Short Communication

Contrasting patterns of adaptive sequence convergence among echolocating mammals

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ABSTRACT

Several recent studies have described genes demonstrating adaptive sequence convergence between echolocating bats and dolphin, suggesting that common selective pressures can induce common molecular changes, even in distantly related species. However, in the case of the auditory genes Otoferlin (*Otof*), Cadherin 23 (*Cdh23*) and Protocadherin 15 (*Pcdh15*), the reported sequence convergence was supported only by incongruent gene and species trees and counts of convergent substitutions. Therefore, it remains unclear whether echolocating bats and dolphin really do demonstrate evidence of adaptive sequence convergence, or whether there is simply a high level of random background convergence in these genes. To address this question, we estimated the number of convergent and divergent amino acid substitutions along all independent branches of a sufficiently deep phylogeny containing between 22 and 32 mammals for each gene, and compared convergence between the two proposed suborders of bat, Yangochiroptera and Yinpterochiroptera, and dolphin. We find no support for convergence between bats and dolphin in the gene *Pcdh15*. For the gene *Otof* we report minimal evidence for convergent evolution only between the Yinpterochiroptera and dolphin. *Cdh23* displayed a high level of convergence between dolphin and the Yinpterochiroptera. In addition, dolphin and certain members of the Yangochiroptera that emit high frequency echolocation calls shared several unique convergent substitutions. These results indicate that the convergent evolution of *Cdh23* was likely driven by selection for hearing above a certain frequency threshold. Moreover, the contrasting patterns of convergence between the two bat suborders and dolphin in all auditory genes studied thus far suggest echolocation may have evolved independently in the Yinpterochiroptera and Yangochiroptera.

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1. Main text

Bats represent one of the most diverse groups of mammals with over 1200 species that inhabit all continents except Antarctica. Bats show multiple examples of unique traits including powered flight, longevity (Brunet-Rossinni, 2004; Podlutsky et al., 2005), vocal imitative learning (Prat et al., 2015), and echolocation. There are only two groups of mammals, bats and toothed whales, known to have evolved echolocation, although not all bats produce laryngeal generated echolocation signals. Historically, the ability to echolocate was used to classify bats into two subgroups; the echolocating microchiroptera and the non-echolocating megachiroptera. However, molecular data now support a different classification in which echolocating bats are paraphyletic (for review, see Jones and Teeling, 2006). Most echolocating bats are now considered part of the suborder Yangochiroptera, with only a few families of echolocating bats grouping with all of the non-echolocators in the suborder Yinpterochiroptera (Teeling et al., 2005). This new classification leads to the possibility that echolocation has independently evolved in the two groups of bats.

In attempting to understand the molecular adaptations associated with the evolution of echolocation in mammals, considerable attention has been given to the outer hair cell motor protein *Prestin*. Given the function of *Prestin* in high frequency hearing and the discovery that the *Prestin* gene tree unites echolocating bats and dolphin (Li et al., 2010), it has been hypothesized that this gene has played a pivotal role in the evolution of echolocation. However, the observed convergent evolution of *Prestin* found in bats and dolphin (Liu et al., 2010) may be due to a specific focus on two closely related families of echolocating bats from the suborder Yinpterochiroptera; the Hipposideridae and the Rhinolophidae. Indeed, two recent studies found no evidence of *Prestin* sequence convergence between dolphin and either of the echolocating bats *Pteronotus parnellii* (Shen et al., 2011), and *Myotis lucifugus* (Thomas and Hahn, 2015), both from the suborder Yangochiroptera, suggesting that sequence convergence with dolphin is limited to the echolocating Yinpterochiroptera.

In addition to *Prestin*, the auditory genes *Cdh23*, *Pcdh15*, and *Otof* have been reported as convergent between echolocating mammals (Shen et al., 2012). However, the potential sequence convergence between bats and dolphin in these genes was supported only by incongruent gene and species trees – echolocating bats and dolphin form a monophyletic group in gene trees based on nonsynonymous substitutions and amino acid sequences - and counts of convergent substitutions between echolocating bats and dolphin. However, for each gene, the monophyletic grouping of echolocating mammals had very low bootstrap support (Shen et al., 2012) and the levels of convergence varied considerably between the two bat suborders. In this study, we employed more rigorous methods to thoroughly examine the level of convergence of these auditory genes. In addition, we included sequences from many more bats than originally studied by Shen et al. (2012). Using the method of Castoe et al. (2009), which provides a more definitive measure of the level of convergence between two species, we estimated the numbers of convergent and divergent substitutions for all pairwise comparisons of independent branches in the given species tree for each gene. This method provides an empirical null distribution of the expected level of convergence. As it has previously been shown that the number of divergent substitutions reliably predict the number of convergent substitutions, excess amounts of convergence between any two species would manifest as an outlier with a disproportionately high ratio of convergent to divergent substitutions.
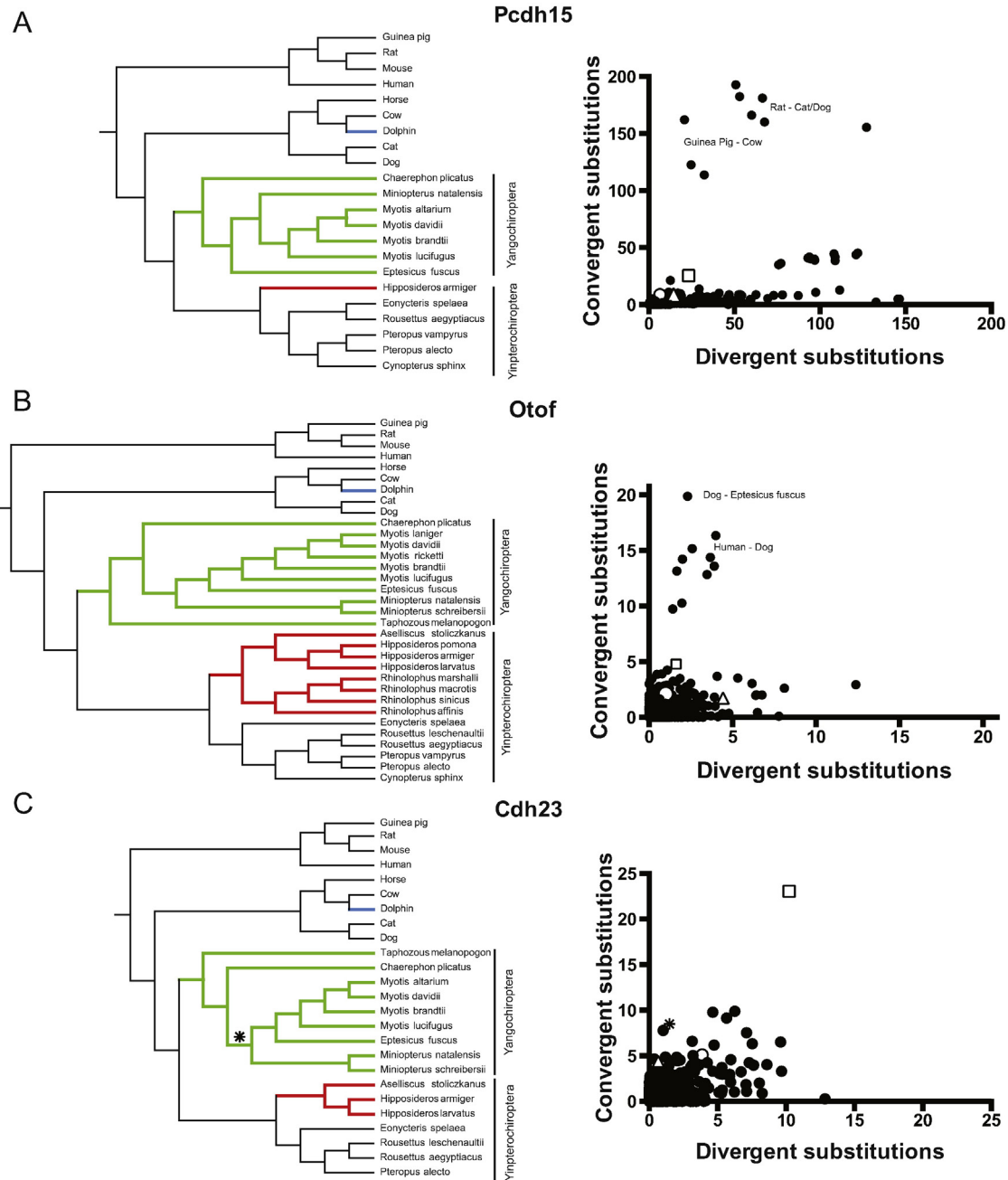
Fig. 1. Evidence for sequence convergence between echolocating mammals. Species trees and plots of posterior numbers of divergent changes versus posterior numbers of convergent changes. Each point represents a pairwise comparison between independent branches of the species tree. For each gene the Yangochiroptera (ancestral green branch) and dolphin (blue branch) comparison is represented by an open triangle, the dolphin and Yinpterochiroptera (ancestral red branch) comparison is denoted by an open square and the Yinpterochiroptera and Yangochiroptera comparison is represented by an open circle. Additionally, for *Cdh23*, the Miniopteridae/Vespertilionidae (branch indicated by an asterisk) and dolphin comparison is indicated by an asterisk. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)