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The evolution of mitochondrial genomes in subterranean caviomorph rodents: Adaptation against a background of purifying selection

Ivanna H. Tomasco *, Enrique P. Lessa

Departamento de Ecología y Evolución, Facultad de Ciencias, Universidad de la República, Iguá 4225, Montevideo 11400, Uruguay

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South American tuco-tucos (*Ctenomys*) and the related coruro (*Spalacopus*) are two rodent lineages that have independently colonised the subterranean niche. The energetically demanding lifestyles of these species, coupled with the hypoxic atmospheres characteristic of subterranean environments, may have altered the selective regimes on genes encoding proteins related to cellular respiration. Here, we examined the molecular evolution of 13 protein-coding genes in the mitochondrial genome of seven caviomorph rodents, including these two subterranean genera and their above-ground relatives. Using maximum-likelihood and Bayesian approaches, we estimated rates of synonymous (dS) and nonsynonymous (dN) substitutions. We found a significantly higher ω ratio (dN/dS) in subterranean groups as compared to their non-subterranean counterparts in 11 of 13 genes, although no ω ratio was larger than 1. Additionally, we applied a method based on quantitative physicochemical properties to test for positive selection. Amino acid changes implicated in radical structural or functional shifts in the protein property were found to be ubiquitous across the phylogeny, but concentrated in the subterranean lineages. Convergent changes were also found between the subterranean genera used in this study and other mammals adapted to hypoxia. The results of this study suggest a link between niche shifts and weak directional (or episodic) selection at the molecular level against a background of purifying selection.

1. Introduction

Mitochondrial DNA (mtDNA) has long been used for phylogenetic reconstruction, phylogeography and the inference of population histories (Avise, 2000; Chapple et al., 2009). It has often been assumed that mtDNA evolves according to the neutral theory, given correlations between global rates of mtDNA evolution, metabolic rate and generation time (Martin, 1995; Martin and Palumbi, 1993). Thus, little attention has been paid to the molecular adaptation of proteins encoded by mitochondria, although variation in protein-coding genes involved in oxidative phosphorylation (OXPHOS) can directly influence metabolic performance (da Fonseca et al., 2008). Despite strong functional constraints, mtDNA may be subject to positive directional selection in response to pressures resulting from greater energy requirements or limited oxygen availability.

Many studies provide support for instances of adaptive selection in mammalian mitochondrial protein-coding genes. Sequence variation in CytB has been correlated with ecological differences among chromosomal races of blind mole-rats (*Spalax ehrenbergi*, Nevo, 1999) and with the metabolic shift in cetaceans relative to their terrestrial ancestors (McClellan et al., 2005). Increasing metabolic demands of an expanding cerebral cortex were suggested by Grossman et al. (2004) to have driven co-adaptation among subunits of OXPHOS proteins during primate evolution, while mitochondrial amino-acid polymorphisms in humans have been shown to improve aerobic capacity and adaptation to different thermal environments (Ballard and Whitlock, 2004; Blier et al., 2001; Dalziel et al., 2006; Grossman et al., 2004; Jobson et al., 2004). In addition, adaptation in COX and CytB genes has been suggested to be due to increased evolutionary rates in anthropoids (Andrews et al., 1998; Adkins and Honeycutt, 1994) and in mammal species adapted to unusual oxygen requirements (da Fonseca et al., 2008; Di Rocco et al., 2006; Luo et al., 2008; Shen et al., 2010; Xu et al., 2005).

Due to the functional importance of mitochondrial genes, purifying selection is the dominant force in their evolution; however, weak and/or episodic positive selection may occur in this

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Abbreviations: ATP, ATP synthase subunits; CytB, cytochrome b; COX, cytochrome c oxidase subunits; ND, NADH dehydrogenase subunits; pK', equilibrium constant; αm, power to be at the middle of the alpha-helix; Br, buriedness; RF, chromatographic index; H, hydropathy; E, long-range nonbonded energy; $R\alpha$, solvent accessible reduction ratio; pHi, isoelectric point; F, mean rms fluctuation displacement; P, polarity; $P\alpha$, alpha-helical tendencies; $R\alpha$, solvent accessible reduction ratio; Pt, turn tendencies; Et, total nonbonded energy; Pr, polar requirement; αn, power to be at the N-terminal; Ns, average number of surrounding residues; Hp, surrounding hydrophobicity; αc, power to be at the C-terminal.

* Corresponding author. Fax: +598 2 525 8617.

E-mail addresses: ivanna@fcien.edu.uy, ivanna.tomasco@gmail.com (I.H. Tomasco), lessa@fcien.edu.uy, enrique.lessa@gmail.com (E.P. Lessa).

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background of strong purifying selection if selective pressures shift, as might happen when oxygen availability decreases.

Fossorial rodents constitute an ideal system to test hypotheses about adaptive evolution driven by ecological shifts. The subterranean niche is characterised by high levels of carbon dioxide and low levels of oxygen (Buffenstein, 2000). Those stresses, coupled with the high energy requirements associated with burrowing (Vleck, 1979), suggest that proteins involved in respiration are likely to have experienced positive directional selection in response to their entry into the fossorial habitat. Under this hypothesis, accelerated rates of replacement, relative to silent, substitutions ($\omega = dN/dS$) in these genes are expected in subterranean organisms.

The sister families Octodontidae and Ctenomyidae provide a unique opportunity to trace the evolution of adaptations related to digging (Lessa et al., 2008). Burrowing for shelter and rearing young are shared characteristics among these families of rodents, but only two extant lineages, *Ctenomys* (tuco-tucos) and *Spalacopus* (coruros), have recently evolved fully subterranean lifestyles and their associated adaptations. Spiny rats (Echimyidae) are largely cursorial and arboreal and represent a suitable outgroup to the ctenomyd-octodontid clade (e.g., Honeycutt et al., 2003; Opazo, 2005). Phylogenetic relationships among the genera are well-established (Opazo, 2005 and references therein) (Fig. 1), which makes it possible to trace the changes associated with the acquisition of subterranean adaptations onto a known phylogeny, as well as to identify and discriminate such adaptations from more general ones associated with fossoriality. Other well-studied subterranean taxa with identified sister groups tend to be very divergent from their sister groups.

Da Silva et al. (2009) found a significantly higher ω ratio in the CytB among independent lineages of subterranean rodents (tuco-tucos, coruros, pocket gophers and mole rats) as compared to their above-ground relatives, suggesting a link between directional selection in this gene and the niche shift to life underground. However, this finding is also consistent with alternative neutralist explanations, such as the relaxation of purifying selection due to reduction of population sizes in subterranean lineages. Mitochondrial genes are completely linked in mammals and thus all share a single evolutionary history. If relaxation of purifying selection due to smaller population sizes is the cause of increased ω in subterranean lineages, the pattern should be observed in all protein-coding genes. Alternatively, an adaptive process might have affected several, but not necessarily all, of these genes. Different patterns of rate variation among lineages are expected if positive selection occurs episodically across the genome. An examination of the pattern of evolution in mitochondrial genomes may thus provide a test of these alternative hypotheses.

Our goal was to test the hypothesis that adaptation to energetically demanding lifestyles, in particular those associated with limited oxygen availability, may involve weak or episodic adaptive change in proteins linked to OXPHOS, including those encoded by mtDNA. To examine this possibility, we analysed 13 mitochondrial protein-coding genes from tuco-tucos and the coruro. We then compared variation in these lineages with that of their non-subterranean counterparts to search for two independent footprints of positive natural selection coinciding with niche shifts.

2. Materials and methods

We selected 13 protein-coding genes from the complete mtDNA of seven caviomorph rodent species, representatives of two related but independent subterranean lineages. Among these were three species of tuco-tucos (*Ctenomys*) and the coruro (*Spalacopus cyanus* HM544133), two non-subterranean relatives (*Octodon degus* HM544134 and *Tympanoctomys barrerae* HM544132), and a spiny rat, (*Proechimys longicaudatus* HM544128), which was used as an outgroup. Tuco-tucos comprise more than 56 species and are considered one of the most rapidly speciating mammalian lineages (Reig et al., 1990). We chose a representative sample of 3 species from this group, in an attempt to capture the diversity of lifestyles and molecular differentiation (Castillo et al., 2005; Parada et al., 2011; Slamovits et al., 2001 and references therein). *Ctenomys rionegrensis* HM544130 is typical of the many low elevation, asocial species of the genus. *Ctenomys leucodon* HM544131 is a species specialised for high altitudes (>4000 m) that was suggested by Osgood (1946) to be a distinct subgenus (*Haptomys*). *Ctenomys sociabilis* HM544129 is a sister to the clade that includes all other *Ctenomys* species (Cutrera and Lacey, 2007; Parada et al., 2011), is social and lives in open burrows (Pearson and Christie, 1985).

The topology of the tree used in all of our analyses was constructed using 12 of the 13 protein-coding genes from the mtDNA genomes of octodontoid rodents obtained in this study. The ND6 gene was excluded because it is encoded by the light-strand, which has a significantly different base composition from the heavy-chain (Gibson et al., 2005). Multiple alignments of the concatenated sequences were generated using MUSCLE (Edgar, 2004). Gaps and ambiguous sites were removed, resulting in a total alignment of 10,775 nucleotides. Bayesian inference methods with Markov chain Monte Carlo (MCMC) sampling were used as implemented in MrBayes (Huelsenbeck et al., 2001; Ronquist and Huelsenbeck,

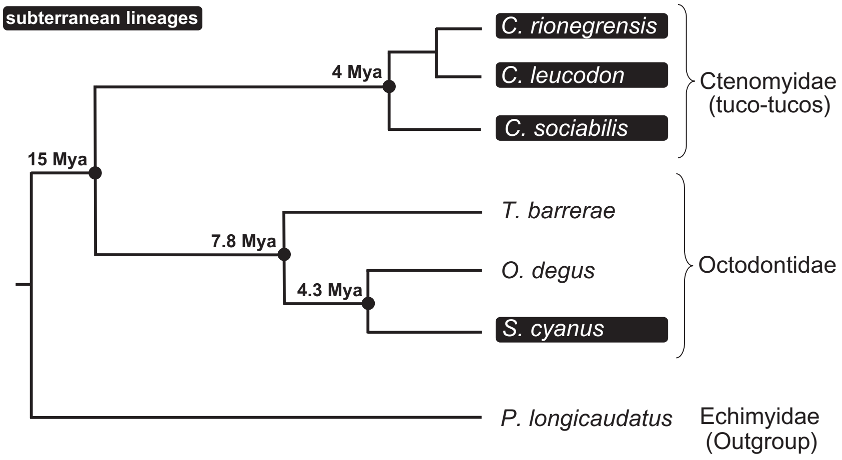

Fig. 1. Phylogenetic relationship among octodontoid rodents included in this study. Divergence times are taken from Opazo (2005) and Verzi (2010). Mya: Millon years ago.