the key finding on which the proposal is based generalizes over stimulus sets and methods of achieving invisibility. If reproducibility does generally correlate with consciousness, then this could provide a useful diagnostic criterion. The hard problem will then be to demonstrate a causal, mechanistic link between the reproducibility of a neural process and its role in consciousness.

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Molecular Evolution: Gene Convergence in Echolocating Mammals

The motor protein prestin confers sensitive and selective hearing in mammals. Remarkably, prestin amino-acid sequences of echolocating dolphins have converged to resemble those of distantly related echolocating bats.

Gareth Jones

Appearances can deceive. Biologists are very familiar with examples of morphological characters that converge independently in response to similar selective pressures, resulting in the evolution of organisms that are very alike in appearance, despite having different ancestry. Such convergent evolution can then confound the reconstruction of evolutionary history. For example, the hedgehog tenrecs of Madagascar were long believed to be close relatives of 'true' hedgehogs, and were placed alongside them in the mammalian order Insectivora because of their extensive morphological similarities, including the possession of spines. Recent and extensive gene sequencing studies have produced a stable tree topology for the higher-level phylogeny of placental mammals, and the tenrecs are now placed in a clade of mammals that diversified in Africa (the superordinal clade Afrotheria), which is phylogenetically distinct from the clade that diversified in the northern supercontinent Laurasia, and which includes 'true' hedgehogs (the Laurasiatheria) [1]. Phylogenies based on gene sequences are often considered as being less susceptible to homoplasy — the possession of similarities that evolved independently in different lineages — than are trees based on morphology, and therefore have the potential to determine evolutionary relationships in a more robust and reliable manner.

Convergence of gene and amino-acid sequences is traditionally considered to be rare. In this issue of *Current Biology*, convergent research by Li *et al.* [2] and Liu *et al.* [3] gives a stunning insight into how gene and protein sequences can be subject to convergent adaptive evolution in similar ways to morphological characters. Their studies are based on prestin, a motor protein found in the outer hair cells of the inner ear of the mammalian cochlea. The expression of prestin correlates with the appearance of outer hair cell electromotility, and prestin differs from classical motors that are driven by enzymes (that require ATP-hydrolysis) by converting voltage to force directly. Consequently, prestin acts several orders of magnitude more quickly than cellular motor proteins, and its contribution to auditory sensitivity in mammals is immense — a targeted deletion of prestin showed a >100-fold (or 40 dB) loss of auditory sensitivity in mice [4,5].

A sensory system that places extreme demands on audition is echolocation. Echolocation involves producing sound — typically, ultrasound frequencies of >20 kHz — and then receiving and analysing echoes that return from objects. Echolocation has attained its greatest sophistication in bats and toothed whales such as dolphins and porpoises where it is used for orientation and often to detect, localise and classify prey [6]. Echolocating animals are complex phenotypically, and show many adaptive specialisations associated with sound production and hearing. Prestin is unique to mammals, and its evolution resulted from positive selection acting on orthologues (solute carrier anion-transport family proteins) since mammals split from a common ancestor with birds [7].

Prestin was believed to be under strong purifying selection and hence became conserved in mammals [7], but recent evidence shows it to have undergone further positive selection in bat species that use specialized constant-frequency echolocation (CF bats), and which have associated sharp tuning in their auditory systems [8]. Moreover, bats that produce echolocation calls in the larynx (laryngeal echolocators) form a monophyletic group in a phylogenetic

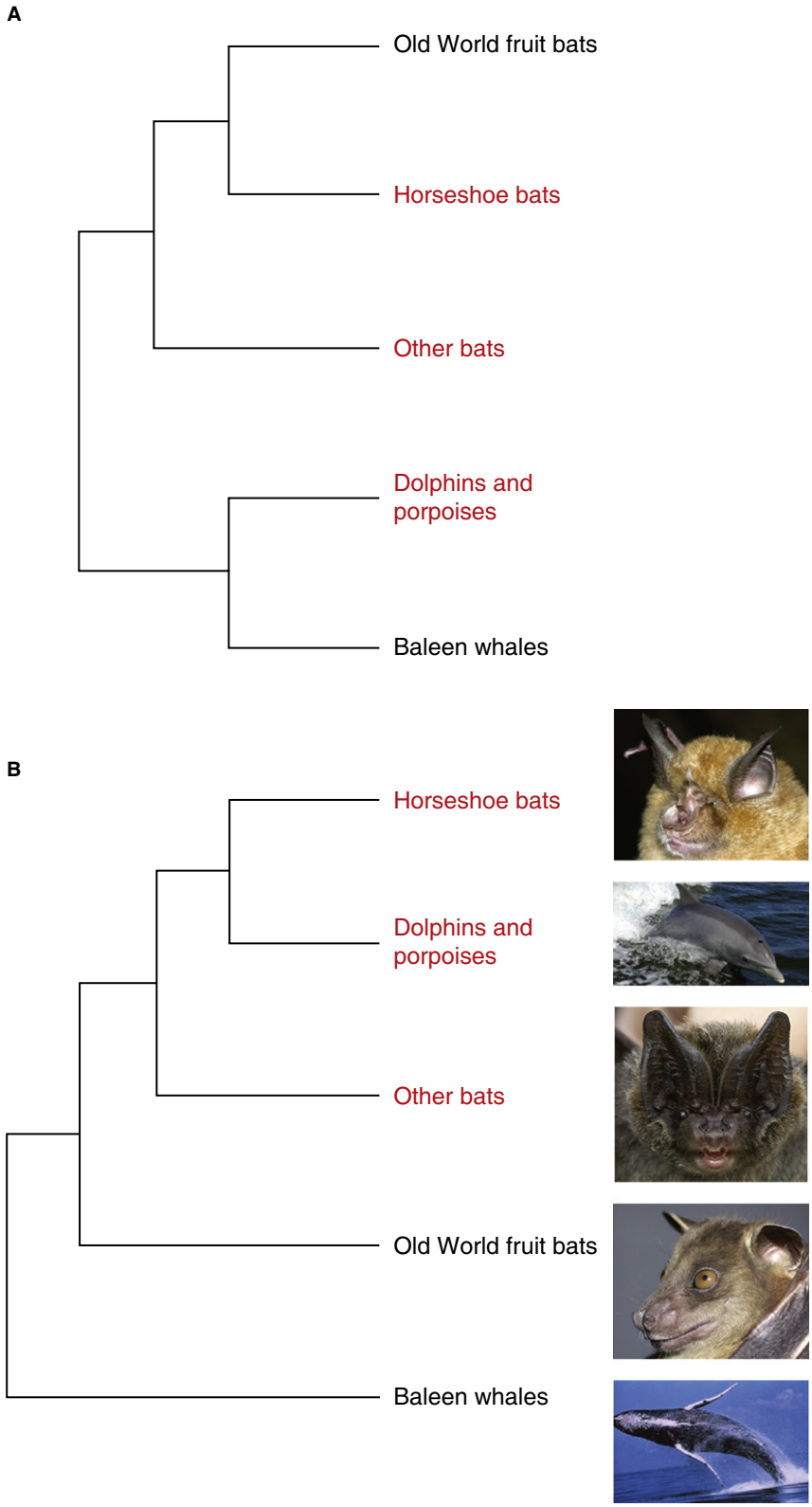

Figure 1. Convergent evolution of prestin sequences in echolocating bats and cetaceans.

(A) The phylogenetic arrangement of Old World fruit bats, horseshoe bats, other bats, dolphins and porpoises, and baleen whales as determined from large-scale molecular sequence analyses. (B) The arrangement that arises from analysis of the *Prestin* gene. Groups highlighted in red show sophisticated biosonar behaviour. In (A) these echolocating taxa are paraphyletic — non-echolocating Old World fruit bats are sister to echolocating horseshoe bats, and echolocating dolphins and porpoises are sister to non-echolocating baleen whales. In (B) all echolocating taxa form a monophyletic group, and dolphins and porpoises are the sister group of horseshoe bats. Photographs are species studied by Liu *et al.* [3] and Li *et al.* [2]. From top to bottom they are the greater horseshoe bat *Rhinolophus ferrumequinum* (G. Jones), the bottlenose dolphin *Tursiops truncatus* (NASA), the Beijing barbastelle *Barbastella beijingensis* (J.R. Flanders), the greater short-nosed fruit bat *Cynopterus sphinx* (G. Jones), and the humpback whale *Megaptera novaeangliae* (NOAA).

tree based on *Prestin* gene sequences [8]. This result conflicts with recent reconstructions of the evolutionary history of bats based on large-scale genetic analyses of both nuclear and mitochondrial DNA, which show that laryngeal echolocators are paraphyletic, with one clade including non-echolocating fruit bats as a sister group to CF bats using sophisticated laryngeal echolocation, the other clade including all other bats that use laryngeal echolocation [9,10]. All bats that use laryngeal echolocation seemingly evolved similarities in prestin because of convergent evolution at amino acid sites of functional importance for echolocation, and hence phylogenetic signals based on functional gene sequences may be misleading when reconstructing the evolutionary history of bats [8].

Even more remarkable is the new finding [2,3] that echolocating dolphins and porpoises show *Prestin* gene sequences that resemble those of echolocating bats. Whales and dolphins belong to the order Cetartiodactyla, and their closest living relatives may be hippopotamuses [11]. Nevertheless, dolphins and porpoises share at least 14 derived amino acid sites in prestin with echolocating bats, including 10 shared with the highly specialised CF bats [2,3]. Consequently, dolphins and porpoises form a sister group to CF bats in a phylogenetic analysis of prestin sequences (Figure 1). This finding is arguably one of the best examples of convergent molecular evolution discovered to date, and is exceptional because it is likely to be adaptive, driven by positive selection [2,3].

Although it appears highly likely that adaptive forces associated with echolocation are driving the molecular evolution of prestin, what might these

selective pressures be? One driver might be the necessity to hear very high frequencies — most bats that emit constant frequency echolocation calls call at high frequencies, as do porpoises and dolphins [12]. Interestingly, sperm whales emit lower frequencies for echolocation [13], and they group with non-echolocating baleen whales in phylogenetic analysis of prestin sequences. However, mice emit [14] and hear [15] ultrasound of relatively high frequencies for communication, and yet their prestin is unspecialised among the mammals.

More broadly, the prestin studies are alerting evolutionary biologists to the issue that genetic data may be susceptible to homoplasy. Evidence for convergent molecular evolution is attracting more attention. Phylogenies based on nucleotide sequences may produce different outcomes according to whether they are based on sites that affect amino acid substitutions to a large extent or not. Although phylogeneticists have long used mitochondrial gene sequences to infer evolutionary relationships, recent studies on reptiles have produced unexpected findings: agamid lizards appear as a sister taxon to snakes in phylogenetic analysis of a 11 kilobase dataset of 13 protein-coding mitochondrial genes [16]. Such an arrangement conflicts with trees based on nuclear genes and morphology, and it is likely that molecular evolution in mitochondria may be susceptible to convergence, at least in reptiles. Indeed, almost 40% of the convergent changes in amino acids in mitochondrial protein-coding genes shared between snakes and agamid lizards may have been driven by metabolic adaptation [16].

A key question is whether convergent, adaptive evolution dominates phylogenetic signals, or whether neutral evolution overrides any convergence driven by natural selection when making phylogenetic inferences [17]. In reptile mitochondrial genes, although molecular convergence is clearly apparent, the specific selective forces driving such convergence are not obvious. The prestin studies [2,3] identify a probable selective pressure — the evolution of ultrasonic echolocation — in driving molecular convergence, and emphasises the necessity of avoiding the use of putative functional genes in estimating evolutionary history. The incorporation of a wide range of genes in phylogenetic analyses will hopefully reduce problems associated with molecular convergence, as convergence in multiple traits may be unlikely, and as more and more neutral sites are incorporated in datasets. Phylogenomic approaches will go some way to circumventing problems arising from molecular convergence, as will careful selection of genetic data that are probably neutral (intron sequences, for example). Even if cases of convergent molecular evolution caused by selection prove to be uncommon, the *Prestin* example emphasises the power of natural selection in driving evolution, even at the molecular level and in complex phenotypes that are associated with specialised behaviours.

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Developmental Evolution: How Beetles Evolved Their Shields

Beetle forewings are modified into hardened structures called elytra. A recent study indicates that the evolution of elytra involved co-opting genes for exoskeleton formation into the wing development gene network of beetles on at least three separate occasions.

Olivier Fédrigo and Gregory A. Wray*

The wings of insects are one of the main reasons for their phenomenal evolutionary success. Ancestrally, flying insects had two pairs of wings, a condition seen in modern dragonflies (Figure 1). Numerous modifications in wing morphology subsequently evolved during the radiation of insects. Several groups, including flies and beetles, use only one pair of wings for flight. In flies, the hindwings are modified into flight-stabilizing structures called halteres, while in beetles the forewings are modified into thick, protective shields called elytra [1]. Beetles are by far the