Title: Comment on "The early origin of a birdlike inner ear and the evolution of dinosaurian movement and vocalization"

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Abstract:

Hanson et al. (Research Article, 7 May 2021, p. 601) claim that the shape of the vestibular apparatus reflects the evolution of reptilian locomotion. Using biomechanics, we demonstrate that semicircular canal shape is a dubious predictor of semicircular duct function. Additionally, we show that inference methods used by Hanson et al. largely overestimate relationships between semicircular canal shape and locomotion.

One Sentence Summary: Semicircular canal shape is not a reliable predictor of behavioral variation.

Main text: Hanson et al. (*1*) analyzed shape variation in the inner ear of reptiles, including birds and extinct archosaurs. For the vestibular apparatus, including the semicircular canals, they found three distinct shape clusters and interpreted them as being functionally related to major locomotory categories, including quadrupedalism, bipedalism/simple flight, and agile flight. However, their interpretations are neither supported by their analyses, nor by mathematical models of semicircular duct function. In particular, we argue that semicircular canal shape has no predictive power for inferring locomotion in reptiles, thereby refuting that its evolution can primarily be explained by locomotory shifts.

The three semicircular ducts of the inner ear are connected, endolymph-filled, toroidal membranous organs, each containing a diaphragm-like structure called the cupula (*2*). Semicircular ducts monitor head rotations and can be modelled as heavily damped torsion pendulums (*3*). The resulting biomechanical models (*2*-*4*) are central to evaluating any link between form and function of these structures. Head motion proprioception being vital (*5*), semicircular duct function is expected to attune to the spectrum of head rotations experienced by an organism (6), suggesting a possible link with behavior, and especially locomotion (7).

Most comparative studies indirectly access semicircular duct structure through the enclosing bony semicircular canals, and many of them (*8*-*10*), including Hanson et al. (*1*), rely on geometric morphometrics to quantify canal shape. However, this widespread approach is flawed. Semicircular canal shape is fundamentally unsuited to predict behavioral signal because shape analyses aggregate morphological signal in a way that is incompatible with biomechanical models. For example, highly different semicircular duct shapes can have the exact same function, illustrated here using a two-dimensional example (Fig. 1A; labyrinths a1, b1 and c1 have different shapes but identical function). Additionally, size differences between similarly shaped semicircular ducts translate into important differences in function that are not captured by shape analysis (Fig. 1A; labyrinths a1, a2 and a3 have near-identical shapes but very different functions). Therefore, results of geometric morphometric shape analyses can be incompatible with those of functional analyses (Fig. 1B, C).

Interpretation of form-function relationships is further complicated by a well-characterized statistical issue associated with comparative datasets (*11*) – that traits of closely-related species tend to be more similar simply because they have a longer duration of shared ancestry. This violates the assumption of ‘ordinary’ (non-phylogenetic) statistical methods, specifically that datapoints represent independent observations, causing high rates of false positives unless data are analyzed using phylogenetic comparative methods (*11*).

Hanson et al. (*1*) do present some phylogenetic comparative analyses (*12*), which return only minimal associations between locomotion and semicircular canal shape (*1*). For example, at various phylogenetic scales the associations of canal shape to key locomotor traits are very weak (flight, $R^2 = 0.026$ [Archosauria]; semiaquatic habits, $R^2 = 0.028$ [Reptilia]; aerial predation, $R^2 = 0.115$ [Neoaves]; i.e., 2.6%, 2.8% and 11.5% of shape variance-explained; Ref. 1, Table 1). Such small portions of shape variation are expected to be functionally negligible. Indeed, according to biomechanical models (2-4), even large shape variations encompassing semicircular duct eccentricities from 0 (fully circular) to 0.87 (highly elliptical) only result in maximum sensitivity differences of 18% when centroid size is kept constant. Similar results could theoretically be achieved by increasing overall size of any semicircular canal by about 9%, which is well within intraspecific variation (*13*).

Hanson et al. (*1*) also present a suite of non-phylogenetic approaches that are the main justification for their conclusions, but are not appropriate for comparative data. These approaches include observation of clusters in morphospace, diagrams showing semicircular canal shapes mapped onto trees with verbal interpretations, and non-phylogenetic statistical tests including linear discriminant analysis. Their linear discriminant analysis suggests that canal shapes can accurately predict the occurrence of flight (86.4–100% reported accuracy), semiaquatic habits (83.2–99.2%) and aerial predation (81.3–99.2%). We argue that these high values are artefacts of the failure to analytically accommodate the biasing effects of shared ancestry (*11*), which allows members of a group to share similar semicircular canal shapes and also similar locomotor traits, without any causal relationship (*11*). We illustrate this by simulating scores for the first ten principal component axes of shape based on landmark data from Hanson et al. (*1*), using Brownian motion and the tree they provided. Running linear discriminant analyses on these dummy shape scores, explicitly uncorrelated with locomotor traits, we obtained jackknifed accuracies of 91% (flight), 99% (bipedalism), 85% (semiaquatic habits) and 85% (aerial predation). This demonstrates that linear discriminant analysis will return a strong association of canal shape with locomotion even when none exists. To address this, we then ran linear discriminant analyses on scores obtained from a phylogenetically transformed principal component analysis (*14*) of the shape data of Hanson et al. (*1*). We found jackknifed accuracies of 40% (flight), 44% (bipedalism), 21% (semiaquatic habits) and 20% (aerial predation). Compare these to a null model accuracy of 50% obtained when classifications are purely guesswork (Fig. 2).

Evidence that canal shape does not provide accurate predictions of locomotor traits was confirmed by another study of the semicircular canals of living and extinct archosauromorphs (*15*). That study reported multiple convergent origins of near-bird-like canal shapes in stem archosaurs (flightless, sprawling quadrupeds), early avemetatarsalians (flightless bipeds), pterosaurs (flying quadrupeds or bipeds), and the closest non-avialan dinosaur relatives of birds (e.g., *Velociraptor*; flightless bipeds). Variation in locomotor styles and agility levels among these taxa further demonstrates that labyrinth shape does not allow reliable predictions of locomotor traits in either extant or extinct species.

In summary, we argue that locomotor predictions made from semicircular canal shape are dubious, at best. Given the absence of strong links between semicircular canal shape and function, it seems more likely that canal shapes are more strongly influenced by spatial and developmental constraints (*9*,*15*). For example, birds have different braincase geometry and relative brain size to other reptiles, providing a non-locomotory explanation of their labyrinth shape. To mitigate this, future studies of semicircular canals should instead focus on biomechanical indices of function when aiming to infer behavioral signal.

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Competing interest:

The authors declare that they have no competing interests.

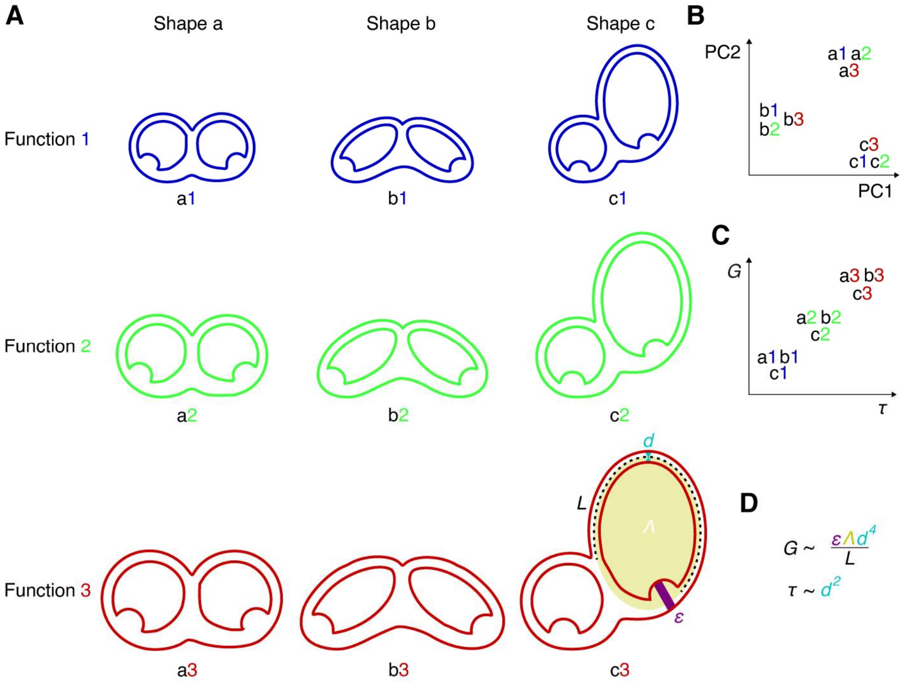

Fig. 1. Illustration of the incongruence between semicircular duct shape and function. (**A**) Diagrams representing idealized anterior and posterior semicircular duct pairs, inspired by existing mammal (a), lizard (b) and bird (c) morphologies. Duct pairs with similar colors have similar function (1,2 or 3), measured as their response speed (τ) and sensitivity (G). Duct pairs of the same column have similar shapes but different sizes. (L) length of the slender portion of a duct. (Λ) area enclosed by a duct torus. (d) diameter of the cross-section of the slender portion of a duct. (ε) deflection factor of the cupula, correlated to its overall size. (**B**) Plot of a principal component analysis of the shape of duct pairs illustrated in (A). Note that specimens cluster by shape, not by function. (**C**) Plot of sensitivity versus response speed for semicircular duct pairs illustrated in (A). Note that specimens cluster by function, not by shape, and that plot (B) and (C) are incompatible. (**D**) Formulae used to calculate semicircular duct sensitivity and response time according to (*4*).

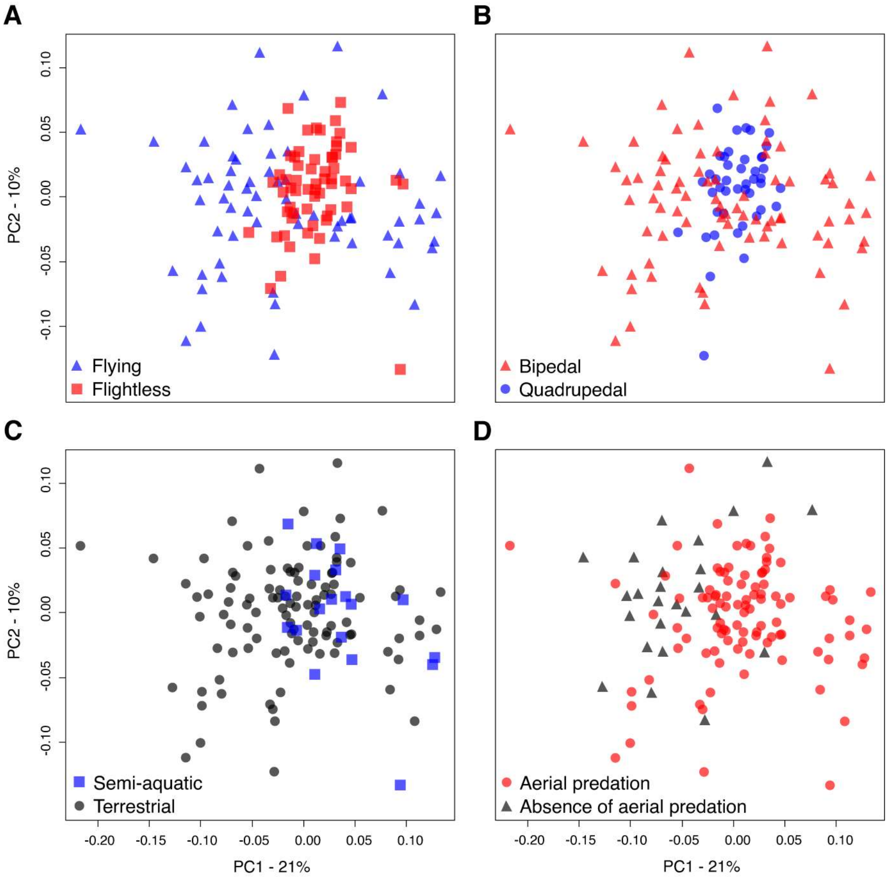

Fig. 2. Phylogenetically transformed principal component analysis of shape data and behavioral signal, showing the absence of locomotor-related clusters when shape data are corrected for phylogeny. (**A**) Flying versus flightless. (**B**) Bipedal versus quadrupedal. (**C**) Semi-aquatic versus terrestrial. (**D**) Presence or absence of aerial predation. The strong overlap of behavioral categories suggests that the clusters reported by Hanson et al (*1*) reflect phylogenetic signal and not behavioral signal.