Mitochondrial tRNA gene translocations in highly eusocial bees

Daniela Silvestre and Maria Cristina Arias
Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo, São Paulo, SP, Brazil.

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.
Received: September 15, 2005; Accepted: January 6, 2006.
small portions of the mitochondrial genome (Macey et al., 1997).

Here, local translocation was observed between the tRNA^{Thr}(W) and tRNA^{Trp}(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^{Ile}(I) 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^{Ser}(S), tRNA^{Glu}(Q) and tRNA^{Trp}(W) 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 Heterodxs macros (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., 2002). 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 (Koulilanos 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 McPherson, 1991). MtDNA fragments were amplified by PCR, using the following pairs of primers: AMB1 [TGATAAAAAGAAATTTTTGA] + Seq41 [CA TATAAGATATTAAAATTC]; Seq9 [GATTCTCATTA TTTCAGG] + Seq3 [GGTATACGTTCAAAATAT TTC]; 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 [TTATATATCTATCCTAT] + ND4F [ATATAATTGACTGTTGTACCA]; Seq32 [AATGCAAGTTATTGATA] + Seq33 [TTTGGTAC CCACCAATTCC]; Seq4 [CAATTCACAATAAAATTAGG AGG] + Seq30 [TCGGATTCCATTGGATT]; mtD29 (Simon et al., 1994) + 16SF (Hall and Smith, 1991); Seq7 [GGAATAAGTCGAAACATAG] + Seq13 [CCCTGATA CAAAAAGGTAC]. 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).

Acknowledgements

We thank Dr Walter Sheppard and Dr Mark Dowton for reading and commenting on earlier versions of this report; Susy Coelho for technical support; Dr Vera Lúcia Imperatriz-Fonseca and the Laboratório de Abelhas of the Departamento de Ecologia do IB-USP for providing the samples and also for instigating us to develop this project. We also thank FAPESP for the graduate fellowship granted to D. Silvestre.

References

Avise JC (1994) Molecular Markers, Natural History and Evolution. Chapman and Hall, New York, 528 pp.

Beagley CT, Okimoto R and Wolstenholme DR (1998) The mitochondrial genome of the sea anemone Metridium senile (Cnidaria): Introns, a paucity of tRNA genes, and a near-standard genetic code. Genetics 148:1091-1108.

Boore JL (1999). Animal mitochondrial genomes. Nuc Acids Res 27:1767-1780.

Boore JL and Brown WM (1998) Big trees from little genomes: Mitochondrial gene order as a phylogenetic tool. Curr Opin Genet Dev 8:668-674.

Boore JL, Collins TM, Stanton D, Daehler LL and Brown WM (1995) Deducing the pattern of arthropod phylogeny from mitochondrial DNA rearrangements. Nature 376:163-165.

Boore JL, Lavrov D and Brown WM (1998). Gene translocation links insects and crustaceans. Nature 392:667-668.

Cantatore P, Gadaleta MN, Roberti M, Saccoone C and Wilson AC (1987) Duplication and remoulding of tRNA genes during the evolutionary rearrangement of mitochondrial genomes. Nature 29:853-855.

Castro LR, Austin AD and Dowton M (2002) Contrasting rates of mitochondrial molecular evolution in parasitic Diptera and Hymenoptera. Mol Biol Evol 19:1100-1113.

Crozier RH and Crozier YC (1993) The mitochondrial genome of the honeybee Apis mellifera: Complete sequence and the genome organization. Genetics 133:97-117.

Dowton M (1999) Relationships among the cyclostome braconid (Hymenoptera, Braconidae) subfamilies inferred from a mi-
Dowton M and Austin AD (1999) Evolutionary dynamics of a mitochondrial rearrangement “hot spot” in the Hymenoptera. Mol Biol Evol 16:298-309.

Engel MS (2001) Monophyly and extensive extinction of advanced eusocial bees: Insights from an unexpected Eocene diversity. Proc Natl Acad Sci 4:1661-1664.

Francisco FO, Silvestre D and Arias MC (2001) Mitochondrial DNA characterization of five species of Plebeia (Apidae, Meliponini): RFLP and restriction maps. Apidologie 32:323-332.

Hall HG and Smith DR (1991) Distinguishing African and European honeybee matrlines using amplified mitochondrial DNA. Proc Natl Acad Sci USA 88:4248-4552.

Hoffmann RJ, Boore JL and Brown WM (1992) A novel mitochondrial genome organization for the blue mussel, Mytilus edulis. Genetics 131:397-412.

Koulianos S, Schmid-Hempel R, Roubik DW and Schmid-Hempel P (1999) Phylogenetic relationships within the corbiculate Apinae (Hymenoptera) and the evolution of eusociality. J Evol Biol 12:380-384.

Lockhart PJ and Cameron SA (2001) Trees for bees. TREE 16:84-88.

Lowe TM and Eddy SR (1997) tRNAscan-SE: A program for improved detection of transfer RNA genes in genomic sequence. Nuc Acids Res 25:955-964.

Macey JR, Larson A, Ananjeva NB, Fang Z and Papenfuss TJ (1997) Two novel gene orders and the role of light-strand replication in rearrangement of the vertebrate mitochondrial genome. Mol Biol Evol 14:91-104.

Martin AP and Palumbi SR (1993) Body size, metabolic rate, generation time, and the molecular clock. Proc Natl Acad Sci USA 90:4087-4091.

Mortiz C, Dowling TE and Brown WM (1987). Evolution of animal mitochondrial DNA. Relevance for population biology and systematics. Ann Rev Ecol Syst 18:269-292.

Okimoto R, MacFarlane JL, Clary DO and Wolstenholme DR (1992). The mitochondrial genome of two nematodes, Caenorhabditis elegans and Ascaris suum. Genetics 130:471-498.

Rokas A and Holland PWH (2000) Rare genomic changes as a tool for phylogenetics. TREE 15:454-459.

Sato A, Nakada K, Akimoto M, Ishikawa K, Ono T, Shitara H, Yonekawa H and Hayashi J (2005) Rare creation of recombinant mtDNA haplotypes in mammalian tissues. Proc Natl Acad Sci USA 102:6057-6062.

Schultz TR, Engel MS and Prentice M (1999) Resolving conflict between morphological and molecular evidence for the origin of eusociality in the “corbiculate” bees (Hymenoptera, Apidae): A hypothesis-testing approach. University of Kansas, Natural History Museum Special Publication 24:125-138.

Sheppard WS and McPherson BA (1991) Ribosomal DNA diversity in Apidae. In: Smith DR (ed), Diversity in the Genus Apis. Westview Press, Oxford, pp 89-102.

Silvestre D (2002) Seqüenciamento e análise do genoma mitocondrial de Melipona bicolor (Hymenoptera, Apidae, Meliponini). Dissertação de Mestrado, Instituto de Bicências, Universidade de São Paulo, São Paulo.

Silvestre D, Francisco FO, Weinlich R and Arias MC (2002) A scientific note on mtDNA gene order rearrangements among highly eusocial bees (Hymenoptera, Apidae). Apidologie 33:355-356.

Simon C, Frati F, Becknbach A, Crespi B, Liu H and Flock P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann Entomol Soc Am 87:651-701.

Staton JL, Daehler LL and Brown WM (1997) Mitochondrial gene arrangement of the horseshoe crab Limulus polyphemus L.: Conservation of major features among arthropod classes. Mol Biol Evol 14:867-874.

Winston ML and Michener CD (1977) Dual origin of highly social behavior among bees. Proc Natl Acad Sci 74:1135-1137.

Associate Editor: Klaus Hartfelder