



Invited reply

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Long-distance dispersal over land by fishes: extremely rare ecological events become probable over millennial timescales

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1. Introduction

Our recent coalescent estimate for the time of colonization of Devil's Hole by *Cyprinodon* pupfish challenged deeply held and widespread assumptions about this species based only on a poor fossil record and a geological calibration from the Middle East [1,2]. Our genome-wide analyses estimated a recent age for the Devil's Hole pupfish (DHP), *Cyprinodon diabolis*, within the past 105–830 years and frequent gene flow among Death Valley populations [1]. This estimate depends, in turn, on a good estimate of the pupfish mutation rate. We estimated this indirectly from the *Cyprinodon* substitution rate, and vertebrate mutation rates are known to vary over at least an order of magnitude [3,4]. However, our initial approach using a time-calibrated phylogenetic concatenated analysis cannot overestimate the rate by more than twofold relative to a gene tree analysis [5]. Furthermore, the unique natural history of DHP—including its miniscule population size, short lifespan, small adult size, high metabolic rate, high environmental temperature and high environmental stressors—all predict a higher mutation rate in this species. Except for their larger effective population sizes [6], this likely applies to *Cyprinodon* pupfishes in general [3–5,7]. We argue that DHP is a prime candidate for exhibiting one of the highest vertebrate mutation rates known [5] and should be further investigated using the gold standard of pedigree sequencing [8,9]. We further argue that a young age for this species does not decrease its conservation value but enhances it due to the evolution of such a unique life history and phenotype in a remarkably short time period.

Knott *et al.* (KEA) [10] do not dispute this young age for DHP, but rather the resulting divergence time estimates for *Cyprinodon* as a whole and its dispersal across North America in the past 25 thousand years (kya) [1]. These ages were based on our internal calibration of the stem age of the Laguna Chichancanab pupfish adaptive radiation to 8 kya, based on fossil evidence and isotopic analyses of multiple cores that indicated that the lake basin was dry prior to that time point [11]. One concern is that this very recent calibration is only appropriate for estimating recent divergent events on the same timescale [12]. Owing to the long-term effects of purifying selection and potentially other forces, the substitution rate over longer time periods (tens to hundreds of thousands of years) is almost certainly slower than the spontaneous mutation rate in each generation [13,14]. Thus, the age of *Cyprinodon* as a whole is almost certainly older than estimated by our time-calibrated phylogenetic analysis based on a recent geological event. This relationship should scale with effective population size as well as other demographic factors (e.g. population bottlenecks [6]) through time, so it is difficult to say exactly how much older *Cyprinodon* may be [15]. We also cannot rule out the possibility that the Chichancanab stem lineage is much older than the endemic basin in which it occurs. However, we originally argued that this is unlikely given the inability of these trophic specialist species to coexist with widespread native Yucatan species such as *Astyanax fasciatus*

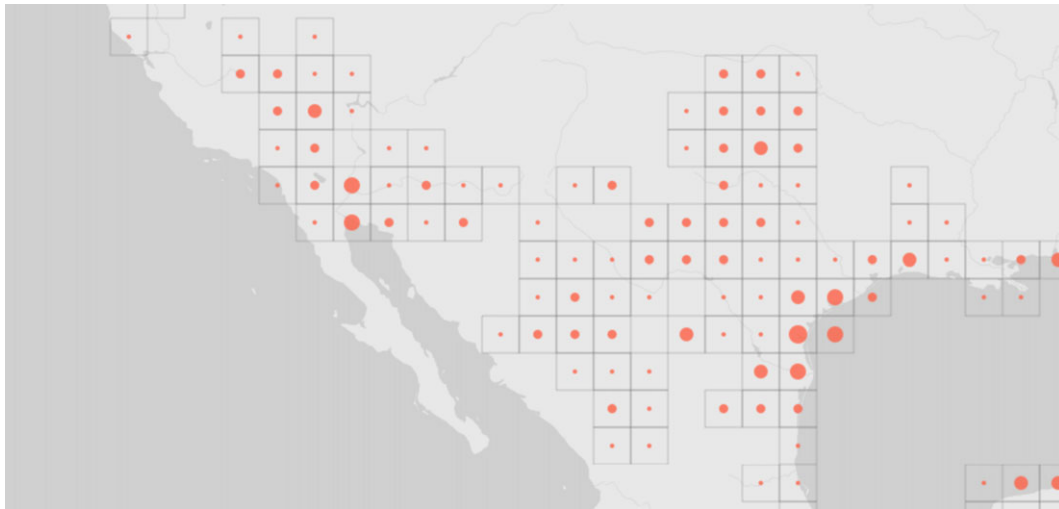


Figure 1. Global Biological Information Facility data for *Cyprinodon* species occurrences within each grid throughout the North American southwest, from the Gulf of Mexico to Death Valley. Note the current widespread occurrences from the Rio Grande to the Gila River and Death Valley drainage basins.

[1,16]. There are also no other isolated brackish lakes known from this region to provide a suitable habitat.

Instead of discussing these legitimate concerns, KEA take the position that all fish require a waterway to disperse, no matter the timescale. They assert that pupfish required a waterway in the last 25 kya connecting the Gulf of Mexico with the Great Basin of California to disperse this far inland. This was specifically questioned by Echelle [17] based on available phylogenetic evidence consistent with our recent study; geological hypotheses that no connections existed between these basins should also be examined (see Discussion below). The broader position that fish dispersal over land is impossible has been refuted countless times, beginning with Darwin, who conducted famous experiments on the ability of aquatic organisms (snails, seeds and plants) to be transported over land by bird vectors. For example, his most famous experiment involved dipping dried duck's feet into an aquarium containing aquatic snails and documenting that they survived out of water for up to 20 h. In the *Origin*, he concluded that 'a duck or heron might fly at least 600 or 700 miles, and would be sure to alight on a pool or rivulet' [18,19]. He did not personally conduct experiments with fish eggs, but corresponded with Sir Humphry Davy who experimented with how long fertilized salmon eggs remained viable after exposure to air, and reported that even a small fish (char) could survive for 72 h barely covered with water [18]. Darwin considered this so important that he made sure it was published by the Royal Society [18,20]. He also collected observations of aquatic animals found on bird's feet in nature throughout his later career, resulting in his final publication [21]. Indeed, given the effort he and colleagues obviously expended on these studies, Darwin certainly would have been quite surprised by KEA's claim that Darwin 'concluded' that aquatic organisms never dispersed long distances over land.

KEA's emphasis on the 3000 km distance from the Gulf of Mexico to Death Valley is misleading. The relevant distance is *between drainage basins*; it should be obvious that fish can swim up rivers because pupfishes have done this throughout their range (figure 1) [22]. For example, the distance between tributaries for one of the proposed routes from the Rio Grande to the Gila River basin is less than

130 km. These mountain passes lie within the flyways of numerous aquatic birds and well within Darwin's proposed range of 600 miles. Furthermore, pupfish eggs are 1–2 mm in diameter and are repeatedly deposited by the female in aquatic plants, algae mats or fine sand/silt [23–25]. They adhere to these plants or fine mud and are desiccation resistant, like those of most cyprinodontiform fishes, a group in which drought-resistant embryonic diapause has repeatedly evolved [26]. 25 kya is a long time; it is wondrous, though not difficult, for us to imagine rare millennial events such as a duck transporting a few eggs stuck to its webbed feet, which maintain a humid environment when tucked into its body during flight [18].

The geological record of ancient inter-basin connections cannot completely account for the observed phylogenetic relationships among Western pupfishes. This is not a novel conclusion of our paper, but has been evident for some time. For example, in a detailed reanalysis of mtDNA sequence divergences, Echelle (pg. 28) noted that 'there is a general paucity of geological support for the inter-basin connections inferred from this study. *This indicates that dispersal across basin divides might have played a greater role than anticipated for the historical biogeography of the group*' (emphasis added). The discrepancy is important because pupfishes 'primarily occupy springs and low-gradient streams on valley floors,' habitats that 'are more likely to reflect geological history than are [those] of most other fish groups in the region' [17]. Thus, 'either our knowledge of (Neogene surface-water connections) is incomplete or pupfish dispersal across basin divides via small, relatively transient, surface-water connections have been more common than expected based on...habitats generally occupied by this group' [17]. 'Dispersal could have been facilitated by headwater stream captures or transient flow across basin divides during extreme rainfall episodes' [17, p. 34]. While Echelle did not consider overland dispersal, the potential explanation we propose, nothing in his analysis precludes this possibility. In any case, KEA to the contrary, there appears to be little argument that non-hydrographic factors likely have been involved in shaping the historical distribution of Western pupfishes.

There are, in fact, numerous examples of isolated water bodies colonized by fishes through long-distance dispersal

over land, including nearly a hundred volcanic crater lake fish species flocks [27–34], all bolide craters [35] and thousands of endorheic lake basins (e.g. [22,36,37]). For example, 18% of the world's landmass drains into endorheic lake basins [38,39]. The most relevant of these is Devil's Hole itself, which was apparently never connected to the surrounding Amargosa River basin in its entire 60 kya history [40]. Similarly, Laguna Chichancanab is an endorheic basin which was never connected to other water bodies; this is also the case for the habitats of several other desert pupfish species [36,41]. We have also personally searched over 30 hypersaline endorheic lake basins across eight islands in the Bahamian archipelago and found pupfish in nearly every lake with salinities below 50 ppt (CH Martin, personal observations 2011, 2013, 2018; [42]). There is also strong circumstantial evidence of trans-oceanic dispersal by cichlid and cyprinodontiform fishes [42–46], which is perhaps more remarkable than over land dispersal across

kilometres of desert. Finally, several alternative mechanisms of fish dispersal have been directly documented, including human introductions, stream capture events and water spouts [47]. Indeed, just a few months ago it rained fishes in Mexico [48]. We agree that fish primarily colonize lakes and rivers via waterways; our point is that there are rare exceptions to this rule.

Finally, we disagree with KEA's claim that a process must be directly observed by humans to be invoked as a potential explanation. Instead, we argue that long-distance dispersal of fishes and their eggs over land is plausible and has repeatedly occurred all over the world.

Data accessibility. This study contains no associated data.

Authors' contributions. C.M. wrote the manuscript. B.J.T. revised and commented on it. Both authors gave final approval for publication.

Competing interests. We declare we have no competing interests.

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