Complete mitochondrial genome of yellow meal worm (*Tenebrio molitor*)

Li-Na LIU[1], Cheng-Ye WANG[2,*]

1. *State Key Laboratory of Genetic Resources and Evolution*, *Kunming Institute of Zoology*, *Chinese Academy of Sciences*, *Kunming* 650223, *China*
2. *Key Laboratory of Cultivating and Utilization of Resource Insects of State Forestry Administration*, *Research Institute of Resource Insects*, *Chinese Academy of Forestry*, *Kunming* 650224, *China*

Abstract: The yellow meal worm (*Tenebrio molitor* L.) is an important resource insect typically used as animal feed additive. It is also widely used for biological research. The first complete mitochondrial genome of *T. molitor* was determined for the first time by long PCR and conserved primer walking approaches. The results showed that the entire mitogenome of *T. molitor* was 15 785 bp long, with 72.35% A+T content [deposited in GenBank with accession number KF418153]. The gene order and orientation were the same as the most common type suggested as ancestral for insects. Two protein-coding genes used atypical start codons (CTA in *ND2* and AAT in *COX1*), and the remaining 11 protein-coding genes started with a typical insect initiation codon ATN. All tRNAs showed standard clover-leaf structure, except for $tRNA^{Ser}$ (*AGN*), which lacked a dihydrouridine (DHU) arm. The newly added *T. molitor* mitogenome could provide information for future studies on yellow meal worm.

Keywords: Tenebrionidae; Mitogenome; Evolution; Resource insect; Yellow meal worm

Mitochondrial genes have been widely used in studies of phylogenetics, phylogeography and molecular diagnostics (Kocher et al, 1989; Simmons & Weller, 2001; Wolstenholme, 1992). The full-length mitochondrial genome (mitogenome) has become an important tool for studies of genome architecture, population genetics, primer design, and molecular evolution (Kim et al, 2009; Lee et al, 2009; Liao et al, 2010; Yukuhiro et al, 2002). Furthermore, mitogenome sequences provide important information for pest control. For example, insecticide resistance in arthropod pest *Tetranychus urticae* is controlled by mtDNA (Van Leeuwen et al, 2008).

Tenebrionidae is a large family of Coleoptera and more than 20000 species have been described worldwide (Bouchard et al, 2011). Despite this large taxonomic diversity, information about the Tenebrionidae mitogenome is still limited. To date, only two complete mitogenomes are available in GenBank (NC_003081: *Tribolium castaneum* and NC_013554: *Adelium* sp. *NCS*-2009). Due to the importance of mitogenomes in population studies and pest control, studies on the mitogenome of tenebrionids are required.

The yellow meal worm (*Tenebrio molitor* L.) is an important resource insect, which has great value as an animal feed additive because of its high protein content. It has also been used in numerous experimental studies (Gomez et al, 2013; Simon et al, 2013). Its relatively large body-size and ease of rearing make it attractive for biological research. An increasing number of researchers have used this beetle as a model system for studies in biology, biochemistry, evolution, immunology and physiology (Dobson et al, 2012; Goptar et al, 2013; Ohde et al, 2013; Prokkola et al, 2013; Yan et al, 2013; Zhu et al, 2013). However, the genetic background of this beetle, in both nuclei and organelles, has not yet been determined, and information is urgently needed.

In this study, we sequenced the whole mitogenome of *T. molitor* for the first time and analyzed its nucleotide composition and major characteristics. The entire mitogenome was found to be 15 785 bp long and harbored 13 protein-coding genes (PCGs), 22 tRNA genes, and

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*Corresponding author, E-mail: cywang11@126.com

2 rRNA genes. The gene arrangement in the *T. molitor* mitogenome was identical to the ancestral insect mitogenome arrangement (Boore et al, 1998). The mitogenome of *T. molitor* is an important addition to the continuing efforts in developing *T. molitor* as a model system for biological studies.

MATERIALS AND METHODS

Sample and DNA extraction

An adult *T. molitor* was collected in Kunming, Yunnan Province, China, on July 29, 2012. The adult insect was one of the descendants from our inbred line, which has been established and maintained in our laboratory since May 2008. All members of this inbred line have one common ancestral mitochondrial genome background. After collection, the fresh materials were immediately preserved in 100% ethanol and stored in a −20 °C refrigerator before genomic DNA extraction. Total genomic DNA was extracted with the WizardTM Genomic DNA Purification Kit, in accordance with the manufacturer's instructions (Promega, USA).

PCR amplification and sequencing

To sequence the complete mitogenome, long PCR primers and some short PCR primers were designed by multiple sequence alignments of all available complete Coleoptera mitogenomes using ClustalX1.8 (Thompson et al, 1997) and Primer Premier 5.0 software. Primer sequence information can be obtained from the authors upon request. Long PCRs were performed using TaKaRa LA Taq polymerase with the following cycling parameters: initial denaturation for 5 min at 95 °C, followed by 30 cycles at 95 °C for 50 s, 50 °C for 50 s, 68 °C for 2 min and 30 s; and a final extension step of 68 °C for 10 min. Short fragments were amplified with TaKaRa Taq polymerase: initial denaturation for 5 min at 94°C, followed by 35 cycles at 94 °C for 1 min, 45−53 °C for 1 min, 72 °C for 2 min, and a final extension step of 72 °C for 10 min. The PCR products were detected *via* electrophoresis in 1.5% agarose gel, and purified using the QIAquick PCR Purification Kit (QIAGEN, USA). Sequencing reaction was performed using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Electrophoresis of purified sequencing products was performed on an ABI-3730 DNA Analyzer (Applied Biosystems).

Gene identification and tRNA structures

Sequences from overlapping fragments were assembled with the neighboring fragments using the SeqMan program included in the Lasergene software package (DNAStar Inc., Madison, Wisc.). Comparison of the DNA or amino acid sequences with homologous regions of known full-length insect mitogenome sequences indicated 13 protein-coding genes (PCGs) and two rRNA genes based on MEGA 5.0 software (Tamura et al, 2011). The nucleotide sequences of the PCGs were translated on the basis of the invertebrate mtDNA genetic code. Transfer RNA gene analysis was conducted based on tRNAscan-SE software v.1.21 (Lowe & Eddy, 1997), and the folding of the predicted tRNA sequences were further confirmed by visual inspection. Finally, the *T. molitor* mitogenome sequence was deposited in GenBank with accession number KF418153.

Sequence analysis

The A+T-content of the whole genome was calculated based on the EditSeq program included in the Lasergene software package. The nucleotide composition at each codon position of the PCGs and codon usage were calculated based on MEGA 5.0. Gene overlaps and intergenic-space sequences were hand-counted.

Nucleotide composition skew was calculated for the PCGs and the whole genome base on the EditSeq program included in the Lasergene software package (DNAStar Inc., Madison, Wisc.), using the following formula proposed by Perna & Kocher (1995): GC-skew=(G–C)/(G+C) and AT-skew=(A–T)/(A+T), where C, G, A and T are the frequencies of the four bases.

RESULTS AND DISCUSSION

General features of *Tenebrio molitor* mitogenome

The complete mitogenome of *T. molitor* was 15 785 bp in length, and consisted of 2 rRNAs (*srRNA* and *lrRNA*), 13 PCGs (*ATP6, ATP8, COI-III, ND1-6, ND4L, CytB*), and 22 tRNAs (Figure 1). Like many other insect mitogenomes, the major strand (J strand) carried most of the genes (9 PCGs and 14 tRNAs), whereas the remaining genes were on the minor strand (N strand) (4 PCGs, 8 tRNAs and 2 rRNA genes) (Figure 1). Gene order and orientation were the same as the most common type suggested as ancestral for insects [18, 23] (Boore et al, 1998; Taanman, 1999).

All genes were closely assembled and only nine intergenic spacers were observed. These intergenic

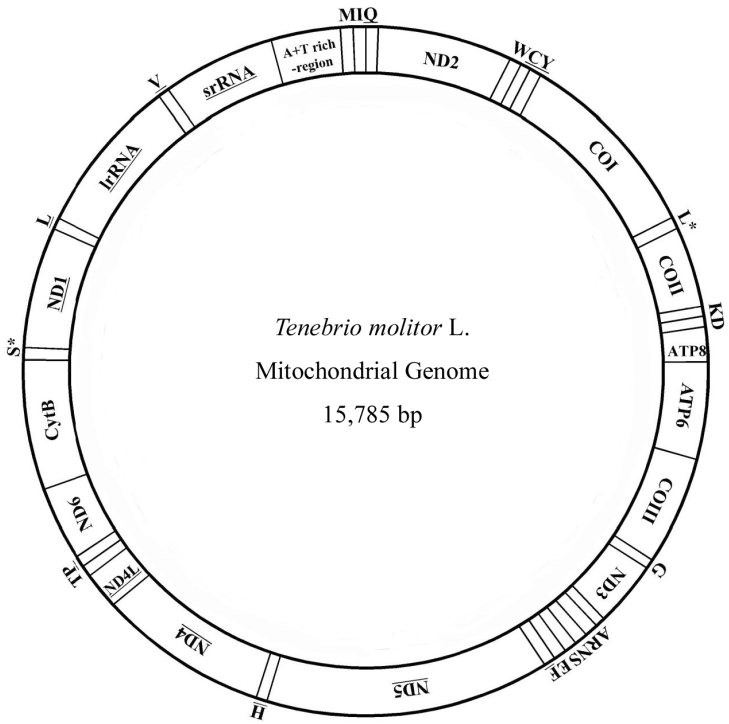


Figure 1 Circular map of the *Tenebrio molitor* mitogenome

CO Ⅰ, CO Ⅱ and COⅢ refer to the cytochrome C oxidase subunits; CytB refers to cytochrome B; ATP6 and ATP8 refer to subunits 6 and 8 of F0 ATPase; ND1-6 refer to components of NADH dehydrogenase. tRNAs are denoted as one-letter symbols consistent with the IUPAC-IUB single letter amino acid codes. Gene names not underlined indicate a clockwise transcriptional direction, whereas underlines indicate a counter-clockwise transcriptional direction. L* and S* denote $tRNA^{Leu}$(UUR) and $tRNA^{Ser}$(UCN), respectively.

spacers were 53 bp in total, with individual sizes ranged from 1 to 22 bp. In addition, there were 36 bp overlapping sequences totally in 15 overlaps between different genes, ranging from 1−7 bp (Table 1).

Comparative analysis with two other published Tenebrionidae mitogenomes of *Tribolium castaneum* and *Adelium* sp. *NCS*-2009 exhibited highly conserved genome architectures including gene order, nucleotide composition, codon usage, and amino acid composition (Friedrich & Muqim, 2003; Sheffield et al, 2009).

Protein-coding genes (PCGs)

Eleven of the 13 PCGs of *T. molitor* used standard ATN (Met) start codon, while the remaining two PCGs used atypical start codons (CTA in *ND2* and AAT in *COX1*) (Table 1). ATP6, ATP8, ND4L, ND6, and CytB genes had the common stop codon TAA; ND1 and ND3 had the stop codon TAG; while ND2, COX1, COX2, COX3, ND5, and ND4 terminated with incomplete stop codon T or TA (Table 1). Similar cases have been found in other insect mitogenomes (Yin et al, 2012). For example, a single T residue was deemed as the stop codon for CO Ⅰ, CO Ⅱ, ND5 and CytB, and TA was deemed as the stop codon for ATP6, ND4, ND4L and ND6 in *Coreana raphaelis* (Kim et al, 2006); similarly, a single T was considered as the stop codon for CO Ⅰ, CO Ⅱ and ND4, and TA residue as the stop codon for ATP6 in *Hyphantria cunea* (Liao et al, 2010). Incomplete stop codons also exist in vertebrate mitochondrial genomes such as in human mitochondria (Andrews et al, 1999). It has been demonstrated that incomplete stop codons can produce functional stop codons in polycistronic transcription cleavage and polyadenylation processes (Ojala et al, 1981).

Transfer RNA and ribosomal RNA genes

Twenty-two tRNA genes (one specific for each amino acid and two for leucine and serine) were identified within the *T. molitor* mitogenome. The 22 tRNA genes were interspersed throughout the coding region and ranged from 60 to 70 bp in length (Table 1). All tRNAs, except $tRNA^{Ser}$ (*AGN*), were folded into typical cloverleaf secondary structures (Figure 2). The unusual $tRNA^{Ser}$ (*AGN*) lacked a dihydrouridine (DHU) arm, which was replaced by a simple loop (Figure 2). Another

unusual feature was the use of TCT as the *tRNA^{Ser}* (*AGN*) anticodon instead of GCT, which is used in most other arthropods. This incomplete *tRNA^{Ser}* structure has also been detected in other insect groups (Kim et al, 2006; Li et al, 2011; Liao et al, 2010; Salvato et al, 2008; Wolstenholme, 1992; Yang et al, 2011).

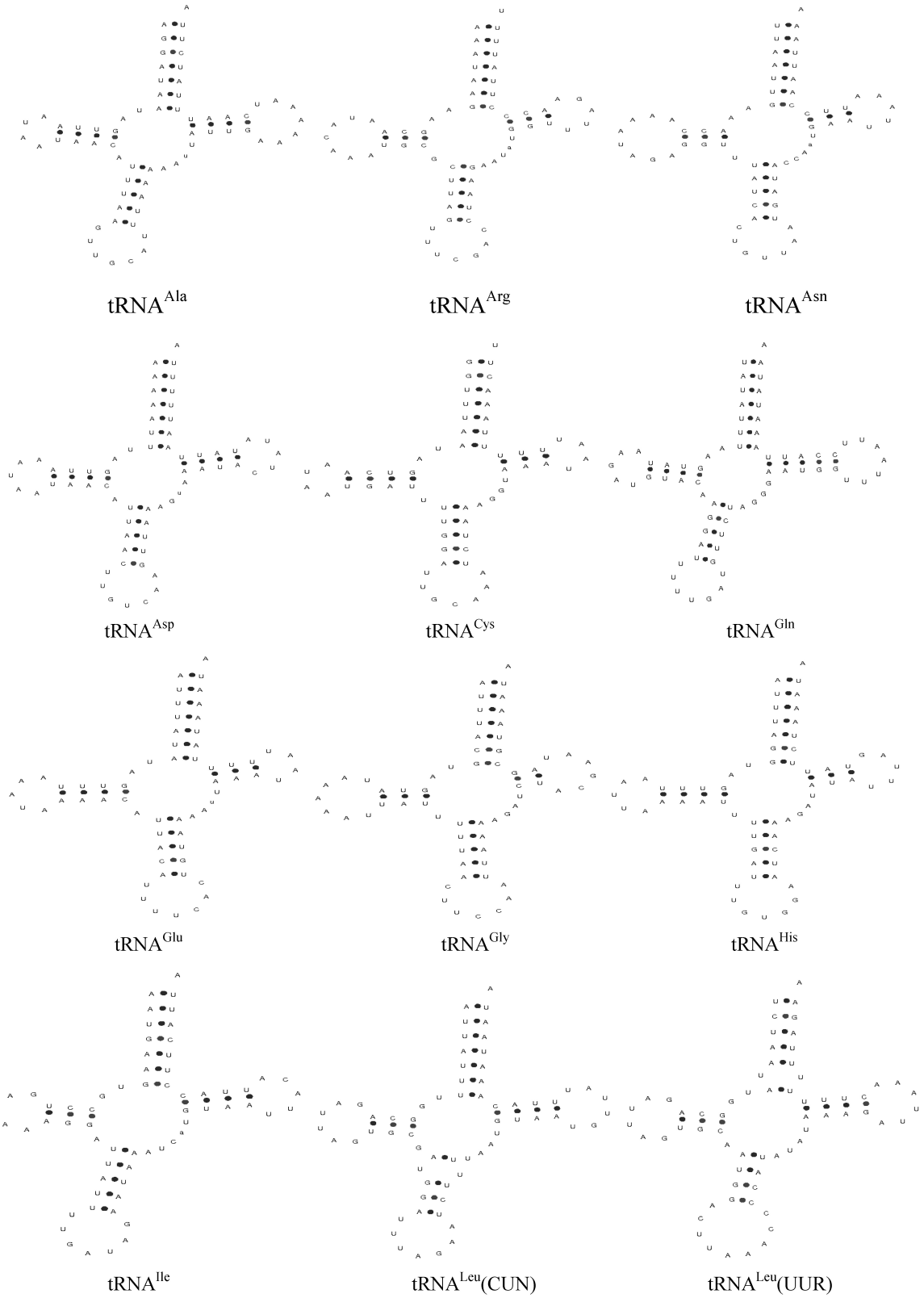
Twenty-two non-Watson-Crick base pairs were observed in the *T. molitor* mt tRNAs, including 17 G-U

Table 1 Summary of *Tenebrio molitor* mitogenome

Gene	Direction	Nucleotide number	Size (bp)	Anticodon	Start codon	Stop codon
tRNA^{Ile}	F	1–64	64	29–31 GAT	–	–
tRNA^{Gln}	R	62–130	69	98–100 TTG	–	–
tRNA^{Met}	F	131–198	68	160–162 CAT	–	–
ND2	F	204–1206	1003	–	CTA(M)	T(AA)$^{\#}$
tRNA^{Trp}	F	1207–1272	66	1240–1242 TCA	–	–
tRNA^{Cys}	R	1295–1355	61	1324–1326 GCA	–	–
tRNA^{Tyr}	R	1356–1421	66	1388–1390 GTA	–	–
COX1	F	1423–2956	1534	–	AAT(M)	T(AA)$^{\#}$
tRNA^{Leu}(UUR)	F	2957–3021	65	2986–2988 TAA	–	–
COX2	F	3022–3709	688	–	–	–
tRNA^{Lys}	F	3710–3779	70	3740–3742 CTT	–	–
tRNA^{Asp}	F	3779–3843	65	3809–3811 GTC	–	–
ATP8	F	3844–3999	156	–	ATA(M)	TAA
ATP6	F	3993–4664	672	–	ATG(M)	TAA
COX3	F	4664–5448	785	–	ATG(M)	TA(A)$^{\#}$
tRNA^{Gly}	F	5448–5509	62	5478–5480 TCC	–	–
ND3	F	5510–5863	354	–	ATT(M)	TAG
tRNA^{Ala}	F	5862–5927	66	5891–5893 TGC	–	–
tRNA^{Arg}	F	5927–5990	64	5957–5959 TCG	–	–
tRNA^{Asn}	F	5990–6054	65	6021–6023 GTT	–	–
tRNA^{Ser}(AGN)	F	6055–6114	60	6077–6079 TCT	–	–
tRNA^{Glu}	F	6116–6177	62	6146–6148 TTC	–	–
tRNA^{Phe}	R	6176–6239	64	6206–6208 GAA	–	–
ND5	R	6240–7953	1714	–	ATA(M)	T(AA)$^{\#}$
tRNA^{His}	R	7951–8013	63	7981–7983 GTG	-	-
ND4	R	8014–9346	1333	–	ATG(M)	T(AA)$^{\#}$
ND4L	R	9340–9627	288	–	ATG(M)	TAA
tRNA^{Thr}	F	9630−9692	63	9660−9662 TGT	–	–
tRNA^{Pro}	R	9693−9758	66	9726−9728 TGG	–	–
ND6	F	9761−10258	498	–	ATT(M)	TAA
CytB	F	10258−11394	1137	–	ATG(M)	TAA
tRNA^{Ser}(UCN)	F	11393−11458	66	11423−11425TGA	–	–
ND1	R	11476−12429	954	–	ATA(M)	TAG
tRNA^{Leu}(CUN)	R	12427−12491	65	12460−12462TAG	–	–
lrRNA	R	12491–13771	1281	–	–	–
tRNA^{Val}	R	13774−13842	69	13810-13812 TAC	–	–
srRNA	R	13844−14615	772	–	–	–
A+T-rich region	R	14616−15785	1170	–	–	–

tRNA abbreviations follow the IUPAC-IUB three letter code.

#: TAA stop codon is completed by the addition of 3' A residues to the mRNA.


tRNA^Ala
tRNA^Arg
tRNA^Asn
tRNA^Asp
tRNA^Cys
tRNA^Gln
tRNA^Glu
tRNA^Gly
tRNA^His
tRNA^Ile
tRNA^Leu(CUN)
tRNA^Leu(UUR)

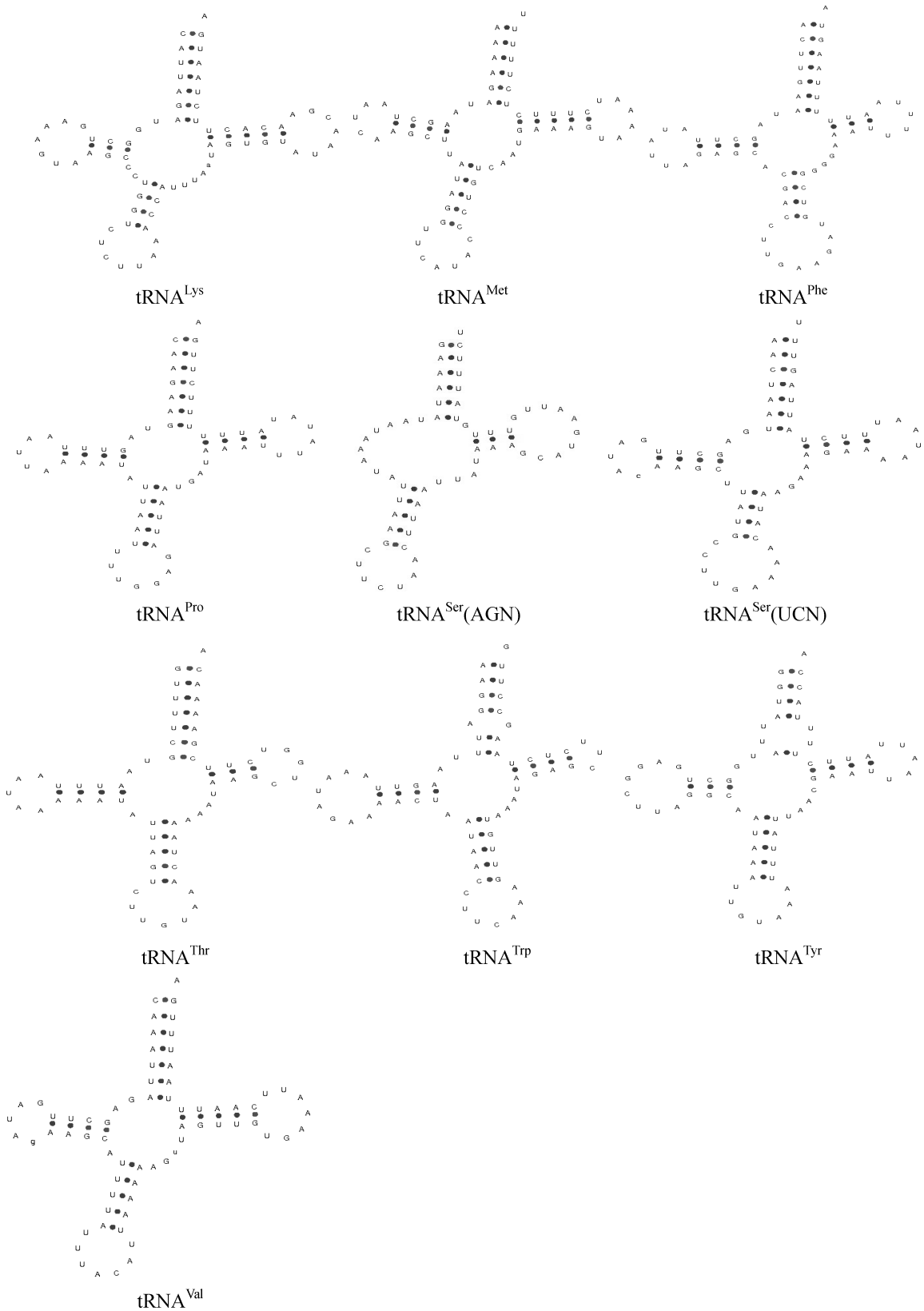


Figure 2 Predicted secondary clover-leaf structure for the 22 tRNA genes of the *Tenebrio molitor* mitogenome

The tRNAs are labeled with abbreviations of their corresponding amino acids. Point (●) indicate Watson-Crick base-pairing. Arms of tRNAs (clockwise from top) are amino acid acceptor (AA) arm, TψC (T) arm, anticodon (AC) arm and dihydrouridine (DHU) arm.

pairs, which form a weak bond in the tRNAs. The remaining five were atypical pairings: one mismatch in $tRNA^{Leu}$ (CUN) (U-U), one in $tRNA^{Leu}$ (UUR) (U-U), two in $tRNA^{Tyr}$ (2 U-U), and one in $tRNA^{Trp}$ (A-G) (Figure 2). These mismatches found in tRNAs can be corrected through RNA-editing mechanisms (Lavrov et al, 2000).

The *lrRNA* and *srRNA* genes of the *T. molitor* mitogenome were 1 281 and 772 bp in length, respectively. As observed in other insects (Boore et al, 1998; Kim et al, 2009), these two genes were located between $tRNA^{Leu}$ (CUN) and $tRNA^{Val}$ as well as between $tRNA^{Val}$ and the A+T-rich region, respectively (Figure 1). The A+T contents of the *lrRNA* and *srRNA* genes were 78.92% and 77.33%, respectively.

A+T-rich region

The 1 170 bp A+T-rich region of the *T. molitor* mitogenome was located between the *srRNA* and $tRNA^{Ile}$ genes (Table 1). Like the two other Tenebrionidae mitogenomes, the A+T-rich region also exhibited the highest A+T content (85.38%) in the *T. molitor* mitogenome. Unlike some insect species (Kim et al, 2006; Yin et al, 2012), the region did not have large tandem repetitive sequences; however, it did have some microsatellite-like repeats (for example, $(T)_{15}$, $(AT)_7$, $(A)_{20}$, and (G)8). The poly-T stretch (15 bp) has been suggested to function as a possible recognition site for the initiation of replication of the mtDNA minor strand (Andrews et al, 1999; Kim et al, 2009). The A+T-rich region in insect mitogenomes is equivalent to the control region of vertebrate mitogenomes, which harbor the origin sites for transcription and replication (Andrews et al, 1999; Yukuhiro et al, 2002; Zhu et al, 2013).

Nucleotide composition and codon usage

The A+T content of the whole *T. molitor* mitogenome coding region was 72.35%, showing an obvious AT mutation bias (Eyre-Walker, 1997), as observed in two other published Tenebrionidae species (Friedrich & Muqim, 2003; Sheffield et al, 2009). The AT-skew value of the major strand was 0.204, indicating the occurrences of As in the major strands were greater than those of Ts. The mean value of the A+T content of the 13 PCGs was 69.27%, with a strong A+T bias. The A+T content at the third codon position (76.30%) was higher than the first (64.75%) and second position (66.77%), indicating that the third codon position was most susceptible to AT mutation bias (Eyre-Walker, 1997).

The relative synonymous codon usage (RSCU) in the *T. molitor* mitochondrial PCGs was investigated and the results are summarized in Table 2. The four most frequently used codons were ATT (Ile), ATA (Met), TTT (Phe), and TTA (Leu), accounting for 25.7% of all codons in the *T. molitor* mitogenome. These four codons were composed of A or T nucleotides, indicating that their biased usage resulted from strong AT mutation bias

Table 2 Codon number and relative synonymous codon usage in *Tenebrio molitor* mitochondrial protein coding genes[a)]

Codon	Count	RSCU	Codon	Count	RSCU	Codon	Count	RSCU	Codon	Count	RSCU
UUU(F)	227	1.34	UCU(S)	99	2.28	UAU(Y)	113	1.47	UGU(C)	25	1.61
UUC(F)	113	0.66	UCC(S)	23	0.53	UAC(Y)	41	0.53	UGC(C)	6	0.39
UUA(L)	198	2.29	UCA(S)	101	2.33	UAA(*)	0	0	UGA(W)	74	1.61
UUG(L)	73	0.84	UCG(S)	11	0.25	UAG(*)	0	0	UGG(W)	18	0.39
CUU(L)	90	1.04	CCU(P)	46	1.39	CAU(H)	31	0.87	CGU(R)	26	1.82
CUC(L)	9	0.1	CCC(P)	18	0.55	CAC(H)	40	1.13	CGC(R)	2	0.14
CUA(L)	131	1.51	CCA(P)	61	1.85	CAA(Q)	50	1.54	CGA(R)	26	1.82
CUG(L)	18	0.21	CCG(P)	7	0.21	CAG(Q)	15	0.46	CGG(R)	3	0.21
AUU(I)	274	1.52	ACU(T)	53	1.07	AAU(N)	116	1.21	AGU(S)	26	0.6
AUC(I)	86	0.48	ACC(T)	40	0.8	AAC(N)	75	0.79	AGC(S)	6	0.14
AUA(M)	252	1.67	ACA(T)	98	1.97	AAA(K)	78	1.46	AGA(S)	66	1.52
AUG(M)	49	0.33	ACG(T)	8	0.16	AAG(K)	29	0.54	AGG(S)	15	0.35
GUU(V)	91	1.69	GCU(A)	48	1.22	GAU(D)	42	1.2	GGU(G)	50	0.94
GUC(V)	14	0.26	GCC(A)	24	0.61	GAC(D)	28	0.8	GGC(G)	20	0.38
GUA(V)	92	1.7	GCA(A)	81	2.05	GAA(E)	53	1.43	GGA(G)	102	1.92
GUG(V)	19	0.35	GCG(A)	5	0.13	GAG(E)	21	0.57	GGG(G)	40	0.75

a) RSCU, relative synonymous codon usage. Letters in brackets are the single-letter amino acid codes. Total number of codons, excluding the stop codons (*)=3696.

(Powell & Moriyama, 1997; Rao et al, 2011).

The total number of non-stop codons (CDs) of PCGs was 3 696. Among all amino acids encoded by the 13 PCGs, Leu (14.04%), Ile (9.74%), Ser (9.38%), and Phe (9.20%) were the four most abundant amino acids, three of which were encoded by the AT-rich codons (see above), suggesting that the AT bias affected amino acid composition (Foster et al, 1997; Min & Hickey, 2007).

SUMMARY

Recently, *T. molitor* has been used as a model to study intoxication response. However, analyzing the transcriptome of *T. molitor* is time-consuming work due to the lack of a sequenced genome (Oppert et al, 2012). A known *T. molitor* mitogenome sequence could accelerate contig assembly and annotation, as well as help to study informative rearrangements in mitochondrial genomes (Timmermans & Vogler, 2012) and perform high resolution phylogenetic analysis of Coleoptera (Kim et al, 2009).

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