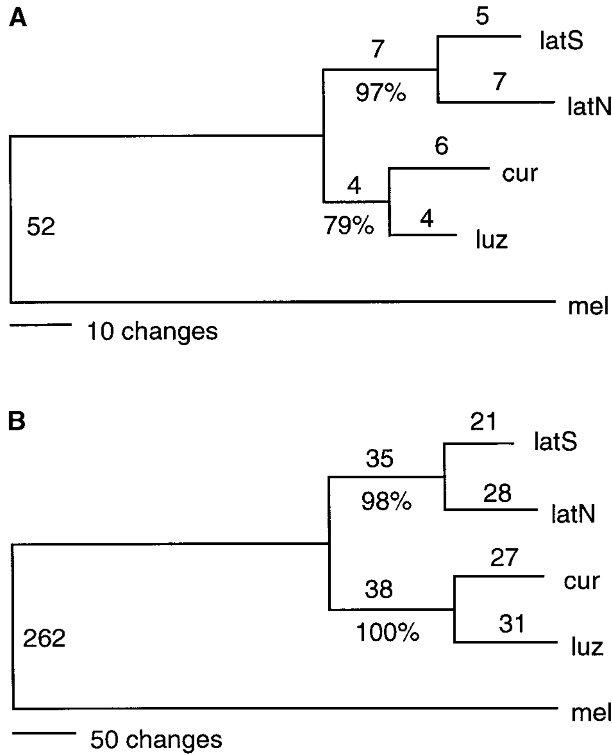

Figure 7.—Maximum parsimony trees of *tyrosinase* gene sequences. Nucleotide sequences were determined for the region indicated by the arrow in Figure 2. This region includes exon 1 (828 bp; nucleotides 3505 to 4332 of the *tyrosinase* gene sequence), intron 1 (439 bp; nucleotides 4333 to 4771), exon 2 (217 bp; nucleotides 4772 to 4988), and part of intron 2 (567 bp; nucleotides 4989 to 5555). The trees were constructed using PAUP (Swofford 1991). *O. melastigma* was used as an outgroup. The species are abbreviated with their first three letters. N and S stand for the Northern Japan and the Southern Japan populations, respectively. The numbers of mutational steps between the nodes are indicated above the branches. The percentages below each branch represent bootstrap values of 100 times. The sequences have been deposited in GenBank with accession nos. AB032694 (latN), AB032695 (cur), AB032696 (luz), and AB032697 (mel). (A) Sequences for exons 1 and 2 were used. (B) The whole sequences determined were used. The mel sequence proved to contain a 0.9-kb insertion in intron 2. This portion was excluded from the calculation.

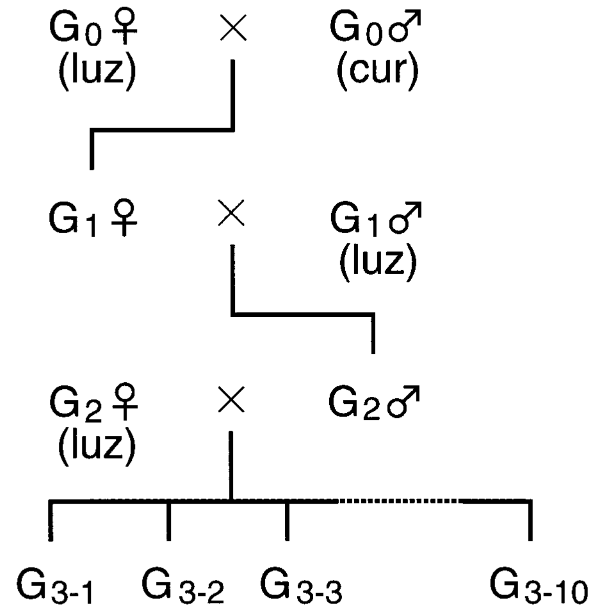

Figure 8.—Mating scheme for linkage analysis of *Tol2* copies. cur and luz in parentheses stand for *O. curvinotus* and *O. luzonensis*, respectively. Fishes of these two species were crossed as shown in the scheme.

the present study, the same method was applied to *O. curvinotus* and the 12 *O. latipes* fish. The results (Figure 5) were the same as those for a single natural population obtained in the previous study: single bands were observed for the three probes of *Tol2* internal regions and these bands were identical in size to those expected for *Tol2-tyr.* Results of PCR using the *Tol2* terminal regions (28 bp from the ends) as primers also indicated no shorter copies (Figure 6). Thus, with the same logic described in Koga and Hori (1999), most, or possibly all, *Tol2* copies carried by these 13 samples have lengths and restriction map structures identical to one another and to *Tol2-tyr.*

Sequencing analysis of ***Tol2*** **copies:** We have previously sequenced five *Tol2* copies randomly chosen from genomic libraries of *O. latipes* and found that they are identical over the entire 4.7-kb element (Koga and Hori 1999). The fish used were samples from the Southern Japan population. In the present study, using the same protocols, we cloned and sequenced one *Tol2* copy from a fish of the Northern Japan population and one from *O. curvinotus.* Differences were found only in the number of consecutive T residues in intron 1 (nucleotides 677 to 705 of the *Tol2-tyr* sequence; see Figure 2): 29 T's in five copies from the *O. latipes* Southern Japan population; 32 T's for the *O. latipes* Northern Japan population; and 33 T's for the *O. curvinotus* sample. Sequences outside the *Tol2* elements, including their target site duplications, differed from one another.

Sequencing analysis of the ***tyrosinase*** **gene region:** The *tyrosinase* gene is a single-copy gene located on one of the chromosomes (Inagaki *et al.* 1994). Its nucleotide sequence is available in GenBank under accession no. AB010101. This sequence was obtained from a fish of the Southern Japan population. To provide a frame of reference for considering the history of *Tol2*, we sequenced part of the *tyrosinase* gene of a fish from each of the Northern Japan population, *O. curvinotus*, *O. luzonensis*, and *O. melastigma*, the last one being used as an outgroup. The phylogenetic trees of the fishes based on the sequences obtained (Figure 7) were consistent with the phylogenetic tree that had previously been obtained (Figure 1).

Distribution of ***Tol2*** **copies in the genome:** *O. latipes*, *O. curvinotus*, and *O. luzonensis* have the same chromosome number, $2n = 48$, and their karyotypes are similar

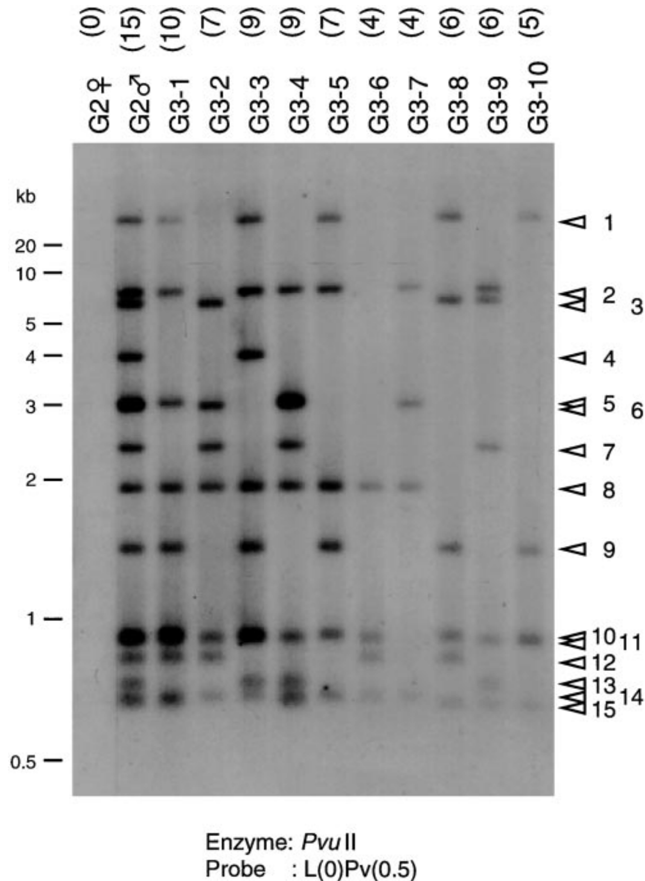

Figure 9.—Southern blot for examination of transmission patterns of *Tol2* copies. Genomic DNAs were prepared from the fishes of generations 2 and 3 (see Figure 8). The numbers shown in parentheses are the numbers of bands in each lane. The male parent (G_2 ♂) exhibits 15 bands, numbered from the top to the bottom along the right margin.

to one another (Uwa 1991). Of the three species, *O. curvinotus* and *O. luzonensis* can be crossed with each other and their hybrid progeny are fertile (Sakaizumi *et al.* 1992). *Tol2* is present in *O. curvinotus* but not in *O. luzonensis.* Using these two species, we made a linkage analysis of *Tol2* copies to ascertain if the copies are dispersed in the genome of *O. curvinotus.* Figure 8 illustrates the mating scheme we employed for this purpose. The first generation of the cross (G_0 to G_1) was conducted to make the copies "heterozygous." The following generation (G_1 to G_2) was to reduce the copy number for better identification of bands in Southern blots because the copy number at generation 1 was not sufficiently low, probably by chance and possibly by transposition. A single male of generation 2 (G_2 ♂) was then crossed to a female of *O. luzonensis* (G_2 ♀), and 10 fish of their progeny (G_3-1 to -10), together with the parents, were analyzed in a Southern blot for transmission patterns of *Tol2* copies between generations 2 and 3. Figure 9 shows the autodiagram obtained. The male parent (G_2 ♂) contains 15 copies, which were transmitted to the progeny without novel insertion events, because new bands were not present in the progeny. The copy numbers in the progeny (G_3) were between 5 and 10, with an average of 6.7. The 15 bands carried by the male parent were numbered from the top to the bottom as shown in the photograph. Fisher's exact probability tests were conducted for all possible combinations of 2 bands out of the 15, and a significant association ($P < 0.01$) was shown only for the combination of bands no. 1 and no. 9. Therefore, the copies represented by these 2 bands appeared to be closely linked to each other on the same chromosome, and the other copies appeared to be transmitted independently of one another. Thus, the *Tol2* copies are dispersed in the genome.

DISCUSSION

Distribution of *Tol2*: *Tol2* was first found in the medaka fish *O. latipes* (Koga *et al.* 1996). Its copies are highly homogeneous even at the nucleotide sequence level (Koga and Hori 1999). One possible explanation of this observation is that *Tol2* is a recently arrived element in the medaka fish genome. With this possibility in mind, we conducted the present Southern blot and PCR analyses of genomic DNAs to study the distribution and structure of *Tol2* in the genus Oryzias. The results show that *Tol2* is present in 2 (*O. curvinotus* and *O. latipes*) of the 10 species (Figure 3B) with identical restriction map structures in both of them (Figure 5). By contrast, restriction map length polymorphisms were observed in the *tyrosinase* gene region (Figure 3A). The discontinuous distribution of *Tol2* within the branch of the fish phylogeny consisting of *O. latipes*, *O. curvinotus*, and *O. luzonensis* (see Figure 1) is also revealing. These results are in accord with the hypothesis that *Tol2* has recently entered the medaka fish genome.

Weak hybridization signals with *Tol2* were observed for zebrafish and chicken. We conducted further Southern blot analysis of zebrafish using probes L(0)Pv(0.5), Pv(0.5)Pv(3.1), and Ps(2.4)Ps(4.3) under the same conditions as for Figure 3B. Similar signals were observed for probe Pv(0.5)Pv(3.1) but not for the other two probes (data not shown). It has been reported (Izsvák *et al.* 1999) that the internal inverted repeats of the *Tol2-tyr* sequence (see Figure 2) are copies of another, short transposable element called *Angel* and that the zebrafish genome contains 10^3 to 10^4 copies of this element. The weak hybridization signals might thus have been due to *Angel* copies and the chicken genome might contain similar repetitive sequences.

Hybridization signals were not observed for the swordtail fish and human samples. *Tol2* thus seems to be absent in these species or else might be too divergent to be detected with Southern blotting. It should be noted that the detectability in the hybridization analysis decreases as the genome size of the target species increases. To overcome this problem, we used 20 μg of genomic DNA for species outside the genus Oryzias,

five times as much as for those within the genus (4 μg). The sizes of haploid genomes are 0.68–0.85 × 10^9 bp for the medaka fish (Tanaka 1995), 1.7 × 10^9 bp for zebrafish (Postlethwait *et al.* 1994), and 3.0 × 10^9 bp for human (Gardiner 1995). Thus, at least for zebrafish and humans, the genome size should not be a significant factor affecting our inference.

Phylogeny of fish species: The relationship among *O. latipes*, *O. curvinotus*, and *O. luzonensis* has been studied from various aspects. Sakaizumi *et al.* (1992) examined electrophoretic patterns of 15 proteins and showed that the number of common alleles is larger between *O. curvinotus* and *O. luzonensis* than between *O. curvinotus* and *O. latipes*, and than between *O. luzonensis* and *O. latipes.* Uwa (1991) constructed a dendrogram of these three species based on their karyotypes, which is consistent with the phylogenetic tree shown in Figure 1. Uwa (1991) and Sakaizumi *et al.* (1992) examined fertility and meiotic segregation in interspecific hybrids and showed that reproductive isolation is complete between *O. latipes* and *O. luzonensis* and between *O. latipes* and *O. curvinotus* but not between *O. curvinotus* and *O. luzonensis.* All findings support the conclusion that *O. luzonensis* is more closely related to *O. curvinotus* than to *O. latipes.*

Molecular phylogenetic studies including the three species have also been completed. Naruse (1996) constructed a phylogenetic tree based on the nucleotide sequence divergence of the mitochondrial 12S ribosomal RNA gene; *O. curvinotus* and *O. luzonensis* clustered with a high bootstrap value of 86%, supporting the clustering of the two species. Another tree using the mitochondrial cytochrome b gene gave the same clustering pattern and exhibited a much higher bootstrap value, 100%, for the clustering of the two species (J. S. Albert and K. Naruse, personal communication). In the present study, we examined the phylogenetic relationship of the three species using a single-copy nuclear gene and obtained the phylogenetic tree shown in Figure 9. Clustering of *O. curvinotus* and *O. luzonensis* is again apparent.

Possibility of horizontal transfer: No deletion copies of *Tol2* were detected, while heterogeneity in size due to the presence of internally deleted, smaller copies is common in the *hAT* family members (Fedoroff *et al.* 1983; Streck *et al.* 1986; Warren *et al.* 1994). High homogeneity was observed not only in a local population (Koga and Hori 1999) but also across the entire species and even between different species (Figures 5 and 6). Although >100 base substitutions have accumulated in the *tyrosinase* gene region (Figure 7), variation in the *Tol2* samples was observed only in the number of consecutive T's in one of its introns. No other variation was found even in the third bases of codons and in introns. This is a strikingly high homogeneity in comparison not only with *hAT* elements but also with more diverse eukaryotic elements including *mariner*, which has been reported to be highly homogeneous (Maruyama and Hartl 1991b; Capy *et al.* 1992). The most likely explanation of virtually no sequence divergence between *O. curvinotus* and *O. latipes* is a recent *Tol2* invasion of one or both of the two species. This explanation involves horizontal transfer of *Tol2* from one species to the other or into the two species from a common source.

An alternative explanation of the sequence homogeneity of *Tol2* is to postulate a strong selective constraint on the *Tol2* sequence. However, this is difficult to reconcile with the fact that *Tol2* contains introns with a total length of 1992 bp (Koga *et al.* 1999) because strong selective pressure can be considered not to work on introns. Another possibility would be to invoke concerted evolution of *Tol2* copies. However, although homogeneity within species may thereby be conceivable, that observed between species is difficult to explain.

A new transposition event was not observed in the last generation of crosses (Figures 7 and 8). Similarly, Southern blot analysis of an inbred strain did not give evidence for transposition in the last few generations (Koga and Hori 1999). Thus, *Tol2* is not an element that moves as frequently as *P* and *mariner* of Drosophila, at least under usual laboratory conditions. However, in medaka fish natural populations, *Tol2* appears to be sufficiently active to create a situation in which *Tol2* copies are dispersed in the genome (Figure 9) and *Tol2* is widespread in the whole distribution area of *O. latipes* (Figure 4). We have demonstrated the activity of *Tol2* in zebrafish by introducing a clone into fertilized eggs and observing its excision by PCR (Kawakami *et al.* 1998; Kawakami and Shima 1999). The same result has also been obtained using *O. luzonensis* (A. Koga and H. Hori, unpublished results). These results indicate the potential of *Tol2* to persist in the genomes of these fishes once introduced into them.

Time and place of horizontal transfer: One of the possibilities about the time of occurrence of the horizontal transfer is that it took place after the collection of the original samples because the materials used in the present study have been maintained as fish stocks for 3–10 years. In other words, it might be that there was transfer of *Tol2* among the laboratory stocks. This possibility, however, is negligible because the two species have been maintained in different places: *O. curvinotus* has been in the Nagoya City Zoological Garden and the *O. latipes* stocks have been in our lab at the University of Tokyo. The two species have not undergone encounter with each other before DNA preparation. Thus, it is more likely that the horizontal transfer occurred in nature.

As to where in nature horizontal transfer occurred, overlapping distribution areas of the two species may be a likely supposition. *O. latipes* is distributed in a wide range in East Asia, from Japan to China. *O. curvinotus* inhabits Southern China and Vietnam. It was examined

whether the habitats of these two species overlap, with samples collected at various locations in China, but none was observed (Uwa and Parenti 1988). However, there is no obvious interruptive natural factor, such as a desert, between the distribution areas of the two species and it is conceivable that there might be an overlap somewhere in Southern China.

Concerning mechanisms involved in the horizontal transfer and also the direction of the horizontal transfer, our results so far obtained provide no suggestions.

Generality of horizontal transfer: Horizontal transfer may be considered general at least for *mariner*/Tc1 family elements because of (1) their presence in diverse organisms (Garcia-Fernàndez *et al.* 1993), (2) independence of the transposition activity from species-specific host factors (Garza *et al.* 1991; Lampe *et al.* 1996; Vos *et al.* 1996; Gueiros-Filho and Beverly 1997; Fadool *et al.* 1998), and (3) accumulation of convincing evidence (Maruyama and Hartl 1991a; Robertson and Lampe 1995). The *hAT* family elements are distributed in a wide range of organisms, comparable to that of *mariner*/Tc1 family elements: they are found in plants (Hartings *et al.* 1991; Tsay *et al.* 1993), fungi (Kempken and Kück 1996), insects (Warren *et al.* 1994; Coates *et al.* 1996), and other invertebrate and vertebrate animals (DeVault and Narang 1994; Bigot *et al.* 1996; Koga *et al.* 1996). Independence of species-specific host factors, at least to a certain extent, is demonstrated by activity in genomes that are free of the elements, as reported for *hobo* (Handler and Gomez 1995; Lozovskaya *et al.* 1996), *Ac* (Baker *et al.* 1986), and *Tol2* (Kawakami *et al.* 1998). Evidence for horizontal transfer from sequence divergence data has been shown for *hobo* (Simmons 1992). The results in this article also suggest horizontal transfer of *Tol2*. Horizontal transfer of *hAT* elements might be frequent, as it appears to be for *mariner*/Tc1 elements.

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