Letter to the Editor

An Unprecedented Major Rearrangement in an Arthropod Mitochondrial Genome

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Until now, the arrangement of protein-coding and ribosomal RNA (rRNA) mitochondrial genes appeared not to vary much within closely related groups of animals (e.g., families). The phylum Arthropoda (insects, crustaceans, arachnids, and their kin) appeared to have a single arrangement, which had remained unchanged for >530 Myr (Staton, Daehler, and Brown 1997). We have found a major gene rearrangement in the mitochondrial genome which unites the cattle tick, *Boophilus microplus,* an arachnid, with some other ticks and which shows that the arrangement of major genes can vary even below the family level within animals.

The arrangement of genes in the mitochondrial genome of animals is usually highly conserved within phyla; thus, when rearrangements occur, they are powerful markers for inferring evolutionary history (Anderson et al. 1981; Brown 1985; Wolstenholme 1992; Boore et al. 1995; Macey et al. 1997; Boore, Lavrov, and Brown 1998). The phylum Arthropoda appeared to exemplify this conservatism (Boore et al. 1995; Boore, Lavrov, and Brown 1998). Until now, rearrangements of protein-coding and ribosomal RNA genes (80%–85% of the genome) have not been reported in arthropods, despite the fact that there have been more studies of the mitochondrial genomes of arthropods than of any other nonvertebrate phylum (see Boore et al. 1995; Boore, Lavrov, and Brown 1998). Only the small transfer RNA (tRNA) genes were known to move around the mitochondrial genomes of arthropods (Boore et al. 1995; Staton, Daehler, and Brown 1997; Boore, Lavrov, and Brown 1998). The uniform arrangement of major genes in each arthropod studied (see the typical arthropod, in fig. 1) indicated that this arrangement was present in the common ancestor of the arthropods and had thus remained unchanged for >530 Myr (Staton, Daehler, and Brown 1997).

We used long polymerase chain reaction (L-PCR; Barnes 1994; Cheng et al. 1994) to amplify the entire mitochondrial genome of the cattle tick. Polymerase chain reaction fragments that contained the 12S and ND2 genes were about 5 kbp longer than expected. Nucleotide sequencing confirmed a major rearrangement of genes in this arachnid (fig. 1). To discover which other arthropods share this rearrangement with the cattle tick, we designed primers that flank the ND5 to cytochrome *b* region in this species (primers 7 and 8; fig. 1). The size of PCR fragments generated using these primers indicated which arthropods have the rearrangement (fig. 2). We found the rearrangement in all hard ticks (Family Ixodidae; 15 species sampled, representing all major genera; Klompen et al. 1996), except for those in the genus *Ixodes* (four species sampled). Further, the rearrangement was not found in soft ticks (Family Argasidae, the other major lineage of ticks), in spiders or scorpions (fig. 2), nor in horseshoe crabs (Staton, Daehler, and Brown 1997); thus, it is a derived feature that distinguishes these genera of hard ticks from all other animals. A rearrangement involving protein-coding or ribosomal mitochondrial genes is unprecendented within a metazoan family. Our discovery is a timely reminder that all gene rearrangements arise in single mitochondrial genomes in single individuals, and thus it is possible to find major differences in gene arrangement among closely related species (as here) or, potentially, even within species.

Key words: Ixodida, *Boophilus microplus*, gene rearrangement, Arthropoda, mitochondrial genome, Arachnida.

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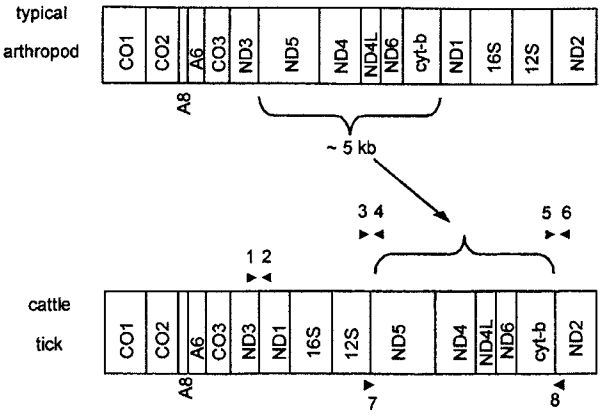

FIG. 1.—Linearized arrangements of protein-coding and rRNA genes in the mitochondrial genomes of a typical arthropod (top) and of the cattle tick, *Boophilus microplus* (bottom). Abbreviations for genes are as follows: 12S and 16S for 12S and 16S rRNAs, ND1–6 and 4L for NADH dehydrogenase subunits 1–6 and 4L, CO1–3 for cytochrome *c* oxidase subunits I–III, cyt-b for cytochrome *b*, and A6 and A8 for ATPase subunits 6 and 8. Nucleotide sequencing of cattle tick mtDNA (primers 1, 2, 3, 4, 5, and 6; GenBank accession numbers AJ006038, AF067440, and AF067441) showed that ND5, ND4, ND4L, ND6, and cyt-b, on the one hand, and ND1, 16S, and 12S, on the other, have swapped position. In *B. microplus,* all genes shown are transcribed from the same strands as in *Limulus polyphemus* (Staton, Daehler, and Brown 1997).

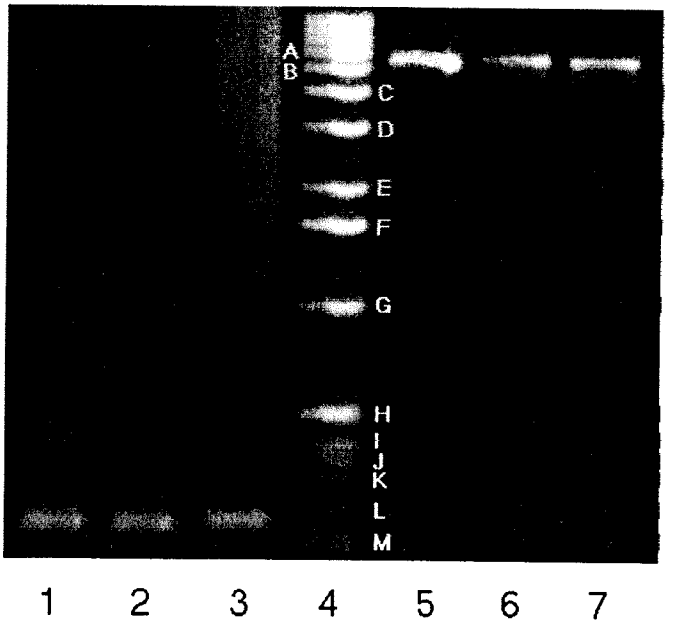

FIG. 2.—Diagnostic L-PCR test for the large rearrangement of protein-coding genes with primers 7 and 8 (see fig. 1). To the left of the size marker (lanes 1, 2, and 3) are three *Ixodes* spp. of hard ticks (Family Ixodidae) that do not have the rearrangement (PCR product ≈ 180 bp), whereas to the right (lanes 5, 6, and 7), there are hard ticks from three other genera that do have the rearrangement (PCR product ≈ 5.5 kbp). Each lane's description follows. Lane 1: *Ixodes auritulus.* Lane 2: *I. pilosus.* Lane 3: *I. holocyclus.* Lane 4: molecular size marker—sizes (in base pairs) of fragments, labeled from top to bottom, are as follows: A, 6,108; B, 5,090; C, 4,072; D, 3,054; E, 2,036; F, 1,636; G, 1,018; H, 517/506; I, 396; J, 344; K, 298; L, 220/201; and M, 154/134. Lane 5: *Amblyomma vikirri.* Lane 6: *Rhipicephalus appendiculatus.* Lane 7: *Boophilus microplus.* In addition to the species shown, PCR tests demonstrated that the hard ticks *Aponomma concolor, Ap. undatum, Am. hebraem, Haemaphysalis longicornis, Ha. humerosa, Hyalomma marginatum, Hy. truncatum, Hy. aegyptium, Dermacentor reticulatus, D. variabilis, Nosomma monstrosum,* and *B. decoloratus* (representing seven genera of hard ticks) also have the rearrangement that we fully characterized for the cattle tick, whereas the hard tick *I. simplex,* the soft ticks (Family Argasidae) *Carios capensis* and *Otobius megnini,* the tetragnathid spider *Nephila plumipes,* and the scorpionid scorpion *Liocheles waigiensis* do not.

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