



Convergence in hearing-related genes between echolocating birds and mammals

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Echolocation, the detection of objects by means of sound waves, has evolved independently in diverse animals. Echolocators include not only mammals such as toothed whales and yangochiropteran and rhinolophoid bats but also *Rousettus* fruit bats, as well as two bird lineages, oilbirds and swiftlets. In whales and yangochiropteran and rhinolophoid bats, positive selection and molecular convergence has been documented in key hearing-related genes, such as *prestin* (*SLC26A5*), but few studies have examined these loci in other echolocators. Here, we examine patterns of selection and convergence in echolocation-related genes in echolocating birds and *Rousettus* bats. Fewer of these loci were under selection in *Rousettus* or birds compared with classically recognized echolocators, and elevated convergence (compared to outgroups) was not evident across this gene set. In certain genes, however, we detected convergent substitutions with potential functional relevance, including convergence between *Rousettus* and classic echolocators in *prestin* at a site known to affect hair cell electromotility. We also detected convergence between Yangochiroptera, Rhinolophidea, and oilbirds in TMC1, an important mechanosensory transduction channel in vertebrate hair cells, and observed an amino acid change at the same site within the pore domain. Our results suggest that although most proteins implicated in echolocation in specialized mammals may not have been recruited in birds or *Rousettus* fruit bats, certain hearing-related loci may have undergone convergent functional changes. Investigating adaptations in diverse echolocators will deepen our understanding of this unusual sensory modality.

genetic convergence | positive selection | avian echolocation | lingual echolocation

Echolocation is an active sensory process used for perceiving the environment via reflected sound waves (1). It has evolved independently in animal lineages that operate in low-light conditions in both terrestrial and aquatic habitats. Echolocation has been particularly well studied in two distinct clades of bats, Yangochiroptera and Rhinolophidea. Most bats produce echolocation calls by vibrating a unique vocal membrane within the larynx, a process termed “laryngeal echolocation”. The origin of laryngeal echolocation in bats remains controversial, although recent evidence suggests a single origin in the last common ancestor of bats (2). Contrary to popular belief, echolocation does not require the production of ultrasonic calls, although most echolocating bats and toothed whales (“odontocetes”) do use ultrasonic frequencies that allow small objects, including prey, to be detected.

Odontocetes, yangochiropterans, and rhinolophoids (here, “classic echolocators”) are known to have multiple laryngeal, auditory, and motor adaptations related to echolocation. Additionally, positive selection or molecular convergence has been detected in several genes linked to hearing and sound production. Genes such as *SLC26A5* (encoding the protein prestin, important for the electromotility of mammalian outer hair cells, which contributes to high-frequency hearing) have received particular attention, and functional convergence between prestin of classic echolocators has been demonstrated (3).

Avian echolocation, by contrast, has received little attention, and whether convergent changes have evolved between birds and echolocating mammals is unknown. Echolocation is much rarer in birds than in mammals, with only three cave-nesting lineages of the ~11,000 birds possessing this ability: *Aerodramus* swiftlets, the closely related but non-sister pygmy swiftlet *Collocalia troglodytes*, and the oilbird *Steatornis caripensis*. As in laryngeally echolocating bats, the click calls used by avian echolocators are generated by vibrating the internal tympaniform membrane found within the avian vocal organ (4); however, avian click calls are lower in frequency than those of bats and whales and are used primarily for navigation, not prey capture. In general, avian echolocation is poorly understood, and to date, morphological or neural specializations have not been conclusively identified in echolocating birds (5, 6).

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Lingually echolocating bats have also been mostly overlooked in echolocation research. Fruit bats of the genus *Rousettus* use their tongues to generate echolocation clicks, a form of echolocation which has long been considered primitive. However, motor cortex specializations facilitating echolocation have recently been identified in *Rousettus*, and they have been shown to perform similarly to yangochiropterans in tasks such as landing accurately and detecting stationary objects in complete darkness (7, 8).

In classic echolocators, patterns of convergence and selection have been examined in genes involved in various aspects of the echolocation process—from sound production (by fast-twitch muscles (9)) to hearing-related changes in the cochlea. Here, we examine whether convergent changes in these genes have also evolved in lesser-studied echolocators such as swiftlets, oilbirds, and *Rousettus* fruit bats.

Results and Discussion

To examine whether echolocation ability relates to the highest frequency an animal can hear, we visualized both these traits across representative birds and mammals. In general, mammals are capable of hearing higher frequencies than are other vertebrates due to cochlear specializations; classic echolocators in particular are capable of hearing higher frequencies than most other mammals. In contrast, lingually echolocating *Rousettus* only hear intermediate frequencies compared to other mammals (Fig. 1B). Echolocating birds also only hear intermediate frequencies compared to other birds (Fig. 1B).

We surveyed the mammalian echolocation literature and identified 16 genes exhibiting positive selection or convergent signals in classic echolocators in earlier studies. A majority of these genes were expressed in the mammalian cochlea (Fig. 1C). As many of these genes have

not been examined in birds, we queried a published cochlea transcriptome of a chicken (10) and confirmed that most inner ear genes (except *NOX3*) were also expressed in the avian inner ear.

Next, using branch-site tests of positive selection, we confirmed strong signals in classic echolocators. In comparison, fewer genes were under positive selection in both the lingually echolocating *Rousettus* as well as in the swiftlet and oilbird (Fig. 1C; only 1 to 3 genes, vs. 7 to 12 in classic echolocators). Because the swift family (Apodidae) includes two of the three lineages of avian echolocators (*Aerodramus* swiftlets and the pygmy swiftlet; genomic data are not yet available for the latter), we included the branch leading to all swifts in our analysis. Interestingly, we recovered more signals of positive selection along this ancestral branch than in either of the other two echolocating birds, suggesting that hearing-related changes might have started evolving early in this family.

Next, using CSUBST (11), a recent method that incorporates a metric (ω_c) which measures error-corrected convergence rates between branch pairs, we confirmed that classic echolocators have higher levels of convergence across the set of 16 genes than do three nonecholocating control mammals (Wilcoxon signed-rank test $P = 0.012$). In contrast, although convergent signals were seen in some loci (most prominently in *DZIP1* and *TMC1* in birds and *SLC26A5* in *Rousettus*; Fig. 2A), neither *Rousettus* ($P = 0.37$) nor nonecholocating birds ($P = 1$ for oilbird and for swiftlet) showed enhanced levels of genetic convergence with classic echolocators relative to controls.

For the loci in which convergence was detected, we examined whether previously identified sites (from studies on classic echolocators) also changed in *Rousettus* or birds. Interestingly, in the most well-studied echolocation gene, *prestin* (*SLC26A5*),

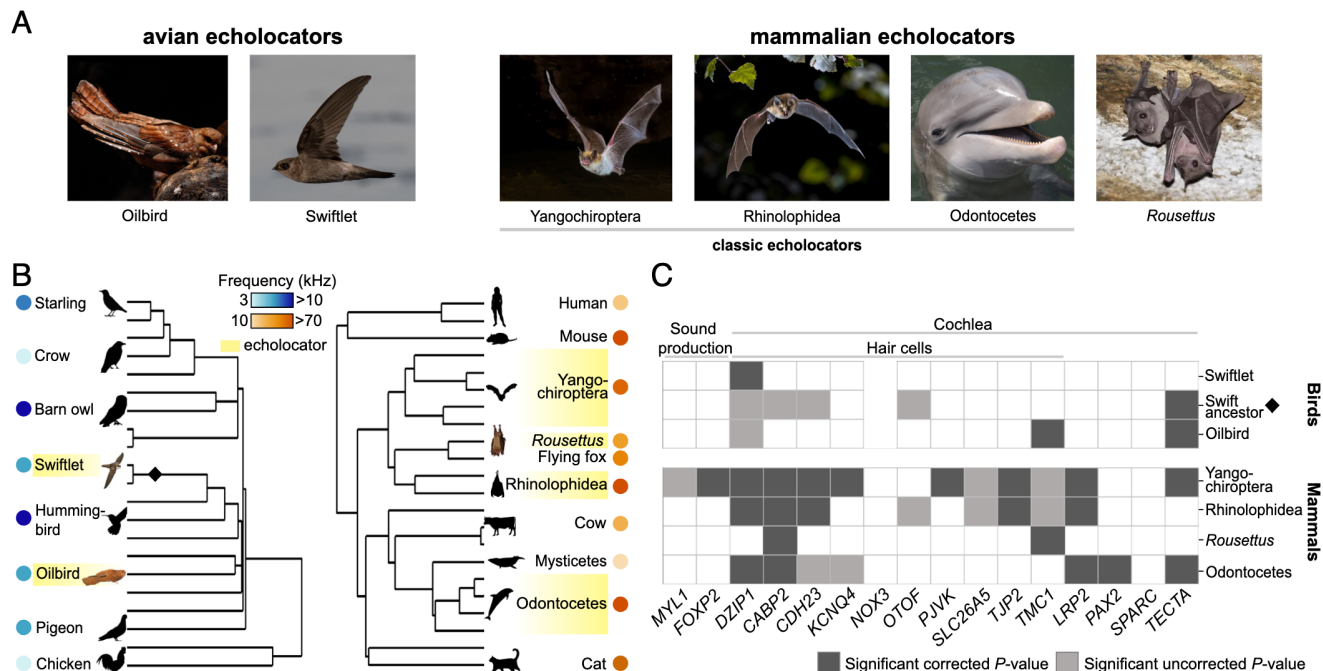


Fig. 1. Signals of positive selection on candidate genes implicated in echolocation ability. (A) Representatives of echolocating birds and mammals; oilbird *Steatornis caripensis*, swiftlet *Aerodramus fuciphagus*, a *Myotis* bat ("Yangochiroptera"), *Rhinolophus ferrumequinum* ("Rhinolophidea"), *Tursiops truncatus* (odontocetes) and fulvous fruit bat *Rousettus leschenaultii*. (B) Placement of echolocating birds (Left) and echolocating mammals (Right) within their respective phylogenies. The highest recorded hearing frequencies of representative birds and mammals are shown; bird hearing ranges are plotted in blue, and mammalian hearing ranges are plotted in orange, with darker colors indicating higher frequencies. The diamond marks the branch leading to all swifts (Apodidae). The tree topologies and species sampling are from an example gene (*CABP2*). (C) Heatmap depicting signals of positive selection in echolocating birds and mammals; gene function or expression location indicated above the heatmap. Bird photos © Samuel Zhang, Shakti Vel; full image credits, see [SI Appendix](#).

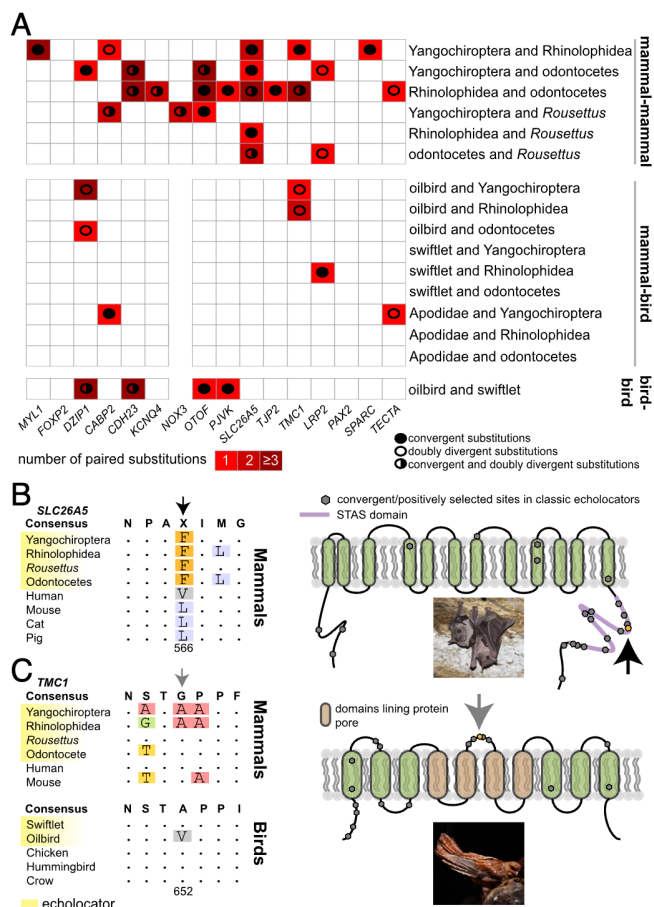


Fig. 2. Convergence between classic and lesser-studied echolocators. (A) Heatmap depicting convergence in candidate genes, indicating number and type of paired substitutions (filled ovals: convergent substitutions to the same amino acid; empty ovals: doubly divergent substitutions—changes at the same site to different amino acids; mixed: both substitution types). (B) Convergence in *SLC26A5* in echolocating mammals. A convergent substitution (L566F, original alignment position 565) in *Rousettus leschanaultii* (photo: Chaitra Ramaiah and Rajesh Puttaswamaiah) is shared with most other classic echolocators. This substitution (orange hexagon, black arrow) is located in the sulfate transporter and anti-sigma factor antagonist (STAS) domain and is known to alter prestin function by affecting hair cell electromotility. (C) Convergence in *TMC1* between echolocating bats and oilbirds. Oilbird (photo: Samuel Zhang) has an amino acid substitution (invariable in other birds screened) at the same site (site 652, original alignment position 478) where there is a convergent substitution between Yangochiroptera and Rhinolophidea (orange hexagon, gray arrow). This site lies between the transmembrane domains that form the functional pore of *TMC1* (cartoon depicts transmembrane domains; intra- and extracellular loops not to scale).

we uncovered a substitution in *Rousettus* (absent in nonecholocating bats) at a site that is known to alter prestin functionality in many classic echolocators (L566F) (3) (Fig. 2B). In addition, we observe convergence in *TMC1*, encoding a pore-forming channel in hair cells that is one of the primary mechanosensory transduction candidates in vertebrates (12), between oilbirds and both yangochiropterans and rhinolophoids. One of the sites driving this signal in oilbirds is at a site within the functional pore-forming domain of the protein that was previously flagged as convergently evolving in classic echolocators (13) (Fig. 2C).

Whether echolocating birds have evolved mechanisms similar to those used by bats and toothed whales to navigate using sound is unknown. We confirm strong signals of convergence across classic echolocators and demonstrate that, across a panel of candidate genes implicated in echolocation, the identified signatures of coding sequence evolution do not necessarily extend to birds or *Rousettus* fruit bats. However, lineage-specific signals of selection and convergence are observed for some hearing-related genes, and testing the functional consequences of changes at known sites, such as those observed in oilbird *TMC1*, may shed light on convergent hearing adaptations that have evolved in cave-dwelling birds and mammals. Examining the underlying genetic and neural mechanisms by which oilbirds and swiftlets echolocate will provide useful insights into echolocation as an orientation strategy and into how convergent sensory processing evolves.

Materials and Methods

Predictions of candidate genes were obtained from NCBI or from draft assemblies. We aligned homologs of these candidate genes from >20 species each of birds and mammals, sampling from major clades across the phylogenies (example shown in Fig. 1B). We included multiple echolocators and outgroups (including other species able to hear high frequencies) in each dataset. Selection testing was performed using aBSREL (adaptive Branch-Site Random Effects Likelihood) (14), and convergence testing was conducted using CSUBST (Combinatorial SUBstitutions) (11). See *SI Appendix*.

Data, Materials, and Software Availability. Alignment files, details on convergent sites, details on echolocation call parameters, gene function data and signals of selection or convergence reported in classic echolocators data have been deposited in Dryad (<https://doi.org/10.5061/dryad.qz612jmm9>) (15).

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