

*Letter to the Editor***A Translocated Mitochondrial Cytochrome *b* Pseudogene in Voles (Rodentia: *Microtus*)****J. Andrew DeWoody,<sup>1,\*</sup> Ronald K. Chesser,<sup>2</sup> Robert J. Baker<sup>1</sup>**<sup>1</sup> Department of Biological Sciences, Texas Tech University<sup>2</sup> Savannah River Ecology Lab and Department of Genetics, University of Georgia, Athens, GA 30602, USA

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**Abstract.** A full-length cytochrome *b* pseudogene was found in rodents; it has apparently been translocated from a mitochondrion to the nuclear genome in the subfamily Arvicolinae. The pseudogene ( $\psi$ *cytb*) differed from its mitochondrial counterpart at 201 of 1143 sites (17.6%) and by four indels. Cumulative evidence suggests that the pseudogene has been translocated to the nucleus. Phylogenetic reconstruction indicates that the pseudogene arose before the diversification of *M. arvalis*/*M. rossiaemeridionalis* from *M. oeconomus*, but after the divergence of the peromyscine/sigmodontine/arvicoline clades some ~10 MYA. Published rates of divergence between mitochondrial genes and their nuclear pseudogenes suggest that the translocation of this mitochondrial gene to the nuclear genome occurred some 6 MYA, in agreement with the phylogenetic evidence.

**Key words:** mtDNA — Cytochrome *b* — Pseudogene — *Microtus* — Rodent — Translocation

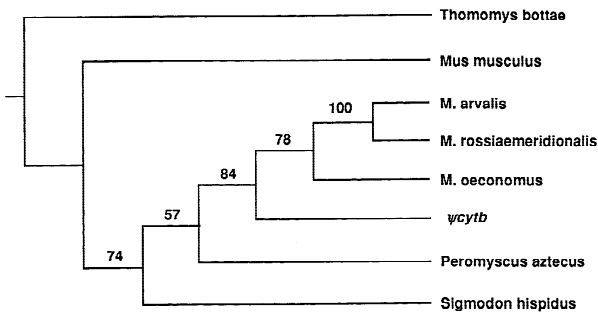
Universal PCR primers that amplify specific mtDNA fragments (Kocher et al. 1989) have been instrumental in genetic studies of natural populations. However, such

universal primers should be used with caution (Smith et al. 1992; Arctander 1995). In addition to the expected PCR product, amplicons may be paralogous or orthologous. Orthology is generally obvious during phylogenetic reconstruction. Less apparent is paralogy, which is usually due to a gene duplication and subsequent transposition. Paralogous events can confound evolutionary analyses and are thus of great interest (Schneider-Broussard and Neigel 1997). Here we describe a novel cytochrome *b* pseudogene that seems to have been translocated from the mitochondria to the nuclear genome of arvicoline rodents.

The entire cytochrome *b* gene was amplified from total genomic DNA of 12 individual voles (*M. arvalis*) using Irwin et al.'s (1991) universal primers L14724 and H15915. Approximately 10 recombinant clones from each individual (a total of 127 clones) were subsequently isolated and sequenced with the L14724 primer. A single clone (designated JAD43) differed dramatically from each of the other 126 clones. New primers were designed and subsequent sequencing revealed that JAD43 differed from the consensus *M. arvalis* cytochrome *b* sequence at 201 of 1143 sites (17.6%) and by four indels, resulting in a total length of 1138 bp for JAD43 (Fig. 1; Genbank Accession No. AF057139). The transition to transversion ratio between clone JAD43 and the consensus *M. arvalis* sequence is almost exactly 2:1. The number of synonymous substitutions per synonymous site ( $K_s$ ) is 0.62

\* Present address: Department of Genetics, University of Georgia, Athens, GA 30602, USA; email: dewoody@arches.uga.edu  
Correspondence to: J.A. DeWoody





**Fig. 2.** Parsimony analysis of 226 informative nucleotides for seven rodent taxa and  $\psi cytb$ . Bootstrap values as obtained by 1000 replicates are shown above each internal node. Distance and maximum-likelihood methods produced trees with identical topologies. Sequences from *P. aztecus* (Sullivan et al. 1997), *T. bottae* (Smith 1998), and *M. musculus* (Bibb et al. 1981) were downloaded from GenBank. Analyses were conducted using test version 4.0d63 of PAUP\*, written by David L. Swofford.

located to the nuclear genome of *M. arvalis* approximately 6 MYA.

Various phylogenetic methods were used to complement this estimate; related taxa were used to polarize the data set in order to determine the number of nucleotide substitutions in each lineage (Li et al. 1981). We used mitochondrial cytochrome *b* sequences from peromyscine, sigmodontine, and arvicoline rodents in our analyses. Parsimony, distance, and maximum-likelihood methods all produced trees of identical topology (Fig. 2). These three lineages split some 8–10 MYA (Adkins et al. 1996; Engel et al. 1998) and the genus *Microtus* arose approximately 6.5 MYA (Chaline and Graf 1988; Robinson et al. 1997), suggesting that the pseudogene arose within this time period. Note that the initial estimate of 6 MYA was based only upon a molecular clock, whereas the phylogenetic estimate of 6.5–10 MYA is based upon sequences from numerous taxa and on the fossil evidence as cited in the references above.

The translocated fragment is likely a larger portion of the mitochondrial genome that encompasses the cytochrome *b* gene. Lopez et al. (1994) reported the transfer of a 7.9 kb mitochondrial fragment to the nuclear genome of the domestic cat. This fragment (termed *Numt*) contains full-length intact pseudogenes for 12S, 16S, ND1, ND2, and CO1, indicating that large portions of the mitochondrial genome (nearly half) can be transferred to the nucleus as one contiguous fragment. It is not difficult to envision a similar scenario for the origin of both *Numt* and  $\psi cytb$  (see Lopez et al. 1994 or Perna and Kocher 1996 for a discussion of mtDNA translocation mechanisms).

Pseudogenes have long been used for the study of rates and patterns of nucleotide substitutions (Li et al. 1981). Further study of  $\psi cytb$  will determine the size and extent of the mtDNA translocation, as well as its phylogenetic placement within the Arvicolinae. While nuclear copies of mitochondrial genes are inherently interesting (Zhang and Hewitt 1996),  $\psi cytb$  is of little practical con-

cern in terms of evolutionary analyses. PCR amplification of  $\psi cytb$  is obviously inefficient; out of a total of 127 clones, only a single isolate contained the pseudogene. Direct sequencing of PCR products instead of cloning would certainly obscure any signal produced by the pseudogene. This is perhaps fortuitous for those studying the mitochondrial genome of voles and their relatives.

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