Behaviors of Cavefish Offer Insight Into Developmental Evolution

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SUMMARY

Many developmental processes have evolved through natural selection, yet in only a few cases do we understand if and how a change of developmental process produces a benefit. For example, many studies in evolutionary biology have investigated the developmental mechanisms that lead to novel structures in an animal, but only a few have addressed if these structures actually benefit the animal at the behavioral level of prey hunting and mating. As such, this review discusses an animal’s behavior as the integrated functional output of its evolved morphological and physiological traits. Specifically, we focus on recent findings about the blind Mexican cavefish, Astyanax mexicanus, for which clear relationships exist between its physical traits and ecosystem. This species includes two morphotypes: an eyed surface dweller versus many conspecific types of blind cave dwellers, some of which evolved independently; all of the blind subtypes derived from eyed surface dwellers. The blind cavefish evolved under clear selection pressures: food is sparse and darkness is perpetual. Simulating the major aspects of a cave ecosystem in the laboratory is relatively easy, so we can use this species to begin resolving the relationships between evolved traits and selection pressures—relationships which are more complex for other animals models. This review discusses the recent advances in cavefish research that have helped us establish some key relationships between morphological evolution and environmental shifts.

INTRODUCTION

Evolutionary processes, such as genetic drift and natural selection, can cause organisms’ developmental processes to evolve. The evolution of such processes can promote morphological and physiological diversity, as well as help an organism adapt to new environmental challenges and ecological niches (Hall, 1999). To understand in detail how developmental processes evolve, we first must identify the selection pressures and molecular mechanisms that elicit these changes—e.g., evolutionary development integrated with functional ecology (reviewed in Irschick et al., 2013). This multi-disciplinary approach is difficult to achieve for most organisms because the environmental conditions that originally led to their developmental changes—and thereby afforded specific selection advantages—are often ambiguous. A few recent studies have begun to reveal the advantages associated with

Abbreviations: [EO] SN, [eye-orbit] superficial neuromast; shh, Sonic Hedgehog; VAB, vibration-attraction behavior
developmental traits, including a feeding advantage for animals with certain jaw morphs or different tooth shapes that depend on an animal’s food sources (Wainwright et al., 1995; Albertson et al., 2001; Kocher, 2004; Alfaro et al., 2005; Laffont et al., 2009; Parsons et al., 2009); increased mechanosensors to compensate for animals with poor visual cues (Yoshizawa et al., 2010); as well as conspicuous (Hert, 1989; Couldridge, 2002) and cryptic pigmentation patterns (Klingenberg, 2010; Linnen, 2013). Yet, these scientific accomplishments also emphasize the vast knowledge gap regarding how animals evolve adaptive traits as their genomes and developmental processes shift. If we can identify an animal that has evolved under clear and simple selection pressures, however, it will be possible to more directly test the advantages of the developmental changes they have evolved to undergo.

The cave ecosystem is characterized by perpetual darkness, the absence of primary productivity, and sparse food resources (Culver, 1982; Culver et al., 2009). These conditions exert significant pressure on cave-dwelling animals such that cave-adapted animals from most major phyla exhibit a remarkable convergence in morphological and physiological changes related to cave life, including features that are both constructive (longer appendages, novel behaviors, elaborate non-visual sensory systems) and regressive (reduced vision and pigmentation) (Culver, 1982; Culver et al., 2009). Some aquatic cave populations, including teleost, have been isolated from their surface-dwelling relatives for such a long time that they have accumulated cave-specific mutations (for examples, see Protas et al., 2006; Gross et al., 2009; Elipot et al., 2014). In contrast, it is relatively difficult to identify the original mutations and selection pressures influencing surface-dwelling animals because they frequently hybridize with other populations and live in complex fauna. In this respect, cave animals are valuable models that we can use to analyze adaptive changes that occur under clearly defined selection pressures. Behavior is a convenient window through which we can observe the selective advantages associated with morphological and physiological traits. For example, better sensitivity of a sensory organ can improve behavioral responses in predator avoidance, prey hunting, or mating, which lead to enhanced survivorship or fecundity. Natural selection then screens animals with the best combination of traits, depending on their fitness (Mayr, 1963; Bateson, 1988; Wcislo, 1989; West-Eberhard, 1989; Gittleman et al., 1996; Wimberger et al., 1996).

This review discusses recent advances in our understanding of behavioral evolution and related morphological and physiological traits in an animal that lives in a habitat with clear selection pressures: the Mexican cavefish, Astyanax mexicanus, an established animal model for studying evolution and development (Mitchell et al., 1977; Wilkens, 1988; Jeffery, 2001, 2008, 2009; Protas et al., 2012; Rétaux et al., 2013). Within the past few million years, at least five independent colonizations by two different migrating waves of eyed-surface-fish ancestors have established 29 geographically isolated Astyanax cavefish populations in northeastern Mexico (Ornelas-Garcia et al., 2008; Bradic et al., 2012, 2013; Strecker et al., 2012; Coghill et al., 2014). After an initial radiation underground, the founder cavefish populations became isolated and evolved independently. Food sources are limited in these caves, and likely consist of small soil crustaceans, micro-organisms, and organic matter present in the water that drops from the cave ceiling (Culver et al., 2009). Depending on the cave, additional nutrient sources for cavefish may come from the guano dropped by bats living at the cave ceiling and organic matter brought in by seasonal flooding (Culver et al., 2009; personal observation). When the habitat of these fish changed from the surface to the cave, the cavefish ancestor evolved regressed eyes, reduced pigmentation or albinism, enhanced oral-pharyngeal morphologies, and expanded nervous systems (Wilkens, 1988; Yamamoto et al., 2000, 2004, 2009; Jeffery, 2005; Protas et al., 2006; Alunni et al., 2007; Franz-Odendaal et al., 2007; Mennet et al., 2007; Rétaux et al., 2008; Atukorala et al., 2013; Rétaux et al., 2013). Despite their 2–6 million year separation from A. mexicanus surface cohorts, the cavefish and surface fish cohorts remain interfertile, allowing us to study the evolution of behavioral, morphological and physiological traits by genetic analysis.

Recent work is revealing the genetic architectures that underlie the behavioral evolution of cavefish species, including the examination of the following behaviors: vibration-attraction behavior (VAB) (Eigenmann, 1909; Hill, 1969; Parzefall, 1983; Yoshizawa et al., 2010, 2012a, 2012b); sleep loss (Duboué et al., 2011, 2012); reduced aggression (Parzefall, 2001; Elipot et al., 2013); loss of schooling and shoaling (Parzefall, 2001; Kowalko et al., 2013a); stabilized feeding posture (Schemmel, 1980; Kowalko et al., 2013b); enhanced chemosensory ability (Protas et al., 2008; Bibiowicz et al., 2013); and reduced circadian rhythms and sleep (Cavallari et al., 2011; Duboué et al., 2011, 2012; Beale et al., 2013). Among these behaviors, VAB is the most-studied, positively selected behavior regarding how it benefits cavefish in their environment, its sensory and genetic basis, and its developmental process. Here, I review the behaviors that have been evolutionarily advantageous and most informative in deciphering how A. mexicanus populations have adapted to their caves.

**PREY DETECTION AND FORAGING BEHAVIOR**

**VAB: Adaptive Cave-Associated Foraging Behavior**

Cavefish exhibit VAB; that is, they swim toward an oscillating object, either in a natural cave pool or in a laboratory (Fig. 1A) (Table 1). VAB represents a potential foraging behavior that has evolved repeatedly in at least three different Astyanax cavefish populations (Fig. 1B) (Parzefall, 1983; Abdel-Latif et al., 1990; Yoshizawa et al., 2010). Considering these populations likely evolved separately under similar ecological conditions (Mitchell et al., 1977; Borowsky, 2008; Ornelas-Garcia et al., 2008; Wilkens, 2010; Bradic et al., 2012; Gross, 2012; Strecker et al., 2012; Coghill et al., 2014), the convergence
of these populations towards VAB suggests the distinct advantage this behavior would have in the cave environment. Yet, the extent of VAB is variable within and among populations, and some individuals of one of the oldest cavefish populations, Pachón, even lack this behavior (Fig. 2A). The converse is true for surface fish: while most surface fish lack VAB, a few individuals exhibit low levels of this behavior (Fig. 2A).

Taking advantage of this variation within populations, the adaptive significance of VAB was tested in competitive prey-capture experiments wherein pairs of fish with and without VAB were fed small amount of vibrating prey: brine shrimp (Fig. 2B). In darkness, cavefish were significantly better at capturing the brine shrimp than surface fish (Fig. 2Ba). The key finding, however, was that surface fish with VAB had significantly more brine-shrimp captures in the dark than surface fish without VAB, a difference that disappeared in the light (Fig. 2Bb). Thus, an individual’s ability to utilize VAB plays a role in foraging and is likely advantageous for survival in dark caves. In wild populations of surface fish, VAB is presumably deleterious because fish with VAB may swim toward predators, such as the nocturnal prawn, at night (Wilson et al., 2004; Yoshizawa et al., 2011). In contrast, VAB is adaptive in cavefish because it increases foraging in an environment with limited food availability, minimal light, and macroscopic predators (Yoshizawa et al., 2010, 2011). Consequently, the VAB already present as a standing variation in ancestral surface populations may have been subject to positive selection once the cavefish ancestors started colonizing caves.

Probing a little deeper reveals hints at how these cave populations have honed VAB to survive. Cavefish VAB occurs at a relatively low frequency range (about 5–50 Hz), with a peak frequency of 35 Hz (Yoshizawa et al., 2010, 2013). These values are within the range that cavefish can detect with their mechanosensory lateral line (20–80 Hz) (Coombs et al., 1989, 2014; Münz, 1989). Interestingly, none of the surface fish, even those with VAB, displayed such behavioral tuning (Yoshizawa et al., 2011). Furthermore, because many crustaceans produce water fluctuations at 30–40 Hz while swimming (Lang, 1980), the tuning of cavefish’s VAB at 35 Hz is a novel trait that is likely adaptive in the cave ecosystem.

The cavefish’s optimized frequency detection system suggests that the lateral-line sensory organ that cavefish use to detect water flow is likely a vibration-sensory receptor that has been enhanced in cavefish compared to surface fish (Schemmel, 1967; Münz, 1989). One type of lateral-line sensory organ is the superficial neuromast (SN), which is composed of ciliated hair cells and a gelatinous cupular matrix (Fig. 3, see below). The SNs of cavefish are larger and more numerous than those of surface fish, and are primarily responsible for VAB, as determined by SN ablation studies and genetic analysis (Schemmel, 1967; Teyke, 1990; Yoshizawa et al., 2010). Although SNs appear throughout the body and are particularly abundant on the cavefish’s head, those SNs located within the orbit of the cavefish’s degenerated eye seem to play a particularly important role in VAB (Yoshizawa et al., 2012b). This suggests that the extra cranial space created with the loss of the cavefish’s eyes is an important event that promoted this novel behavior. As there was no difference in the number of eye-orbit SNs (EO SNs) among surface fish—even in fact, no EO SN was observed in surface fish—their appearance in cavefish did not arise through the selection of a standing phenotypic variation (Yoshizawa et al., 2012b); instead, there must be a distinct evolutionary path linking VAB and EO SN evolution.

Cavefish eyes start degenerating around 36 hr post-fertilization, but no significant increases in any SNs (including EO SNs) are detectible, relative to surface fish, until 2 months
| Behavior                                      | Function                             | Morphological/physiological bases                                                                 | First appearance of behavioral difference | References                                                                                          |
|----------------------------------------------|--------------------------------------|---------------------------------------------------------------------------------------------------|-------------------------------------------|-----------------------------------------------------------------------------------------------------|
| Low feeding angle                            | Increase foraging efficiency (??)    | No obvious association with morphological traits including jaw shape and taste bud number         | Assayed at 4.5 mpf or older              | Kowalko et al. (2013b) and Schemmel (1980)                                                           |
| Vibration attraction behavior (VAB)          | Increase foraging efficiency         | Lateral line superficial neuromast at the eye orbit                                              | 3 mpf, peak at young adult (<2 years old) | Abdel-Latif et al. (1990); Parzefall (1983) and Yoshizawa et al. (2010, 2012a, 2012b, 2014)          |
| Loss of aggressive behavior and its shift to foraging | Increase foraging efficiency (??)    | Increased serotonergic neurons at the hypothalamus                                                | Assayed at 3 mpf                         | Burchards et al. (1985); Elipot et al. (2012) and Espinasa et al. (2005)                             |
| Wall-following                               | Spatial recognition                  | Lateral line (superficial/canal neuromast, pharmacological inhibition)                           | Assayed at adult stage. Possibly appears 3–4 mpf | Coombs et al. (2010); de Perera (2004); Hassan (1989); Hassan et al. (1992); Windsor et al. (2008); and Windsor et al. (2010) |
| Loss of Schooling                            | No need to avoid predator or group foraging (may be neutral) | Eye and one genetic factor                                                                         | Assayed at 4.5 mpf or older              | Kowalko et al. (2013a) and Parzefall and Fricke (1991)                                              |
| Loss of male preference                      | No preference in mate choice (may be neutral) | Visual cue (eye)                                                                                  | Assayed at adult stage                  | Plath et al. (2006)                                                                                  |
| Chemosensory response                        | Increase foraging efficiency         | Positively associated with naris size                                                             | Unknown: Assayed at adult stage          | Bibliowicz et al. (2013) and Protas et al. (2008)                                                   |
| Foraging behavior (??)                       | Increase foraging efficiency (??)     | Increased jaw width/taste bud number                                                              | Unknown: morphological difference at 3 dpf | Varatharasan et al. (2009) and Yamamoto et al. (2009)                                               |
| Reduced sleep                                | Increase foraging efficiency (??)     | Noradrenergic system (pharmacological inhibition)                                                | Assayed at 21 dpf                       | Duboué et al. (2011, 2012)                                                                           |
| Reduced circadian rhythm                     | Increase DNA damage repair; Increase foraging efficiency (??); Reduced O₂ consumption | Sustained high expression level of per2 clock repressor; Note: Attenuated circadian rhythm is not enough to reduce O₂ consumption | Assayed at adult stage                    | Beale et al. (2013); Moran et al. (2014)                                                             |

Sf, surface fish; Cf, cavefish; mpf, months post fertilization; dpf, days post fertilization; ??, no direct evidence.
post-fertilization (Yoshizawa et al., 2010). This ontogeny suggests that eye degeneration itself does not induce EO SN formation. Many aspects of eye degeneration are controlled by increased Hedgehog signaling in cavefish (Yamamoto et al., 2004; Jeffery, 2005), so a surface fish was engineered to overexpress shh by mRNA injection so that its eyes would also degenerate to test the relationship between eye retention and EO SN development; these shh-overexpressing fish did not develop EO SN (Yoshizawa et al., 2012b). Therefore, neither eye degeneration alone nor excess Hedgehog signaling in early development induces EO SN formation or enlargement; note that tests that ask if Hedgehog signaling plays a role later in development, when EO SNs first appear at 2 months old, have not yet been conducted.

Although there is no shared developmental process between eye degeneration and EO SN formation, ontogenetic analysis and a comparison using different cave populations could help establish the relationship between VAB and EO SN evolution. The first approach was to determine how the timing of VAB onset correlates with the development of EO SNs in surface fish versus Pachón cavefish by studying fish at 2, 3, and 4 months post-fertilization and in the young-adult stage (~1-year-old). Cavefish EO SN quantity gradually increases between 2 months and the first year of age (Fig 3); this temporally correlates with the appearance and enhancement of VAB, which is first detectible between 2 and 3 months post-fertilization (Fig. 3A). Interestingly, another cavefish population, Tinaja, presents with a weak level of VAB, similar to the amount that some individual surface fish display, and does not have EO SNs even though their eyes degenerate (Fig. 3C). This difference further supports the correlation between cavefish VAB and EO SN evolution, and also reinforces that EO SNs are not directly attributable to eye degeneration.

While EO SN ontogeny corresponds with VAB cavefish, there is no correlation between VAB and the total number of SNs in an individual. While Pachón and Tinaja cavefish have significantly more SNs in their infraorbital region than surface fish do (Fig. 3C), the development of these organs is not genetically correlated with the level of VAB, and their ablation does not detectably affect VAB (Yoshizawa et al., 2012b). These observations further emphasize the model that the EO SN, as a minor group of sensory organs, plays an important role in facilitating VAB whereas other SNs in the infraorbital region may contribute to other sensations, such as hydrodynamic imaging (see below, and also Has-san, 1989; Montgomery et al., 2001; Windsor et al., 2008a, 2010a; Coombs et al., 2010). Development of the EO SN may therefore have evolved as a consequence of positive selection for the enhancement of an adaptive behavior, indicating that studying behavioral traits can help reveal distinct roles for SNs residing in different cranial areas.

Considering the importance of the EO SNs in VAB, and thus the cavefish's ability to forage, it is surprising that VAB and EO SNs emerge so late in development. Such timing may occur for two reasons: (1) the developmental process might be constrained or (2) their foraging preference might change with age. To address the first possibility, Wada et al. (2010) investigated SN development in zebrafish. The SNs first form at the edge of a developing intramembranous bone in the cranial region (the operculum, at 4–5 days post-

Figure 2. Significance of VAB in feeding, as determined by competitive prey-capture experiments. (A) VAB levels measured as the number of approaches. Surface fish, n = 54 (gray area); cavefish, n = 52 (orange area). Vertical dashed line represents the cut-off value of 4 approaches used to classifying fish with (>4 approaches) and without (<4 approaches) VAB using a stimulus of 50 Hz. (B) Prey-capture competition assays. Bars show the proportion of strikes at prey between pairs of surface fish (black fish cartoons) and cavefish (orange fish cartoons) with or without VAB during a 1-min assay period in darkness (left bars) and in light (right bars). A total of eight pairs of cavefish versus surface fish (Ba), and five pairs of surface fish with versus without VAB (Bb) in the dark and light are shown. Values are mean ratio of strikes ± 95% confidence intervals of the mean. *, P < 0.05; **P < 0.01. For details about the method, please see Yoshizawa et al. (2010).
Figure 3. Ontogeny of VAB and EO SNs in surface fish versus Pachón and Tinaja cavefish. (A) VAB ontogeny measured as the number of approaches. (B) EO SN development in surface fish and cavefish. Values are means ± standard errors. Surface fish, n = 10 at 2 months post-fertilization (mpf), = 9 at 3 mpf, = 10 at 4 mpf, and = 12 at ~1-year-old; Pachón cavefish, n = 9 at 2 mpf, = 8 at 3 mpf, = 9 at 4 mpf, and = 11 at ~1-year-old; Tinaja cavefish, n = 7 at ~1-year-old. Pachón cavefish significantly increased VAB and EO SN quantity, whereas Tinaja cavefish were indistinguishable from surface fish in both VAB level and EO SN number at 1 year old (one-way ANOVA followed by planned-contrast analysis at 1 year old). **, P < 0.01; ***, P < 0.001; N.S., not significant. (C) Example fluorescence images of DASPEI-vital staining of surface fish and two cavefish. Thick, white dotted lines indicate the infraorbital canal, and white arrowheads indicate EO SNs that were counted in (B). Thin, white dotted line encloses the neuromasts in an adjacent region on the cranium, the third infraorbital bone (previously denoted as the third suborbital bone, SO-3) (Yoshizawa et al., 2010, 2012a, 2012b). Grey dotted lines indicate the eyes. Magnification is the same for all frames. Scale bar, 1.0 mm.
fertilization). In Astyanax, however, the second and third infraorbital (suborbital) bones, which underlie the infraorbital SNs and EO SNs, develop in cavefish around 2–3 months post-fertilization (Yamamoto et al., 2003). Therefore, the number of SNs in cavefish may only be able to increase after the infraorbital bone forms, meaning that the development of EO SN — and thereby VAB — is developmentally constrained until the infraorbital bone forms at 2–3 months post-fertilization. The second reason for the late development of VAB and EO SN — that cavefish foraging preferences change over time — derives from the hypothesis that cavefish larvae and adults may have different foraging preferences, possibly to reduce competition with one another. Cavefish adults quickly respond and move toward a water surface that is disturbed by fallen water droplets or bat guano (likely via VAB), but they also scavenge for food, perhaps relying on chemosensory inputs originally adapted for bottom feeding. In contrast, relatively small, younger fish scavenge for food exclusively at the bottom, again possibly using chemosensation (Parzefall, 1983; personal and S. Retaux’s observation at the cave Los Sabinos). In the laboratory, however, large cavefish occasionally eat smaller cavefish, suggesting that small, younger fish cannot compete with mature adults. Therefore, the late development of EO SN and VAB may offer small, young fish a better chance to survive by avoiding the hazards of larger, hungry conspecifics that are using VAB to forage just beneath the water’s surface.

While it is difficult to determine which of these possibilities — developmental constraints or an ecological advantage — is responsible for the late development of VAB and EO SNs, genetic experiments have helped. By overexpressing or knocking down the gene(s) responsible for EO SN development using available transgenic and/or genome editing methods (e.g., TALEN and CRISPR technologies) (Gaj et al., 2013; Hwang et al., 2013; Elipot et al., 2014; Ma et al., 2015), and performing gene-regulatory-network analysis (Gavin-Smyth et al., 2013), we will gain insight to the relationship between EO SN development and the timing of dermal-bone formation. Deciphering which gene(s) are responsible for VAB will — through in situ hybridization and/or immunohistochemistry techniques — help reveal if the novel sensory tuning occurs at the level of first projection, the octavolateralis nucleus, or higher, such as at the torus semicircularis area. It is unlikely that sensory tuning is achieved at the neuromast level, however, since the sensitivity estimation based on the morphometrics of neuromasts does not peak at 35 Hz (Yoshizawa et al., 2014). Nevertheless, the foraging advantage associated with VAB gives us a new way to resolve the driving forces for the evolution of other morphological traits, including cranial SN, intramembranous bone, and also neural connections in the central nervous system.

Evolution of Foraging-Related Traits Through the Pleiotropy of Hedgehog Signaling

The pleiotropic effects of enhanced Hedgehog signaling promote eye degeneration as well as increases in jaw size and number of taste buds in Astyanax cavefish (Table 1) (Yamamoto et al., 2004, 2009; Jeffery, 2005). Given the necessary redistribution of attention to the non-visual senses, selection for these other constructive traits, which are fostered by Hedgehog signaling, during cavefish evolution may have accelerated eye loss as a developmental tradeoff. No one has tested if these gustatory traits (larger jaws, more taste buds) improve cavefish’s foraging abilities. Nevertheless, an enlarged jaw is likely beneficial in the darkness, possibly as an adaptation to bottom feeding, and carrying more taste buds likely improves foraging because they could help navigate to food in the dark. To understand the adaptive significance of these morphological and sensory shifts, however, we must perform behavioral tests, such as food-competition experiments. For example, behavioral analyses showing that these gustatory traits are advantageous would support the hypothesis that oral-pharyngeal shifts can promote eye reduction through pleiotropy. If having a large jaw and more taste buds does not enhance foraging efficiency, however, these traits would constitute another example of “not all evolved traits are adaptive” (see below).

Hedgehog signaling is also required for other critical developmental processes, beyond cavefish eye degeneration and a few gustatory traits. During development, enhanced Hedgehog signaling increases the number of migratory cells that enter the olfactory bulbs (Menuet et al., 2007) and serotonergic neurons at the hypothalamus (Elipot, 2013). While the former may represent a positive behavioral response linked to the chemical stimulus of food (Table 1) (Bibliowicz et al., 2013), the latter is an example of a change in behavioral “strategy.” For instance, having an enhanced serotonergic system in the anterior hypothalamus and the raphe nucleus redirects attacking behavior towards foraging behavior. Attacking behavior is frequently used to establish a hierarchical relationship between individuals in surface dwelling fish (Magurran, 1993; Elipot et al., 2013), but this does not occur in cave populations, possibly because A. mexicanus cavefish lack schooling behavior (Elipot et al., 2013; Kowalko et al., 2013b; see below). Indeed, cavefish have higher serotonin levels and more serotonergic neurons at the hypothalamic anterior paraventricular nucleus and hindbrain raphe than their surface counterparts; these anatomical and endocrine changes are believed to have shifted cavefish behavior from attacking to foraging (Elipot et al., 2013). Another study reported that cavefish have higher serotonin levels in the brain because of mutations in the serotonin degradation enzyme, monoamine oxidase (mao) — which again promotes foraging behavior (Table 1) (Elipot et al., 2014). The cavefish’s heightened foraging is likely adaptive to the cave environment, where there is little food and few predators: fish eat on a “first come, first served” basis. In fact, enhanced foraging behavior has converged in independently-evolved cave populations (Elipot et al., 2013).

Yet even though cavefish have higher numbers of serotonergic neurons in their hypothalamus by 1 week of age (Elipot et al., 2013), there is no obvious difference in attacking behavior between cavefish and surface fish at
that age (personal observation). This may be because fish at this young age are too immature to establish hierarchical positions, or because some unknown benefit exists for having an enhanced serotonergic system in the larval stage. Overall, if we could better understand the difference in the “aggression” neural circuits between cavefish and surface fish, and their relationship with the number of serotonergic cells in 1-week-old juveniles, we would gain much-needed insight to the evolution of cavefish’s foraging behavior. In general, this behavioral shift in cavefish from attacking to foraging provides the first example of a likely behavioral advantage underlying their enhanced Hedgehog signaling, whose trade-off is eye development (c.f. Yamamoto et al., 2009).

Evolution of Foraging Behavior Without Obvious Morphological Shifts

Many behaviors have evolved in the company of morphological changes, yet behavior can also evolve without obvious anatomical adaptations—e.g., feeding posture. When in the dark, surface fish feed at a steep angle of ~90° relative to a substrate. In contrast, multiple cave populations feed at a much shallower angle of ~45° (Table 1) (Schemmel, 1980). Although no one has yet determined how low-angle feeding is advantageous in the dark (though this could be demonstrated in a competition assay), three A. mexicanus cave populations (Pachón, Tinaja, and Los Sabinos) (Kowalko et al., 2013b and personal observation) and other benthic feeders (for example, see Ferry-Graham et al., 2002) all perform low-angle feeding, suggesting that this behavior may be advantageous for foraging at the bottom of caves. Since jaw morphology is frequently associated with feeding (Wainwright et al., 1995; Albertson et al., 2001; Kocher, 2004; Alfaro et al., 2005; Parsons et al., 2009), it was surprising when a set of studies suggested that mate preference depends on visual cues and on a non-visually-related genetic factor (Kowalko et al., 2013a). Yet, the same genetic factor encoded at this allele actually promotes schooling in cavefish compared to surface fish (Table 1) (Kowalko et al., 2013a). Such a finding suggests that the absence of cavefish schooling is mainly due to eye loss, which is supported by ablation of the lateral-line sensory system. Although it was once suggested that the lateral line controls schooling behavior (Partridge et al., 1980), ablating the lateral line didn’t affect schooling in either surface fish or cavefish (Kowalko et al., 2013a). Therefore loss of schooling behavior may simply be a consequence of eye loss—which could be beneficial, as opposed to deleterious, because being solitary may provide a better chance to find the rare food sources. A test of food-finding ability using eyed, schooling individuals and eyed, non-schooling individuals—e.g., those that Kowalko et al. (2013a) isolated among F₂ hybrids from a surface fish × cavefish cross—should reveal an advantage for the loss of schooling under sparse-food condition.

Mating preferences cover another social behavior. Female surface fish prefer to mate with large males; in dark environments, though, this preference disappears, suggesting that male preference depends on visual cues (Table 1) (Plath et al., 2006). Yet even in the dark, two out of six cavefish populations evolved a preference for large males (Micos and Yerbaniz; others are Molino,
Pachón, Piedras, and Curva cavefish), suggesting the evolution of a non-visual mate preference (Plath et al., 2006). Such a preference could be a consequence of adaptations and reliance on the mechanosensory lateral-line system, which can sense the vortices produced by fish (Bleckmann et al., 1991). It would therefore be interesting to investigate if the frequency stimulus of vortices produced by large males (~100 Hz) (Bleckmann et al., 1991) attracts females and stimulates their release of gonadotropin releasing hormone (GnRH) to initiate reproductive behavior (Hofmann, 2006). Such an investigation could then explore how the reproduction process adapted after cavefish were no longer able to use visual sensory inputs.

NAVIGATION WITHOUT VISUAL INFORMATION

Another behavior cavefish exhibit is wall-following, which is thought to function in space recognition and avoiding collisions with cave walls (Table 1) (Hassan 1989; De Perera, 2004; Windsor et al., 2008, 2010a, 2010b; Coombs et al., 2010). This behavior has evolved in all Astyanax cavefish populations, and likely helps them navigate in the absence of visual cues. As mentioned, the cavefish’s lateral-line system senses hydrodynamic changes in the flow field of water caused by objects in the cave (Hassan 1989, 1992; Coombs et al., 2010). The ontogeny of this behavior is not clear, although it seems to appear by 3–4 months post-fertilization (personal observations). Because numbers of infraorbital SNs significantly increase after 2 months post-fertilization (Yoshizawa et al., 2010), wall-following behavior may largely depend on this sensory organ. Furthermore, wall-following behavior may be tightly associated with the ability to recognize and navigate spaces in the absence of visual cues (De Perera, 2004; Holbrook et al., 2009). The part of the nervous system most likely involved in space recognition is at a region homologous to the hippocampus, in the lateral part of the doralis of the telencephalon, where spatial memories form (Lo et al., 2002). It would therefore be interesting to find out how cavefish establish spatial memory that is only based on temporal information from hydrodynamic stimuli.

ON THE EVOLUTION OF BEHAVIOR

For the most part, cave animals adeptly confront the challenges of sparse food and perpetual darkness. Consequently, many researchers have associated cave animals' traits with these selection pressures without actually testing the advantages of these evolved traits, thereby leading to misunderstanding or an overemphasis of the significance of the evolution of these traits. For example, the significant increase of infraorbital SN was first predicted as the receptor for adaptive VAB, but the minor sensory population of EO SNs turned out to be the major receptors for VAB (Yoshizawa et al., 2010, 2012b). This case study reminds us to consider a classic criticism of the adaptive program: not all evolved traits are adaptive (Gould et al., 1979). The A. mexicanus cavefish system makes it easy to avoid such pitfalls by permitting laboratory studies in simulated cave environments, which are easy to replicate with dark and food-deprived conditions.

Some interesting, classic evolutionary questions could be answered by surveying behavioral and morphological phenotypes in the A. mexicanus system, such as: Do behavioral adaptations emerge before or after morphological changes? Since behavior can be drastically modified by a simple change—such as a hormone concentration in the central nervous system (Kobayashi et al., 1999)—the evolution of behaviors has been assumed to precede morphological and sensory evolution (reviewed in West-Eberhard, 1989). Yet, multiple studies in the cockroach, moth, silkworm, and mouse suggest that changes in the expression level of some sensory receptors is enough to trigger adaptive shifts of behaviors (Jacobs et al., 2007; Sakurai et al., 2011; Leary et al., 2012; Wada-Katsumata et al., 2013). In A. mexicanus, there are complex trends among cave-associated behaviors. A few behavioral traits, including VAB and stabilized feeding angle, may have preceded morphological or sensory evolution because there are standing phenotypic variations of these behaviors among the cavefish and their related surface-dwelling cohorts. Thus, during the initial steps of the adaptation process, individual cavefish ancestors that expressed these cave-associated behaviors were at a selective advantage because they did not require extreme morphological/sensory changes to adjust to the cave environment (Yoshizawa et al., 2010, 2011, 2012; Kowalko et al., 2013). In contrast, cavefish behaviors that require eye regression, including loss of schooling (Kowalko et al., 2013) and wall-following (personal observation of eye-ablated surface fish), may have emerged after the morphological loss of the eye. Furthermore, loss of pigment, another morphological trait, may have also induced behavioral changes, including higher locomotor activity and/or sleep loss, because a null mutation of the ocellocutaneous albinism II (oca2) gene increases the production of dopamine and noradrenaline, two neurotransmitters that affect sleep and locomotor behaviors (Duboué et al., 2011, 2012; Bilandžija et al., 2013). Oca2 could therefore work in a pleiotropic manner at the morphological and behavioral level. In addition, well-known pleiotropic Hedgehog signaling controls both morphology/sensory system (eye regression, widening jaw, increase of taste buds) and behavior (behavioral shift from aggression to foraging via increase of serotonergic neurons in the hypothalamus) (Yamamoto et al., 2004, 2009; Elipot et al., 2013). Thus, traits controlled by pleiotropic genes could have evolved concurrently as a result of the mutation of these genes.

In summary, the cavefish serves as an excellent model to study the evolution of multiple morphological and behavioral traits because it has provided evidence that morphological and behavioral traits evolved through complex manners. The latest A. mexicanus behaviors studied include: enhanced prey capture ability in 25-day-old cavefish in the dark (Espinasa et al., 2014); feeding control via appetite-related hormones (Penney et al., 2014); and loss
of circadian rhythm and sleep (Duboué et al., 2011, 2012; Beale et al., 2013; Moran et al., 2014; Yoshizawa et al., 2015). The next frontier in cavefish research will be to identify more of the genes and mutations involved in the adaptation to the cave environment, thereby establishing a field where genetics, ontogeny, neuroscience, phylogeny, and ecology are integrated.

The recent development of powerful tools has led to a wealth of important information we can use to unravel evolutionary mysteries, including available genome sequences (AstMex102 from the Ensembl genome browser, at www.ensembl.org); available embryology techniques that can modify gene expression (Yamamoto et al., 2000, 2004, 2009; Gross et al., 2009; Bilandžija et al., 2013); defined embryonic and larval stages (Hinaux et al., 2011); transcriptome datasets (Gross et al., 2013; Hinaux et al., 2013); and defined evolutionary relationships among populations (Omelas-García et al., 2008; Bradic et al., 2012; Gross, 2012; Bradic et al., 2013). Further advances in transgenic capabilities (Elipot et al., 2014) and genomic engineering methods, such as TALEN and CRISPR technologies (Gaj et al., 2013; Hwang et al., 2013), allow us to perform more directed genetic studies. Such technology is helping to fill the technical gap between the A. mexicanus system—with its clear selection pressures and many cave-adapted traits—and other model animal systems—with their historical knowledge base. With new information and better experimental techniques, we can further exploit A. mexicanus as an evolutionary vertebrate model, which will ultimately allow us to comprehensively understand the evolutionary processes through which genomic and developmental shifts produce enhanced or co-opted adaptive traits.

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REFERENCES

Abdel-Latif H, Hassan ES, von Campenhausen C. 1990. Sensory performance of blind Mexican cave fish after destruction of the canal neuromasts. Naturwissenschaften 77:237–239.

Albertson RC, Kocher TD. 2001. Assessing morphological differences in an adaptive trait: A landmark-based morphometric approach. J Exp Zool 289:385–403.

Alfaro ME, Bolnick DI, Wainwright PC. 2005. Evolutionary consequences of many-to-one mapping of jaw morphology to mechanics in labrid fishes. Am Nat 165:E140–E154.

Alunni A, Menuet A, Candal E, Penigault JB, Jeffery WR, Rétaux S. 2007. Developmental mechanisms for retinal degeneration in the blind cavefish Astyanax mexicanus. J Comp Neurol 505:221–233.

Atukorala AD, Hammer C, Dufton M, Franz-Ödendaal TA. 2013. Adaptive evolution of the lower jaw dentition in Mexican tetra (Astyanax mexicanus). EvoDevo 4:28.

Beale A, Guibal C, Tamai TK, Klotz L, Cowen S, Peyric E, Reynoso VH, Yamamoto Y, Whitmore D. 2013. Circadian rhythms in Mexican blind cavefish Astyanax mexicanus in the lab and in the field. Nat Commun 4:2769.

Bibliowicz J, Atié A, Espinasa L, Yoshizawa M, Blin M, Hinaux H, Legendre L, Pére S, Rétaux S. 2013. Differences in chemosensory response between eyed and eyeless Astyanax mexicanus of the Rio Subterráneo cave. EvoDevo 4:25.

Bilandžija H, Ma L, Parkhurst A, Jeffery WR. 2013. A potential benefit of albinism in Astyanax cavefish: Downregulation of the oca2 gene increases tyrosine and catecholamine levels as an alternative to melanin synthesis. PLoS One 8:e80823.

Bleckmann H, Breithaupt T, Blickhan R, Tautz J. 1991. The time course and frequency content of hydrodynamic events caused by moving fish, frogs, and crustaceans. J Comp Physiol A 168:749–757.

Borowsky R. 2008. Restoring sight in blind cavefish. Curr Biol 18:R23–R24.

Bradic M, Beerli P, Garcia-de Leon FJ, Esquivel-Bobadilla S, Borowsky RL. 2012. Gene flow and population structure in the Mexican blind cavefish complex (Astyanax mexicanus). BMC Evol Biol 12:9. doi: 10.1186/1471-2148-12-19.

Bradic M, Teotónio H, Borowsky RL. 2013. The population genomics of repeated evolution in the blind cavefish Astyanax mexicanus. Mol Biol Evol 30:2383–2400.

Cavallari N, Frigato E, Vallone D, Fröhlich N, Lopez-Olmeda JF, Foà A, Berti R, Sánchez-Vázquez FJ, Bertolucci C, Foulkes NS. 2011. A blind circadian clock in cavefish reveals that opsins mediate peripheral clock photoreception. PLoS Biol 9:e1001142.

Coghill LM, Darrin Hulsey, Garcia J, de Leon, Johnson FJ. 2014. Next generation phylogeography of cave and surface Astyanax mexicanus. Mol Phylogenet Evol 79:368–374.

Coombs S, Bleckmann H, Fay RR, Popper AN. 2014. The lateral line system. New York: Springer.

Coombs S, Görner P, Münz H. 1989. The mechanosensory lateral line. New York: Springer-Verlag.

Coombs S, Patton P, Windsor S. 2010. Active wall following by Mexican blind cavefish (Astyanax mexicanus). J Comp Physiol A Neuroethol Sens Neural Behav Physiol 196:853–867.

Coulard VCK. 2002. Experimental manipulation of male egg-spots demonstrates female preference for one large spot in Pseudotropheus lombavdoi. J Fish Biol 60:726–730.

Culver DC, Pipan T. 2009. The biology of caves and other subterranean habitats. Oxford: Oxford University Press.
Culver DC. 1982. Cave life, evolution and ecology. Cambridge: Harvard University Press.

De Perera TB. 2004. Fish can encode order in their spatial map. Proc Biol Sci 271:2131–2134.

Duboué ER, Borowsky RL, Keene AC. 2012. β-adrenergic signaling regulates evolutionarily derived sleep loss in the Mexican cavefish. Brain Behav Evol 80:233–243.

Duboué ER, Keene AC, Borowsky RL. 2011. Evolutionary convergence on sleep loss in cavefish populations. Curr Biol 21:671–676.

Eigenmann CH. 1909. Cave vertebrates of America: A study in degenerative evolution. Washington DC: The Carnegie Institution of Washington.

Elipot Y, Hinaux H, Callebert J, Launay J-M, Blin M, Rétaux S. 2014. A mutation in the enzyme monoamine oxidase explains part of the Astyanax cavefish behavioural syndrome. Nat Commun 5:3647.

Elipot Y, Hinaux H, Callebert J, Rétaux S. 2013. Evolutionary shift from fighting to foraging in blind cavefish through changes in the serotonin network. Curr Biol 23:1–10.

Elipot Y, Legendre L, Père S, Sohm F, Rétaux S. 2014. Astyanax transgenesis and husbandry: How cavefish enters the laboratory. Zebrafish 11:291–299.

Espinasa L, Bibliowicz J, Jeffery WR, Rétaux S. 2014. Enhanced prey capture skills in Astyanax cavefish larvae are independent from eye loss. EvoDevo 5:35.

Ferry-Graham LA, Wainwright PC, Westneat MW, Bellwood DR. 2002. Mechanisms of benthic prey capture in wrasses (Labridae). Mar Biol 141:819–830.

Franz-Odendaal TA, Ryan K, Hall BK. 2007. Developmental and morphological variation in the teleost craniofacial skeleton reveals an unusual mode of ossification. Exp Zool B Mol Dev Evol 308B:709–721.

Gaj T, Gersbach C a, Barbas CF. 2013. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol 31:397–405.

Gavin-Smyth J, Wang Y-C, Butler I, Ferguson EL. 2013. A genetic network conferring canalization to a bistable patterning system in Drosophila. Curr Biol 23:2296–2302.

Gittleman JL, Anderson CG, Kot M, Luh H-K. 1996. Phylogenetic liability and rates of evolution: A comparison of behavioral, morphologic and life history traits. In: Martins EP, editor. Phylogenies and the comparative method in animal behavior. New York: Oxford University Press. pp 166–205.

Gould SJ, Lewontin RC. 1979. The spandrels of San Marco and the panglossian paradigm: A critique of the adaptationist programme. P Roy Soc Lond B Bio 205:581–598.

Gross JB, Furterer A, Carlson BM, Stahl BA. 2013. An integrated transcriptome-wide analysis of cave and surface dwelling Astyanax mexicanus. PLoS One 8:e56659.

Gross JB. 2012. The complex origin of Astyanax cavefish. BMC Evol Biol 12:105. doi: 10.1186/1471-2148-12-105

Hall BK. 1999. Evolutionary Developmental Biology. 2nd ed. New York: Springer.

Hassan ES, Abdel-Latif H, Biebricher R. 1992. Studies on the effects of Ca ++ and Co ++ on the swimming behavior of the blind Mexican cave fish. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 171:413–419.

Hert E. 1989. The function of egg-spots in an African mouthbrooding cichlid fish. Anim Behav 37:726–732.

Hill LG. 1969. Feeding and food habits of the spring cavefish, Chologaster agassizi. Am Midl Nat 82:110–116.

Hinaux H, Pottin K, Chalhoub H, Pere S, Elipot Y, Legendre L, Rétaux S. 2011. A developmental staging table for Astyanax mexicanus surface fish and Pachon cavefish. Zebrafish 8:155–165.

Hinaux H, Poulain J, da Silva C, Noirot C, Jeffery WR, Casane D, Rétaux S. 2013. De novo sequencing of Astyanax mexicanus surface fish and Pachón cavefish transcriptomes reveals enrichment of mutations in cavefish putative eye genes. PLoS One 8:e53553.

Hofmann H a. 2006. Gonadotropin-releasing hormone signaling in behavioral plasticity. Curr Opin Neurobiol 16:343–350.

Holbrook RL, de Perera TB. 2009. Separate encoding of vertical and horizontal components of space during orientation in fish. Anim Behav 78:241–245.

Hwang WY, Fu Y, Reyon D, Maeder ML, Tsai SQ, Sander JD, Peterson RT, Yeh J-RJ, Joung JK. 2013. Efficient genome editing in zebrafish using a CRISPR-Cas system. Nat Biotechnol 31:227–229.

Irsichick DJ, Albertson RC, Brennan P, Podos J, Johnson N a, Patek S, Dumont E. 2013. Evo-devo beyond morphology: From genes to resource use. Trends Ecol Evol 28:267–273.

Jacobs GH, Williams GA, Cahill H, Nathans J. 2007. Emergence of novel color vision in mice engineered to express a human cone photopigment. Science 315:1723–1725.

Jeffery WR. 2001. Cavefish as a model system in evolutionary developmental biology. Dev Biol 231:1–12.

Jeffery WR. 2005. Adaptive evolution of eye degeneration in the Mexican blind cavefish. J Hered 96:185–196.

Jeffery WR. 2008. Emerging model systems in evo-devo: Cavefish and microevolution of development. Evol Dev 10:265–272.

Jeffery WR. 2009. Evolution and development in the cavefish Astyanax. Curr Top Dev Biol 86:191–221.

Klingenberg CP. 2010. Evolution and development of shape: Integrating quantitative approaches. Nat Rev Genet 11:623–635.
Kobayashi M, Nakanishi T. 1999. 11-ketotestosterone induces male-type sexual behavior and gonadotropin secretion in gynogenetic crucian carp. Gen Comp Endocrinol 115:178–187.

Kocher TD. 2004. Adaptive evolution and explosive speciation: The cichlid fish model. Nat Rev Genet 5:288–298.

Kowalko JE, Rohner N, Rompani SB, Peterson BK, Linden T, Yoshizawa M, Kay EH, Weber J, Hoekstra HE, Jeffery WR, Borowsky R, Tabin CJ. 2013a. Loss of schooling behavior in cavefish through sight-dependent and sight-independent mechanisms. Curr Biol 23:1874–1883.

Kowalko JE, Rohner N, Linden TA, Rompani SB, Warren WC, Borowsky R, Tabin CJ, Jeffery WR, Yoshizawa M. 2013b. Convergence in feeding posture occurs through different genetic loci in independently evolved cave populations of Astyanax mexicanus. Proc Natl Acad Sci USA 110:16933–16938.

Laffont R, Renvoisé E, Navarro N, Albert P, Montuire S. 2009. Morphological modularity and assessment of developmental processes within the vole dental row (Microtus arvalis, Arvicolinae, Rodentia). Evol Dev 11:302–311.

Lang HH. 1980. Surface wave discrimination between prey and nonprey by the backswimmer Notonecta glauca L. (Hemiptera, Heteroptera). Behav Ecol Sociobiol 6:233–246.

Leary GP, Allen JE, Burger PL, Lugubilin JB, Linn CE, Macallister IE, Kavanaugh MP, Wanner KW. 2012. Single mutation to a sex pheromone receptor provides adaptive specificity between closely related moth species. Proc Natl Acad Sci USA 109:14081–14086.

Linnen CR, Poh Y-P, Peterson BK, Barrett RDH, Larson JG, Jensen JD, Hoekstra HE. 2013. Adaptive evolution of multiple traits through multiple mutations at a single gene. Science 339:1312–1316.

Lo JC, Vargas JP, Broglio C, Go Y. 2002. Spatial memory and hippocampal pallium through vertebrate evolution: Insights from reptiles and teleost fish. Brain Res Bull 57:499–503.

Ma L, Jeffery WR, Essner JJ, Kowalko JE. 2015. Genome editing using TALENs in blind Mexican cavefish, Astyanax mexicanus. PLoS One in press.

Magurran AE. 1993. Individual differences and alternative behaviours. In: Pitcher TJ, editor. The Behaviour of Teleost Fishes. 2nd Ed. London: Chapman & Hall. pp 441–477.

Mayr E. 1963. Animal Species and Evolution. 1st ed. Cambridge: Belknap Press.

Menuet A, Alunni A, Joly JS, Jeffery WR, Rétaux S. 2007. Expanded expression of sonic hedgehog in Astyanax cavefish: Multiple consequences on forebrain development and evolution. Development 134:845–855.

Mitchell RW, Russell WH, Elliott WR. 1977. Mexican eyeless characin fishes, genus Astyanax: Environment, distribution, and evolution. Texas: Texas Tech Press.

Montgomery JC, Coombs S, Baker CF. 2001. The mechanosensory lateral line system of the hypogean form of Astyanax fasciatus. Environ Biol Fishes 62:87–96.

Moran D, Softley R, Warrant EJ. 2014. Eyeless Mexican cavefish save energy by eliminating the circadian rhythm in metabolism. PLoS One 9:e107877.

Ornelas-Garcia CP, Dominguez-Dominguez O, Doadrio I. 2008. Evolutionary history of the fish genus Astyanax; Girard (1854) (Actinopterygii, Characidae) in Mesoamerica reveals multiple morphological homoplasies. BMC Evol Biol 8: 340. doi: 10.1186/1471-2148-8-340

Parsons KJ, Albertson RC. 2009. Roles for Bmp4 and CaM1 in shaping the jaw: Evo-devo and beyond. Annu Rev Genet 43:369–388.

Partridge BL, Pitcher TJ. 1980. The sensory basis of fish schools: Relative roles of lateral line and vision. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 135:315–325.

Parzefall J. 1983. Field observation in epigean and cave populations of Mexican characid Astyanax mexicanus (Pisces, Characidae). Mém Biospéolé 10:171–176.

Parzefall J. 2001. A review of morphological and behavioural changes in the cave molly, Poecilia mexicana, from Tabasco, Mexico. Environ Biol Fishes 62:263–275.

Penney CC, Volkoff H. 2014. Peripheral injections of cholecystokinin, apelin, ghrelin and orexin in cavefish (Astyanax fasciatus mexicanus): Effects on feeding and on the brain expression levels of tyrosine hydroxylase, mechanistic target of rapamycin and appetite-related hormones. Gen Comp Endocrinol 196:34–40.

Plath M, Rohde M, Schröder T, Taebel-Hellwig A, Schlupp I, . 2006. Female mating preferences in blind cave tetras Astyanax fasciatus (Characidae, Teleostei). Behaviour 143:15–32.

Protas M, Jeffery WR. 2012. Evolution and development in cave animals: From fish to crustaceans. Wiley Interdiscip Rev Dev Biol 1:823–845.

Protas M, Tabansky I, Conrad M, Gross JB, Vidal O, Tabin CJ, Borowsky R. 2008. Multi-trait evolution in a cave fish, Astyanax mexicanus. Evol Dev 10:196–209.

Protas ME, Hersoy C, Kochanek D, Zhou Y, Wilkens H, Jeffery WR, Zon LI, Borowsky R, Tabin CJ. 2006. Genetic analysis of cavefish reveals molecular convergence in the evolution of albinism. Nat Genet 38:107–111.

Rétaux S, Casane D. 2013. Evolution of eye development in the darkness of caves: adaptation, drift, or both? EvoDevo 4:26.

Rétaux S, Pottin K, Alunni A. 2008. Shh and forebrain evolution in the blind cavefish Astyanax mexicanus. Biol Cell 100:139–147.

Sakurai T, Mitsuno H, Haupt SS, Uchino K, Yokohari F, Nishioka T, Kobayashi I, Sezutsu H, Tamura T, Kanzaki R. 2011. A single sex pheromone receptor determines chemical response specificity of sexual behavior in the silkmoth Bombyx mori. PLoS Genet 7:e1002115.

Schemmel C. 1967. Vergleichende Untersuchungen an den Hautsinnesorganen ober- und unterirdisch lebender Astyanax-Formen. Z Morph Tiere 61:255–316.
Schemmel C. 1980. Studies on the genetics of feeding behavior in the cave fish Astyanax mexicanus f. anoptichthys. An example of apparent monofactorial inheritance by polygenes. Z Tierpsychol 53:9–22.

Strecker U, Hausdorf B, Wilkens H. 2012. Parallel speciation in Astyanax cave fish (Teleostei) in Northern Mexico. Mol Phylogenet Evol 62:62–70.

Teyke T. 1990. Morphological differences in neuromasts of the blind cave fish Astyanax-hubbsi and the sighted river fish Astyanax-mexicanus. Brain Behav Evol 35:23–30.

Wada H, Ghyse A, Satou C, Higashijima S-I, Kawakami K, Hamaguchi S, Sakaizumi M. 2010. Dermal morphogenesis controls lateral line patterning during postembryonic development of teleost fish. Dev Biol 340:583–594.

Wada-Katsumata A, Silverman J, Schal C. 2013. Changes in taste neurons support the emergence of an adaptive behavior in cockroaches. Science 340:971–975.

Wainwright PC, Richard BA. 1995. Predicting patterns of prey use from morphology of fishes. Environ Biol Fishes 44:97–113.

Wcislo WT. 1989. Behavioral Environments and Evolutionary Change. Annu Rev Ecol Syst 20:137–169.

West-Eberhard MJ. 1989. Phenotypic plasticity and the origins of diversity. Annu Rev Ecol Syst 20:249–278.

Wilkens H. 1988. Evolution and genetics of epigean and cave Astyanax-fasciatus (Characidae, Pisces)—Support for the neutral mutation theory. In: Hecht MK, Wallace B, editors. Evolutionary Biology. New York: Plenum Publishing Corporation. pp 271–367.

Wilkens H. 2010. Genes, modules and the evolution of cave fish. Heredity (Edinb) 105:413–422.

Wilson EV, Thomas RQ, Evans LM. 2004. Macrobrachium as a possible determinant of Astyanax fasciatus distribution in a neotropical lowland stream. Dartmouth Stud Trop Ecol 2004:99–102.

Wimberger PH, de Queiroz A. 1996. Comparing behavioral and morphological characters as indicators of phylogeny. In: Martins EP, editor. Phylogenies and the comparative method in animal behavior. New York: Oxford University Press. pp 206–233.

Windsor S, Mallinson G, Montgomery JC. 2008. Hydrodynamic imaging by blind Mexican cave fish (Astyanax fasciatus). Comp Biochem Physiol A Mol Integr Physiol 150:S79–S79.

Windsor SP, Norris SE, Cameron SM, Mallinson GD, Montgomery JC. 2010a. The flow fields involved in hydrodynamic imaging by blind Mexican cave fish (Astyanax fasciatus). Part II: Gliding parallel to a wall. J Exp Biol 213:3832–3842.

Windsor SP, Norris SE, Cameron SM, Mallinson GD, Montgomery JC. 2010b. The flow fields involved in hydrodynamic imaging by blind Mexican cave fish (Astyanax fasciatus). Part I: Open water and heading towards a wall. J Exp Biol 213:3819–3831.

Yamamoto Y, Byerly MS, Jackman WR, Jeffery WR. 2009. Pleiotropic functions of embryonic sonic hedgehog expression link jaw and taste bud amplification with eye loss during cavefish evolution. Dev Biol 330:200–211.

Yamamoto Y, Espinasa L, Stock DW, Jeffery WR. 2003. Development and evolution of craniofacial patterning is mediated by eye-dependent and -independent processes in the cavefish Astyanax. Evol Dev 5:435–446.

Yamamoto Y, Jeffery WR. 2000. Central role for the lens in cave fish eye degeneration. Science 289:631–633.

Yamamoto Y, Stock DW, Jeffery WR. 2004. Hedgehog signalling controls eye degeneration in blind cavefish. Nature 431:844–847.

Yoshizawa M, Ashida G, Jeffery WR. 2012a. Parental genetic effects in a cavefish adaptive behavior explain disparity between nuclear and mitochondrial DNA. Evolution 66:2975–2982.

Yoshizawa M, Goricki S, Soares D, Jeffery WR. 2010. Evolution of a behavioral shift mediated by superficial neuromasts helps cavefish find food in darkness. Curr Biol 20:1631–1636.

Yoshizawa M, Jeffery W, Van Netten S, McHenry M. 2014. The sensitivity of lateral line receptors and their role in the behavior of Mexican blind cavefish (Astyanax mexicanus). J Exp Biol 217:886–895.

Yoshizawa M, Jeffery WR. 2011. Evolutionary tuning of an adaptive behavior requires enhancement of the neuromast sensory system. Commun Integr Biol 4:89–91.

Yoshizawa M, Robinson BG, Duboué ER, Masek P, Jaggard JB, O’Quin KE, Borowsky RL, Jeffery WR, Keene AC. 2015. Distinct genetic architecture underlies the emergence of sleep loss and prey-seeking behavior in the Mexican cavefish. BMC Biol in press.

Yoshizawa M, Yamamoto Y, O’Quin KE, Jeffery WR. 2012b. Evolution of an adaptive behavior and its sensory receptors promotes eye regression in blind cavefish. BMC Biol 10:108.