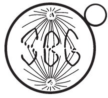

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Short Communication

Mitochondrial tRNA gene translocations in highly eusocial bees

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Abstract

Mitochondrial gene rearrangement events, especially involving tRNA genes, have been described more frequently as more complete mitochondrial genome sequences are becoming available. In the present work, we analyzed mitochondrial tRNA gene rearrangements between two bee species belonging to the tribes Apini and Meliponini within the "corbiculate Apidae". Eleven tRNA genes are in different genome positions or strands. The molecular events responsible for each translocation are explained. Considering the high number of rearrangements observed, the data presented here contradict the general rule of high gene order conservation among closely related organisms, and also represent a powerful molecular tool to help solve questions about phylogeny and evolution in bees.

Key words: mtDNA, *Melipona bicolor*, mitogenomics, gene order, eusociality.

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Animal mitochondrial DNA (mtDNA) is a circular molecule of approximately 16 kb that codifies for 13 proteins, 22 transfer RNAs (tRNA) and 2 ribossomal subunits (Moritz *et al.*, 1987). This genomic content is considered conservative, although some exceptions have been described among the Nematoda (Okimoto *et al.*, 1992), Mollusca (Hoffmann *et al.*, 1992) and Cnidaria (Beagley *et al.*, 1998). The gene order or placement of these genes in the mitochondrial molecule is also considered stable, mainly among closely related organisms (Moritz *et al.*, 1987).

The mitogenomics era has considerably increased the number of entire mitochondrial genome sequences available. Comparisons of whole genomes are now used to infer phylogenetic relationships, and such studies are also contributing to the understanding of the molecular evolution of this genome (Boore *et al.*, 1998). It has been demonstrated that the gene order is actually more flexible than postulated before, especially for tRNA genes, which are more likely to undergo translocations than the other mitochondrial genes. However, such events are still rare and have been reported among organisms belonging to different taxonomic families or orders.

In the class Insecta, the mtDNA gene order reported for *Drosophila yakuba* and other species is considered to be plesiomorphic (Boore, 1999) and has been used to detect and infer mtDNA gene rearrangements in other organisms (Boore *et al.*, 1995; Rokas and Holland, 2000). Within the order Hymenoptera, Dowton and Austin (1999) described two clusters of tRNA genes that independently underwent several rearrangements among families of wasps and other hymenopteran groups. These authors also suggested that the great number of mitochondrial rearrangements in Hymenoptera could be associated with accelerated rates of sequence evolution, indicating that hymenopteran mitochondrial genomes may be particularly plastic and possibly useful to study rearrangement mechanisms.

In the present work, we analyzed mitochondrial tRNA gene rearrangements between two bee tribes, Apini and Meliponini within the "corbiculate Apidae", completing our previous analyses (Silvestre *et al.*, 2002). Nearly 80% of the mitochondrial genome of *Melipona bicolor* was sequenced and analyzed (Silvestre, 2002; Genbank accession number NC_004529). Comparing the mitochondrial gene order of *M. bicolor* and *Apis mellifera* (Crozier and Crozier, 1993), eleven tRNA genes were found or inferred to be located in different positions or on different strands (Figure 1). The number of genes involved in the rearrangements between these two tribes of the subfamily Apinae was much higher than that usually found between pairs of Diptera families (http://www.jgi.doe.gov/programs/comparative/MGA_Source_Guide.html).

Local translocation, defined as an exchange of positions within a group of tRNA genes, appears to be the most common type of event among mitochondrial gene rearrangements (Boore and Brown, 1998; Dowton and Austin, 1999), probably caused by the duplication and deletion of

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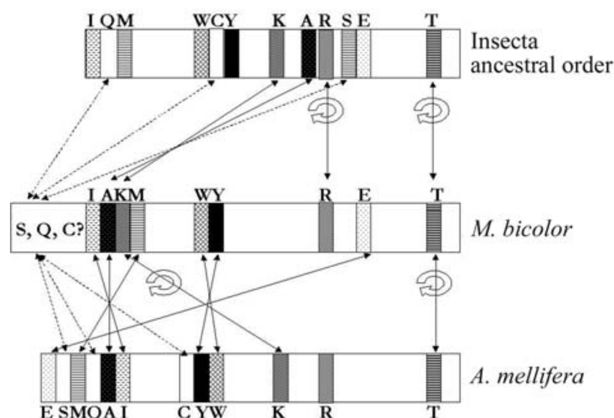

Figure 1 - Schematic representation of mitochondrial tRNA gene rearrangements between *Apis mellifera* and *Melipona bicolor*. Only tRNA genes presenting a different gene order are represented. The putative ancestral gene order of Insecta (Boore, 1999) is also represented here for comparison. Each gene is identified by the letters of the universal code of the amino acid carried by the correspondent tRNA. The thin solid arrows indicate translocations, and the thick circular arrows are symbols of strand inversions. The dotted arrows indicate the possible positions of the tRNA genes not present in the sequenced portion of *M. bicolor* genome (S,Q and C).

small portions of the mitochondrial genome (Macey *et al.*, 1997).

Here, local translocation was observed between the $tRNA^{Trp}$(W) and $tRNA^{Tyr}$(Y) genes and also among the $tRNA^{Met}$(M), $tRNA^{Ile}$(I) and $tRNA^{Ala}$(A) genes (Figure 1). The latter group of tRNA genes flanks the control region and is considered a "hot spot" for gene rearrangements (Boore and Brown, 1998), as are the tRNA genes located between ND3 and ND5 genes (Boore, 1999). Interestingly, the $tRNA^{Glu}$(E) translocation took place between these two regions. In *A. mellifera*, it is located close to the control region, while in *M. bicolor* it is located between ND3 and ND5. It has been hypothesized that the latter region contains a second origin of replication-transcription, for the light strand of mtDNA, which may justify the high frequency of rearrangements (Boore, 1999).

The $tRNA^{Thr}$(T) gene is located in the same position on both bee genomes, but it is transcribed by opposite strands (Figure 1). It can be explained by a simple inversion caused by intramolecular recombination (Dowton and Austin, 1999). This result is consistent with recent observations of recombination in animal mtDNA (Sato *et al.*, 2005).

The $tRNA^{Lys}$(K) gene of *M. bicolor* is translocated and inverted in relation to *A. mellifera* (Figure 1). The mechanism to explain that change is more speculative, but there are at least two hypotheses. The first would be a combination of both phenomena cited above: duplication-deletion and intramolecular recombination. Another explanation could be the illicit primer function of a tRNA (Cantatore *et al.*, 1987). The tRNA would be used as a primer to replicate the molecule from an alternative point and would not be excised from the final product. The $tRNA^{Lys}$(K) gene has been reported to undergo rearrangement in hymenopteran mtDNA, however none of the four species of bees previously studied have the same arrangement as *M. bicolor* (Dowton and Austin, 1999).

The genes for $tRNA^{Ser1}$(S), $tRNA^{Gln}$(Q) and $tRNA^{Cys}$(C) were not located in the sequenced region of *M. bicolor* mtDNA. Considering that the mitochondrial gene content is conserved, they could be located in two regions: either between the 12S gene and the control region, or between the control region and $tRNA^{Ile}$(I). The second option is more likely (Figure 1), since this arrangement is most common in many other genomes.

In comparisons of mitochondrial nucleotide sequences, the molecular clock based on rates of divergence in *Drosophila* has been used to scale differences on a timeline of 2% divergence per one million years (Avise, 1994). But, when comparing mitochondrial gene order, there is no molecular clock to infer the frequency of those rare and less explained translocation events (Boore and Brown, 1998).

Thus, to comparatively analyze mitochondrial rearrangements, it is necessary to consider some ancestral and derived gene orders (Boore and Brown, 1998). Among arthropods, the ancestral gene order is presumed to be that of *Limulus polyphemus*, a chelicerate (Staton *et al.*, 1997). The gene order considered plesiomorphic in the insect-crustacea clade has only one tRNA gene translocation when compared to *L. polyphemus* and can be found in many species of insects and crustacea, including *Drosophila yakuba* (Boore, 1999).

The mitochondrial gene arrangement of *A. mellifera* requires a minimum of eight translocations of the *D. yakuba* genome. Considering the data presented here for *M. bicolor*, the number may be smaller: five translocations and two local inversions. We speculate that the high number of tRNA rearrangements observed between these two bee species belonging to the same subfamily (Apinae), and also in comparison to *D. yakuba*, may be explained by their mitochondrial activity, whereas the high concentrations of free oxygen radicals in cells with higher metabolic rates should be a major cause of DNA damage, as postulated by Martin and Palumbi (1993). However, nothing has been demonstrated so far.

Rearrangements of mtDNA gene order, involving one tRNA gene, were recently described in parasitic wasps of several Braconidae subfamilies (Dowton, 1999), another hymenopteran group. The "Hemipteroid group" (Hemiptera, Psocoptera, Thysanoptera and Phthiraptera) was also analyzed and many rearrangements were found, including protein-coding genes (Shao *et al.*, 2001). The wallaby louse *Heterodoxus macropus* (Phthiraptera) has nine protein-coding genes in different positions relative to the ancestral insect arrangement, four inversions, and 22 translocated tRNA genes.

It is clear that gene order rearrangements are more frequent in Hymenoptera and Hemiptera than in Diptera,

but the molecular and evolutionary events that are responsible for this high rearrangement frequency remain to be investigated. As most translocations have been found in parasitic hymenopterans, Dowton and Austin (1999) hypothesized that there was a relation between the parasitic lifestyle and the dynamic of mtDNA changes. However, that idea cannot explain the same phenomenon occurring in a great number of free-living hemipteroid insects (Shao *et al.*, 2001) or in the mitochondrial genome of *M. bicolor*. In fact, Castro *et al.* (2002) investigated this question specifically, and found no association between rearrangement rate and parasitism.

Recent studies about mitochondrial gene rearrangements have pointed to the analysis of gene order as a source of strong characters to reconstruct phylogenetic relationships. The strength of these characters is based on the fact that the abundance of potential arrangements makes convergence very unlikely and homology more certain, while the arrangements themselves are considered selectively neutral (Boore and Brown, 1998). Based on the statements above regarding the differences in the tRNA gene order, we found them promising as molecular markers for the study of evolutionary and phylogenetic questions on bees, such as the origin of their social behavior. The long-standing question about the number of independent origins of social behavior (single or dual) in the family Apidae has been investigated by several researchers. This controversial issue has been tentatively addressed by studies on morphology, behavior (Winston and Michener, 1977; Engel, 2001), and DNA sequence data (Koulianos *et al.*, 1999; Schultz *et al.* 1999; Lockhart and Cameron, 2001); however, no conclusive answer has been found so far.

Although the fact itself that Apini and Meliponini show a different tRNA gene order may suggest that a highly eusocial behavior arose twice in the family Apidae, this statement deserves further investigation. We will only be able to affirm this after analyzing the mtDNA gene order of other bee tribes, particularly the "corbiculate" Bombini (primitively eusocial) and Euglossini (from solitary to primitively eusocial).

Melipona bicolor individuals were collected and stored at -80 °C until DNA extraction. Total DNA was extracted from thoraces, using a phenol-chloroform protocol (Sheppard and McPheron, 1991). MtDNA fragments were amplified by PCR, using the following pairs of primers: AMB1 [TGATAAAAGAAATATTTTGA] + Seq41 [CATATAAAGATTAAAATTC]; Seq9 [GATTTCCATTAATTTCAGG] + Seq3 [GGTATTAACGTTCAAATAATTTC]; mtD19 (Simon *et al.*, 1994) + mtD22 (Simon *et al.*, 1994); Seq18 (Francisco *et al.*, 2001) + 8467F (Francisco *et al.*, 2001); 5612R (Francisco *et al.*, 2001) + tPheF (Francisco *et al.*, 2001); 8321R [TTATATATCTAATTCTAT] + ND4F [ATAAATTATGAACTTGGTCATCA]; Seq32 [AATGCAGTTGCTATTGATA] + Seq33 [TTTTGATGGACCCAAATTC]; Seq4 [CAATTCCAAATAAATTAGGAGG] + Seq30 [TCGAGTTCCATTTGATTT]; mtD29 (Simon *et al.*, 1994) + 16SF (Hall and Smith, 1991); Seq7 [GGAATAAGTCGTAACATAG] + Seq13 [CCCTGATACAAAAGGTAC]. The PCR conditions were: an initial denaturation step of 94 °C/5 min, followed by 35 cycles of 94 °C/60 s, annealing at 42 °C/80 s, and elongation at 64 °C/120 s, followed by an additional final extension step of 64 °C for 10 min. The PCR products were analyzed in 0.8% agarose gels, stained with ethidium bromide, visualized and photographed under UV light. PCR fragments were cloned in pGEM-T Easy plasmid (Promega) and cycle-sequenced with BigDye Terminator (Applied Biosystems) following the manufacturer's protocols. Sequences were aligned and analyzed using the free software package GeneRunner (Hastings Software, www. generunner.com) and the tRNA genes were located using the online application tRNAScan-SE (Lowe and Eddy, 1997).

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