Intraspecific rearrangement of mitochondrial genome suggests the prevalence of the tandem duplication-random loss (TDLR) mechanism in *Quasipaa boulengeri*

Yun Xia¹, Yuchi Zheng¹*, Robert W. Murphy² and Xiaomao Zeng¹*

**Abstract**

**Background:** Tandem duplication followed by random loss (TDRL) is the most frequently invoked model to explain the diversity of gene rearrangements in metazoan mitogenomes. The initial stages of gene rearrangement are difficult to observe in nature, which limits our understanding of incipient duplication events and the subsequent process of random loss. Intraspecific gene reorganizations may represent intermediate states, and if so they potentially shed light on the evolutionary dynamics of TDRL.

**Results:** Nucleotide sequences in a hotspot of gene-rearrangement in 28 populations of a single species of frog, *Quasipaa boulengeri*, provide such predicted intermediate states. Gene order and phylogenetic analyses support a single tandem duplication event and a step-by-step process of random loss. Intraspecific gene rearrangements are not commonly found through comparison of all mitochondrial DNA records of amphibians and squamate reptiles in GenBank.

**Conclusions:** The intraspecific variation in *Q. boulengeri* provides insights into the rate of partial duplications and deletions within a mitogenome, and reveals that fixation and gene-distribution in mitogenomic reorganization is likely non-adaptive.

**Keywords:** Mitochondrial gene order, tRNA, Mitogenomics, Intermediate mitogenomic rearrangement, Random gene loss

**Background**

The order of mitochondrial (mt) genes in metazoans varies greatly [1, 2] and the molecular drivers that explain the underlying evolution are subject to debate [3, 4]. The most widely invoked model involves tandem duplication of mt genes followed by the random loss of one copy (TDRL) [5–8]. However, duplication and non-random loss may result from the transcripational polarities of genes and their positions in the genome, as two models describe: tandem duplication and non-random loss (TDNL) [9]; and dimer-
randomly lost. Despite the increasing number of taxa known to have gene rearrangements, few mitogenomes exhibit intermediate states that could point to this evolutionary mechanism, even though pseudogenes and residues of tandemly duplicated sequences may provide indirect evidence for an intermediate step in genomic rearrangement [3, 6, 16].

If gene deletion occurs randomly, then populations should have mitogenomes with varying gene-orders that consist of alternative arrangements of duplicated genes. Such alternative gene arrangements have been reported in only a few closely-related lineages or species [16, 17]. These interspecific occurrences support the TDRL model, yet no information exists as to when and where the rearrangements occurred and how they subsequently dispersed within a species. Investigations at a lower (intraspecific) level may be necessary to understand the evolution of mitochondrial gene rearrangements.

Intraspecific rearrangements of mitogenomes are rarely reported in vertebrates. Many species have structural diversity in their control region (CR), but all of these involve non-coding sequences. Gene order diversity within a species is known only from asexual squamates [7, 14, 18], an amphibiaenid [19] and a bird [20]. In these cases, gene rearrangements that qualify as potential intermediate states involve either a large number of genes adjacent to the CR or the formation of pseudogenes [3, 19]. In addition, gene rearrangements in the mitogenomes of asexual squamates results from multiple independent duplications and lack the random loss of alternative states [7].

High levels of gene rearrangement have been reported from amphibians, especially among so-called modern frogs [21–23]. A hotspot of gene rearrangement has been reported in the “WANCY” region (trnW, trnA, trnN, origin of light strand replication (O_L), trnC, and trnY) [6, 24]. Because many amphibians have gene rearrangements in their WANCY region [17, 25, 26], reorganizations in this group facilitate testing hypotheses on how gene-rearrangement occurs. Each of the above hypotheses predicts a unique arrangement of five short tRNA genes in the WANCY region (Fig. 1), which can be compared with the results of sequencing.

Herein, we report a diversity of rearrangements in the frog *Quasipaa boulengeri* and the discovery of intermediate states. To test the hypotheses of mechanisms of gene rearrangement, we investigate the origins and evolution of the rearrangement by 1) determining the structure of the rearranged region for each type, 2) speculating on the steps resulting in observed gene rearrangements, and 3) placing each type on an inferred phylogeny and estimating the time at which each rearrangement arose. We supplement this with an *in silico* approach using mitochondrial gene orders from GenBank data. By using a custom Perl script (mtGordV0.5.pl), we explore the frequency of occurrence of intraspecific rearrangements in Amphibia and Squamata, which have high diversities in mitogenomic rearrangements.

**Results and Discussion**

**Intraspecific rearrangements**

We obtained 290 samples from 28 localities for *Quasipaa boulengeri* (Fig. 2 and Additional file 1: Table S1). Sequences from a region encompassing *nad2* to *cox1*, which includes the WANCY hotspot, revealed gene-organizations atypical of vertebrates. Stable secondary structures of tRNA genes and the absence of premature stop codons in *nad2* and *cox1* authenticated the sequencing of mtDNA. The WANCY region in this frog differed from the typical organization by having a long noncoding sequence that ranges in size from 473 bp to 925 bp. Further, gene annotation identified different positions for the gene and its copy, even within a single population. The details of gene organization of the WANCY region for each sample were listed in Additional file 2: Table S2.

Annotation identifies four kinds (types) of gene rearrangements (Fig. 3a). The typical gene order of the trnW–trnY block is trnW, trnA, trnN, O_L, trnC, and trnY (WANCY). Unlike the other types, where the O_L is located before trnN (Fig. 3a), in Type I, the O_L occurs after trnN, separated by an intergenic spacer (IGS or noncoding region). In Type II, two trnA occur with an IGS located between trnAI and O_L, another IGS occurs between O_L and trnA2, and another IGS between trnN and trnC. The gene organization of Type III and Type IV are similar to Type II, but Type III lacks trnAI and Type IV lacks trnA2, respectively. Except for the reorganizations of trnA, trnN, and O_L, all other tRNAs and protein-coding genes have positions and lengths typical of the vertebrate mitogenome (Fig. 3a; Additional file 2: Table S2).

**Sequential process of TDRL**

All four types of gene rearrangement in *Q. boulengeri* involve trnA, trnN, O_L and trnC. The IGSs reveal tandem duplications in the WANCY region. These residues identify pseudogenes of trnA, trnN and trnC, whose sequences are similar to corresponding tRNAs. Additional file 3: Figure S1 shows the primary sequence of trnA and trnN for each variant. The secondary structures of these genes fold into typical stem-and-loop structures (Additional file 4: Figure S2). Each type of variant has only one trnA and trnN, except in Type II, which has two trnAs, both of which form stable clover-leaf structures. Thus, these genes are paralogs created by gene duplication. Residues are very similar to trnA and trnN, but have a loss of function owing to secondary structures or a mutation on the anticodon position.
TDRL [5, 16] best explains the gene rearrangement in *Q. boulengeri*, and the diversity of rearrangements rejects the alternative hypotheses of non-random loss (TDNL and DMNL), recombination, and DRRL. Genes of the same polarity do not cluster together, and the finding of alternative loss of duplicated genes, as seen in comparison of Types III and IV, contradicts non-random loss models (TDNL or DMNL). The absence of different tandem duplication junction points and no variation in the number of tandem repeats (VNTR) and this does not support the recombination model. We cannot reject the hypotheses that unequal crossing over of intermolecular recombinations were subtly inserted in front of *trnA* and behind *trnC*, but tandem duplication would essentially be a consequence of this recombination. Further, no concerted evolution rejects intramolecular recombination, because the two copies of *trnA* in type II differ (Additional file 3: Figure S1, and Additional file 4: Figure S2). Finally, analyses reject the hypothesis of DRRL due to the absence of two control regions in the mitogenome of *Q. boulengeri* [26].

The TDRL hypothesis remains the only viable explanation and our results conform to its predictions (Fig. 3b).
The hypothesized duplicated region in the mitogenome of *Q. boulengeri* includes *trnA*, *trnN*, O L, and a partial fragment of *trnC*. Slipped-strand mispairing, imprecise termination or recombination have been proposed to explain mitogenomic duplications [5, 7, 15]. Regardless of the molecular mechanism, tandem duplication mutations will yield two copies each of *trnA*, *trnN*, O L, and *trnC*. Subsequent random loss appears to have occurred at least twice independently in the mitogenome of *Q. boulengeri*. First, rearrangement Type I involves the loss of O L1, *trnC1*, *trnA2*, and *trnN2*. Second, loss involves *trnN1*, *trnC1*, and O L2 in Type II. The retention of two copies of *trnA* in Type II is direct evidence for the randomness of loss because alternative losses occur in Type III and Type IV, which have the same gene order as Type II (*trnA1* has been lost in Type III as compared to *trnA2* in Type IV). Rather than a result of selection for one or other alternative, loss of one copy of *trnA* appears to have occurred by chance alone.

The sequencing *cox1* and *cob* for 290 individuals (Additional file 1: Table S1) identifies the origin of the initial tandem duplication event and the stepwise process of random loss when viewed in terms of the phylogenetic relationships of all types of gene rearrangement. The concatenated alignment contains 1463 nucleotide positions (*cox1*: 626 bp; *cob*: 837 bp) without stop codons. Maximum likelihood (ML) and Bayesian inference (BI) reconstructions obtain similar tree topologies for the four types of rearrangement (Fig. 3c). All haplotypes cluster together by type and with moderate to strong levels of nodal support, except for Type III, which is paraphyletic. Analyses recover the group Type II + Type III + Type IV, and roots it as the sister-group of Type I. Type II forms the sister-group of Type III + Type IV and some samples of Type III unite with Type IV. The phylogenetic analyses and gene-order strongly indicate a single tandem duplication event and stepwise random loss in *Q. boulengeri*. The clustering of Types II–IV suggests that they share the same primary TDRL process (Fig. 3b, c), and that Type I represents an independent random loss. The monophyly of types I, II and IV indicate a single origin of each rearrangement type. Paraphyly of Type III suggests two parallel random losses are responsible for the same gene rearrangement. The associations of Type IIIa, IIIb and IV indicate that they shared a recent common ancestor, but independently
lost one duplicated copy of trnA. The possession of both copies of trnA indicates that Type II represents the intermediate state.

Although mitochondrial gene rearrangements are not uncommon among related taxa, recognized intermediate steps of gene-order rearrangements are rare, and their presence can suggest evolutionary mechanisms [7, 27]. Most intermediate states appear as pseudogenes or residues of tandemly duplicated sequences rather than as two functional gene copies [3, 8, 16]. The intermediate state of Type II, leading either to Type III or Type IV, is an example of the random loss of one trnA gene.

Alternative loss-types have been observed among closely related species, e.g. alternative losses of trnH in the anuran Babina [17, 28]. However, alternative losses have been reported rarely within a single species and even more so within a population. The finding of alternative losses in Q. boulengeri is the first observation of an intermediate state involving two functional gene copies, and, simultaneously, the loss of alternative types in a vertebrate mt genome.

The occurrence of TDRL in Q. boulengeri corresponds to the view that reorganization of the mitochondrial genome is nonadaptive [17, 29]. Our results indicate that the hotspot of gene rearrangement is adjacent to the origin of light-strand replication [6]. Homoplastic mitochondrial rearrangements are contiguous in the genome or they locate around the origin of replication [6, 30, 31]. Under these conditions, mitochondrial gene-orders appear susceptible to convergent or parallel evolution because of...
functional constraints or selective pressures [32–34]. However, such evolution does not occur in Q. boulengeri. The gene-order and phylogenetic analyses indicate a single tandem duplication event followed by independent losses (Fig. 3), which implies that random loss and gene-order are not involved in adaptive evolution [17].

Evolution of mitogenomic rearrangement

A time-calibrated phylogeny constructed using Bayesian inference estimates the recency of mitogenomic rearrangements in Q. boulengeri (Fig. 3c). The initial diversification (Type I) dates to about 0.8 Ma and divergence among the other three types ranges from 0.4 to 0.6 Ma, suggesting that the duplication and fixation of these rearrangements can occur quite quickly. Combing interspecific rearrangement data, we summarize the rates of mitogenomic duplication and loss (Additional file 5: Table S3). This suggests that post duplication, the alternative loss types can occur in 0.2–5 Ma.

To explore how many intraspecific rearrangements existed, we gather both in silico and experimental evidence to detect the gene order in highly rearranged groups. First, our in silico approach obtains mitochondrial gene-order information (mtGordV0.5.pl; Additional file 6: Software) from GenBank in Amphibia and Squamata, two groups with high diversities in mitogenome rearrangement. Analyses discover that intraspecific rearrangements are rare, but they exist. Two cases occur in Squamata, one in asexual squamates with multiple origins of duplication [7], and another in an amphibiaenian with alternative loss types varying among populations [19]. No intraspecific rearrangements in Amphibia exist in data from GenBank.

Our sequences of the WANCY fragment in multiple populations of the frogs Odorrana schmackeri and Amolops mantzorum detected that this hotspot region differed from the typical vertebrate arrangement [17]. These species do not exhibit intraspecific variation in gene-order, yet evidence for variation in losses may be represented by the residues of pseudogenes (data not shown).

Intraspecific studies may provide new insights into the high incidence of rearranged mitochondrial genomes. Above the species level, rates of mitogenomic partial duplication have been found to be high, and multiple duplication events can facilitate gene-rearrangement [7, 16, 21]. However, duplications may not occur frequently within a species. The rarity of this may reflect selection or functional constraints that prevent fixation, and may shed light on the limits of intraspecific diversity of mitogenomes. It could also owe to the dearth of investigations of intraspecific mitogenomic reorganization. We predict that mitochondrial metagenomic skimming by next-generation sequencing [35, 36] will detect additional cases of intraspecific rearrangements.

Random loss within duplicated regions could occur repeatedly, and the rate of duplication excision may be relatively high. At the intraspecific level, random loss occurred independently many times both in Q. boulengeri and the lizard Bipes bipurus (Fig. 3c, Additional file 5: Table S3). At the interspecific level, the sibling frog genera Babina and Odorrana share the same duplication of genes, but the pathways of deletion differed among species [17, 28]. The loss of a duplicated region and fixation could occur in short evolutionary time (0.2 Ma, Additional file 5: Table S3). Deletion of a redundant gene-copy may happen rapidly due to functional constraints and the compactness of the metazoan mitogenome, facilitating the formation of pseudogenes or the complete deletion of redundant genes [27, 37]. A functionally redundant duplicate gene copy may not persist long in a population because deleterious mutations can accumulate and cause the redundant gene to become nonfunctional [38].

Nonadaptive forces, such as genetic drift or bottlenecking, may drive the fixation and dispersal of mitogenomic reorganizations. The low effective population size of the mitogenome leaves it vulnerable to bottlenecks and genetic drift, which can drive the fixation of large-scale genomic modifications [16, 39–41]. Quasipaa boulengeri resides in localized montane areas, mainly in rocky streams [42]. Its highly specific habitat may limit gene flow and result in a pattern structured by genetic drift. The upper and midstream tributaries of the Yangtze River, including some areas in Chongqing, Guizhou and Hubei, have populations containing two or more sympatric types of rearrangements (Fig. 2). This area may be the original source of the gene rearrangements, or may represent areas of secondary contact. Both scenarios explain the distribution of types. Historical demographic analyses in this area point to dispersal events for Q. boulengeri [42]. TDRL may have first occurred in this area, followed by dispersal to other places. However, the single origin of each type suggests independent fixations of alternative arrangements, in which case secondary contact could also explain the pattern.

Conclusion

The initial stages of gene rearrangement are difficult to observe in nature, which limits our understanding of the evolutionary mechanism. Intraspecific or population level investigations may represent intermediate states and fixation of initial rearrangement, and if so they potentially shed light on the evolutionary dynamics. Here, we found mitogenomic rearrangements diversity in a single frog species, Quasipaa boulengeri. Intermediate state and alternative losses types were observed in this frog, which provide direct evidence of tandem duplication and random loss model for mitochondrial gene
rearrangement. The intraspecific variation in *Q. boulengeri* provides insights into the rate of partial duplications and deletions within a mitogenome, and reveals that fixation and gene-distribution in mitogenomic reorganization is likely non-adaptive. Our observation may shed light on the investigations of intraspecific mitogenomic reorganization.

**Methods**

**Samples and Sequence Amplification**

A total of 290 samples from 28 localities were used. Frogs were collected from 2006 to 2013, and Fig. 1 and Additional file 1: Table S1 detail the localities. Tissue samples, including liver, muscle, and tadpoles were stored in 95% ethanol at −20 °C in the Chengdu Institute of Biology, Chinese Academy of Sciences (CIB). The CIB Animal Care and Use Committee approved all procedures.

We sequenced the hotspot of gene rearrangement, from *nad2* to *cox1*, of mtDNA for *Quasipaa boulengeri*, which included the WANCY region [26]. Two other fragments, *cox1* and *cob*, were sequenced for population genetic and phylogenetic analysis. For *cox1*, we added published sequences (GenBank No. JX629572–JX629667) for phylogenetic analysis [43]. The sample and sequence information were provided in Additional file 1: Table S1. The PCR primers for the three fragments were those of Kurabayashi and Sumida [44] and Qing et al. [43]. To avoid Numt (nuclear copies of mtDNA genes), we designed a pair of primers to confirm amplification of the WANCY region: 5059 F-3, 5’- TTCTTTTACTTAC GACTGACAT -3’; 6399R-2, 5’- ATGCCCTGGCAGCT AAACTGGAAGAG-3’. PCR amplification, sub-cloning, and sequencing followed Xia et al. [17]. All newly obtained sequences were examined by checking for the presence of premature stop codons (pseudogenes).

**Gene Annotation and Time-Tree Analyses**

The tRNA genes were identified by using both tRNAscan-SE v.1.21 (http://lowelab.ucsc.edu/tRNAscan-SE) and MITOS (http://mitos.bioinf.uni-leipzig.de). To avoid misannotated tRNA genes, we predicted the secondary structure for each. We extracted and aligned the duplicated tRNA genes and their pseudogene residues.

We aligned sequences of each fragment using ClustalW in MEGA6 [45]. DnaSP v.5.10 [46] was used to determine DNA polymorphisms and divergences. To estimate the time-tree, we constructed phylogenies using *cox1* and *cob*, and partitioned these genes by codon position. Six species of *Quasipaa*, including *Q. verrucospinosa* (KF199147), *Q. shini* (KF199148), *Q. yei* (KJ842105), *Q. spinosa* (FJ432700), *Q. jiulongensis* (KF199149) and *Q. exilispinosa* (KF199151), were chosen as outgroup taxa. The best-fit substitution model for each partition was estimated using the Akaike information criterion (AIC) implemented in PartitionFinder v1.1.1 [47]. The best model of each partition was chosen for maximum likelihood (ML) and Bayesian inference (BI) analyses, which were performed with RAxML BlackBox web-servers (http://phylobench.vital-it.ch/raxml-bb/index.php) [48] and MrBayes v.3.1 [49], respectively.

BI as implemented in BEAST2 v.2.1.2 [50] was used to obtain an ultrametric time-tree for *Q. boulengeri*. Each locus was assigned its own partition with unlinked substitution model but with linked clock and tree models. We assumed a substitution rate ranging from 0.65 to 1.00% per Ma for the *cox1* and *cob* based on evolutionary rates commonly proposed for frogs [42, 51, 52]. Lacking fossil evidence, we calibrated our phylogeny using the published divergence time to the most recent common ancestor (TM RCA) between the *Q. jiulongensis* and *Q. exilispinosa* of about 9 Ma [53]. We ran BEAST for 20 million generations while logging trees every 1000 generations for a total of 20,000 trees. We determined a 10% burn-in length using Tracer v.1.5 and retained the maximum clad e credibility tree using TreeAnnotator v.2.1.2.

A Perl script named mtGordV0.5.pl was written by YZ to obtain the gene-orders of mitochondrial records deposited in GenBank, based on the annotation of the sequence. Records were downloaded together as a single file, which was used as the input file of the script. For each record with more than one gene, items in the order of accession number, sequence length, species name, gene names in their original order, and total number of genes were saved in an individual line to the output file. Items were separated from each other by a tab. The script was applied to two major groups of vertebrates, amphibians and squamate reptiles. For amphibians, all 126,638 mitochondrial records were downloaded on 02 Nov 2015, and the output file contained 17,559 records. For squamates, all 110,064 records were downloaded on 25 Sep 2015, and the output file contained 21,045 records. The output files were opened using Microsoft Excel and records were aligned according to species names. The records were manually checked for intraspecific and intrageneric cases of random loss of genes after duplication. As the script did not include all variation of annotations for all mitochondrial genes, errors from missing genes were expected for a small number of records. However, when a potential case was detected, the related GenBank full records were carefully checked. More importantly, this script made such a scan possible, analyses could be conducted within a reasonable amount of time, a few days for each group in our case, and it could be applied to other groups of taxa. Regarding the speed of the script itself, the data for squamates were processed within 3 min on a ThinkPad X200 laptop computer.
Additional files

Additional file 1: Table S1. Summary of sample localities and mitochondrion regions sequenced for *Quassia* boulengeri. (XLS 84 kb)

Additional file 2: Table S2. The gene organization of “WANCY” region for each sample. (XLS 77 kb)

Additional file 3: Table S3. Time-scale of mitogenomic duplication and random loss. (PDF 209 kb)

Additional file 4: Figure S1. The primary structure of tRNA and trnV for each variant. (PDF 161 kb)

Additional file 5: Figure S2. The secondary structures of of tRNA and trnV fold into typical stem-and-loop structures. (DOCX 22 kb)

Additional file 6: Software. The Perl script mtGordV0.5.pl with its readme file and example input and output files. (ZIP 5.35 kb)

Abbreviations

BI: Bayesian inference; cob: Cytochrome b; cox1: Cytochrome c oxidase subunit 1; CR: Control region; DMINL: Dimer-mitogenome and non-random loss; DRRL: Double replications and random loss; IGS: Intergenic spacer; ML: Maximum likelihood; mt: Mitochondrial; O L: Origin of light strand replication; TDNL: Tandem duplication and non-random loss; TDRL: Tandem duplication and random loss; WANCY: trnW, trnA, trnK, O L, trnC, and trnY

Acknowledgments

We thank Liyan Qing, Shujun Zhang, Xiang Shan for collecting the samples. We gratefully acknowledge technical support from the laboratory of XZ.

Funding

This research was supported by the National Natural Sciences Foundation of China (NSFC–31272282, 31372181, 31401960, 31572243), by the China Postdoctoral Science Foundation (2014 M552386) and West Light Foundation of The Chinese Academy of Sciences.

Availability of data and materials

The nucleotide sequence dataset for this research article is available in the GenBank with accession numbers shown in Additional file 1: Table S1.

Authors’ contributions

XY carried out molecular lab work and conceived the project and analyzed the data. YX wrote the Perl script. YX, RWM, and XZ wrote the paper. XZ and RWM finalized the manuscript. All authors have read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The tissue samples collection and DNA extraction were approved under the Ethics approval and consent to participate Not applicable.

The authors declare that they have no competing interests.

Competing interests

Authors’ contributions

RWM finalized the manuscript. All authors have read and approved the final manuscript.

Acknowledgments

We thank Liyan Qing, Shujun Zhang, Xiang Shan for collecting the samples. We gratefully acknowledge technical support from the laboratory of XZ.

Funding

This research was supported by the National Natural Sciences Foundation of China (NSFC–31272282, 31372181, 31401960, 31572243), by the China Postdoctoral Science Foundation (2014 M552386) and West Light Foundation of The Chinese Academy of Sciences.

Availability of data and materials

The nucleotide sequence dataset for this research article is available in the GenBank with accession numbers shown in Additional file 1: Table S1.

Authors’ contributions

XY carried out molecular lab work and conceived the project and analyzed the data. YX wrote the Perl script. YX, RWM, and XZ wrote the paper. XZ and RWM finalized the manuscript. All authors have read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The tissue samples collection and DNA extraction were approved under the Chengdu Institute of Biology (CIB) Animal Care and Use Committee.

Author details

1Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China. 2Centre for Biodiversity, Royal Ontario Museum, 100 Queen’s Park, Toronto, ON M5S 2C6, Canada.

Received: 19 July 2016 Accepted: 17 November 2016

Published online: 24 November 2016

References

1. Boore JL. Animal mitochondrial genomes. Nucleic Acids Res. 1999;27:1767–80.
2. Dovton M, Cameron SL, Dowavic JJ, Austin AD, Whiting MF. Characterization of 67 mitochondrial tRNA gene rearrangements in the

hymenoptera suggests that mitochondrial tRNA gene position is selectively neutral. Mol Biol Evol. 2009;26:1607–17. doi:10.1093/molbev/msp072.
3. Macey JR, Schulte JA, Larson A, Papenfuss TJ. Tandem duplication via light-strand synthesis may provide a precursor for mitochondrial genomic rearrangement. Mol Biol Evol. 1998;15:71–5.
4. Shao R, Barker SC, Mitani H, Takahashi M, Fukunaga M. Molecular mechanisms for the variation of mitochondrial gene content and gene arrangement among chigger mites of the genus *Leptotrombidium* (Acari: Acariformes). J Mol Evol. 2006;63:251–61. doi:10.1007/s00239-005-0196-y.
5. Boore JL. The duplication/random loss model for gene rearrangement exemplified by mitochondrial genomes of deuterostome animals. In: Sankoff D, Nadeau J, editors. Comparative Genomics. Dordrecht: Kluwer Academic Publishers; 2000. p. 133–47.
6. 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;23:227–34. doi:10.1093/molbev/msj025.
7. Fujita MK, Boore JL, Moritz C. Multiple origins and rapid evolution of duplicated mitochondrial genes in parthenogenetic geckos (Heteronotia binoei; squamata, gekkonidae). Mol Biol Evol. 2007;24:2775–86. doi:10.1093/molbev/msm212.
8. Shi W, Gong L, Wang SY, Mao XG, Kong XY. Tandem Duplication and Random Loss for mitochondrial genome rearrangement in *Symphurus* (Teleostei: Pleuronectiformes). BMC Genomics. 2015;16:355.
9. Lavrov DV, Boore 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;19:163–9.
10. Shi W, Dong XL, Wang ZM, Mao XG, Wang SY, Kong XY. Complete mitochondrial gene sequences of four flatfishes (Pleuronectiformes) reveal a novel gene arrangement of L-strand coding genes. BMC Evol Biol. 2013;13:173.
11. Dovton M, Campbell NJH. Intramitochondrial recombination—is it why some mitochondrial genes sleep around? Trends Ecol Evol. 2001;16:269–71. doi:10.1016/S0169-5347(01)02182-6.
12. Kurabayashi A, Sumida M, Yonekawa H, Glaw F, Vences M, Hasegawa M. Phylogeny, recombination, and mechanisms of stepwise mitochondrial genome reorganization in mantellid frogs from Madagascar. Mol Biol Evol. 2008;25:874–91. doi:10.1093/molbev/msn031.
13. Shi W, Mao XG, Kong XY. A novel model of double replications and random loss accounts for rearrangements in the Mitogenome of *Samariscus latus* (Teleostei: Pleuronectiformes). BMC Genomics. 2014;15:330.
14. Moritz C, Brown W. Tandem duplications in animal mitochondrial DNAs: variation in incidence and gene content among lizards. Proc Natl Acad Sci U S A. 1987;84:7183–7.
15. Macey JR, Larson A, Ananjeva NB, Fang ZL, Papenfuss TJ. Two novel gene orders and the role of light-strand replication in rearrangement of the mammalian genome. Mol Biol Evol. 1997;14:991–104.
16. Mueller RL, Boore JL. Molecular mechanisms of extensive mitochondrial gene rearrangement in plethodontid salamanders. Mol Biol Evol. 2005;22:2104–12. doi:10.1093/molbev/msi204.
17. Xia Y, Zheng YC, Miura I, Wong PB, Murphy RW, Zeng XM. The evolution of mitochondrial genomes in modern frogs (*Neobatrachia*): nonadaptive evolution of mitochondrial genome reorganization. BMC Genomics. 2014;15:691.
18. Moritz C. Evolutionary dynamics of mitochondrial DNA duplications in parthenogenetic geckos, Heteronotia binoei. Genetics. 1991;129:221–30.
19. Macey JR, Papenfuss TJ, Kuehl JV, Fourcade HM, Boore JL. Phylogenetic relationships among amphibian reptiles based on complete mitochondrial genomic sequences. Mol Phylgenet Evol. 2004;33:22–31. doi:10.1016/j.ympev.2004.05.003.
20. Sammler S, Kretzraer V, Havenstein K, Tiedemann R. Intraspecific Rearrangement of Duplicated Mitochondrial Control Regions in the Luzon Tarictic Hornbill *Penelopides mariana* (Aves: Bucerotidae). J Mol Evol. 2013;77:199–205. doi:10.1007/s00239-013-9591-y.
21. Kurabayashi A, Yoshihikawa N, Sato N, Hayashi Y, Oumi S, Fujii T, et al. Complete mitochondrial DNA sequence of the endangered frog *Odorrana ishikawaensis* (family Ranidae) and unexpected diversity of mt gene arrangements in ranids. Mol Phylogenet Evol. 2010;56:543–53. doi:10.1016/j.ympev.2010.01.022.
22. Kurabayashi A, Sumida M. Afroarabian mitochondrial genomes: genome reorganization, gene rearrangement mechanisms, and evolutionary trends of duplicated and rearranged genes. BMC Genomics. 2013;14:6363.
23. Zhang P, Liang D, Mao RL, Hills DM, Wake DB, Cannatella DC. Efficient sequencing of Anuran mtDNAs and a mitogenomic exploration of the
phylogeny and evolution of frogs. Mol Biol Evol. 2013;30:1899–915.
doi:10.1093/molbev/msr091.
24. Fonseca MM, Harris DJ. Relationship between mitochondrial gene
rearrangements and stability of the origin of long strand replication.
Genet Mol Biol. 2008;31:566–74.
25. Iriani I, Mauro DS, Abascal F, Öhler A, Vences M, Zardoya R. The origin
of modern frogs (Neobatrachia) was accompanied by acceleration in
mitochondrial and nuclear substitution rates. BMC Genomics. 2012;13:626.
26. Shan X, Xia Y, Zheng YC, Zou FD, Zeng XM. The complete mitochondrial
genome of Quispispa boulengi (Anura: Dicroglossidae). Mitochondr DNA.
2014;25:83–4. doi:10.3109/19401736.2013.78203.
27. Kumazawa Y, Miura S, Yamada C, Hashiguchi Y. Gene rearrangements in
gekkonid mitochondrial genomes with shuffling, loss, and reassignment of
tRNA genes. BMC Genomics. 2014;15:930.
28. Kakehashi R, Kurabayashi A, Oumi S, Katsuren S, Hoso M, Sumida M.
Mitochondrial genomes of Japanese Babina frogs (Ranidae, Anura):
unique gene arrangements and the phylogenetic position of genus Babina.
Genes Genet Syst. 2013;88:59–67.
29. Bousau B, Brown JM, Fujita MK. Nonadaptive evolution of mitochondrial
polymorphisms by translocation in the parasitic wasp Nasonia.
Science. 2006;311:1727–30. doi:10.1126/science.1118884.
30. Oliveira D, Montoya J, Attardi G. tRNA punctuation model of RNA processing
in human mitochondria. Nature. 1981;290:470–4.
31. Mindell DP, Sorenson MD, Dimcheff DE. Multiple independent origins of
mitochondrial gene order in birds. Proc Natl Acad Sci U S A. 1998;95:10693–7.
32. Satoh T, Sato Y, Masuyama N, Miya M, Nishida M. Transfer RNA gene
arrangement and codon usage in vertebrate mitochondrial genomes: a
new insight into gene order conservation. BMC Genomics. 2010;11:479.
33. Detal A, Hinsinger DD, Utage J, Debruyne R, Thomas M, Denys GP, et al.
Fishing for barcodes in the Torrent: from COI to complete mitogenomes
on NGS platforms. DNA Barcodes. 2015;3:170.
34. Crampton-Platt A, Timmermans MJTN, Gimmel ML, Kutty SN, Cockerill TD,
Dettai A, Hinsinger DD, Utage J, Debruyne R, Thomas M, Denys GP, et al.
Mitochondrial Metagenomics of a Bornean Rainforest Sample. Mol Biol Evol.
2014;31:83–94. doi:10.1093/molbev/msu265.
35. Babbucci M, Basso A, Scupola A, Patarnello T, Negrisolo E. Is It an Ant or
a Butterfly? Convergent Evolution in the Mitochondrial Gene Order of
Hymenoptera and Lepidoptera. Genome Biol Evol. 2014;6:3326–43.
doi:10.1093/gbe/evu265.
36. Ojala D, Montoya J, Attardi G. tRNA punctuation model of RNA processing
in human mitochondria. Nature. 1981;290:470–4.
37. Lynch M, Koskella B, Schaack S. Mutation pressure and the evolution of
genome size. Evolution. 2011;65:2706–11.
38. Harris EE. Nonadaptive processes in primate and human evolution.
Am J Phys Anthropol. 2010;143:13–45. doi:10.1002/ajpa.21499.
39. Lynch M, Kojima B, Schaal B. Mutation pressure and the evolution of
organellar genome architecture. Science. 2006;311:1727–30. doi:10.1126/
science.1118884.
40. Oliveirea D, Raychoudhury R, Lavrov DV, Werren JH. Rapidly evolving
mitochondrial genome and directional selection in mitochondrial genes in
the parasitic wasp Nasonia (Hymenoptera: Pteromalidae). Mol Biol Evol.
2008;25:267–80. doi:10.1093/molbev/msn159.
41. Chong RA, Mueller RL. Low metabolic rates in salmonanders are correlated
with weak selective constraints on mitochondrial genes. Evolution. 2013;67:
894–9. doi:10.1111/j.1558-5646.2012.01830.x.
42. Yan F, Zhou WW, Zhao HT, Yuan ZY, Wang YY, Jiang K, et al. Geological
events play a larger role than Pleistocene climatic fluctuations in driving the
genetic structure of Quispispa boulengi (Anura: Dicroglossidae). Mol Ecol.
2013;22:1120–33. doi:10.1111/mec.12153.
43. Qiong LY, Xia Y, Zheng YC, Zeng XM. A De Novo Case of Floating Chromosomal
Polymorphisms by Translocation in Quispispa boulengi (Anura, Dicroglossidae).
PloS One. 2012;7:e46163. doi:10.1371/journal.pone.0046163.
44. Kurabayashi A, Sumida M. PCR primers for the neobatrachian mitochondrial
genome. Current Herpetology. 2009;28:1–11. doi:10.3105/018028:0101.
45. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular
Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol. 2013;30:2725–9.
doi:10.1093/molbev/mst107.
46. Librado P, Rozas J. DnaSP v5: a software for comprehensive analysis of
DNA polymorphism data. Bioinformatics. 2009;25:1451–2. doi:10.1093/
bioinformatics/btp187.