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Convergent sequence evolution between echolocating bats and dolphins

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Cases of convergent evolution — where different lineages have evolved similar traits independently — are common and have proven central to our understanding of selection. Yet convincing examples of adaptive convergence at the sequence level are exceptionally rare [1]. The motor protein Prestin is expressed in mammalian outer hair cells (OHCs) and is thought to confer high frequency sensitivity and selectivity in the mammalian auditory system [2]. We previously reported that the *Prestin* gene has undergone sequence convergence among unrelated lineages of echolocating bat [3]. Here we report that this gene has also undergone convergent amino acid substitutions in echolocating dolphins, which group with echolocating bats in a phylogenetic tree of *Prestin*. Furthermore, we find evidence that these changes were driven by natural selection.

The ability of some bats and all toothed whales to produce sonar pulses and process the returning echoes for prey detection and orientation (echolocation) is a spectacular example of phenotypic convergence in mammals. Echolocation requires exceptionally high frequency hearing and, though echolocating whales and bats generate their calls differently, their cochleae show multiple convergent anatomical features [4]. In particular, the cochlear OHCs in both taxa are shorter and stiffer than in other mammals [4], and this inferred adaptation for processing ultrasound is supported by audiograms that reveal correspondingly higher frequency thresholds [5].

To test whether convergent changes in bat *Prestin* genes have also occurred in echolocating whales, we sequenced the entire gene in a range of echolocating toothed whales and non-echolocating baleen whales, as well as additional bats (see Table S1 in the Supplemental Data available on-line with this issue). Trees based on nucleotide alignments from this larger dataset strongly supported the accepted species tree topology, albeit with the clustering of echolocating bats reported earlier [3]. However, in trees based on amino acid sequences, constructed using a range of different phylogenetic methods, we found that the echolocating dolphins now formed a well-supported group with echolocating horseshoe and Old World leaf-nosed bats (node posterior probability = 0.99 or 0.94 depending on the analysis), members of which emit Doppler-sensitive signals dominated by a constant frequency (CF) component [6] (Figure 1A). Intriguingly, the addition of the sperm whale, which appears to echolocate at much lower frequencies [7], was seen to decrease support for this convergent signal, leading to the cetaceans and bats both forming monophyletic groups. The extent of sequence convergence between bats and whales was thus not sufficient to unite these clades when non-dolphin odontocetes were included in the analysis.

We assessed the support for phylogenetic convergence between echolocating dolphins and bats by
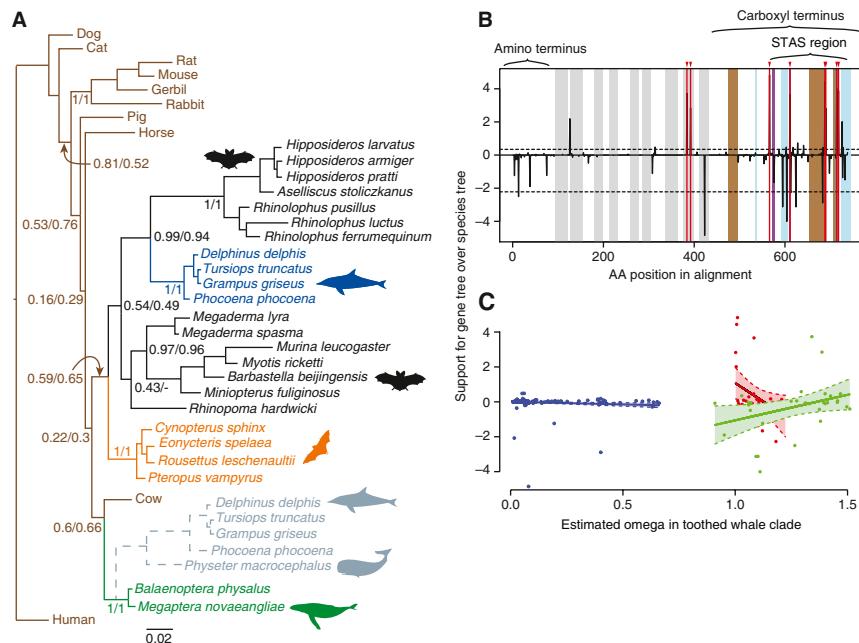

Figure 1. Evidence of sequence convergence in the *Prestin* gene between dolphins and bats.

(A) Bayesian phylogeny of Prestin based on amino acid data. Values at important nodes are posterior probabilities under two different models (see Supplemental Data). Colours and drawings indicate the groups (baleen whales, toothed whales, fruit bats and echolocating bats) referred to in the text. The grey broken lines indicate the alternative position of toothed whales when the sperm whale (*Physeter macrocephalus*) was included in the analysis. (B) Relative support for gene tree and species tree topologies for different sites along the *Prestin* gene sequence. Values are the difference between site-wise negative log likelihood scores for the constrained tree minus the site-wise negative log likelihood scores for the convergent tree. Positive values indicate more support for the gene tree than the constrained tree, negative values indicate more support for the constrained tree. Red lines with arrows indicate the 8 sites showing statistically significant convergence based on simulations. Domains of the Prestin protein are indicated: in addition to those labelled on the figure, grey bands indicate transmembrane domains, blue bands coil domains, brown bands α-helices, and purple bands charge clusters. The first α-helix is transmembrane. (C) Relationship between ω and support for the convergent topology (as in B above) for sites in three categories — blue points are sites under purifying selection across the tree, red those under neutrally across the tree, and green those with different omega values in the toothed whale clade than in other parts of the tree. Shaded regions are 95% confidence intervals for the regression lines (see also Table S2).

comparing likelihood values at each site on a tree constrained to show monophyly of the bats and of the cetaceans and on our maximum-likelihood amino acid tree. We found that support for the convergent tree was distributed along the entire coding sequence (Figure 1B) and, therefore, was not caused by gene conversion. To assess the statistical support for our results, we generated a null distribution of 100,000 simulated sites that were allowed to evolve randomly on the constrained tree under the best-fitting model of evolution. These results confirmed that the total support for the convergent tree was significantly greater (p < 0.001) than expected under this model.

To identify sites showing unexpected support for convergence, we used the same simulations to calculate the chance of observing site-wise likelihood values higher than we observed at each site, calculating the false discovery rate to correct for multiple testing. This approach identified eight sites showing unexpected support for convergence (q < 0.05; Figure 1B). Comparing amino acid sequences between echolocating whales and bats and their respective reconstructed ancestors revealed additional convergent sites. In total, echolocating whales collectively shared 14 derived amino acids with echolocating bats, including ten with CF bats (see Supplemental Data). Four of these sites (G167S, I384T, A565S and E700D) were unique to CF bats and echolocating whales, though A565S was only seen in the sperm whale. We also found six additional replacements that could also be convergent, but which we could not confirm with certainty because of unresolved ancestral states (see Supplemental Data). The dichotomy between convergent and parallel evolution may be of little relevance [8], but we note that most, but not all, convergent sites were parallel changes from the same ancestral amino acid residue. No significant relationship was found between the rate of amino acid substitution at a site and support for the convergent topology, confirming that long-branch attraction artefacts do not explain the signal we have observed.

To investigate the causes of this convergent signal, we estimated rates of nucleotide substitutions that changed the amino acid (non-synonymous rate) or left no change (synonymous rate) at each site, for different parts of the species tree. The ratio of non-synonymous to synonymous rates (ω) measures the strength of selection at a site, from 0 for strong purifying selection to greater than 1 for positive selection. For sites that have evolved neutrally, we found no relationship between ω and the support for convergence, whereas sites under purifying selection showed a significant negative relationship between ω and support for convergence. However, the ω for sites showing a shift in functional constraint in the echolocating whales correlated significantly with support for convergence (p ~ 0.018), and, of 33 sites predicted to fall into this category, 31 (94%) had ω values greater than one (Figure 1C, Table S2). Finally, none of these relationships were significant when sites were modelled as varying in ω within the bats showing convergence.

Our results suggest that the observed sequence convergence between dolphins and CF bats in *Prestin* has been driven by adaptive evolution of the dolphin gene. To date, no specific anatomical parallels have been drawn between these groups, perhaps with the exception of the fine tuning of the cochlear basal membrane in the porpoise (*Phocoena*) [9]. However, detailed comparisons of OHC length and fine-scale structure in cetaceans and bats are lacking [4], and could provide clues given the high frequency hearing thresholds recorded in dolphins and some CF bats. Regardless, our findings of adaptive sequence convergence between two highly divergent groups that share a complex phenotype is unprecedented, and suggests sequence convergence may be more common than previously suspected.

Supplemental Data
Supplemental data are available at http://www.cell.com/current-biology/supplemental/S0960-9822(09)02073-9

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