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## Evolution: Drift Will Tear Us Apart

That the widely scattered geographical distribution of some animals could be due to continental drift is a neat idea. Now, cave animals provide evidence for extreme long-term persistence on continents drifting apart.

Florian Maderspacher

It was the 6th of January 1912, epiphany in Christian calendars, when a 31-year-old man stepped up on the stage to address the German Geological Society's annual assembly in Frankfurt. He was a geological nobody. Instead of rocks, he had studied clouds and the ice of Greenland, and what he had to say sounded ludicrous: "New Ideas on the Formation of Large-Scale Structures of the Earth's Crust". Why new ideas? Everyone knew that the continents had always been where they were in 1912. Yes, sea levels had risen and fallen, mountains emerged; and what about the animals whose identical fossils had been found on either side of the Atlantic? Well, they obviously had crossed on a now submerged land bridge. The idea that the young meteorologist by the name of Alfred Wegener put forward was that the continents had drifted into their current place from the break-up of a large landmass that once comprised all current continents. To him, this explained everything — from the shapes of continents fitting together like puzzle pieces to the similar species found on either side of uncrossable oceans. Yet, the audience ridiculed his idea as a 'fever fantasy', and it wasn't widely accepted until decades after Wegener's untimely death in 1929. A hundred years on, Wegener doesn't need our support anymore. But if he did, the phenomenon of cave animals becoming separated by continents drifting apart that Maria M. Bauzà-Ribot and colleagues [1] describe in this issue of *Current Biology* would surely please him.

Think of it as a birthday gift to his theory.

Bauzà-Ribot and her team [1] investigated the phylogeny of a small family of cave-dwelling amphipods. Amphipods are a fairly large group of crustaceans (around 7,000 species), but to describe them as 'inconspicuous' would be a massive understatement — let's just say they look like small shrimp. Amphipods have a penchant for living in caves: more than ten percent of amphipods, in Europe nearly half, are stygobionts [2], meaning they dwell in subterranean waters (Styx was the mythological underworld river ancient Greeks had to cross to get to 'the other side', and coincidentally also the name of a 70s prog-rock band that is itself an example of long-term persistence).

The particular family they studied is the Metacrangonyctidae (the cumbersome name befits their cryptic nature and I promise this is the only time I will spell it out here), which comprises two genera of about 35 species, all of which live in caves. No disrespect, but these are your run-of-the-mill stygobionts: blind, pale, with long antennae. What is, however, noteworthy is where their caves are located, namely on opposite sides of the Atlantic [3]: they inhabit caves in Morocco, Mallorca, Elba and Fuerteventura, as well as on the Caribbean island of Hispaniola (Figure 1).

Bauzà-Ribot and colleagues [1] generated a phylogeny of 16 species in their family based on entire mitochondrial genomes and some nuclear data. Strangely, all of the island species form a monophyletic group, meaning they all descended from

a common ancestor, despite their very scattered distribution. Even the one species living on the Canary island of Fuerteventura is more closely related to species on islands thousands of miles away than to species on the relatively nearby Moroccan mainland. That such related species should live in caves separated by a vast ocean is weird, because it is at odds with the usually fairly narrow geographic ranges of cave animals, yet the same pattern is seen with a few other kinds of cave crustaceans [4,5]. How can this be?

### Vicariance Vindicated

The distribution of any biological entity — be it a species, a group of species, or a population — can be



Figure 1. Spelunking for stygobionts. Brackish waters in Cova de Cala Varques, located on the eastern coast of Mallorca. This body of brackish waters, separated from the underlying sea-water by a major halocline, is a typical habitat for the amphipod *Metacrangonyx longipes* (Photo: Tomeu Cañellas).

due to either dispersal or vicariance. Vicariance means that the distribution of said entity is caused by its ancestral range (or that of its evolutionary precursors) being split up by ecological or geological events. For a cartoon view, imagine two closely related species on either side of a chain of mountains. Either, members of one species at one point crossed the mountains and gradually evolved into a new species on the other side; or, the range of the ancestors spanned all of the extant range, but became split by the rise of the mountains. Dispersal is 'going places', vicariance is 'wait and see'.

A neat example of vicariance is the Isthmus of Panama: many marine animals on either side of the isthmus look remarkably similar, and molecular analyses have confirmed that their divergence times fit roughly with the time the isthmus formed, some 3 million years ago [6]. Geologically speaking, though, this happened only 'last week', so people have been keen to find older patterns, in particular traces of the erstwhile southern supercontinent of Gondwana [7]. 150 million years ago, Gondwana began fragmenting into today's South America, Africa, Madagascar, India, Australia and Antarctica. Song birds, for instance, evolved on Gondwana [8], but their current worldwide distribution has been mainly shaped by their subsequent dispersal and diversification. But the distribution of other species that couldn't disperse easily across seas, such as some frogs, still carries Gondwanan signatures [9]. Oh, and if you're looking for a good example of dispersal, well, just look at your own species!

Vicariance has been associated with cave animals, from the very time naturalists first started dragging them into the light [10]. Darwin already referred to cave animals as "wrecks of ancient life", and in its most extreme, progressionist version, the idea was that the less sophisticated animals of ancient times had survived concealed in caves while their surface relatives had been displaced by newer, better creatures. This pastiche view is obsolete now, but vicariance does make intuitive sense for cave animals [4]. For a start, caves can be quite cut-off from the outside world, and the very adaptations to cave life, in particular blindness, don't help in getting around either.

The overall pattern of cave biogeography seems to fit this notion: of the over 20,000 or so cave species, most are known from only a single or at best a few neighbouring caves [2]. And, in most cases, the closest living relatives are other cave animals, often from nearby caves. The question then is how these species did arise. Ultimately, all cave animals come from surface ancestors. But are the actual species we see today derived from cave species dispersing into nearby caves or did they come from surface species that invaded different caves in their range independently?

The latter seems to be the case in the diving beetles of the Western Australian desert (how's that for an oxymoron?). These beetles, of which there are nearly 100 species, evolved from ancestors that invaded water-filled underground limestone cavities when surface waters dried up due to climate change between 5 and 10 million years ago, and they did so in at least 87 independent instances, with some subsequent diversification underground [11–13]. Their distribution in individual caverns scattered over hundreds of kilometres is thus largely a reflection of their wiped out ancestors' range.

By comparison, the geographical distance between the cave amphipods studied by Bauzá-Ribot and colleagues [1] is much, much larger. What is clear is that this family, all freshwater-dwelling, descended from marine ancestors that might have become separated from their conspecifics, for instance through a drop in sea level [14]. But how did sister species end up on either side of the Atlantic? One idea was that these lineages might have become separated at a time when the Atlantic was much smaller than it is today, and existed only as the western opening of an ancient, now vanished ocean called 'Tethys' [5].

### The Wrecks of Tethys

The Tethys ocean existed for well over 100 million years. It covered most of what is now the Middle East and India, stretching east of the all-in-one super-continent of Pangaea. As Pangaea began breaking up around 175 million years ago, the Tethys extended westward between the aforementioned Gondwana and the Northern super-continent of Laurasia (Northern America, Europe, Asia). Later, as Africa pushed north, the

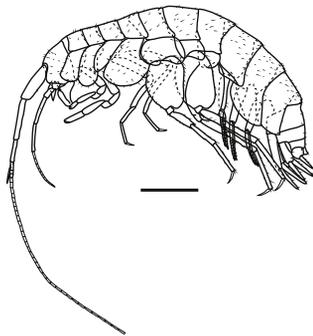
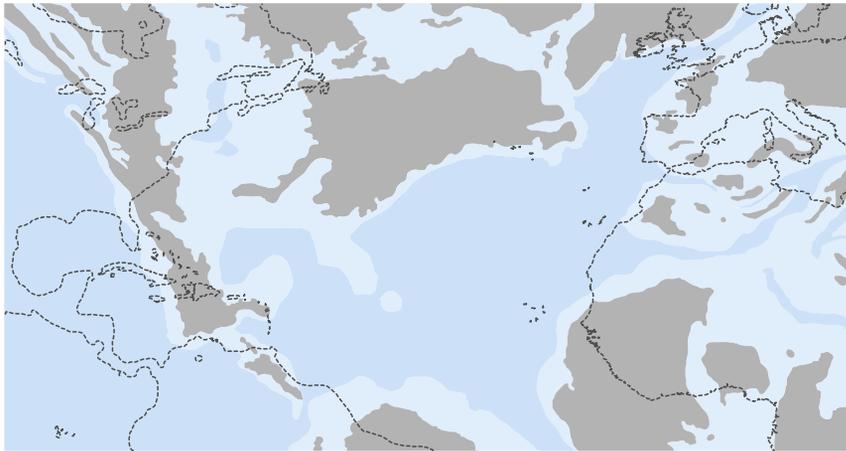
Tethys ocean shrank, leaving a sea whose remnants today are the Mediterranean, the Black sea, the Caspian sea and the Aral lake, in whose current drying up you can, if you wish, hear the death cries of Tethys.

The idea of transatlantic amphipods being the distant echoes of an ancestor that inhabited the once continuous shores of a long gone ocean, ripped apart by the drift of the continents, surely is an evocative one, but how can it be tested? Morphology — animals from either side of the Atlantic do look very alike (Figure 2) — can be misleading. This is especially true in the case of cave animals, which regardless of the branch of the tree of life they stem from often are blind, pale and have elongated appendages. So, similitude may betray common habitat much more than common ancestry.

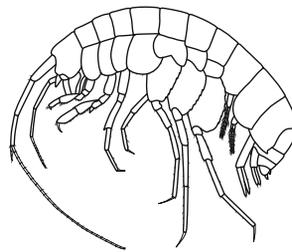
What's needed is some sort of time component. The idea that time can be inferred from the rate of genetic changes that accumulate between two lineages is an old one. But rates can vary over time [15] and depend on other factors, such as population size, thus confounding the picture; so, what is needed is a way of linking the branching points in the phylogeny to the geological events in question. In the context of the Panama example: if the lineages split before or at the time the isthmus formed, it argues for vicariance, while if they split after the event, it would argue for subsequent dispersal.

Bauzá-Ribot and colleagues [1] used different calibration points: first, the two lineages on the neighbouring islands of Mallorca and Menorca cannot be older than when these islands split from mainland Spain, around 16 million years ago, and not younger than the Mediterranean sea level drop that separated the two islands 5.5 million years ago; second, two sister species living on the Atlas mountains in Morocco cannot be younger than when these mountains rose from the sea (from 37 to 25 million years ago). With these calibration points, the authors arrive at a divergence time for all of their major lineages between 80 and 95 million years ago and the island clades around 79–77 million years ago, making this a very old group indeed.

At around this time, Africa and South America were much closer together (Figure 2). The continuous



*Metacrangonyx samanensis*



*Metacrangonyx longipes*

Current Biology

Figure 2. Tracking Tethys.

Top: map of the North Atlantic, about 90 million years ago in the late Cretaceous, when the major lineages of Metacrangonyctidae had begun to diversify. At this time, the shallow coastal waters (light blue) in which their ancestor may have dwelled began to fragment, and the Western Tethys gave way to the Atlantic. (Modified with permission from Ronald Blakey, Colorado Plateau Geosystems Inc.) Bottom: *Metacrangonyx samanensis* living in caves on the Caribbean island of Hispaniola (scale bar: 0.5 mm; reproduced after [3] with permission from Contributions to Zoology) and *M. longipes* from Mallorca.

shallow coastal zone that had framed Tethys and that may have been the range of the Ur-Metacrangonyctid (there I said it again) had begun to fragment. This makes it plausible that the lineages lost contact and began to diversify in place, as the tectonic plates they were located on drifted apart. The alternative scenario, that these lineages arose in one place and later crossed the ocean, seems unlikely given the time frame and the deep waters that separated them.

Now, the avid reader will have noted an obvious problem: the islands which these animals inhabit now weren't around 80 million years ago. The larger Caribbean islands are no more than 40 million years old, and Fuerteventura is only about half that

age. So clearly, the ancestors of the current lineages must have lived either on now perished islands, many of which were scattered in the nascent Atlantic, or they populated other coastal habitats before becoming cave animals.

As it happens, another impressive instance of long-term vicariance across continents has just surfaced [16]. A molecular phylogeny of gobies, one of largest families of fish, revealed that two groups of blind cave goby, one living in Madagascar, the other in Northwestern Australia, are each other's closest relatives. And what's more, their estimated divergence time fits roughly with the break-up of eastern Gondwana in the mid-Cretaceous. This family tree was built with fewer genetic data and

calibration points than that of the cave amphipods, and there is the off chance that the ancestors may have dispersed later across the forming Southern Indian ocean; but nonetheless, this is possibly another striking case of cave animals being dragged along by drifting continents.

Obviously, dating biological and geological events that lie so deep in the past is fraught with uncertainties. But, a relatively recent dispersal of these animals can most likely be ruled out. And although we don't know for how long these animals have actually lived in place since the time of Tethys, a topical sea over which pterosaurs flew and where over the breaking waves the calls of dinosaurs could be heard is awe-inspiring. Alfred Wegener surely would have liked it.

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## Light Perception: More Than Meets the Eyes

**Larval zebrafish lacking eyes and pineal organ show elevated activity levels and undirected light-seeking behaviour upon loss of illumination. This behaviour, termed dark photokinesis, is mediated by hypothalamic deep brain photoreceptors expressing melanopsin.**

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The proverbial man on the street would unhesitatingly agree that the eyes are the major site for light perception in vertebrates. Indeed, they are the only structure responsible for image-forming vision and control most observable visual behaviours. There are, however, other light-sensitive structures outside of the eyes, which are much less appreciated though common, at least in nonmammalian vertebrates (Figure 1). The best known example is the pineal complex, which, in nonmammalian vertebrates, is directly light sensitive and governs circadian entrainment of physiological functions, including the linking of sleep–wake cycles to light and dark periods [1,2]. In addition to the pineal complex, dermal melanophores of nonmammalian vertebrates contain photoreceptors which control skin colour depending on ambient light levels [3]. And as early as the beginning of the 20<sup>th</sup> century, behavioural experiments in fish and birds had already suggested the existence of deep brain photoreceptors located in the hypothalamus as a further site for light perception in nonmammalian vertebrates [4,5].

Considering the mostly light-impermeable skull of vertebrates and the location of the hypothalamus deep within the brain, this certainly would not be the first place to look for light-sensitive structures. Consequently, not only the existence

of deep brain photoreceptors, but also their exact location within the brain, their identity, and the light-sensing pigments involved have been a matter of debate for over a century. Some of these questions have now been definitively answered in a series of elegant experiments, reported in this issue of *Current Biology* by Fernandes *et al.* [6]. These authors show that zebrafish larvae lacking the well-established light-sensitive structures — eyes and pineal organ — still react to a sudden loss of illumination with an elevated locomotor activity and an undirected light-seeking behaviour. They further demonstrate that this behaviour is triggered by a group of neurons in the preoptic area of the hypothalamus and that the light-sensing pigment responsible for mediating this reaction is melanopsin.

Fernandes *et al.* [6] observed that zebrafish larvae exposed to a sudden loss of illumination, and simultaneously presented a target light in one part of their swimming arena, display some distinct behaviours. First, they perform an orientation maneuver (termed O-turns) towards the target light. Second, a series of slower turns (termed R-turns) brings them closer to the target light, where they finally aggregate. Surprisingly, this behaviour is not completely abolished if the larval eyes are surgically removed. Such enucleated larvae fail to perform O-bends, and hence fail to orient themselves towards the target light, but they still display an increased rate of R-turns which will ultimately let them

aggregate in the illuminated portion of the arena.

This aggregation is achieved in a stochastic manner. Upon loss of illumination, blinded zebrafish larvae become hyperactive until they reach the illuminated site by chance. At this point, their activity decreases, thereby ‘trapping’ the larvae in a bright zone. A robust measure for the hyperactivity induced by a loss of illumination (dark photokinesis) is the visual motor response assay, where gross locomotor activity of many larvae in relation to the illumination can be measured in parallel. Because dark photokinesis can be triggered in enucleated larvae, the pineal organ — given its known light sensitivity in teleosts — suggests itself as the relevant structure and thus was the first organ the authors tested for its involvement in the observed reaction. Surprisingly, dark photokinesis could not be abolished by a genetically targeted ablation of the pineal complex, neither in intact, nor in enucleated larvae which accordingly lacked both eyes and pineal organ.

Fernandes *et al.* [6] therefore searched for visual pigments that are expressed in neither the eyes nor the pineal gland, as potential light receptors mediating photokinesis. They considered two promising candidates: teleost multiple tissue opsin a (*tmtopsa*) and the invertebrate-like opsin melanopsin 4a (*opn4a*), both expressed in a domain in the hypothalamus defined by the orthopedia (*otpa*) transcription factor. Indeed, dark photokinesis was absent in *otpa* mutant fish, a finding which was confirmed by transgenic ablation of *otpa*-expressing neurons. The *tmtopsa* gene product could be ruled out as a candidate, as its level was not reduced in *otpa* mutants; the authors thus concluded that melanopsin expressed in