A novel model of double replications and random loss accounts for rearrangements in the Mitogenome of Samariscus latus (Teleostei: Pleuronectiformes)

Wei Shi, Xian-Guang Miao and Xiao-Yu Kong*

Abstract

Background: Although more than one thousand complete mitochondrial DNA (mtDNA) sequences have been determined in teleostean fishes, only a few gene rearrangements have been observed, and genome-scale rearrangements are even rarer. However, flatfishes (Pleuronectiformes) have been identified as having diverse types of mitochondrial gene rearrangements. It has been reported that tongue soles and the blue flounder mitogenomes exhibit different types of large-scale gene rearrangements.

Results: In the present study, the complete mitochondrial genome of another flatfish, Samariscus latus, was sequenced, and genome-scale rearrangements were observed. The genomic features of this flounder are different from those of any other studied vertebrates, including flatfish species too. The mitogenome of S. latus is characterized by the duplication and translocation of the control region (CR). The genes located between the two CRs are divided into two clusters in which their relative orders are maintained.

Conclusions: We propose a "Double Replications and Random Loss" model to explain the rearrangement events in S. latus mitogenome. This model consists of the following steps. First, the CR was duplicated and translocated. Subsequently, double replications of the mitogenome were successively initiated from the two CRs, leading to the duplication of the genes between the two CRs. Finally, one of each pair of duplicated genes was lost in a random event.

Keywords: Flatfish, Flounder, Mitochondrial recombination, Gene order, Molecular revolution

Background

The vertebrate mitochondrial genome typically codes for 37 genes, including 13 protein-coding genes, 22 transfer RNAs (tRNAs) and two ribosomal RNAs (rRNAs). These genes are arranged on a highly compact circular genome [1-3]. The order of the genes was initially considered conserved among vertebrates. However, with more than 1700 complete mitochondrial DNA (mtDNA) sequences currently determined, mtDNA gene rearrangements have been found in several groups, including birds [4-6], reptiles [7,8], amphibians [9,10] and fishes [11-13].

Gene rearrangements in animal mtDNA can be explained using four available models. (1) The Recombination model, which is characterized by the breakage and rejoining of the participating DNA strands [14], has been used to account for changes in the mitochondrial gene order in frogs, birds, mussels and other organisms [15-17]. (2) Another commonly accepted hypothesis is the Tandem Duplication and Random Loss (TDRL) model, which posits that rearrangements of the mitochondrial gene order have occurred via tandem duplications of some genes, followed by the random deletion of some of the duplications [18,19]. This model is widely used to explain gene rearrangements in vertebrate mtDNA [4,11,20,21]. (3) The other two models are seldom used; one is the Tandem Duplication and Non-Random Loss (TDNL)
model proposed by Lavrov et al. [22], and the other is the tRNA miss-priming model [23,24].

Fishes constitute the vertebrate group with the largest number of determined complete mtDNA sequences (1107 species as of July 2013; http://www.ncbi.nlm.nih.govigenomes). However, only a few gene rearrangements have been observed in fish [12,13,25-27], and genome-scale rearrangements are rare [11]. Flatfish (Pleuronectiformes) have been found to be a diversified group for mitogenome rearrangements. Studies of the Cynoglossus semilaevis and Paraplagusia japonica (tongue soles, Cynoglossidae) mitogenomes have discovered the translocation of their control regions and a tRNA gene inversion [31]. However, no gene rearrangements have been detected in soles (Soleidae), the closest family to the Cynoglossidae [28-30]. This interesting event has also occurred in two other species belonging to close families Bothidae and Paralichthodidae. The mitogenome of Crossorhombus azureus (blue flounder) exhibits genome-scale gene rearrangements, whereas the gene order of Pseudorhombus cinnamoneus is intact [31]. These findings imply that the origin of gene rearrangements in different groups of Pleuronectiformes was likely a case of “polyphyly”. Due to the short divergence time between the fishes with and without gene rearrangements, many residual traces of these rearrangements may have been preserved; the mechanism of the rearrangement events may be inferred from these traces. Kong et al. [12] developed a model of inverse duplication and the deletion of redundant genes to explain gene rearrangements in tongue soles. To account for the gene rearrangements in blue flounder, Shi et al. [31] advanced the Dimer-Mitogenome and Non-Random Loss model (DMNR) that inferred the course of gene rearrangement in the blue flounder based on the typical gene order.

In the present study, the complete mitogenome of the deep-body righteye flounder, Samariscus latus, was sequenced, revealing genome-scale rearrangements. The gene order of this flounder is different from those of tongue soles, blue flounder and all other vertebrate species reported so far and none of the gene rearrangement models can account for this event properly. Thus, we deduced a novel mechanism of gene rearrangement for this flounder’s mitogenome.

Methods

Ethics statement

The fish specimen used in the present study was marine captured and purchased from Dasi seafood market in Keelung, Taiwan. The species was not involved in the endangered list of IUCN (http://fishdb.sinica.edu.tw/chi/species.php?id=382768). Specimen collection and maintenance were performed in strict accordance with the recommendations of Animal Care Quality Assurance in Taiwan and Chinese Mainland.

Sampling, DNA extraction, PCR and sequencing

The specimen of S. latus was collected from Taiwan. A portion of the epaxial musculature was excised from fresh specimen and was immediately stored at ~70°C. Total genomic DNA was extracted using the SQ Tissue DNA Kit (OMEGA) following the manufacturer’s protocol. Based on alignments and comparisons of complete mitochondrial sequences of flatfishes, dozens of primer pairs were designed for the amplification of the mtDNA genomes (Additional file 1: Table S1). More than 30 bp of overlapping fragments between tandem regions were used to ensure the correct assembly and integrity of the complete sequence.

PCR was performed in a 25-μl reaction volume containing 2.0 mM MgCl₂, 0.4 mM of each dNTP, 0.5 μM of each primer, 1.0 U of Taq polymerase (Takara, China), 2.5 μl of 10x Taq buffer, and approximately 50 ng of DNA template. The PCR cycling conditions included an initial denaturation at 95°C for 3 min followed by 30–35 cycles of denaturation at 94°C for 45 s, annealing at 45–55°C for 45 s, and elongation at 68–72°C for 1.5–5 min. The reactions were completed by a final extension at 72°C for 5 min. The PCR products were purified with the Takara Agarose Gel DNA Purification Kit (Takara, China) and were used directly as templates for cycle sequencing reactions. Sequence-specific walking primers were designed for both strands of each fragment and were employed for sequencing with an ABI 3730 DNA sequencer (Applied Biosystems, USA). The mtDNA sequences of S. latus have been submitted to GenBank under the accession number KF494223.

Sequence analysis

The sequenced fragments were assembled to create complete mitochondrial genome using CodonCode Aligner v3 and BioEdit v7 [32]. During the processing of large fragments and walking sequences, regular manual examinations were performed to ensure the reliable assembly of the genome sequence. The annotation and boundary determination of protein-coding and ribosomal RNA genes were performed using NCBI-BlAST (http://blast.ncbi.nlm.nih.gov). Transfer RNA genes and their secondary structures were identified using trNAscan-SE 1.21 [33], with the cut-off values set to 1 when necessary.

Results and discussion

Novel gene order in S. latus mitogenome

The complete mitogenome of S. latus is 18,706 bp in length. The genome includes two rRNA genes, 24 tRNA genes, 13 protein-coding genes, two control regions (CR1 and CR2) and one 376-bp noncoding region (NC).
Most of the genes are encoded on the H-strand, except for ND6 and ten of the tRNA genes (including the copied tRNA-C and -Y) (Table 1, Figure 1).

The genome-scale mitogenome rearrangements in S. latus make the mitogenome of this species differ greatly from other known vertebrate mitogenomes. The typical gene order of vertebrate mitogenomes and that of S. latus mitogenome are shown in Figure 1A and F, respectively. The genes of the typical genome are numbered consecutively from 1 (tRNA-F) to 38 (CR) (Figure 1A). Compared with the typical mitogenome, that of S. latus has undergone extensive changes to produce the following novel order: 1, 2, 3, 4, 5, 6, 7, 8, 38, (14, 15, 17, 20, 26, 30, 32, 33, 34, 35, 36), (9, 10, 11, 12, 13, 16, 18, 19, 21, 22, 23, 24, 25, 27, 28, 29, 31), 38, 14, 15, 37 and 38′ (Figure 1F). The rearrangement of S. latus mitogenome exhibits the following unique features: The first eight genes (1–8) retain the original position and order, followed by the insertion of a CR (38, designated CR1). The typical order (9–36) is largely changed into two gene clusters: an 11-gene cluster (14, 15, 17,...36) and a 17-gene cluster (9, 10,...16..31), in both clusters the typical relative orders of the genes are maintained (from small to large). The other CR-C′Y fragment follows (designated CR2, C′Y′). The site of the last tRNA gene, tRNA-P (37), is unchanged, and the end is a noncoding fragment (NC, designated 38′).

Intergenic regions and control regions

The S. latus mitogenome contains 19 intergenic regions, nine of which are over 50 bp in length (an uncommon occurrence). Given the parsimonious nature of the vertebrate mitogenome, the presence of so many long intergenic regions is surprising. We hypothesize that the reason for this phenomenon is that the intergenic regions are likely to be the residual sequences of genes that degenerated during the process of gene rearrangement. Moreover, three large noncoding regions of CR1, CR2 and NC are longer than 300 bp. The NC is located at the typical CR position, between the tRNA-F and tRNA-P genes, but it shows no sequence similarity to CRs in other flatfishes. Two CR fragments, CR1-C′Y (between tRNA-Q (8) and tRNA-S1 (17)) and CR2-C′Y′ (between tRNA-L2 (31) and tRNA-P (37)), are identical in sequence. Compared with the sequences of other flatfish CRs, CR1 and CR2 exhibit the typical tripartite structures of the CR: terminal associated sequences (TAS), central conserved sequence blocks (CSB-D, E), a pyrimidine tract (PY region) and conserved sequence blocks (CSB-2, 3) [34-37] (Additional file 2: Figure S1). The structures of CR1 and CR2 support the opinion that these two fragments are indeed the control regions same as other fishes except that S. latus mitogenome possesses two identical control regions.

Gene rearrangement mechanism of S. latus mitogenome

Mitogenome rearrangements have been reported in flatfishes (two tongue soles and blue flounder) [12,27,31]. The rearrangement of tongue sole mitogenomes is characterized by tRNA gene inversion, whereas that of the blue flounder is characterized by an L-strand coding gene cluster. However, the characteristics of S. latus rearrangements are completely different from those of the above flatfishes; therefore, there is little possibility that the gene order of S. latus mitogenome was derived from that of these flatfishes. Thus, the models mentioned above [12,31] that explain the mtDNA rearrangements in these flatfishes do not perfectly suit S. latus mitogenome.

Some models, such as recombination [14] and TDRL [18,19], might help explain the mechanism of S. latus mitogenome rearrangements. However, several unique features of S. latus rearrangements prevent the application of these models to this species. For example, the recombination model requires that several (more than five) recombination events occurred during the course of rearrangement. Moreover, gene rearrangements caused by recombination are unusual in the teleost fishes. The Tandem Duplication and Random Loss Model (TDR) might have the applicability for gene rearrangements in S. latus mitogenome [18,19], however, this model fails to highlight the specific features of S. latus mitogenome, such as gene clusters separated by CRs and its remnants. As for the tRNA miss-priming [23,24] and TDNL models [22], no obvious corresponding characteristics exist in S. latus mitogenome.

The ancestral gene order of S. latus mitogenome is most likely the typical vertebrate order because the mtDNA of all other flatfishes (more than ten species) follows the typical order. Therefore, we propose a “Double Replications and Random Loss (DRRL)” model to describe the rearrangement events that changed the ancestral gene order to that observed in S. latus mitogenome (Figure 1A). The hypothesized intermediate steps from a typical gene order to that of S. latus gene arrangement are as follows. First of all, the CR was duplicated to CRI and CRII, and then the CRI was translocated into the IQM (7–9) tRNA gene cluster. These events produced a genome with two CRs (CRI and CRII), both of which contained origin of heavy (OH) strand replication (OH1 and OH2) and either of which could serve as the mitochondrial origin of replication (Figure 1B). Second, mitochondrial replication (replication1, RP1) was first initiated at OH1 (Figure 1B, Figure 2A). Subsequently, after the replication elongation passed through OH2 (Figure 2B), the other replication (replication2, RP2) began at OH2 (Figure 1C, Figure 2C). Both replications terminated at OH1 (the 5′ end of the nascent strand of RP1; Figure 1C, Figure 2D). The closure event of the circular mtDNA connected the 5′ end of the
## Table 1 Organization of *S. latus* mitochondrial genome

| From   | To     | Size | Anticodon | Start Codon | Stop Codon | Strand | Intergenic Region |
|--------|--------|------|-----------|-------------|------------|--------|-------------------|
| tRNA\(^{Phe}\) (F) | 1 | 68 | GAA | H | 0 |
| 12 s rRNA | 69 | 1020 | 952 | H | 0 |
| tRNA\(^{Glu}\) (M) | 1021 | 1092 | 72 | TAC | H | 0 |
| 16 s rRNA | 1093 | 2805 | 1713 | H | 0 |
| tRNA\(^{Met}\) (L1) | 2806 | 2879 | 74 | TAA | H | 0 |
| ND1 | 2880 | 3854 | 975 | ATG | H | 3 |
| tRNA\(^{Val}\) | 3926 | 3997 | 72 | TTG | L | 0 |
| tRNA\(^{Asp}\) | 4892 | 4958 | 67 | TGC | H | 0 |
| tRNA\(^{Ile}\) | 5107 | 5177 | 70 | TAC | H | 88 |
| tRNA\(^{Lys}\) | 5263 | 5337 | 75 | CAC | L | 85 |
| tRNA\(^{Arg}\) | 5381 | 5450 | 68 | TCG | L | 0 |
| tRNA\(^{Ser}\) (S1) | 5539 | 5605 | 67 | GCT | H | 75 |
| ND2 | 5681 | 7519 | 1839 | 613 | ATG | TAG | H | −4 |
| ND6 | 7516 | 8037 | 522 | 174 | ATG | TAA | L | 0 |
| tRNA\(^{Cys}\) (C) | 8038 | 8106 | 69 | TTC | L | 0 |
| CYTB | 8111 | 9251 | 1141 | 380 | ATG | T | H | 0 |
| tRNA\(^{Glu}\) (L) | 9252 | 9321 | 70 | TGT | H | 86 |
| tRNA\(^{His}\) (M) | 9408 | 9476 | 69 | CAT | H | 0 |
| ND2 | 9477 | 10521 | 1045 | 348 | ATG | T | H | 0 |
| tRNA\(^{Pro}\) (N) | 10522 | 10591 | 70 | TGC | H | 1 |
| tRNA\(^{Glu}\) (A) | 10593 | 10660 | 68 | TGC | L | 1 |
| tRNA\(^{Asp}\) (C) | 10662 | 10734 | 73 | GTT | H | 59 |
| COI | 10794 | 12344 | 1551 | 517 | TAA | H | 22 |
| tRNA\(^{His}\) (D) | 12367 | 12434 | 68 | GTC | GTG | H | 5 |
| COII | 12440 | 13138 | 699 | 233 | ATG | AGA | H | 62 |
| ATP8 | 13201 | 13368 | 168 | 56 | ATG | TAA | H | −10 |
| ATP6 | 13359 | 14042 | 684 | 228 | ATG | TAA | H | −1 |
| COIII | 14042 | 14827 | 786 | 262 | ATG | TAA | H | −1 |
| tRNA\(^{Cys}\) (C') | 14827 | 14896 | 70 | TCC | H | 0 |
| ND3 | 14897 | 15247 | 351 | 117 | ATG | TAA | H | 70 |
| ND4L | 15318 | 15614 | 297 | 99 | ATG | TAA | H | −7 |
| ND4 | 15608 | 16081 | 1374 | 458 | ATG | TAA | H | 7 |
| tRNA\(^{Ser}\) (H) | 16989 | 17057 | 69 | GTG | H | 16 |
| tRNA\(^{Ser}\) (L2) | 17074 | 17147 | 74 | TAG | H | 0 |
| CR2 | 17148 | 18046 | 899 | H | 0 |
| tRNA\(^{Glu}\) (C) | 18047 | 18113 | 67 | CAC | L | 0 |
| tRNA\(^{Glu}\) (Y) | 18114 | 18181 | 68 | GTA | L | 78 |
| tRNA\(^{Ser}\) (P) | 18260 | 18330 | 71 | TGG | L | 0 |
| NC | 18331 | 18706 | 376 | H | 0 |

CR1, CR2: control region 1 and control region 2. NC: noncoding region. tRNA\(^{Phe}\): tRNAPhe(TAA); tRNA\(^{Val}\): tRNAVal(GAG); tRNA\(^{Asp}\): tRNAsAsp(TAG); tRNA\(^{Glu}\): tRNA\(^{Glu}\)(TGA); tRNA\(^{Ser}\): tRNA\(^{Ser}\)(GCT); tRNA\(^{Cys}\)(C), tRNA\(^{Cys}\)(Y): a copy of tRNA\(^{Cys}\) and tRNA\(^{Cys}\). Intergenic spacers are located between the feature on the same line and that on the following line; a negative number indicates an overlap.
Figure 1 The proposed mechanism of the rearrangement of the ancestral gene order to that of *S. latus* mitogenome. Protein-coding genes and CRs are indicated by boxes, and tRNA genes are indicated by columns. Genes labeled above the diagram are encoded by the H-strand and the others by the L-strand. OH indicates the origin of replication for the H-strand; the direction of replication is shown by arrows. Consecutive numbers (1–38) indicate the gene order. The dark boxes indicate the control regions (CRs) and noncoding region (NC). (A) The ancestral mitogenome with 37 genes and one CR. (B) The ancestral CR was duplicated to CRI and CRII, and then CRII translocated to the position between 12S and ND1. (C) After RP1 passed through OH1, replication2 (RP2) began at OH2. Both replications terminated close to OH1 (namely, the 5’ end of the nascent strand of RP1). (D) Double replications led to the duplication of one CR and 29 genes. One of each copied gene pair was lost randomly. Dark gray boxes indicate the degenerated genes.
nascent strand of RP1 to the 3′ end of RP2. The connection of the 5′ end of RP2 to the 3′ end of RP1 could be completed by another circular closure event or by an mtDNA repair mechanism afterwards (Figure 1C, Figure 2E). The double replications resulted in a copy of 29 genes (indicated by subscript 1 & 2 to the letters) from tRNA-M (9) to tRNA-P (37), with three CRs (CRI, CRII and CRIII) in the mtDNA (Figure 1D; Figure 2F). In subsequent evolutionary events, one of each of the 29 duplicated gene pairs was randomly lost (Figure 1D; gray boxes). The three CRs followed different courses: CRI retained its function, while CRII and CRIII degenerated into two noncoding regions: NC (38′) and an 86-bp intergenic region between tRNA-T (36) and tRNA-M (9)

Figure 2 A duplication caused by double replications. (A) A mitochondrial replication event (RP1) was initiated at OH1. (B) RP1 passed through OH2. (C) RP2 was initiated at OH2. (D) Both RP1 and RP2 terminated at OH1, while the nascent H-strand of RP1 was replaced by that of RP2. (E) The connection of the 3′ end of RP2 to the 5′ end of RP1, and the connection of the 5′ end of RP2 to the 3′ end of RP1. (F) The duplication was made permanent in the next round of replication.
(Figure 1E). Finally, the fragment of CRI- \( C_1 \cdot Y_1 \) (Figure 1E) were duplicated once, which were respectively named CR1-\( C \cdot Y \) and CR2-\( C \cdot Y \), followed by the latter translocated to a position between \( tRNA-L2 \) (31) and \( tRNA-P \) (37) (Figure 1F). These genome-scale rearrangements resulted in the mitogenome of \( S. \) latus containing two CRs, 24 tRNAs, one 376-bp noncoding region and several long intergenic regions (Figure 1F, Table 1).

Details of the model

The evidence supporting the proposed model (Figure 1E-F) is the presence of completely identical sequences in CR1-\( C \cdot Y \) and CR2-\( C \cdot Y \). This finding indicates that both fragments were derived from the same original template during a very recent duplication and translocation event. Based on \( tRNA-C \), \( Y \) is located between \( tRNA-Q \) and \( tRNA-SI \) in the typical vertebrate mitogenome, we infer that CR1-\( C \cdot Y \) is CRI- \( C_1 \cdot Y_1 \) at the original template location (Figure 1E) and CR2-\( C \cdot Y \) is a copy that then translocated to the site between \( tRNA-L2 \) and \( tRNA-P \). More specifically, the recent ancestral gene order for \( S. \) latus mitogenome (Figure 1F) is 1, 2, 3, 4, 5, 6, 7, 8, 38, (14, 15, 17, 20, 26, 30, 32, 33, 34, 35, 36), (9, 10, 11, 12, 13, 16, 18, 19, 21, 22, 23, 24, 25, 27, 28, 29, 31, 37, 38′), as illustrated in Figure 1E.

Both the copies of the original duplication of the CR (CRI and CRII, Figure 1A to B) and CR-C-Y (CRI-\( C_1 \cdot Y_1 \) and CR2-\( C \cdot Y \), Figure 1E to F) can be explained by TDRL or other possible mechanisms, however, there is no clear indication to confirm which one is more suitable for those duplication events. The duplication and translocation of CR is relatively common in gene-rearranged mitogenomes of metazoan, such as thrips, frogs, amigdas, parrots, and so on [4,10,38,39], which also occurred in teleost fishes [11].

The gene order depicted in Figure 1E clearly indicates that the genes from 9 to 38 were divided into two clusters (14, 15, 17,...,36 and 9, 10,...,16,...,38′), each of which retained the conserved relative gene order, from small to large. It is reasonable to assume that the two clusters were derived from a duplicated DNA fragment that spanned genes 9 to 38 in the typical gene order. There are three distinctive differences between the proposed “Double Replications” process (Figure 2) and normal mitochondrial replication. One difference is the origination of the double replications from two CRs (CRI and CRII) in one mitogenome (Figure 2A-C). This is a possible process because there have been similar events in dimeric mitogenomes (two monomeric mitogenomes linked head-to-tail) [40]. Clayton has studied these kind of events and detailed the process of “Double Replications” in unicircular dimeric mouse mtDNA with double CRs. Second, both replications (RP1 and RP2) terminated at OH1; after originating from OH1, RP1 did not terminate at the first encountered OH (OH2) (Figure 2D). The reason for this phenomenon is as follows: When RP1 proceeded to OH2, RP2 did not begin, and the relevant proteins or RNA primer did not bind to OH2 (Figure 2B). Therefore, there was no termination signal [40,41], and RP1 could bypass this region and normally terminated at OH1, where the proteins and RNA primer were bound when RP1 started. RP2 also terminated at OH1 because the first termination signal encountered by RP2 was also the one at OH1 (Figure 2D). Finally, the circular closure event of the double replications was different from normal replication, which the four ends (the 3′ and 5′ ends of the nascent H-strands of RP1 and RP2) needed to be closed in pairs (Figure 2D). Because the initiation of RP2 occurred later than that of RP1, the nascent H-strand of RP1 would have been replaced by that of RP2 from CR II to CRI. As a result, only the 5′ end of RP1 and the 3′ end of RP2 would remain bound at CRI on the template L-strand, and the simplest means of connecting the ends would be the circular closure event at CRI (Figure 2D). The remaining two ends, the 5′ end of RP2 bound to CRII and the freed 3′ end of RP1, could be joined by the other circular closure event at CRII or by mitochondrial repair [42] (Figure 2E). These double replications would represent a feature specific to triple CRs (Figure 1D, Figure 2F). As in \( S. \) latus mitogenome, the three CRs (CRI, CRII and CRIII) developed into fully functional CRs (CR1 and CR2), a degenerated CR (NC), and an 86-bp intergenic region between \( tRNA-T \) and \( tRNA-M \), respectively; the latter has been almost entirely lost during evolution.

DRRL model is different from the TDRL by the following features. First are the fixed replication origin points (OHs) but not the random sites as in the TDRL; second is the duplicated region which should start from an OH and end at another OH. Indeed, the DRRL model still needs more experimental evidence to verify. Nonetheless, every step of the model follows the nature and rule of normal mitochondrial replications. And both replications (RP1 and RP2) in our model initiated from the normal initiation sites, and terminated at the first met R-loop (the double strands of DNA and RNA primer) sites [3,43,44]. Existing other models and explanations are not comparable on this point (No violate the nature and rule of mitochondrial replications).
Conclusions
In summary, we determined the complete mitochondrial genome of a flatfish, S. latus. The genes of this mitogenome are extensively rearranged. The mitogenome is characterized by the duplication and translocation of the control region. The genes located between the two CRs are divided into two clusters in which their relative orders are maintained. We proposed a “Double Replications and Random Loss” model to explain the rearrangement events in S. latus mitogenome. First of all, the CR was duplicated and translocated. Double replications of the mitogenome were successively initiated from the two CRs, leading to the duplication of the genes between the two CRs. Lastly, one of each pair of duplicated genes was lost in a random event.

Data accessibility
DNA sequences: Genbank accessions KF494223.

Additional files
Additional file 1: Table S1. The primers used for fragment amplification in S. Latus flatfish mitogenomes.
Additional file 2: Figure S2. Aligned sequences of nine flatfishes CRs and the two CRs of S. Latus.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
WS collected datasets, carried out partial experiments, and drafted the manuscript. XGM carried out partial experiments. XYK directed the whole research work and revised the manuscript. All authors read and approved the final manuscript.

Acknowledgments
This work was supported by the Natural Science Foundation of China (grant numbers 30870283, 31071890 and 41206134).

Received: 12 September 2013 Accepted: 25 April 2014 Published: 9 May 2014

References
1. Boore J: Animal mitochondrial genomes. Nucleic Acids Res 1999, 27:1767–1780.
2. Clayton DA: Replication and transcription of vertebrate mitochondrial DNA. Annu Rev Cell Biol 1991, 7:453–478.
3. Shadel GS, Clayton DA: Mitochondrial DNA maintenance in vertebrates. Annu Rev Biochem 1997, 66:409–435.
4. Schmitzger EE, Tavares ES, Gonzales LA, Eberhard JR, Miyaki CY, Sanchez JJ, Hernandez A, Mueller H, Graves GR, Fleischer RC, Wright TF: Multiple independent origins of mitochondrial control region duplications in the order Psittaciformes. Mol Phylogenet Evol 2012, 64(2):342–356.
5. Bensch S, Harlid A: Mitochondrial genomic rearrangements in songbirds. Mol Biol Evol 2000, 17(1):107–113.
6. Verkuil YI, Piersma T, Baker AJ: A novel mitochondrial gene order in shorebirds (Scolopacidae, Charadriiformes). Mol Phylogenet Evol 2010, 57(1):411–416.
7. Mueller RL, Boore JL: Molecular mechanisms of extensive mitochondrial gene rearrangement in plethodontid salamanders. Mol Biol Evol 2005, 22(10):2104–2112.
8. Okajima Y, Kumaizawa Y: Mitochondrial genomes of acrodont lizards: timing of gene rearrangements and phylogenetic and biogeographic implications. BMC Evol Biol 2010, 10:141.
9. Macey JR, Larson A, Ananjeva NB, Fang Z, Papenfuss TJ: Two novel gene orders and the role of light-strand replication in rearrangement of the vertebrate mitochondrial genome. Mol Biol Evol 1997, 14(1):91–104.
10. Sano N, Kurabayashi A, Fuji T, Yonekawa H, Sumida M: Complete nucleotide sequence of the mitochondrial genome of Schlegel’s tree frog Rhacophorus schlegelli (family Rhacophoridae): duplicated control regions and gene rearrangements. Genes Genet Syst 2005, 80(3):213–224.
11. Inoue JS, Miya M, Tsukamoto K, Nishida M: Evolution of the deep sea gulp eel mitochondrial genomes: large-scale gene rearrangements originated within the eels. Mol Biol Evol 2003, 20(1):1917–1924.
12. Iong X, Dong X, Zhang Y, Shi W, Wang Z, Yu Z: A novel rearrangement in the mitochondrial genome of tongue sole, Cynosoglossus semilaevis: control region translocation and a tRNA gene inversion. Genome 2009, 52(12):975–984.
13. Ponce M, Infante C, Jimenez-Cantizano RM, Perez L, Manchado M: Complete mitochondrial genome of the blackspot seabream, Pagellus bogaraveo (Sparidae), with high levels of length heteroplasmy in the WANCY region. Gene 2008, 409(1–2):44–52.
14. Lunt DH, Hyman BC: Animal mitochondrial DNA recombination. Nature 1997, 387(6630):247.
15. Sammiller S, Bleidorn C, Tiedemann R: Full mitochondrial genome sequences of two endemic Philippine hornbill species (Aves: Bucerotidae) provide evidence for pervasive mitochondrial DNA recombination. BMC Genomics 2011, 12:55.
16. Ladoukakis ED, Zouros E: Recombination in animal mitochondrial DNA: evidence from published sequences. Mol Biol Evol 2001, 18(11):2127–2131.
17. Kurabayashi A, Sumida M, Yonekawa H, Law F, Vences M, Hasegawa M: Phylogeny, recombination, and mechanisms of stepwise mitochondrial genome reorganization in mantellid frogs from Madagascar. Mol Biol Evol 2008, 25(5):874–891.
18. Arndt A, Smith MJ: Mitochondrial gene rearrangement in the sea cucumber genus Cucumaria. Mol Biol Evol 1998, 15(8):1099–1106.
19. Moritz C, Bowling TE, Brown WM: Evolution of animal mitochondrial-DNA - relevance for population biology and systematics. Annu Rev Ecol Syst 1987, 18:269–292.
20. San Mauro D, Gower DJ, Zardoya R, Wilkinson M: A hotspot of gene order rearrangement by tandem duplication and random loss in the vertebrate mitochondrial genome. Mol Biol Evol 2006, 23(1):227–234.
21. Arner SA, Kumazawa Y: The mitochondrial genome of the lizard Calotes versicolor and a novel gene inversion in Asian Draco amagids. Mol Biol Evol 2007, 24(6):1330–1339.
22. Lavrov DV, Boone JL, Brown WM: Complete mtDNA sequences of two millipedes suggest a new model for mitochondrial gene rearrangements: duplication and nonrandom loss. Mol Biol Evol 2002, 19(2):163–169.
23. Cantatore P, Gadaleta MN, Roberti M, Saccone C, Wilson AC: Duplication and remoulding of tRNA genes during the evolutionary rearrangement of mitochondrial genomes. Nature 1987, 329(6142):853–855.
24. Jacobs HT, Heribert ER, Rankine J: Sea urchin egg mitochondrial DNA contains a short displacement loop (D-loop) in the replication origin region. Nucleic Acids Res 1989, 17(22):9849–9865.
25. Mabuchi K, Miya M, Satoh TP, Weisrock MW, Nishida M: Gene rearrangements and evolution of tRNA pseudogenes in the mitochondrial genome of the parrotfish (Teleostei: Perciformes: Scaridae). J Mol Evol 2004, 59(2):287–297.
26. Kit JS, Jung SO, Hwang DS, Lee YM, Lee JS: Unusual mitochondrial genome structure of the freshwater goby Odontobutis platycephala: rearrangement of tRNAs and an additional non-coding region. J Fish Biol 2008, 73(2):414–428.
27. Gong L, Shi W, Wang Z-M, Xiao X-G, Kong X-Y: Control region translocation and a tRNA gene inversion in the mitochondrial genome of Paraplagusia japonica (Pleuronotociformes; Cynoglossoidae). Mitochondrial DNA 2013, 24(6):671–673.
28. Shi W, Jiang JX, Xiao XG, Kong XY: The complete mitochondrial genome sequence of Heteromycteris japonicus (Pleuronectiformes: Soleidae). Mitochondrial DNA 2013, 1:1–2.
29. Manchado M, Catanese G, Ponce M, Funes V, Infante C: The complete mitochondrial genome of the Senegal sole, Solea senegalensis Kaup.

Shi et al. BMC Genomics 2014, 15:352
http://www.biomedcentral.com/1471-2164/15/352
Page 8 of 9
Comparative analysis of tandem repeats in the control region among soles. DNA Seq 2007, 18(3):169–175.

30. Wang SY, Shi W, Wang ZM, Gong L, Kong XY: The complete mitochondrial genome sequence of Aesopia cornuta (Pleuronectiformes: Soleidae). Mitochondrial DNA 2013, 21:1–2.

31. Shi W, Dong XL, Wang Z-M, Miao X-G, Wang S-Y, Kong X-Y: Complete mitogenome sequences of four flatfishes (Pleuronectiformes) reveal a novel gene arrangement of L-strand coding genes. BMC Evol Biol 2013, 13:173.

32. Hall TA: BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 1999, 41:95–98.

33. Lowe TM, Eddy SR: tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 1997, 25(5):955–964.

34. Nesbo CL, Arab MO, Jakobsen KS: Heteroplasmy, length and sequence variation in the mtDNA control regions of three percid fish species (Perca flavilatilis, Aserina cernua, Stizostedion lucioperca). Genetics 1998, 148(4):1907–1919.

35. Ravago RG, Monje VD, Junio-Menez MA: Length and sequence variability in mitochondrial control region of the milkfish, Chanos chanos. Mar Biotechnol (NY) 2002, 4(1):40–50.

36. Guo XH, Liu SJ, Liu Y: Comparative analysis of the mitochondrial DNA control region in cyprinids with different ploidy level. Aquaculture 2003, 224(1–4):25–38.

37. Mjelle KA, Karlsen BO, Jorgensen TE, Moum T, Johansen SD: Halibut mitochondrial genomes contain extensive heteroplasmic tandem repeat arrays involved in DNA recombination. BMC Genomics 2008, 9:10.

38. Arner SA, Kamazawa Y: Mitochondrial genome of Pogona vitticeps (Reptilia: Agamidae): control region duplication and the origin of Australasian agamids. Gene 2005, 346:249–256.

39. Yan DK, Tang YX, Xue XF, Wang MH, Liu FQ, Fan JQ: The complete mitochondrial genome sequence of the western flower thrips Frankliniella occidentalis (Thysanoptera: Thripidae) contains triplicate putative control regions. Gene 2012, 506(1):117–124.

40. Clayton DA: Replication of animal mitochondrial DNA. Cell 1982, 28(4):693–705.

41. Fernandez-Silva P, Enriquez JA, Montoya J: Replication and transcription of mammalian mitochondrial DNA. Exp Physiol 2003, 88(1):41–56.

42. Pinz KG, Bogenhagen DF: Efficient repair of abasic sites in DNA by mitochondrial enzymes. Mol Cell Biol 1998, 18(3):1257–1265.

43. Brown TA, Tkachuk AN, Clayton DA: Native R-loops persist throughout the mouse mitochondrial DNA genome. J Biol Chem 2008, 283(S2):36743–36751.

44. Clayton DA: Mitochondrial DNA replication: what we know. IUBMB Life 2003, 55(4–5):213–217.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at www.biomedcentral.com/submit