

Mitochondrial Sequence Evolution in Spiders: Intraspecific Variation in tRNAs Lacking the TΨC Arm

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Analyses of mitochondrial DNA sequences from three species of *Habronattus* jumping spiders (Chelicerata: Arachnida: Araneae) reveal unusual inferred tRNA secondary structures and gene arrangements, providing new information on tRNA evolution within chelicerate arthropods. Sequences from the protein-coding genes NADH dehydrogenase subunit I (ND1), cytochrome oxidase subunit I (COI), and subunit II (COII) were obtained, along with tRNA^{Val}, tRNA^{Leu(CUN)}, and large-subunit ribosomal RNA (16S) sequences; these revealed several peculiar features. First, inferred secondary structures of tRNA^{Val} and, likely, tRNA^{Leu(CUN)}, lack the TΨC arm and the variable arm and therefore do not form standard cloverleaf structures. In place of these arms is a 5–6-nt T arm-variable loop (TV) replacement loop such as that originally described from nematode mitochondrial tRNAs. Intraspecific variation occurs in the acceptor stem sequences in both tRNAs. Second, while the proposed secondary structure of the 3' end of 16S is similar to that reported for insects, the sequence at the 5' end is extremely divergent, and the entire gene is truncated about 300 nt with respect to *Drosophila yakuba*. Third, initiation codons appear to consist of ATY (ATT and ATC) and TTG for ND1 and COII, respectively. Finally, *Habronattus* shares the same ND1-tRNA^{Leu(CUN)}-16S gene arrangement as insects and crustaceans, thus illustrating variation in a tRNA gene arrangement previously proposed as a character distinguishing chelicerates from insects and crustaceans.

Introduction

Animal mitochondrial genomes contain a number of unusual features that distinguish them from other related prokaryote genomes and from the nuclear genomes of eukaryotes. Only in animal mitochondrial genomes have tRNAs that do not have the canonical cloverleaf secondary structure been discovered (reviewed in Dirheimer et al. 1995). In addition, the genetic codes of metazoan mitochondria are highly modified and utilize unusual initiation codons (reviewed in Wolstenholme 1992). Furthermore, while metazoan mitochondria typically have 22 tRNA, 13 protein-coding, and two ribosomal RNA genes, the arrangement of these genes differs greatly among taxa (Wolstenholme 1992). For taxa in which few mitochondrial-based molecular studies have been reported, it is probable that new evolutionary patterns may yet be discovered. The Arachnida is one such understudied group.

Much of our understanding of mitochondrial evolution has resulted from examination of complete sequences of mitochondrial genomes and from use of partial mitochondrial sequences to reconstruct phylogenetic relationships of groups of organisms. Less work has focused on interpreting patterns of mitochondrial sequence variation found within a single species or population. Therefore, examination of intraspecific sequence variation may also provide new insight into molecular evolutionary processes.

Comparative studies of patterns of molecular evolution among phylogenetically diverse taxa have al-

lowed inferences about the secondary structure of gene products, especially for the structurally complex ribosomal RNA genes (reviewed in Gutell 1993, 1996). Regions of evolutionarily conserved secondary-structure motifs may suggest functional importance of a region in the correct functioning of the RNA or protein. Lack of conservation in a region may lead us to ask how the organism can tolerate mutations in that region. Mitochondria may be particularly susceptible to an accumulation of mutations, and mitochondrial tRNAs have been shown to accumulate deleterious mutations relative to their nuclear tRNA counterparts (Lynch 1996). Mitochondrial tRNAs that lack the TΨC arm have been found in both nematodes (e.g., Wolstenholme et al. 1987; Keddie, Higazi, and Unnash 1998) and land snails (e.g., Terrett, Miles, and Thomas 1996). Yamazaki et al. (1997) suggested that pressure to minimize the size of the mitochondrial genome may have resulted in truncation of these tRNAs. Macey et al. (1997) proposed that replication slippage may cause loss of tRNA arms. However, the mechanisms that allow these apparently deleterious mutations to exist while still maintaining functionality of the tRNAs are not fully known, although relaxation of selective constraints is probably important (Kumazawa and Nishida 1993). One possible mechanism that may allow deleterious mutations to accumulate in mitochondria is RNA editing (Börner et al. 1997), whereby RNA transcripts are “edited” so that they differ from the nucleotide sequence they were transcribed from. In this way, nucleotide mismatches that occur in stem regions of tRNAs may be corrected.

In this paper, I report on molecular evolutionary patterns observed in six mitochondrial genes of *Habronattus* jumping spiders (Araneae: Salticidae): NADH dehydrogenase subunit I (ND1), cytochrome oxidase subunit I (COI) and subunit II (COII), tRNA^{Val}, tRNA^{Leu(CUN)}, and the gene encoding 1/n of ribosomal RNA (1-rRNA, or 16S). All of these genes except COI and COII were truncated with respect to *Drosophila*

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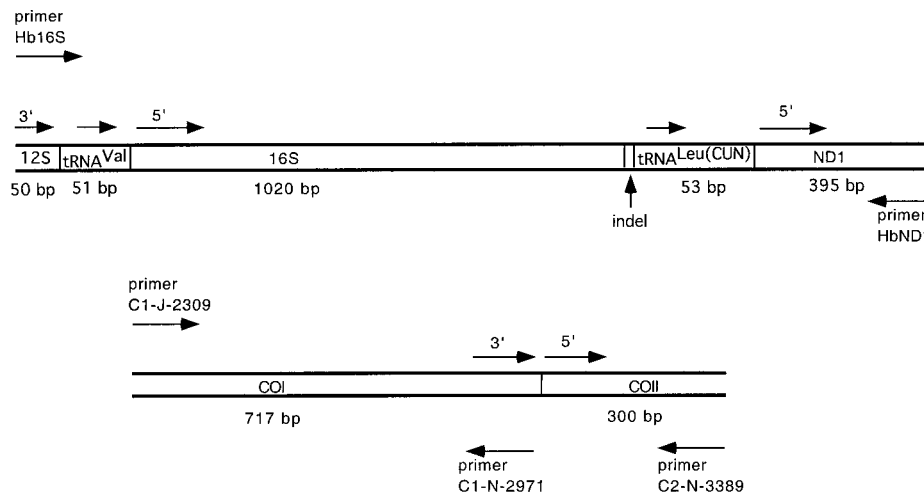


FIG. 1.—Mitochondrial gene arrangement in *Habronattus*. The direction of transcription is given by arrows. The number of nucleotides sequenced for each region is given below the gene, with primer names and locations as illustrated.

yakuba. DNA sequences from individuals from multiple populations within two species were then compared, and the secondary structures of both tRNA genes and 16S rRNA were then deduced. The inferred secondary structure of tRNA^{Val} lacks the TΨC arm, and that of tRNA^{Leu(CUN)} most likely also lacks this arm. Both tRNAs have mispairings and intraspecific sequence variation in their acceptor stems, suggesting both relaxed selection against deleterious mutations and a potential role for RNA editing. Finally, I discuss tRNA gene arrangements in the mitochondria of these arachnids relative to other arthropods, with one region sharing an identical arrangement with insects and the other being identical with respect to chelicerates.

Materials and Methods

DNA Extraction

Fourteen individuals of *Habronattus oregonensis* were collected from five mountain ranges in southeast Arizona and one location in northern California, eight *Habronattus pugillis* individuals were collected from five ranges in southeast Arizona, and one individual of *Habronattus geronimo* was collected from southeast Arizona, for a total of 23 individuals.

Total genomic DNA was isolated using a modified SDS extraction protocol. Four legs from each spider were placed in 200 μ l lysis buffer (100 mM Tris [pH 9.1], 50 mM EDTA, 0.5% SDS, 50 mM NaCl, 200 μ g proteinase K/ml) in a microfuge tube and ground using a disposable pestle. The DNA was incubated at 45°C for 3–5 h, phenol-chloroform extracted, ethanol precipitated, and resuspended in 100 μ l water.

Mitochondria-enriched DNA was isolated from one *H. pugillis* individual and two *H. oregonensis* individuals using a modified mitochondrial DNA preparation. Either 10 eggs or the abdomen from a single spider was ground in cold STE (250 mM sucrose, 30 mM Tris [pH 8.0], 50 mM EDTA pH 8.0) in 100- μ l glass homogenizers. Cell debris and nuclei were removed by pelleting at 3,500 rpm, 4°C for 8 min. The supernatant was then

transferred to a clean microfuge tube and spun at 12,000 rpm, 4°C, for 20 min to pellet the mitochondria and remaining nuclei. The pellet was resuspended in TE (pH 8.0), and membranes were lysed by addition of one tenth of a volume of 10% SDS, followed by the addition of one tenth of a volume of 5 M potassium acetate. The mixture was allowed to sit overnight at 0°C to precipitate the nuclear DNA and then was then centrifuged at 12,000 rpm, 4°C, for 30 min. The supernatant containing mitochondrial DNA was then treated with proteinase K, phenol-chloroform extracted, ethanol precipitated, and resuspended in water.

PCR and Sequencing

The mitochondrial genes encoding ND1 and 16S ribosomal RNA were amplified by the polymerase chain reaction (PCR) using primers designed specifically to amplify *H. oregonensis* and *H. pugillis*. The primer HbND1 (5'-TGAGCTACTCTTCGAATAGC-3') corresponds to positions 12257–12276 on the J strand in *Drosophila yakuba* mitochondrial ND1 (Clary and Wolstenholme 1985). The primer Hb16S (5'-TTACGGAAGTGCACATATCG-3') corresponds to positions 14226–14245 on the N strand of *D. yakuba*, located in the small-subunit ribosomal RNA (12S) coding region (see fig. 1). Amplification by PCR was performed in 50 μ l reactions, using final concentrations of 0.52 μ M of each primer, 3 mM MgCl₂, 0.2 mM of each dNTP, 1 \times PCR buffer (as supplied by the manufacturer of the *Taq* polymerase), 1 U *Taq* polymerase, and 1 μ l DNA. The amplification conditions were denaturation at 95°C for 30 s, annealing at 58°C for 1 min, and a 1-min extension at 72°C for 35 cycles.

The 3' end of COI and the 5' end of COII were amplified using the primer C1-J-2309 (5'-TTTATGCTATAGTTGGAATTGG-3'), designed by M. Hedin (personal communication) based on sequences from multiple species of *Habronattus*, and the universal primer C2-N-3389 (Simon et al. 1994). The names of the primers correspond to their positions in the mitochondrial se-

quence of *D. yakuba* (Clary and Wolstenholme 1985). PCR solution conditions identical to those for ND1–16S amplification were used, except that the primers were at a final concentration of 0.4 μ M. The cycling conditions for these reactions were 95°C for 30 s, 50°C for 1 min, and 72°C extension for 1 min, for 35 cycles.

The PCR products were purified either by excising the PCR band from a 1% agarose gel, followed by GeneClean purification, or by Qiagen QIAquick PCR spin column purification to remove primers and unincorporated dNTPs.

The PCR products were sequenced manually with the GibcoBRL dsDNA Cycle Sequencing system end-labeling kit or on an ABI automated sequencer at the University of Arizona sequencing facility. Both of the PCR amplification primers were used to sequence ND1 through 16S. The 5' end of COII was sequenced using the PCR primer C2-N-3389, but the 3' end of the adjacent COI gene was sequenced using the internal primer C1-N-2971 (5'-ATTGAGTAAAAGTATGATC-3'). With the exception of primer C2-N-3389, the universal primers as described in Simon et al. (1994) do not work well or do not work at all in these spiders, so it was necessary to design all primers specifically for *Habronattus*.

Either the PCR products were sequenced in only one direction or there was little overlap of sequences from both directions, and therefore two measures were taken to assess the reliability of the sequence data. All sequences were manually aligned in SeqApp, version 1.9 (Gilbert 1994), and all sites differing from the other sequences were reexamined to see if the base had been correctly scored. Because the individuals are closely related (intrapopulation and intraspecific samples), this type of polymorphism data allows assessment of sequencing accuracy. All ambiguous bases were scored with the appropriate IUBMB symbol. Second, although most sequences were obtained with an ABI automated sequencer, 12 of the sequences were obtained both manually and with the automated sequencer for the 5' end of ND1 through the 3' end of 16S. There was complete agreement among all sequences. Additionally, the ND1–16S PCR fragment from one individual (*H. geronimo*) was sequenced in both directions by M. Hedin, and the sequences were found to be concordant (M. Hedin, personal communication).

Nucleotide sequences among *Habronattus* species and amino acid sequences among arthropods were both aligned manually. *Habronattus* ND1, COI, and COII sequences were translated with MacClade, version 3.0 (Maddison and Maddison 1992), using the *Drosophila* mitochondrial genetic code (de Bruijn 1983) and aligned with sequences from representatives of two major groups of arthropods: the insect *D. yakuba* (X03240) and the chelicerate *Ixodes hexagonus* (AF081828). Initial attempts to align *Habronattus* and *D. yakuba* 1-rRNA nucleotide sequences using the program Blast, version 2.0 (Altschul et al. 1997), failed to align sequence motifs predicted to be conserved between the two species, even with a variety of different gap penalties. Therefore, alignment of 1-rRNA sequences among arthropods re-

quired multiple steps. First, 1-rRNA sequences were manually folded into their presumed secondary structures to look for structural motifs conserved across multiple organisms. The proposed 1-rRNA structures for *D. yakuba* (Gutell and Fox 1988) and the nematode *Caenorhabditis elegans* (Gutell 1994) and partial structures for the chelicerates *Haemaphysalis cretica* and *Cupiennius salei* (Huber et al. 1993; Black and Piesman 1994) were used for comparisons. Conserved structural motifs were also identified by examining regions of little or no sequence polymorphism within *Habronattus*. Final alignment of *Habronattus* and *D. yakuba* 1-rRNA sequences was done manually using a beta test version of MacClade, version 4.0 (test version 4.0a12 by Maddison and Maddison), based on conserved stem and loop regions present in the folded secondary structures. Sequences encoding tRNA-like structures were searched for by comparing *Habronattus* sequences with each of the 22 anticodon arms reported for the chelicerate *Limulus polyphemus* (Staton, Daehler, and Brown 1997) using the program Blastn, version 2.0.9 (Tatusova and Madden 1999). Folding of DNA sequences coding for tRNA-like structures was performed manually. Calculation of A+T content and pairwise sequence divergence was performed using the beta version of PAUP*, version 4.0d64 (Swofford 1998).

Results and Discussion

Nucleotide Sequences

Sequences were obtained in one direction for ND1 through the 3' 16S end of the PCR fragment spanning the region of the mitochondria containing the 5' end of ND1, tRNA^{Leu(CUN)}, 16S, tRNA^{Val}, and part of 12S (fig. 1), from a total of 23 individuals. A subset of 18 of these individuals were also sequenced in the opposite direction, yielding 3' 12S through 5' 16S sequences. The entire length of the PCR product was approximately 1,580 bp, and sequences from opposite strands overlapped for about 40 bp in five individuals of *H. oregonensis*, yielding five full-length sequences of 16S. GenBank accession numbers for these 23 sequences are AF239933–AF239955. Partial sequences from the 3' end of COI and the 5' end of COII were obtained for three *H. oregonensis* individuals (GenBank accession numbers AF241476–AF241478).

Alignment of the ND1–16S gene sequences from *Habronattus* resulted in a single region containing an indel of 4 nt, a simple TAAT repeat. This indel is located between 16S rRNA and tRNA^{Leu(CUN)} and is present only in individuals of *H. pugillis* collected from two geographic locales. Other single- or few-nucleotide indels are present in 16S, but there are no indels in ND1, COI, and COII sequences.

The increasing number of reports of mitochondrial sequences that have been transposed to the nucleus (e.g., Smith, Thomas, and Patton 1992; Sunnucks and Hales 1996; Zhang and Hewitt 1996) makes it important to ascertain that mitochondrial sequences obtained from total DNA extracts are indeed from mitochondria. Several factors provide confidence that these sequences are not

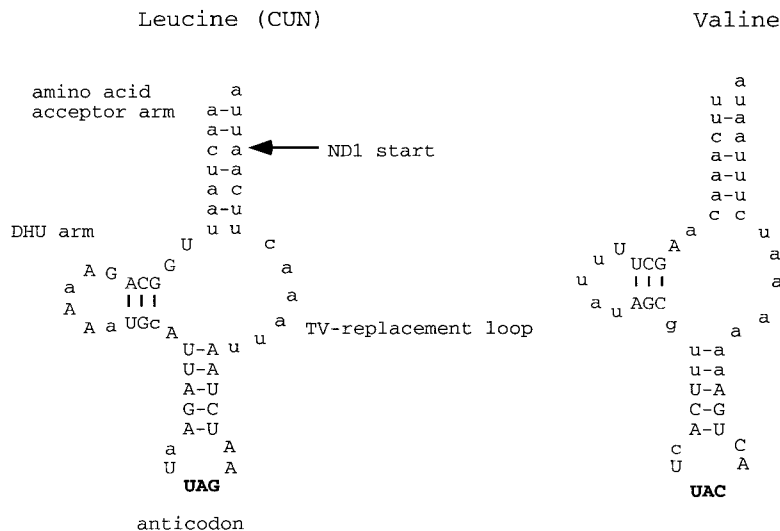


FIG. 2.—Proposed secondary structures for $\text{tRNA}^{\text{Leu(CUN)}}$ and tRNA^{Val} based on DNA sequence data from *H. oregonensis* individual 4R (Hbor4R). Dashes represent Watson-Crick bonds. Bold letters denote the anticodon; capital letters indicate that the nucleotide is conserved with respect to *Limulus polyphemus* (Staton, Daehler, and Brown 1997).

from mitochondrial regions that were transposed to the nucleus and subsequently became pseudogenes. First, PCR amplifications yielded a single band. Second, sequences did not have many ambiguous bases, as would be expected if the primers amplified multiple different copies from the two genomes. Third, sequences appeared to encode functional products: no termination codons were present within the translated protein-coding sequences, and the RNA-coding sequences folded into plausible secondary structures. There was no sequence variation in either of the tRNA regions corresponding to the anticodon arms (which may be expected to be highly conserved) among the 23 individuals that were sequenced. Finally, sequences obtained from mitochondrial DNA extractions were identical to sequences from total genomic DNA extractions.

tRNA Secondary Structure

Two tRNA-like sequences were identified based on their sequence similarity with *L. polyphemus* anticodon arms, using BLAST searches. When these were folded into their putative secondary structures (fig. 2), tRNA^{Val} and $\text{tRNA}^{\text{Leu(CUN)}}$ appeared to be truncated and to lack the variable arm and the T Ψ C arm found in the canonical tRNA. Instead, these arms are replaced by a TV-replacement loop, as previously described for nematodes (e.g., Wolstenholme et al. 1987; Okimoto and Wolstenholme 1990) and some molluscs (e.g., Terrett, Miles, and Thomas 1996; Yamazaki et al. 1997). Even though these structures differ from the cloverleaf-shaped tRNAs found in the mitochondria of other non-Araneae chelicerates (Staton, Daehler, and Brown 1997; Black and Roehrdanz 1998; but see Campbell and Barker 1999), there is almost complete conservation of the anticodon arm and of the DHU arm in $\text{tRNA}^{\text{Leu(CUN)}}$ between *Habronattus* and *L. polyphemus* (fig 2). tRNA^{Val} also shows sequence conservation of the anticodon arm and the DHU stem with *L. polyphemus*, but to a lesser degree.

However, there are complementary base changes present in the anticodon stem, where the sequences differ from one another. Sequences both upstream of the DHU arm and downstream of the anticodon arm show little sequence homology with the comparable *Limulus* tRNAs.

Alternative cloverleaf structures are possible for the *Habronattus* $\text{tRNA}^{\text{Leu(CUN)}}$, but these require either more mismatches in the acceptor stem or mismatches in the T Ψ C stem for each of the three spider species. Some individuals of *H. pugillis* (e.g., Hb1BQ) possess a sequence that potentially could form a stable cloverleaf; however, other *H. pugillis* sequences would require multiple mismatches in the T Ψ C stem. If this same cloverleaf structure was imposed on the *H. oregonensis* sequences, the acceptor and T Ψ C stem would show many mismatches. Additionally, a longer $\text{tRNA}^{\text{Leu(CUN)}}$ requires either extensive overlap with the downstream ND1 gene (20 bp) or that ND1 be truncated 6–7 amino acids with respect to *D. yakuba* (fig. 3a). This much overlap between adjacent mitochondrial genes encoded on the same strand has been found only among the ATPase6 and ATPase8 genes, for which it is proposed that translation initiation of ATPase6 occurs within the ATPase8 gene (reviewed in Wolstenholme 1992). A mechanism that could account for this much overlap between a tRNA and a protein-coding gene has not previously been described. Therefore, rather than posit both extensive gene overlap and intraspecific and intrageneric variation in tRNA structure, it is most parsimonious to conclude that $\text{tRNA}^{\text{Leu(CUN)}}$ is truncated in all three *Habronattus* species. A cloverleaf structure seems even less likely for tRNA^{Val} , as immediately downstream of the sequence encoding the 3' amino acid acceptor arm is a region with high intra- and interspecific sequence variation (fig. 3b). Folding this sequence into a structure resembling a cloverleaf would require that the T Ψ C and amino acid acceptor arms be highly divergent both among populations and among *Habronattus* species.

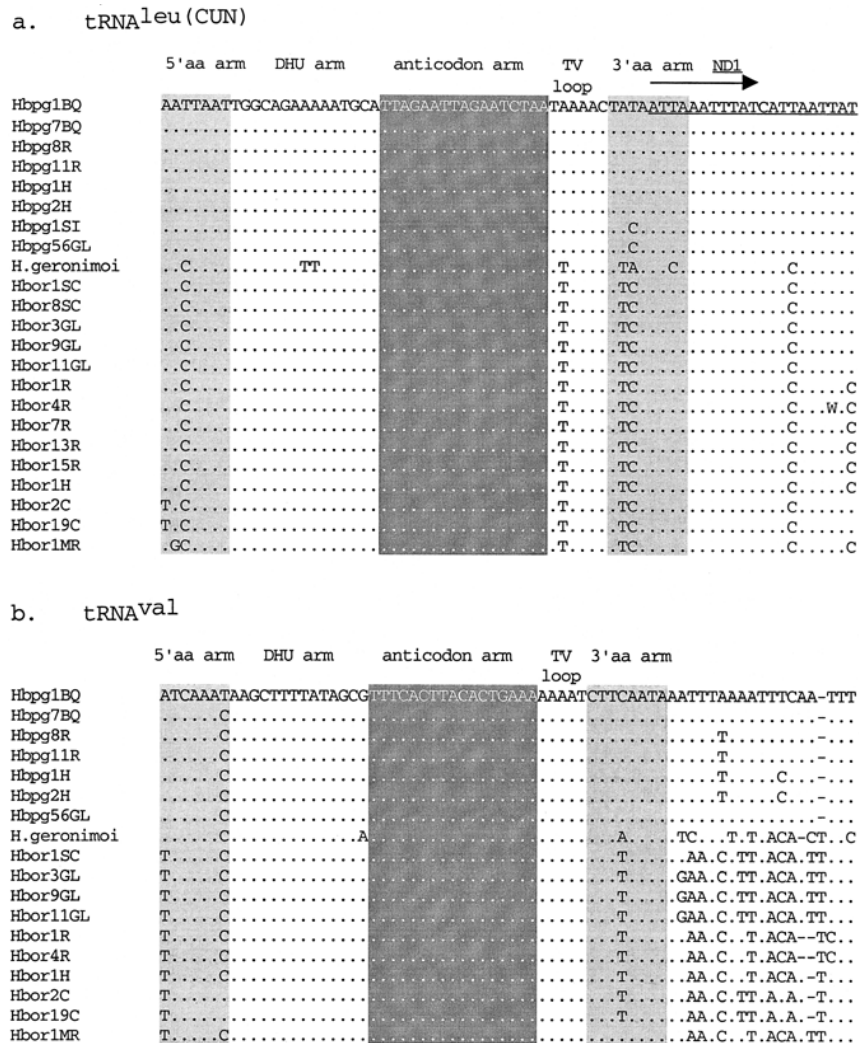


FIG. 3.—Aligned *Habronattus* sequences corresponding to (a) the $\text{tRNA}^{\text{Leu(CUN)}}$ gene and the 5' end of the adjacent ND1 gene (underlined; arrow indicates direction of transcription), and (b) the tRNA^{Val} gene. Light gray shading denotes the amino acid acceptor stem, and dark gray shading denotes the anticodon arm. Nucleotides downstream of the tRNAs are shown to illustrate the polymorphism present within a species. IUBMB symbols denote ambiguous bases, dots denote nucleotides identical to the top sequences, and dashes indicate gaps introduced to align the adjacent 16S sequences.

Furthermore, to maintain a cloverleaf structure would require either a large number of unusual base-pairings in both the T Ψ C and amino acid acceptor stems, or substantial editing of the transcripts. Thus, cloverleaf structures seem unlikely for both tRNAs, although RNA analysis is required to confirm the proposed unusual tRNA secondary structures.

The tRNA secondary structure proposed for *Habronattus* may not be unusual among spiders, however. Mitochondrial sequences for $\text{tRNA}^{\text{Leu(CUN)}}$ for spiders outside Salticidae have been reported by Hedin (1997a, 1997b). Spiders in the genus *Nesticus* (Araneae: Nesticidae) have $\text{tRNA}^{\text{Leu(CUN)}}$ sequences (AF004653, U40523, but incorrectly annotated in GenBank) that can be folded into secondary structures lacking the T Ψ C arm, although there is complete conservation of the anticodon arm with *Habronattus*. Both Nesticidae and Salticidae are in the derived suborder Araneomorphae of the order Araneae; it remains unclear if the more basal

Mygalomorphae spiders share this attribute. Because very few mitochondrial sequences have been published for Araneae, our knowledge of arachnid tRNAs should grow with sampling of both more of the mitochondrial genome and additional taxa.

Reports on ticks (Campbell and Barker 1999) and snakes (Kumazawa et al. 1996) show severe reduction of T Ψ C arms in some mitochondrial tRNAs. Kumazawa et al. (1996) proposed that selective pressure to shorten the T Ψ C arm is balanced by selective pressure to maintain T Ψ C loops because of functional interaction with DHU loops. Even for nematode tRNAs completely lacking the T Ψ C arm, Watanabe et al. (1994) have shown that tertiary interactions between the TV-replacement loop and the DHU arm occur, suggesting that these tRNAs maintain their functionality.

It seems unlikely that either of the tRNAs described here is nonfunctional, because there is complete conservation of their anticodon arms among all three species

in a region that should be free to accumulate mutations had it lost functionality. Therefore, the fact that 23 spiders from 12 different populations contain mismatches in their tRNA acceptor stems suggests that a mechanism must exist to allow these tRNAs to function. Posttranscriptional RNA editing of nucleotides in the amino acid acceptor stem has been shown to occur in mitochondrial tRNAs (Lonergan and Gray 1993) from a diverse array of metazoans (e.g., Börner et al. 1997) and has been suggested to be widespread in tRNAs from metazoan mitochondria (Yokobori and Pääbo 1995a). In most cases, it has been found that RNA editing restored base-pairings in the tRNA acceptor stem that were not present in the DNA sequence. In addition to being associated with mismatches in the nucleotide sequence of the acceptor stem, RNA editing in metazoans has been found to occur when there is overlap between the tRNA acceptor stem and protein-coding genes encoded on the same strand of the mitochondria (Yokobori and Pääbo 1995a, 1995b). Habronattus tRNA^{Leu(CUN)} exhibits both overlap with the adjacent ND1 gene and mismatches in the acceptor stem (fig. 3a), attributes consistent with a DNA region that may be expected to undergo RNA editing. Known editing mechanisms (Yokobori and Pääbo 1995a, 1995b) may correct some of the mismatches found in the acceptor stems of both of these tRNAs. While experiments have not been conducted to ascertain if RNA editing occurs in Habronattus mitochondria, it is interesting to note that if it does occur, there must be editing mechanisms that can act fairly generally within species, as intraspecific variation in nucleotide mismatches is present within at least two Habronattus species.

l-rRNA Secondary Structure

The inferred secondary structure of the 3' half of the 16S molecule in *H. oregonensis* has many structural features in common with the *D. yakuba* mitochondrial l-rRNA (Gutell and Fox 1988). These two taxa share highly conserved stem-and-loop-region sequence motifs (fig. 4) and show 67% sequence similarity for the last 553 bp of the 3' end of the gene. These same regions are conserved in other chelicerates for which the 3' end of 16S has been sequenced (Huber et al. 1993; Black and Piesman 1994). The putative rRNA transcription termination sequence was identified in the tRNA adjacent to the 3' end of 16S. The stop signal for mitochondrial 16S transcription is believed to be the conserved heptamer TGGCAGA (Valverde, Marco, and Garesse 1994). This sequence is located at positions 8–14 of the adjacent tRNA^{Leu(CUN)} gene, where it is also located in insects (Valverde, Marco, and Garesse 1994).

While the 3' end of 16S shares both structural and sequence similarities with *D. yakuba*, the first 463 bp of the 5' region of the 16S molecule shares only about 40% sequence similarity. Although some conserved structural motifs are present (e.g., the GTPase center), when Habronattus 16S was folded, it was not possible to fold the entire 5' end. The published secondary structure of the 5' end of the l-rRNA of *D. yakuba* was left unstructured

in the regions immediately upstream and downstream of the GTPase center (Gutell and Fox 1988). Therefore, it is unclear how much these taxa differ in their secondary structures in these regions of the l-rRNA.

Overall, Habronattus l-rRNA sequences are substantially shorter (by >300 nt) than the *D. yakuba* sequence. This approximately 1,000-nt gene is the shortest mitochondrial l-rRNA gene reported for any chelicerate. Truncated l-rRNA genes have also been found in the mitochondria of nematodes that have tRNAs that lack the TΨC arm by Wolstenholme et al. (1987) and Okimoto and Wolstenholme (1990). These authors suggested that correct functioning of tRNAs with a TV-replacement loop may require changes in ribosomal RNA structure. When sequences are aligned based on secondary structures, the mitochondrial l-rRNA of *C. elegans* (Gutell 1994) and Habronattus both appear to be truncated in similar regions, upstream of the GTPase center with respect to *D. yakuba*. There is not a high degree of sequence similarity (50%) between Habronattus and *C. elegans*, even for the structurally conserved 3' half of the gene, suggesting that very different sequences may still encode similar structures. These findings suggest that there may have been convergence on a similar secondary structure upstream of the GTPase center and are therefore consistent with the idea put forth by Wolstenholme et al. (1987) and Okimoto and Wolstenholme (1990). However, the observation of truncation in similar regions between Habronattus and *C. elegans* may simply be a coincidence, as pulmonate gastropods with truncated mitochondrial tRNAs do not necessarily have truncated l-rRNAs (Terrett, Miles, and Thomas 1996). Instead, it is possible that the truncation of these Habronattus tRNA, l-rRNA, and ND1 genes reflects a trend toward minimization of the mitochondrial genome, consistent with the idea of Yamazaki et al. (1997).

There are alternative explanations for the apparently unusual sequence lengths and secondary structures of both 16S and the tRNAs. First, these sequences may be pseudogenes that are nonfunctional. This seems unlikely, both for the reasons described above and because within Habronattus, regions with little or no sequence polymorphism correspond to regions that are highly conserved across very divergent taxa.

Another alternative explanation for the apparent truncation is that the genes are fragmented and the remainders of the genes lie elsewhere within the mitochondrial genome. Although this type of mitochondrial genome rearrangement has been found in green algae (e.g., Nedelcu 1997), it has never been reported for metazoans.

Protein-Coding Genes, Initiation Codons, and A+T Bias

The three protein-coding genes sequenced for Habronattus appear to be almost as, or more, divergent from the chelicerate *I. hexagonus*, as they are from the more distantly related *D. yakuba*. The last 238 amino acids of COI have sequence similarities of 63% with *D. yakuba* and 68% with *I. hexagonus*. The first 98 amino

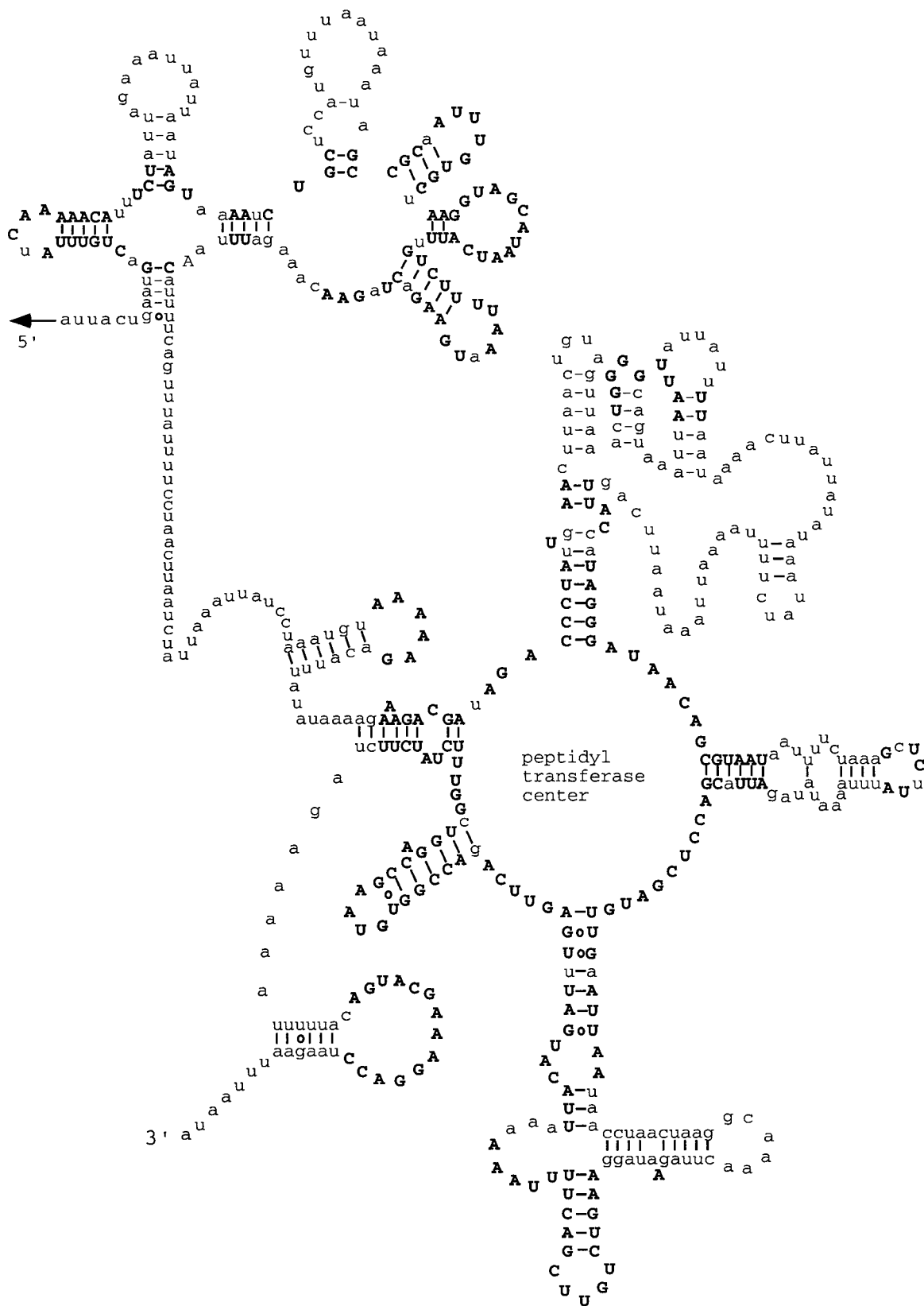


FIG. 4.—Proposed secondary structure of the 3' half of *Habronattus oregonensis* 16S rRNA, based on the mitochondrial DNA sequence. Bold capital letters correspond to nucleotides conserved with respect to *Drosophila yakuba* (Gutell and Fox 1988). Dashes represent Watson-Crick bonds, and circles represent T-G bonds. The sequence from *H. oregonensis* individual 4R (Hbor4R), for which there was the most overlap of sequences in two directions, is depicted here.

ND1 amino acids

D.yakuba	MEFILSLIGSLLLIICVLSVAFLTLLERKVLGYIQIRKGNPKVGLMGIQPFCDAIKLFTEKQTYPLLSN-YLSYYISPIFSLFSLFVVMC
I.hexagonus	MISYLSYIFLIMCVLSVAFFTLERKILGYIHIRKGNPKVGFIVGFQPFSDAIKLFSEKEMMMFYIN-MIFYIISVLLIIMMSLWFL
Hbor4R	IKFIINYLILVLSIILSVAFYTLERKILGYIQIRKGNPKVGMGILQPFSDALKLFENKLLSSENTNFMISYITPALSFLLISMLIPII
D.yakuba	MPFFVKLYSFNLGGLFLLCCTSLGVYIVMVGWSSNSNYA
I.hexagonus	FSWDYNQILLIEYGMVLLCISLSLVVILFCGWSSNSKYA
Hbor4R	LFNNSSLYDNKHNILLFFLILSSISAYSILLIGWSSNSKYS

COII amino acids

D.yakuba	MSTWANLGLQDSASPLMBQLIFFHDEHALLILVMIITVLVGYLMFMLFFNNYVNRFLHGLTETIWTILPAIILLFIALPSLRLLYLDEINEPSVTLK
I.hexagonus	MVTWSNIMFSNSNSPIMBQMIFFHDSMLTIMMITIITLYMIINIMMNSFSSRFIMEGQEIETIWTIIPAITLIFIAFSLRLLYMLDESFTPSITIK
Hbor7R	LPVWGSLYFQDGVSSVMEHLIFFHDYTMIMIMIMFLVGYMLIGAYVQGSYNLGLFEGQELMIWTIVLPAVFLVFIAPFSLRLLYLMEESESYDMTIK
Hbor8SC	LPVWGSLYFQDGVSSVMEHLIFFHDYTMIMIMIMFLVGYMLIGAYVQGSYNLGLFEGQELMIWTIVLPAVFLVFIAPFSLRLLYLMEESESYDMTIK

FIG. 5.—Alignment of amino acids from translated partial sequences of *Habronattus oregonensis* ND1 and COII with *Drosophila yakuba* (Clary and Wolstenholme 1985) and *Ixodes hexagonus* (Black and Roehrdanz 1998). The sequences begin at the 5' ends of the genes. Dashes denote gaps inserted to improve the sequence alignment; shaded areas denote amino acids conserved across these three species of arthropods.

acids of COII (fig. 5) have sequence similarities of 48% with *D. yakuba* and 46% with *I. hexagonus*. This gene has been shown to have a high degree of divergence both within Collembola (Fрати et al. 1997) and across multiple orders of insects (Liu and Beckenbach 1992); therefore, the low amino acid sequence similarity within Chelicerata is not surprising. The first 130 amino acids of *Habronattus* ND1 (fig. 5) have sequence similarities of 45% with *D. yakuba* and 42% with *I. hexagonus*. Despite the low sequence similarity between these two chelicerates, they both appear to have the 5' end of ND1 truncated by three amino acids with respect to *D. yakuba*.

The first codon of ND1 in *Habronattus* appears to be ATT (in *H. oregonensis* and *H. pugillis*) and ATC (in *H. geronimoi*). Although these may not be typical initiation codons, it appears that all ATN codons can act as transcription initiators in mitochondria (Bibb et al. 1981; Wolstenholme 1992), possibly due to wobble in the third position of the codon (Barrell et al. 1980). A much less common initiation codon appears to occur in COII of all three *Habronattus* species, where TTG is found in the position corresponding to the first codon of *D. yakuba* and *I. hexagonus*. A typical initiation codon is not found in close proximity. While TTG has not previously been reported as an initiation codon in arachnids, its use as an initiation codon has been reported in the mitochondria of nematodes (Okimoto, Macfarlane, and Wolstenholme 1990; Okimoto et al. 1992).

Sequenced regions of the *Habronattus* mitochondria show a high A+T bias, ranging from 71% for COI to 78% for 16S. A high A+T bias is typical of most arthropod mitochondrial genomes, and these values are similar to those reported for *D. yakuba* (Clary and Wolstenholme 1985), although they are slightly lower than the 83% for 12S rRNA in Tetragnatha spiders (Croom, Gillespie, and Palumbi 1991).

Gene Arrangement

There has been an apparent gene rearrangement in the location of tRNA^{Leu(UUR)} in the mitochondria of the

Habronattus lineage of arachnids as compared with the chelicerates *L. polyphemus* (Boore et al. 1995; Staton, Daehler, and Brown 1997), *Pandinus imperator* (Boore, Lavrov, and Brown 1998), and *I. hexagonus* (Black and Roehrdanz 1998). Only a single tRNA—that coding for tRNA^{Leu(CUN)}—is located between 16S and ND1 in *Habronattus* (fig. 1). In contrast, *Limulus*, *Pandinus*, and *Ixodes* share the arrangement 16S-tRNA^{Leu(CUN)}-tRNA^{Leu(UUR)}-ND1. It has been suggested that mitochondrial gene order is conserved in arthropods and that phylogenetic relationships among arthropods can be inferred from their tRNA gene arrangements (reviewed in Boore 1999). Boore et al. (1995) suggested that the translocation of tRNA^{Leu(UUR)} from between 16S and ND1 to its location in insects and crustaceans between COI and COII represented a shared derived character that offered strong support for an insect-crustacean clade that excluded chelicerates.

The fact that tRNA^{Leu(UUR)} is not located between COI and COII in *Habronattus* argues for an independent translocation of this tRNA. Mitochondrial sequences for this region reported by Hedin (1997a, 1997b) for spiders in the family Nesticidae share the same ND1-tRNA^{Leu(CUN)}-16S gene arrangement as *Habronattus*, but until further taxa are sampled, it will remain unclear exactly when during the diversification of spiders this translocation occurred.

Recent reports by Black and Roehrdanz (1998) and Campbell and Barker (1998) indicate that mitochondrial gene order among other arachnids (hard ticks) also varies from that described for *L. polyphemus* and other chelicerates. Cases of variation in mitochondrial tRNA gene arrangements previously believed to be conserved have been found in multiple divergent taxa (e.g., birds [Mindell, Sorenson, and Dimcheff 1998] and insects [Haucke and Gellissen 1988]). Therefore, it appears that tRNAs are more evolutionarily mobile than originally thought. Further sampling of mitochondria within Araneae will help elucidate the extent of both gene rearrangements and gene truncation in this little-known group.

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