Molecular structure of the *copia*-like retrotransposable element *Yokozuna* on the W chromosome of the silkworm, *Bombyx mori*

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(Received 30 August 1998, accepted 14 December 1998)

We discovered a novel retrotransposable element, designated *Yokozuna*, on the W chromosome of *Bombyx mori*. The size of this element is 4738 bp, including a 208-bp long terminal repeat (LTR) on one side and a 183-bp LTR on the other. This retrotransposable element is flanked by a 5-bp target site duplication, TAATT. *Yokozuna* contains a single long open reading frame (ORF) and the whole deduced amino acid sequence of ORF reveals strong homology with *copia* of *Drosophila*. Moreover, an alignment analysis of the reverse transcriptase (RT) sequences suggested that the *Yokozuna* element is the first *Bombyx* retrotransposable element belonging to the *Ty1-copia* group. The number of *Yokozuna* per haploid genome is approximately four copies. Although *Yokozuna* was discovered on the W chromosome, it is not specific for the W chromosome.

INTRODUCTION

The sex chromosomes of the silkworm, *Bombyx mori*, are designated ZW (equivalent to XY) for the female and ZZ (XX) for the male (Tanaka, 1916). Femaleness is determined by the presence of a single W chromosome irrespective of the number of autosomes or of Z chromosomes, as shown by the observation that even ZZW and ZZZW individuals in the triploid and tetraploid states, respectively, are normal females (Hasimoto, 1933). Therefore, it is presumed that the female-determining gene, *Fem*, is present on the W chromosome of *B. mori*. Although 400 or more visible mutations have been placed on linkage maps in *B. mori* (Doira, 1992; Goldsmith, 1995), no gene for a morphological character has yet been found on the normal W chromosome. Therefore, finding a DNA sequence on the W chromosome would be an important breakthrough for further analysis of the W chromosome.

Recently, we identified five randomly amplified polymorphic DNAs (RAPDs) that are female specific. One of the five, designated Female-218 RAPD, is specific to the female on translocation-carrying W chromosomes of

B. mori (Abe et al., 1995, 1996; Ohbayashi et al., 1996); three of the five, designated W-Kabuki, W-Samurai and W-Kamikaze RAPDs, are specific to the female on the “true” W chromosomes of *B. mori*; and one of the five, designated W-Yamato RAPD, is specific to the female on the W chromosome of *Bombyx mandarina* (Abe et al., 1998b). A computer search for deduced amino acid sequences of W-Kabuki, W-Samurai, W-Kamikaze, and W-Yamato RAPDs revealed that all four amino acid sequences show homology to the previously reported amino acid sequences of retrotransposable elements (Abe et al., 1998b).

To further analyze the W chromosome of *B. mori*, we constructed a genomic DNA lambda phage library and picked the lambda phage clone containing the W-Kabuki RAPD sequence by using a PCR strategy. We then sequenced the 18.1-kb DNA insert in which we discovered a novel *copia*-like long terminal repeat (LTR) retrotransposable element, designated *Yokozuna*. Here we describe the novel *copia*-like retrotransposable element, *Yokozuna*, from the W chromosome of *B. mori*.

MATERIALS AND METHODS

Insect. The TW+P strain of *B. mori* was used in this

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study. TW+P, which is congenic to the J137 ($+^p/+^p$) strain with respect to the W chromosome, was constructed by crossing a C137 (Dp [2; W] $+^p, p/p$) female with a male of the J137 strain and backcrossing the resulting F₁ females to J137 males. $+^p$ represents the normal larval marking gene and p (plain) represents the recessive allele; the p/p larva is white. The TW+P strain has been maintained by repeated backcrossing of the females (ZT [W; 2] $+^p$) to males of the J137 strain.

Genomic lambda phage library construction and screening. Two females of the TW+P strain were used for genomic lambda phage library construction. Genomic DNA was extracted from larval posterior silk glands by the method of Bender et al. (1983). The DNA was further purified by phenol/chloroform extraction and was ethanol precipitated three times. The DNA was digested with *Bam*HI for 90 min and ligated into λ EMBL3 (Stratagene). This ligation mix was packaged in vitro using a Gigapack II kit (Stratagene) according to the manufacturer's instructions, and plated onto *Escherichia coli* XL1-BlueMRA (P2).

We predicted that conventional screening of lambda phage libraries by hybridization would be difficult because the W-Kabuki RAPD sequence, based on a homology search, may contain two different elements. Thus, the resulting lambda phage library was screened by a locus-specific PCR strategy as described below. Initially, we determined the titer of the lambda phage library by serial dilution. In the initial round of screening, we made ten 9 cm plates, each containing nearly 10,000 plaques. Plates were removed from the incubator, covered with 8 ml SM buffer, and stored at 4°C for 16 h to elute the lambda phage. Aliquots of 0.3 ml SM buffer containing phage particles from each plate were heated at 95°C for 5 min to elute lambda phage DNAs and then 1 μ l was used as a template for PCR. The PCR protocol was that used by Abe et al. (1998a). To detect the W-Kabuki RAPD as a sequence characterized amplified region (SCAR) marker (Paran and Michelson, 1993), PCR reactions with the Kabuki-1A primer (5'-CCGCATCTACCTAACTCAGC-3') and Kabuki-2 primer (5'-CGCATCTACCATACTGCTCA-3') consisted of 40 cycles: 94°C for 1 min, 50°C for 2 min, 72°C for 3 min, and a final extension of 10 min at 72°C. Female genomic DNA was amplified for positive controls. The SM buffer containing W-Kabuki was used in subsequent rounds. Similarly, during the second and third rounds of screening, we made ten plates containing nearly 1000 and 100 plaques, respectively. Finally, we made twenty plates, each containing nearly 20 plaques, and randomly picked 200 plaques by microspurtle and dropped each into 1 ml of SM buffer. Three of 200 plaques were positive. One of these positive plaques, named K-1/3, was sequenced.

DNA sequencing and computer analysis. Standard DNA sequencing techniques were carried out according to

Sambrook et al. (1989). DNAs were sequenced using the Dye Terminator Cycle sequencing kit (Perkin Elmer ABI 377). The BLAST program (Altschul et al., 1990) was used to search for sequence similarities with known DNA (blastn) or protein (blastx) sequences. An amino acid sequence alignment was conducted with the CLUSTAL W (Thompson et al., 1994) and modified by visual inspection. A protein distance matrix was calculated from the aligned sequences (Fig. 4), where the insertions and deletions were ignored, by using Dayhoff's PAM amino acid substitution matrix (Dayhoff et al., 1978). A phylogenetic tree was constructed from this matrix using the Neighbor-Joining method (Saitou and Nei, 1987). From this, the tree in Figure 5 was constructed. To estimate confidence limits, 100 replicates of bootstrap analyses were performed using the programs of PHYLIP (Felsenstein, 1989). The nucleotide sequence of *Yokozuna* will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases with the accession number AB014676.

Construction of silkworm bacterial artificial chromosome library. A bacterial artificial chromosome (BAC) library for *B. mori* was constructed under collaboration with Dr. Pieter de Jong's laboratory (Osoegawa et al., 1998; Mita et al., unpublished). Silkworm high molecular DNA from the nuclei of posterior silk gland cells of 5th instar larvae of strain p50 was prepared by embedding it in agarose as described by Ioannou and de Jong (1996). The nuclei isolation of posterior silk gland cells was described previously (Ichimura et al., 1985). Preelectrophoresis and partial digestion of silkworm genomic DNA with *Eco*RI and size fractionation were carried out to isolate 150–200-kb DNA fragments as described in the paper (Osoegawa et al., 1998). These fragments were ligated with *Eco*RI-digested and dephosphorylated pBACe3.6 vector. The conditions of ligation and transformation were also followed as described in the paper. The resultant BAC library of *B. mori* represents an estimated 11-fold redundancy of coverage of the *B. mori* genome with an average insert size of more than 170 kb.

Hybridization of BAC filter. The hybridization of a BAC high density replica (HDR) filter was carried out by the ECL hybridization method (Amersham). The hybridization conditions and labeling protocol were exactly followed per the manufacturer's directions.

Genomic Southern blots and hybridization. Genomic DNAs were prepared from posterior silk glands and digested with restriction endonucleases. Then, these DNA samples were electrophoresed in 1.0% agarose gels and blotted onto nitrocellulose filters (Biodyne plus membrane; Pall Biosupport Division) in 20 $\times$ SSC (1 $\times$ SSC is 0.15 M NaCl plus 15 mM sodium citrate) by capillary transfer, and were fixed by exposing to UV light. The hybridization solutions

contained 50% formamide, $6 \times$ SSC, 0.5% SDS, and 100 μ g/ml denatured salmon testes DNA. The Yoko-1 (nt 342–nt 2595) was used as a probe labeled with [α - 32 P]dCTP (ICN) by the random priming method using Random Primer DNA Labeling Kit Version 2 (Takara). Hybridiza-

tion was performed at 42°C. The membrane was washed two times with $2 \times$ SSC, 0.1% SDS for 10 min at 42°C, and then for 15 min at 50°C under the same conditions.

RESULTS

Cloning of the *Yokozuna* element. We used a PCR strategy to pick a lambda phage clone, K-1/3, which contains the W-Kabuki RAPD sequence (Fig. 1) and we sequenced the DNA insert. This K-1/3 clone contained 18.1 kb insertional DNA. Based on a blastn and a blastx search, we determined that this 18.1-kb insertional DNA is composed primarily of clusters of three retrotransposable elements, one retroposon, and one functionally unknown insertion. Each element, namely a non-LTR retrotransposable element BMC1 (Ogura et al., 1994), a retroposon *Bm1* (Adams et al., 1986), functionally unknown inserted DNA (designated as FUI), and a novel *copia*-like LTR retrotransposable element, *Yokozuna*, was inserted into a novel *gypsy*-*Ty3*-like LTR retrotransposable element, *Kabuki* (unpublished) (Fig. 2). In the FUI, no significant ORFs were obtained, despite the presence of LTR-like sequences found at both ends.

Structure of *Yokozuna* LTR. The *Yokozuna* retrotransposable element was found to be 4738 bp in length, with two LTRs (Fig. 3). The 5'- and 3'-LTRs were 208 bp

Fig. 1. Amplification patterns of genomic DNA from males and females of the TW+P strain; the DNA from lambda clone K-1/3, which contains the W-Kabuki RAPD sequence; and another lambda clone, N-K2, which lacks the W-Kabuki RAPD sequence. M: molecular size marker, λ -EcoT14 digest. The numbers at the left indicate base pairs. The arrowhead indicates the W-Kabuki RAPD amplified as a SCAR marker by using a set of primers, Kabuki-1A and Kabuki-2.

Fig. 2. Schematic view of the lambda clone K-1/3. This map is based upon DNA sequence information. Each block indicates a transposable element and each box with or without an arrow indicates one LTR or LTR-like sequence of each retrotransposable element. The *Yokozuna* element located in a novel LTR retrotransposable element *Kabuki* is shown flanked by its LTRs. The transcriptional orientation (arrows over or under each boxes) of the *Yokozuna* and BMC1 is opposite that of the *Kabuki* element. FUI, functionally unknown inserted DNA with two LTR-like sequences. The W-Kabuki RAPD sequence is extended from *Kabuki* to FUI. Restriction site, *Bam*HI. Cys, cysteine and histidine motif; Pro, protease domain; Int, integrase domain; RT, reverse transcriptase domain; and RH, RNase H domain.

cacataaattggttgagataatttttagaattctgtgcactactctgtgacctatataattgtagt	140
cccttccggctgcgtatataaattggtgcccgacttcttctgtgtaataattttgccaaagaaaatccaat	290
cgaaaagtcaaggaggaagacaaaaatcacaaaaaatttacttggcaactaaaatggcggacagcggt	440
madddasrklpeftgtgnfstwifrisclleek	590
tgcaaaagacaaattggaactggcataaacagaggaataattgaaactcagaaaattcaaaaaagaa	740
ckeaiedgiteeilksekfkfkddakarslilvncslsdkhleyvrsgtsagsake	890
atgatcgaaaacttacgaaaaatttcaaaagaaaagtaccttattccggcttatagctgcaggaagaaat	1040
mienlrkifkkrkstlsalylvvrklstllklcdpsvlelsdhnfnfndmlirql	1190
gaaactggaaagcagctaaatgaacagcaaggtgtgccacttactacttgccctgacagctacacatac	1340
etgstvneqdkvchlrlttmpdpsyntttitaleltnvelttteyvkksklldae	1490
ctaaaaaaccaaaataattcaattggaatcagagatgcacacagcttctcagttgctttaaattggt	1640
lknqnnsmsesqdgadahsflscgckcgktghkaiqcrtgthlmrgqhrqgkmgngv	1790
tatttcaacgggaactacagactctctagggcaacggcagagacttcaattccaggtacggcagagct	1940
yfnsgnttrdfrfyrngngdrfnpgngngfrgnfrnsgnrpgrnvrqsgsrssrdtilq	2090
ggaaaaagtgccttaagtggagaaaatccattttctttgttgcagtgaaaatgtactacttctgtgat	2240
gksalsesernpfsfvaseenvlsvdvynedikfiiidsagasqnlvidsyekym	2390
tcaaaattgaaatatttagaaactaaaactaagatttatgttgcattggtcaatatttggttcaagt	2540
snieylelletktkiyvncangqylfslscgkglilnvtkgynclptkiealelavlklshn	2690
atatattctgttataaaatttataagaaagaaaattctgtcagatttcagataaaattctgtatac	2840
ilsvkklllekkgnsvrfhknsvsiaiagnniiygtmnlnslyainltnleqc	2990
tattcgataaataatgataaagacttatggcataaaaaggttaggacatgcttaatagaaaacact	3140
ysinnndkdlwhkrlghanrhkrhklklklkpisekpcgicvegkstrlpfstt	3290
cccagcccgagatcaagattatagcgagactatcacacagatataatcaggaccataaaatttctc	3440
pkprskykiyelihthdisgpinipltltnevyfhtiiddythfcevyllqrk	3590
agtgagcgaacccgataggctctatagaattgttaatagaattgaaagacaaaatagaattgtaagt	3740
seattldrlielevyvnrmerqieackevkikirsdnngseefknecltnkfckdkgtciltq	3890
tttactttggcttatalgcccagctcaaaaggvtttcagaaacgcattgaacagaaaattatttaac	4040
ftlpyspqsnsgvsrmermnriynrartlliesglpkltlwgeavrvctavycttn	4190
agatgtccattcgccaactttcaaacccagcagagaaaattgttggaaaacaaagacttaacagac	4340
rcpsssnfqtptaekmfngnkdltrrlrifgskswvhiipkqdkllqrakemrl	4490
gtaggatatagcccaattggatattcgcttatgggacccctacagataaaagktatagtgctcaag	4640
vgyspngyryrlwdptssnkvivrsrdvridetqltlnyketeeekhnikgeteyed	4790
gaattaatacaaaatgatgatactgagaaggacacagaaggtgaccaagagataagaataacctaat	4940
elinqnddtekdtegdqdeiricpnenendnmqhdrripdktnkefvsknqddde	5090
gaaaaataaaaattgaaataaaaaggtagaaataataacagagatcaggaagagaaaatccgaaa	5240
eneklnkkgrinikykylitrsGREIRKPKNleeydlndlniayclslsgdpqefe	5390
gatgcaattcatgacaagactgggaaaaagcaataaagacagaataaaattcacagtgaaaattag	5540
daihdkdwekaijktelnsqvkleitweeattlpmgskaidtkwvfrtkqngt	5690
aagagacgctgatttagtgacgaaggttttcagcaacagacaatgataatcattatgctccggtag	5840
krallravltagakgfqqqnndhnyapvawklstirlmmlslavqlldslskqldvpt	5990
ttcttaaatggaagattaaacagactgtctataataaattgtcccaaggaattgaaattagtgaga	6140
flngklndnvYikcpkgmeisegkvllklkralyglgleapkcwnqrfhnfv	6290
actcagaaagggtttgtacaattcacacgatgatttatgtttatagtaaagagaaaaatttggat	6440
tqkqgfvyqsqhldlcllygkgkikiwlllyvddlilylgnssnemikelekefkfvkn	6590
ttaggagaagtacatacaatttttaggaattggagtaacaagaagacagacttgaaaataagaca	6740
lgevhyqllylglevtrtedsleirqteiaikrlllekymqdcksktpein	6890
aattgaaacatttccatcaccatcagataataattgaagtacccttcaagcaataataggacttt	7040
nvniipspsdiievvpfkeliylgsllyiavnssrpdiaayavsfslsrfldkptqe	7190
ttatggacagcaagtaagagaggtitacgtttatgaaggaactcttcacagggacttgatttaga	7340
lwtasrkvrlyrlklygtctshqglilyrkteeytkliaysdadwadardtrdkrst	7490
ggctgtgcttatatatcaaggccagaacttaataacttggttctctagaaagcaaacatgtacgg	7640
gcailyvgqgnlittwfsrskqtctalstaeaeYVAGAHSMSELihikgilsdi	7790
ctaagctactagtatcatttttagtttgacaaactcagagtacaaaatttgatacaaaaactcag	7940
lstsiisahlldndnqsstikaliqnqmnttkaashieikyhyiakdliylkklivs	8090
atctcatatgtgcctacaaaatgaaaattatctctgatataatttacaaaactctcaactattt	8240
isyvptnneisidiftkssstiqkhnYfcsklnlv*	8390
agcattatttattatcttaagagaatttaatttagataaatttttagtaataatttttaagttaac	8540
tcatattacatttataattttgttggcaaattttaacagctgtgaaactctataaattttagggg	8690
attataaaatgtactatcattctccttccgcttgcgttaataatgttgcggctgtttgtgataa	8840
ttttagttagtctgtacataactttagttagttagttagttagttagttagttagttagttagt	8990

Fig. 3. Complete nucleotide sequence of the *Yokozuna* element from clone K-1/3. Lower case letters correspond to flanking sequences with the target site duplication double underlined. The LTRs begin and end with the underlined nucleotides TG and TA, respectively. The deduced amino acid residues of the single ORF are shown below the nucleotide sequence. Underlined amino acid residues correspond to conserved sequence motifs identified in other *Ty1-copia* group elements: one cysteine-histidine motif of *gag*-like genes, DSGA and ILS conserved motifs of proteases, H-X₄-H-X_N-C-X₂-C and D, D(35)E motifs of integrases, the seven motifs of reverse transcriptase sequences, and a domain of RNase H from EYT to DIFTK. Sequences within the boxes correspond to the presumed promoter site (TATAAT) and poly(A) signal (AATAAA). The underlined nucleotide sequence adjacent to 3'-LTR is a polypurine tract.

and 183 bp in length, respectively, and were identical except for a deletion (or an insertion) of 25 bp. Since only one *Yokozuna* clone was sequenced, it remains to be determined which LTR length is major in *Yokozuna*. However, there was a presumed promoter site (5'-TATAAT-3') and a poly(A) signal (5'-AATAAA-3') in both LTRs. A region adjacent to the 5'-LTR seems likely to be a tRNA primer-binding site (PBS). However, data base screens for DNA sequence identities were not complementary to any tRNA. On the other hand, a region adjacent to the 3'-LTR of *Yokozuna* contained a short polypurine tract nine bp in length (8/9). LTRs of many retrotransposable elements have a short inverted repeat (5'-TG – CA-3') at the ends. However, the LTRs of *Yokozuna* differ from other retrotransposable element LTRs in that they do not terminate in CA.

ORF of *Yokozuna*. DNA sequencing of the *Yokozuna* element revealed a single ORF which occupies nearly the entire region between the LTRs. This ORF extends from position nt 342 to 4289, starting with the amino acid (aa) initiation codon (methionine); the length of the ORF in the *Yokozuna* was 1316 amino acids (Fig. 3).

The *Yokozuna* ORF contained five regions with sequence similarity to functional domains found in the ORFs of the *Ty1-copia* group of LTR retrotransposable elements. The first domain was a putative nucleic acid binding domain (Covey, 1986). This domain of *Yokozuna* contained one putative cysteine and histidine motif (C-X₂-C-X₄-H-X₄-C) (Cys), located at aa 202, found in retroviral *gag* genes. The second domain was a potential protease domain (Pro) located immediately downstream of the *gag*-like domain. The conserved residues of this domain, "DSGA" followed by "ILS", are similar to those identified in *copia* (DSGA – LMS) and TY912 (DSGA – LLS) (Doolittle, 1989). The third identifiable domain was an integrase (Int) located immediately downstream from the potential protease domain. An H-X₄-H-X₁₇-C-X₂-C motif seems to correspond to a conserved motif of retroviral integrase (H-X₃-H-X₂₂-C-X₂-C) (Johnson et al., 1986). Also found was a highly conserved amino acid domain of integrase, referred to as D,D(35)E, and composed of two invariant aspartate (D) residues and a glutamate (E) residue consistently separated by 35 amino acids (Kulkosky et al., 1992). The fourth domain was a potential reverse transcriptase (RT) domain, containing the seven motifs identified in all reverse transcriptase sequences (Xiong and Eickbush, 1988, 1990). The (final) fifth domain was a potential RNase H domain (RH) (Doolittle, 1989) located immediately downstream from the RT domain. The order of the five domains in the ORF of *Yokozuna*, Cys-Pro-Int-RT-RH, from 5' to 3', was identical to that within retrotransposons of the *Ty1-copia* group.

Copy number of *Yokozuna* in the genome. In a pre-

liminary experiment, we characterized the BAC library with a BmeE75 cDNA fragment, which was expected to correspond to a single copy gene, as a probe in the genome of *B. mori*. Nine positive clones were detected. Thus, this BAC library represented most of the *B. mori* genome at the expected redundancy of coverage. To estimate the copy number of *Yokozuna* in the genome, we performed Southern hybridization using BAC library filters and the Yoko-1 probe. Approximately 46 positive clones were detected in 36,864 clones. From this result, the number of *Yokozuna* per haploid genome was approximately four copies.

To estimate the difference of the copy number in the genome between male and female, we performed Southern blot hybridization using the Yoko-1 as a probe. The genomic hybridization pattern did not vary in either sex. This result indicates that *Yokozuna* is not specific to the W chromosome, but is present on the autosomes as well.

Phylogenetic relationship of *Yokozuna* to other retrotransposable elements. A blastn search for DNA sequence similarity revealed that the DNA sequence of *Yokozuna* shows homology with many *copia*-like elements. For example, nt sequences 1822–2248 (Int domain), 3034–3347 (RT domain), and 4077–4336 (RH domain) exhibit nt identity of 54% with nt sequence 1837–2263, 57% with nt 3178–3491 of *Drosophila koepferae copia* element DNA (GenBank accession No. X96971), and 53% with nt 4422–4681 of *D. melanogaster copia* element (accession No. M11240; Mount and Rubin, 1985), respectively (figure not shown).

A blastx search for deduced aa sequence similarity revealed that the aa sequence of *Yokozuna* ORF shows homology with *Drosophila copias*. For example, the highest level of homology was seen between the deduced aa sequence throughout the whole of the ORF of *Yokozuna* and the aa sequence of *copia* from *D. melanogaster* (accession No. M11240). The identity (similarity) between the two deduced aa sequences was calculated to be 30.8% (57.5%) when a gap was counted as one substitution (figure not shown). A phylogenetic tree of retrotransposable elements has been constructed by comparison of deduced amino acid sequences of the RT domain (Xiong and Eickbush, 1990). The amino acid sequence of the RT domain of *Yokozuna* was compared to those of *Ty1-copia* group elements and the *gypsy* element (Fig. 4). Amino acid alignment revealed amino acid identities (similarities) of 35.0% (65.5%), 32.2% (59.6%), 37.7% (64.7%), 34.9% (63.6%), and 18.3% (45.1%), with *copia*, 1731, *Tnt1*, *Ta1*, and *Ty1*, respectively (Fig. 4). The phylogenetic tree in Figure 5 indicates that *Yokozuna* is comparatively closer to the *copia* family than to *Ty1*. Figure 5 suggests that *Yokozuna*, *copia*, 1731, and *Tnt1/Ta1* all diverged during a relatively short time, and have since been evolving independently over a long period, being that they are equally

Fig. 4. Amino acid sequence alignment of RT-related sequences of the *Yokozuna*, other *Ty1-copia* group elements and *gypsy* elements. This figure was generated by performing a CLUSTAL W alignment and modified by visual inspection based upon Xiong and Eickbush (1990). Identical residues that are present in at least two of the seven amino acid sequences are highlighted by black boxes, while similar residues are highlighted by gray boxes. Numbers to the right indicate the number of residues from the 5' end of the total ORF containing the RT sequences. *Yokozuna*, *Yokozuna* on the W chromosome of *B. mori*; *copia* and 1731, *copia* and 1731 of *D. melanogaster* (Mount and Rubin, 1985; Fourcade-Peronnet et al., 1988); *Tnt1*, *Tnt1* of *Nicotiana tabacum* (Garvey et al., 1990); *Ta1*, *Ta1* of *Arabidopsis thaliana* (Voytas and Ausubel, 1988); *Ty1*, *Ty1* of *Saccharomyces cerevisiae* (Clare and Farabaugh, 1985); *gypsy*, *gypsy* of *D. melanogaster* (Marlor et al., 1986), and from RT ① to RT ⑦ seven motifs identified in the RT sequence.

Fig. 5. Phylogenetic tree of the amino acid sequences of seven motifs in the RT domain of *Yokozuna* and other *Ty1-copia* group elements. The *gypsy* element from *Drosophila melanogaster* was used as an outgroup. The scale means corrected genetic divergences. The numbers above the branches are bootstrap values per 100 replicates.

distant from each other.

DISCUSSION

We discovered that *Yokozuna* on the W chromosome of *B. mori* is a retrotransposable element belonging to the *Ty1-copia* group. The central region of this element contains a single ORF. This ORF contains a *gag*-like domain, a protease domain, an integrase domain, a reverse transcriptase domain, and an RNase H domain. Analysis of the DNA sequence and the deduced amino acid sequence of the ORF shows that *Yokozuna* is most similar to *copia* of *Drosophila*, which represents the *Ty1-copia* group. It remains to be investigated whether *Yokozuna* is active transcriptionally or transpositionally in vivo or in vitro and whether virus-like particles of *Yokozuna* are formed, as observed in *D. copias* (Shiba and Saigo, 1983; Yoshioka et al., 1992). Although *Yokozuna* was discovered on the W chromosome, it is not specific to the W chromosome.

All four aa sequences of the four RAPDs identified on the W chromosomes of *B. mori* and *B. mandarina* show homology to aa sequences of retrotransposable elements (Abe et al., 1998b). Furthermore, a complete full-length

BMC1 retrotransposable element (Abe et al., 1998a), a retroposon Bm1 and a novel *copia*-like *Yokozuna* were tandemly inserted into a novel *gypsy-Ty3*-like retrotransposable element (unpublished). Thus, it is likely that many retrotransposable elements and their derivatives have accumulated on the W chromosome. Because crossing over is restricted to males in *B. mori* and because the W chromosome is transmitted only through the female line, the W chromosome is recombinationally isolated from the Z chromosome or the autosomes. The W chromosome would be expected to change (evolve) more slowly than other chromosomes and the inserted transposable elements would not likely be changed compared to other chromosomes.

Elements of the *Ty1-copia* group are present in a wide range of species from yeast (*Sacchromyces*), with the *Ty1* elements, to *Drosophila*, with the *copia* elements, and include algae and many plants (Capy, 1998). Phylogenetic analysis has provided a body of data that suggests that horizontal transfer of the *Ty1-copia* group retrotransposons between different host species has occurred in the past (Flavell, 1993; Eickbush, 1995). Although it remains possible that significant levels of horizontal transfer of the *Ty1-copia* group retrotransposons occurs between species, in light of recent work it now seems unlikely (Flavell et al., 1997). *Yokozuna*, to our knowledge, seems to be the first full-length retrotransposable element identified belonging to the *Ty1-copia* group in Lepidoptera. It will be interesting to isolate *Ty1-copia*-like retrotransposable elements from a greater number of species in this family. It will be useful in consideration of horizontal transfer and evolution of the *Ty1-copia* group of retrotransposable elements.

This work was supported in part by CREST (Core Research for Evolutional Science and Technology) of Japan Science and Technology Corporation (JST).

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