Evolution: Blinking through deep time

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Abstract. Blinking is everywhere in the tetrapods and almost nowhere in aquatic animals. But how and why did it arise? A new study looks at this through the eyes of a mudskipper, fish that stay for long periods on land and blink.

As comfortable as we are as landlubbers, it's hard for us to appreciate the manifold changes that coming up on to land forced upon our ancestors 375 million years ago. Some of these changes are written in our bones, like rib cages. Our need for these early in our life on land is not entirely clear, but one idea is that they helped keep our internal organs from being squashed while pushing the initially stubbily-limbed bodies of tetrapods over ground[1]—unnecessary in the neutral bouyancy of water. Others are written in glands that secrete fluids that can't be assumed to be present on land, like the salivary to lubricate food, and the lacrimal, harderian, and meibomian eye glands[2].

With both gravity and large density differences between the body and surrounding air to contend with for land-based animals, not only are internal organs pressed down, but so too are airborne particles. There's a constant rain of dust to foul all surfaces it meets. Those who wear eyeglasses live the consequent need for perpetual wiping. Engineers of Mars science rovers, which regularly have their billion-dollar missions terminated by an accumulating layer of martian dust on their solar panels, probably wish for rover-sized eyelids and tears to do what every tetrapod has evolved for wiping off their own photon-hungry surfaces: blink.

Imagine the awkwardness of life in the absence of blinking—having to, say, spit on our shirt cuffs before using them to wipe the dust off our eyes. Incidentally, that's what our spineless land-based friends over on the other branch of the animals have to do. The praying mantis dabs their femoral brush with oral mucus before they start "eye-grooming" with their raptorial forelegs[3,4]. Pity the mantis that just walked over a ghost pepper. Similarly, the jumping spider *Portia*, a 1 cm long animal with the acuity of a cheetah[5], turns their paired mouthparts (pedipalps) into windshield wiper blades if you happen to dab their eyes with dental silicone (Ximena Nelson, personal communication).

While it's clear that blinking in terrestrial vertebrates has to respond to the rain of small visual occlusions on to their eyes, it's not the only function of the blink. The presence of evoked blinks to protect the eye, and wetting the cornea to aid the diffusion of oxygen into this unvascularized tissue, is also important. But when and how did blinking arise over evolutionary time? For example, the protective function of the blink is something that some chondrichthyans and teleosts accomplish through a form of eye retraction.

It seems impossible to answer this question given blinking's lack of fossilizable correlates. A creative approach was taken by Aeillo and colleagues recently[6] in a study of blinking in the mudskipper, a teleost which can spend the better part of a day out of water. Besides giving insights into blinking's origins, Aiello and colleagues show us what aspects of this behavior are constant despite very different starting points 425 million years ago when tetrapods and teleosts last shared a common ancestor.

First, using a clever set of experiments they showed quantitative evidence that the mudskipper's blink fulfills the same functional roles as tetrapod blinking: protection, wetting, and debris clearance. But while the mudskipper blink achieves the same ends, it's done in an entirely different way from how tetrapods blink. Aiello et al. found that, unlike the tetrapods with their three varieties of eye glands, mudskippers do not have any glands uniquely dedicated to wetting the eyes. Instead, they found that mudskippers execute a body roll on their typically wet substrates more often when conditions cause their eyes to dry more rapidly.

Then their is the motor pattern of mudskipper blinking. While tetrapods move an eyelid while keeping the eye steady, mudskippers flip that around, like brushing teeth by holding the brush steady while shaking your head. Rather than move an eyelid over a stationary eye, they move their eye down through the stationary head using their eye's retractor bulbi muscle. A stretchy and moist "dermal cup" passively wipes the eye on retraction, and again on release. Finally, spontaneous blinks in the mudskipper occur slower than evoked blinks, as is found in tetrapods. All of this, as the article details, is occurring with different muscles through different cranial nerves between fish and tetrapods. In the end, the mudskipper's eye is moistened, cleared, and protected. One of my favorite details of the work is how they measured the effectiveness of the blink in clearing the eye of debris. They identified that dried brine shrimp eggs have about the same diameter as sand in the mudskipper's environment, and dusted these evenly on to the eye surface, measuring the fraction cleared on blinking.

Aiello et al. take advantage of comparisons to the mudskipper's nearest relatives, other oxudercids which do not come out of the water and do not blink. This, coupled with the early purely aquatic stage of mudskipper life (prior to becoming amphibious) also being blinkless point toward terrestrialization itself as the most likely driving force for the blink.

Like flying in the insects, pterosaurs, birds, and bats, convergently evolved features help us understand what features of blinking are more or less optional. The terrestrial tetrapod eye achieved a massive 100x increase in range, or million-fold increase in total volume scanned visually, with emergence from water[7]. We found this was due to a combination of a tripling in absolute eye size and the switch to the much more transparent medium of air[7]. Paralleling the presence of blinking and eye size increase in tetrapods, a co-author of that work, Lars Schmitz, has collected preliminary evidence of increased eye size in mudskippers compared to purely aquatic oxudercids[8]. Although a clear determination awaits a time-calibrated phylogeny, the relative increase is ≈1.4-fold, very similar to the relative increase we found in early tetrapods of 1.42–1.53[7].

Evolutionary considerations[7,9,10], computational modeling[11,12], and experimental efforts[12,13] suggest that the combination of longer visual ranges and the right kind of habitat may have eventually fueled selection for planning circuits in the brains of certain tetrapods. But terrestrialization's consequences for visual acuity brings its own constraints. One is the need for a very smooth, flatter, and constantly cleared cornea due to the higher refraction at the air-eye

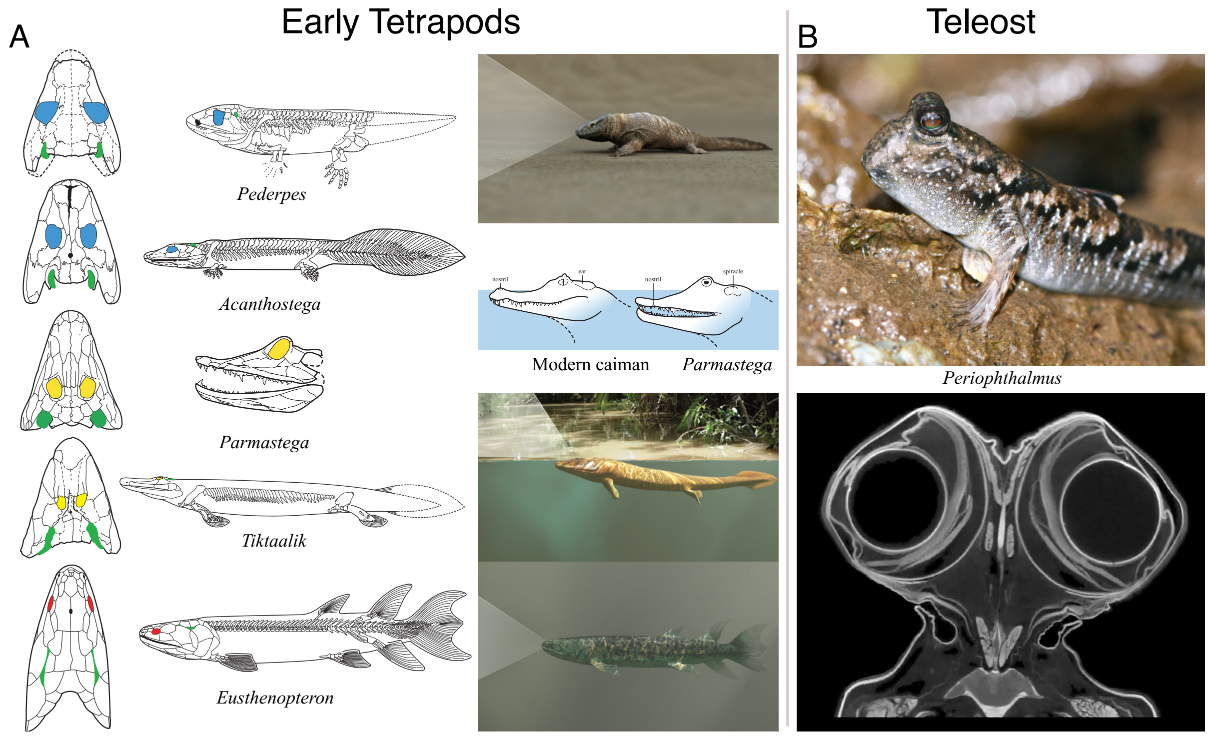

Fig. 1: **a.** Some early tetrapods (from *Eusthenopteron* at ≈385 Mya to *Pederpes* at ≈348 Mya) showing progressive dorsalization of orbits, modified from[10]. Image of caiman and *Parmastega* from[14], Extended Data Figure 8, courtesy Per Ahlberg. **b.** Top: Image of *Periophthalmus argentilineatus* by Christa Rohrbach (CC BY-NC-SA 2.0). Note propping up with pectoral fins, aided in this case by a bump on the substrate. Bottom: A cross-section of the mudskipper *Periophthalmus barbarus*, from Aiello et al. Movie S5[6]. A person of a certain age could be excused for seeing a family resemblance between this image and an Earth-stranded extraterrestrial from an early ‘80s movie.

interface and typically higher acuities. Another is that if the head is flat on the ground, any near-field clutter is likely to obscure your vision, no matter how large your eyes are and how transparent the new aerial viewing medium is. This generates a need to raise the eyes off the ground.

There is evidence that an early tetrapod, *Acanthostega gunnari*, had the same retractor bulbi muscle that mudskippers use to pull down their eye in performing their blink. This raises the possibility that the blink mechanism seen in the mudskipper was also what the tetrapod lineage started with[6]. But we see the second constraint come in to play in the early tetrapods as well, with a migration of the eyes from their fish-like position on the sides of the skull to the top of the skull[7], Fig. 1**a**. Here’s where the tetrapod’s innovations in limbs begin to raise possibilities perhaps out of reach for teleosts. Whereas tetrapods elevate their visual systems with a combination of placing the orbits higher on the skull *and* raising the body off the ground with limbs, mudskippers have to mostly resort to orbit dorsalization[15] for getting a better view. Aiello et al.’s μCT of their heads (Fig. 1**b**) indicates that this strategy may have gone as far as it can in this group of animals.

Overall, the demands of living in a medium 800 times less dense than water generates an intricate series of knock-on effects across many aspects of vertebrate life. Things formerly suspended in the water column now rain down upon your eyes. Suddenly light can travel—in the case of starlight—many light years before being absorbed, instead of a few body lengths in water. But taking advantage of this visual richness requires constant clearing of the eye, among other important roles of blinking, as well as related changes such as larger eyes that are raised off the ground—whether or not you come to it as a fish or a tetrapod. Aiello and colleagues give strong reason to believe that it's the mudskipper's penchant for staying out of the water that led to blinking, and thus imply that the same is true for tetrapods. Furthermore, blinking, like flight, is a behavior with a limited set of good solutions for the aerial eye. Combining careful quantification of extant analogs of a target behavior across several taxa together with their phylogenetic relationships presents the exciting prospect that the evolutionary origin of other behaviors may be inferred in the future.

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