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REVIEW

Cavefish as a Model System in Evolutionary Developmental Biology

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The Mexican tetra *Astyanax mexicanus* has many of the favorable attributes that have made the zebrafish a model system in developmental biology. The existence of eyed surface (surface fish) and blind cave (cavefish) dwelling forms in *Astyanax* also provides an attractive system for studying the evolution of developmental mechanisms. The polarity of evolutionary changes and the environmental conditions leading to the cavefish phenotype are known with certainty, and several different cavefish populations have evolved constructive and regressive changes independently. The constructive changes include enhancement of the feeding apparatus (jaws, taste buds, and teeth) and the mechanosensory system of cranial neuromasts. The homeobox gene *Prox 1*, which is expressed in the expanded taste buds and cranial neuromasts, is one of the genes involved in the constructive changes in sensory organ development. The regressive changes include loss of pigmentation and eye degeneration. Although adult cavefish lack functional eyes, small eye primordia are formed during embryogenesis, which later arrest in development, degenerate, and sink into the orbit. Apoptosis and lens signaling to other eye parts, such as the cornea, iris, and retina, result in the arrest of eye development and ultimate optic degeneration. Accordingly, an eye with restored cornea, iris, and retinal photoreceptor cells is formed when a surface fish lens is transplanted into a cavefish optic cup, indicating that cavefish optic tissues have conserved the ability to respond to lens signaling. Genetic analysis indicates that multiple genes regulate eye degeneration, and molecular studies suggest that *Pax6* may be one of the genes controlling cavefish eye degeneration. Further studies of the *Astyanax* system will contribute to our understanding of the evolution of developmental mechanisms in vertebrates.

***Key Words: Astyanax mexicanus*; cavefish; evolution; development; eye degeneration; model system.**

INTRODUCTION

The recent resurgence of evolutionary developmental biology is a consequence of two major factors. First, the analytical value of comparative developmental biology has been rediscovered; that is, valuable insights are obtained by comparing developmental processes in closely related organisms. Second, it is now understood that diverse metazoan animals can employ the same or similar genes to control key developmental processes, such as germ cell formation, gastrulation, axis determination, segmentation, muscle, limb, and eye formation, and many others. Despite these molecular similarities, embryos of closely related species can develop into larvae or adults with distinct morphologies. A case in point is the variety of morphotypes exhibited by congeneric marine invertebrate larvae (Jägersten, 1972; Strathmann, 1978; Raff, 1996; Jeffery, 1997).

In generating developmental novelty, organisms have evolved different ways to use the same genes. Examples of such strategies include gene duplication and functional divergence (Holland *et al.*, 1994), changing the expression pattern of single copy genes by mutating their *cis*-regulatory regions, or modifying their *trans*-acting control factors (Sucena and Stern, 2000), and co-opting entire gene cascades for new functions (Keys *et al.*, 1999). These modifications are likely to involve complex enterprises, such as rewiring of genetic circuitry underlying developmental pathways (Arnone and Davidson, 1997).

The understanding of how regulatory genes mediate the evolution of development should be simplified by studying closely related species with distinct developmental differences, or even better, a single species in the process of evolutionary diversification. Experimental models of this

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kind have been useful for studying evolutionary developmental biology in invertebrates (Jeffery and Swalla, 1992; Raff, 1992; Schneider *et al*., 1992; Sucena and Stern, 2000; and others), but are not well developed in vertebrates.

I review here the teleost *Astyanax mexicanus*, a single species with surface and cave-dwelling forms, as a model system to study the evolution of developmental mechanisms. This review will describe the current understanding of constructive and regressive developmental changes that have occurred during the evolution of blind cavefish from a sighted surface fish ancestor.

ADVANTAGES OF CAVE ANIMALS

It is worthwhile to discuss some of the advantages that cave animals offer for research in evolutionary developmental biology. Cave animals are sometimes dismissed as entirely degenerate and unable to provide useful information on evolutionary novelty. This is a false assumption. Actually, cave animals have evolved many constructive features, including longer life spans, specialized appendages, hypersensitive olfactory systems, and revamped gustatory and mechanosensory systems (Poulson and White, 1969; Culver, 1982). Thus, both constructive and regressive developmental processes and possible tradeoffs and compensations between them can be investigated in cave animals.

Because cave animals are derived from surface-dwelling ancestors, the polarity of evolutionary change is known with certainty. For example, it is clear that eyes have been lost and that embryonic eye primordia are degenerate vestiges rather than emerging novelties in cave animals. Knowledge of the direction of developmental changes reduces the degree of dependence on phylogenetic hypotheses to reconstruct the evolutionary history of cave animals.

The surface-dwelling ancestors of many cave forms have become extinct, leaving their underground derivatives as the sole representatives of the taxon. When the ancestral form has remained extant, a direct comparison can be made between the ancestral and derived developmental states. This relationship exists in a few cases, in the isopod *Asellus aquaticus* (Kosswig and Kosswig, 1940), the amphipod *Gammarus minus* (Gootch and Hetrick, 1979), and *Astyanax mexicanus*, but could be more common than generally appreciated (Laing *et al*., 1976; Almeida-Toledo *et al*., 1992).

Surface-dwelling species have occasionally invaded caves more than once in their evolutionary history and have given rise to multiple cave-adapted lineages. Each of these lineages represents a replicate experiment in evolutionary developmental biology. The *Astyanax* system is a classic example of this phenomenon (Mitchell *et al*., 1977). The existence of independently derived cave forms in *Astyanax* provides an opportunity to examine the developmental mechanisms underlying parallelism and convergence.

The perpetual darkness of caves leads to limitations in food, spatial orientation, and reproduction, which have generated a suite of specific adaptations. Thus, the evolution of development in cave animals can be studied in the context of specific environmental changes and their corresponding adaptive responses.

THE MEXICAN TETRA

The Mexican tetra *Astyanax mexicanus* consists of the eyed surface form (surface fish), which is widely distributed in northeastern Mexico and south Texas, and at least 29 different eyeless cave populations (cavefish; Figs. 1A and 1B). The surface form and six different cave forms are currently maintained as breeding populations at the University of Maryland (Jeffery and Martasian, 1998). *Astyanax* is easy to maintain in the laboratory, breeds readily, and exhibits many of the other favorable attributes that have made the zebrafish an excellent model system in developmental biology (Driever *et al*., 1994). These features include external fertilization, large numbers of spawn, transparent eggs and embryos, rapid development, a 3–6 month generation time, and the opportunity of genetic analysis. The surface and cave forms are completely interfertile; matings can be made between surface fish and cavefish as well as among the different cave-dwelling populations (Sadoglu, 1957; Wilkens, 1971). The Mexican tetra and the zebrafish are members of the Order Cypriniformes (Fig. 1C). Other teleosts commonly used as models in developmental biology and related areas, such as the pufferfish *Fugu* (Brenner *et al*., 1993) and the medaka (Ishikawa, 2000), are more distant relatives of the cypriniformes. This relationship allows technologies to be shared and genes already cloned in zebrafish to be obtained from *Astyanax* in short order.

The first *Astyanax* cavefish were discovered in limestone caverns in northeastern Mexico (Hubbs and Innes, 1936; Alvarez, 1946, 1947). Although initially described as three different species (*Anoptichthys jordani*, *A. antrobius*, and *A. hubbsi*, respectively) in a unique genus, the interfertile surface and cave forms are now considered as morphotypes of the same species, *Astyanax mexicanus* (Avise and Selander, 1972; Mitchell *et al*., 1977; Kirby *et al*., 1977). More recently, 26 additional cavefish populations have been reported with varying degrees of eye and pigment reduction (Wilkens and Burns, 1972; Mitchell *et al*., 1977). The antiquity of these cavefish populations is estimated to be from 10,000 to 1 million years (Avise and Selander, 1972; Chakraborty and Nei, 1974; Mitchell *et al*., 1977). Genetic (Wilkens, 1971), biogeographic (Mitchell *et al*., 1977), and phylogenetic (Borowsky and Espinaza, 1997; Dowling *et al*., 2001) evidence suggests that some of the cavefish populations evolved independently. For example, a cross between two geographically isolated cavefish populations can result in F1 progeny with a greater degree of eye development than exhibited by either parent (Wilkens, 1971), indicating that mutations in different genes are involved in eye regression.

TABLE 1
Constructive and Regressive Changes in *Astyanax* Cavefish

Feature	Change	Reference(s)
Larval jaw	Constructive	Figs. 2B, 2C
Maxillary teeth	Constructive	D. Stock (personal communication)
Taste buds	Constructive	Breder and Rasquin (1943); Schemmel (1967, 1974); Jeffery *et al.* (2000)
Cranial neuromasts	Constructive	Schemmel (1967); Teyke (1990); Jeffery *et al.* (2000)
Telencephalon	Constructive	Peters *et al.* (1993)
Eyes	Regressive	Cahn (1958); Zilles *et al.* (1983); Langecker *et al.* (1995)
Cornea	Absent	
Iris	Absent	
Anterior chamber	Absent	
Lens	Degenerate	
Posterior chamber	Absent	
Neural retina	Small and distorted	
RPE	Rudimentary	
Sclera	Small	
Optic nerve	Small degenerate	
Optic Tectum	Regressive	Voneida and Fish (1984); Peters *et al.* (1993)
Infraorbital Bones	Constructive	Breder (1944); Alvarez (1946); Jeffery and Yamamoto (2000)
Pineal Gland	Regressive (partial)	Omura (1975); Herwig (1976); but also see Tabata (1982)
Vertebrae	Regressive	Wilkens (1988)
Scales	Regressive	Wilkens (1988)
Pigmentation	Regressive	Breder and Rasquin (1943); Rasquin (1947); Wilkens (1970a); McCauley and Jeffery (2000)
Egg size (Yolk)	Constructive	Hüppop and Wilkens (1991)
Fat content	Constructive	Rose and Mitchell (1982)
Metabolism	Regressive	Hüppop (1986)
Schooling behavior	Regressive	Parzefall and Fricke (1991)
Aggressive behavior	Regressive	Burchards *et al.* (1985)
Circadian activity	Regressive	Wilkens (1988)

EVOLUTIONARY CHANGES IN CAVEFISH

The constructive and regressive changes in cavefish are described in Table 1. Other features are unmodified in cavefish. For example, the olfactory (Riedel and Krug, 1997) and auditory (Popper, 1970) systems are not enhanced to compensate for blindness. Table 1 should not be considered to be inclusive, however, because many parameters, particularly those related to embryonic development, have not been studied in detail.

General Developmental Features

Cave animals usually have much larger eggs that develop more slowly than their surface counterparts (Poulson and White, 1969). However, *Astyanax* cavefish eggs are only slightly (15%) larger in diameter and their rate of development is virtually the same as surface fish (Hüppop and Wilkens, 1991). Most of the increase in egg volume is accounted for by yolk, although there are no differences in yolk composition in the two types of eggs. Until the beginning of eye formation (see below), there are no visible differences between surface and cavefish embryos. The early developmental stages are comparable to those of zebrafish (Langeland and Kimmel, 1997): epiboly begins about 4 h; a tailbud is formed by 10 h; somitogenesis occurs between 10 and 24 h, and embryos hatch at 24 h after fertilization. The first constructive and regressive features appear just before hatching and become increasingly evident as cavefish embryos develop into young fry.

Constructive Changes

Feeding apparatus. The jaws, taste buds, and teeth have been refined or increased in number in cavefish (Table 1). The cavefish larval jaw is wider and more protruding than the surface fish jaw (Figs. 2B and 2C). Jaw modifications are accompanied by larger maxillary bones, which ossify earlier during cavefish development (Fig. 2C), an example of heterochrony. Cavefish also have 3–4 teeth per maxillary bone, whereas 1–2 maxillary teeth are present in surface fish (Table 1). These modifications in feeding structures have converged in different cavefish populations and are likely to be under the influence of strong selective pressure.

Taste buds can be detected in *Astyanax* by their characteristic rosette morphology and by staining with antibodies against the Ca^{+2}-binding protein calretinin (Fig. 2D) or the homeodomain transcription factor Prox 1 (Fig. 2F; Jeffery *et al.*, 2000). The first recognizable taste buds are formed

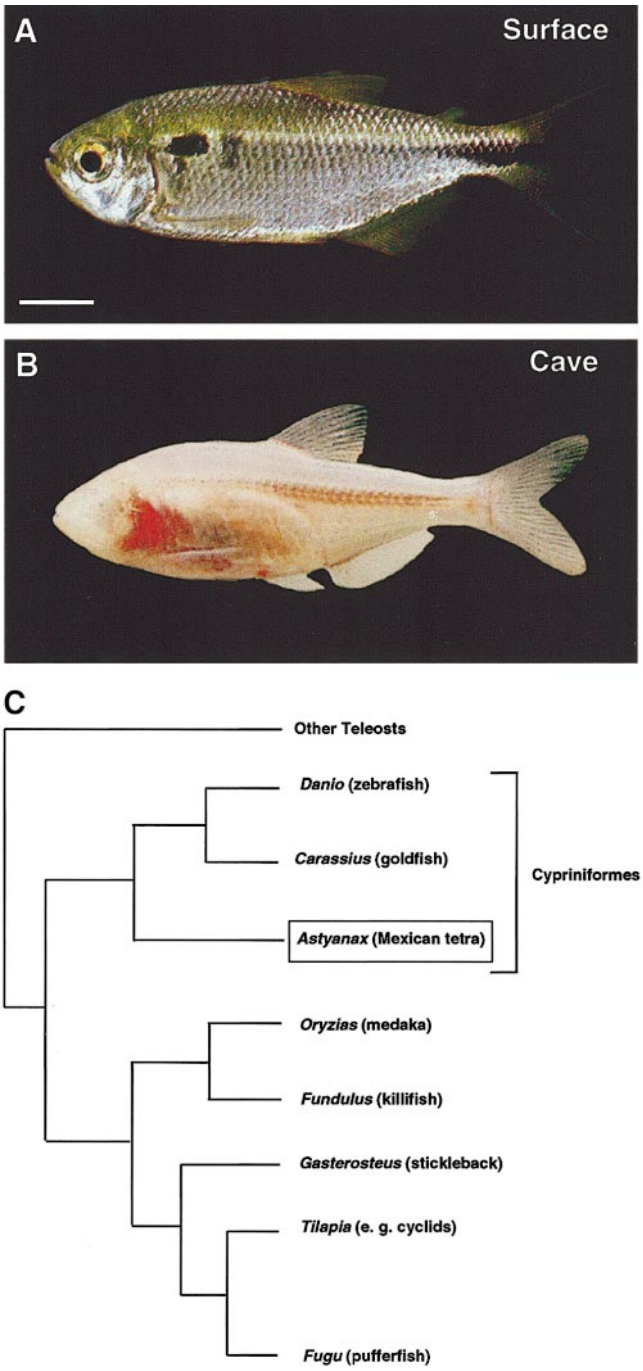

FIG. 1. *Astynanx mexicanus.* Eyed surface fish (A) and blind cavefish (B). Scale bar: 1 cm. From Jeffery *et al.* (2000). (C) The phylogenetic relationship of teleosts commonly used in developmental biology. See Lauder and Liem (1989) and Nelson (1994) for more detailed phylogenies.

about 2 days after fertilization, before the mouth opens and fry begin to feed. There are no differences in the structure of individual taste buds in surface fish and cavefish (Jeffery *et al.*, 2000). In adult surface fish, taste buds are present in the mouth and pharynx, on the lips, and to a lesser extent on the external surface of the head. In cavefish, the number of taste buds is increased several fold in all these areas, but they are especially prevalent on the ventral surface of the head, perhaps explaining the efficiency of cavefish in bottom feeding (Schemmel, 1980). The enhancement in taste-bud number is matched by an increase in the size of the forebrain (Table 1), which contains the teleost gustatory center.

Genetic studies indicate that multiple genes control taste bud expansion in cavefish (Schemmel, 1967, 1974, 1980). The *Prox 1* gene is expressed in developing taste buds and may function in the genetic pathway leading to gustatory development. In other vertebrates, *Distalless-3* (*Dlx-3*), *sonic hedgehog*, *patched*, and *Gli1* (Morasso *et al.*, 1995; Bitgood and McMahon, 1995; Hall *et al.*, 1999) are expressed during taste-bud development, although these genes have yet to be studied in *Astyanax* taste buds.

The oral taste receptor cells develop intrinsically from the endodermal epithelia in vertebrates (Northcutt and Barlow, 1998). In *Drosophila*, the *Prox 1* homologue *prospero* is involved in a fate specification process involving lateral inhibition by Notch and its ligands (Artavanis-Tsakonas *et al.*, 1995). The expression of *Prox 1* in *Astyanax* taste buds brings up the possibility that the Notch specification system may be involved in expanded taste bud development in cavefish.

Neuromasts. Cavefish exhibit an increased number of cranial neuromasts relative to their surface fish counterparts (Table 1; Fig. 2E). The cavefish neuromasts are also larger and have longer cupulae (Teyke, 1990), the sensory hair cell-containing elements that are responsive to environmental perturbations. In contrast to distantly related cavefish (*Amblyopsis spelaea*; Poulson and White, 1969), however, *Astyanax* cavefish apparently have not enhanced their lateral line systems (Wilkens, 1988). In fact, the lateral line and cranial neuromast systems are interrupted in *Astyanax* cavefish (Wilkens, 1988). The cranial neuromasts are formed concurrently with taste buds early in development and their sensory hair cells express the *Prox 1* gene (Jeffery *et al.*, 2000). There is about a twofold increase in the number of Prox 1 expressing hair cells in cavefish cranial neuromasts (Figs. 2G and 2H). The lateral fate specifying mechanisms involving Prox 1 also may be adjusted during cavefish neuromast development.

Craniofacial structure. Craniofacial morphology is altered in cavefish with changes especially notable in infraorbital bone structure (Fig. 2A). Surface fish have seven infraorbital bones, which are morphologically similar in all specimens. In contrast, different cavefish populations show changes in the size, shape, and number of infraorbital bones, which may be related to their antiquity. The third infraorbital bone (see arrowheads in Fig. 2A) is most highly modified in different cavefish populations, ranging from fusion with the fourth infraorbital bone to separation into as many as 10 individual infraorbital bones (Alvarez, 1947). The infraorbital

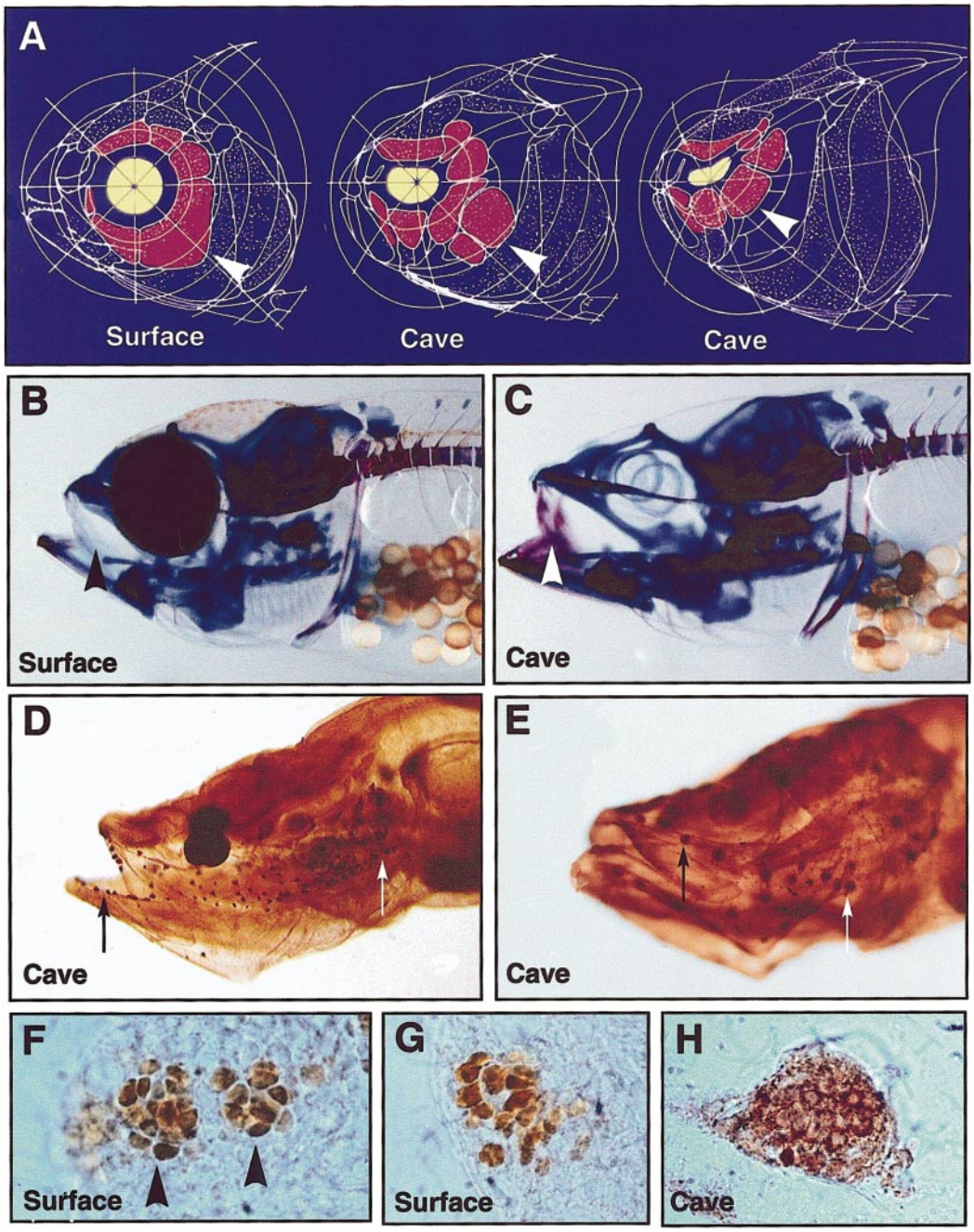

FIG. 2. Constructive evolutionary changes in cavefish craniofacial skeleton (A), jaws (B–C), taste buds (D, F), and head neuromasts (E, G, H). (A) Increase in number and morphological divergence of infraorbital bones (red) in cavefish. The transformation of the craniofacial skeleton from the surface fish to the cavefish phenotypes is shown by polar coordinates centered on the orbit (yellow). White arrowheads show the third infraorbital bone(s). Adapted from Breder (1944). (B, C) Alcian blue (cartilage) and alizarin red (bone) stained surface fish (B) and cavefish (C) fry showing jaw protrusion and premature ossification of the maxilla (arrowheads) in cavefish. (D) Taste buds stained with anti-calretinin (arrows) in a cavefish fry. (E) Head neuromasts stained with Prox 1 antibody (arrows) in a cavefish fry. (F) Prox 1 expression in surface fish taste bud primordia (arrowheads). (G, H) Prox 1 expression in the sensory hair cells of surface fish (G) and cavefish (H) cranial neuromasts. (D–H) From Jeffery *et al.* (2000).

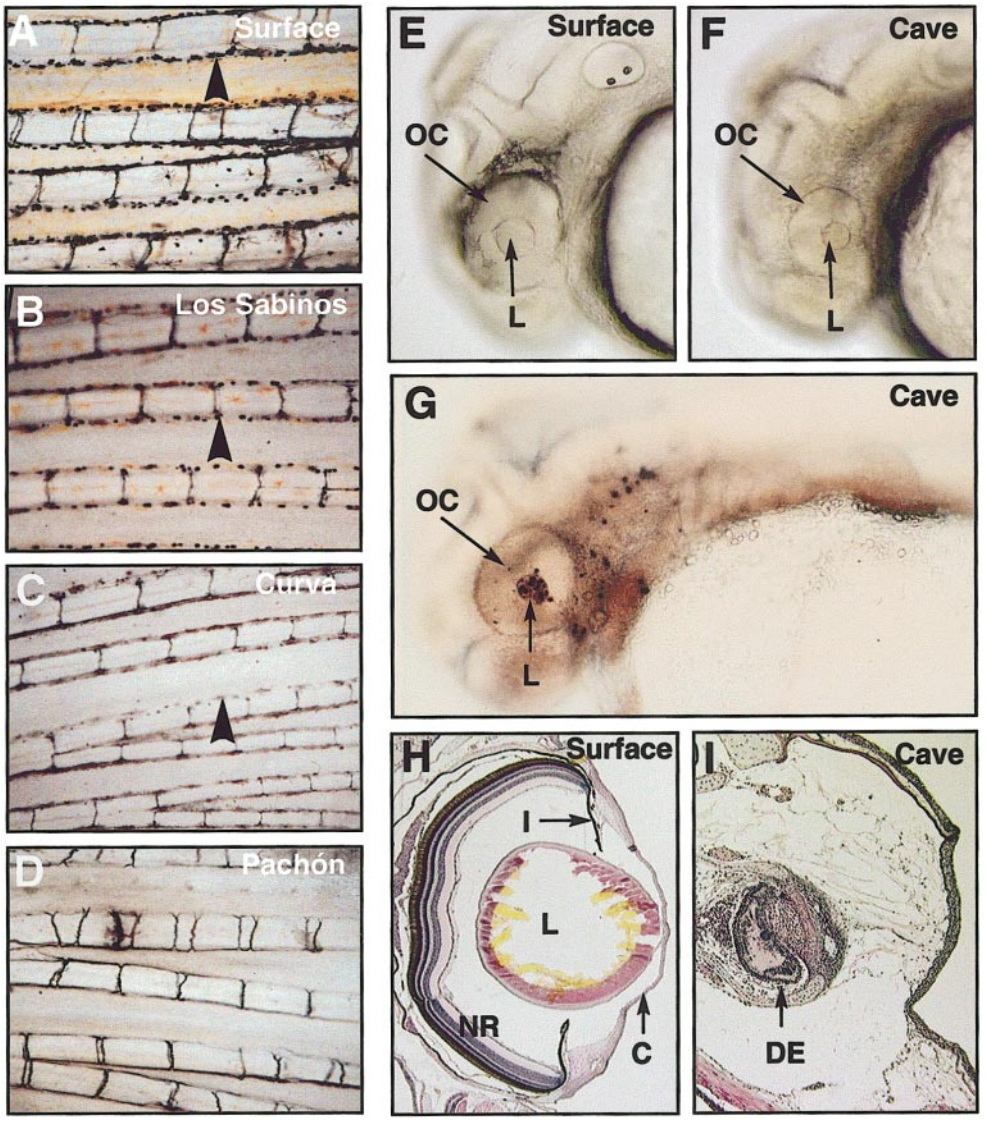

FIG. 3. Regressive evolutionary changes in cavefish pigmentation and eyes. (A–D) Pigment regression. Tail fin rays in surface fish (A) and three cavefish populations (B–D). Los Sabinos (B), Curva (C), and Pachón (D) cavefish showing progressive loss of melanophores (arrowheads). (E–F) Eye primordia of 24-h-old surface fish (E) and cavefish (F) embryos, showing the diminished cavefish eye primordium. From Yamamoto and Jeffery (2000). (G) A cavefish embryo showing lens apoptosis detected by the TUNEL assay. From Jeffery and Martasian (1998). (H, I) Structure of normal and degenerate eyes in the surface fish (H) and cavefish (I) adults. From Yamamoto and Jeffery (2000). OC, optic cup; L, lens; I, iris; NR, neural retina; C, cornea; DE, degenerate eye.

bones are dermal bones that are probably induced late in development by interactions between cranial neural crest and underlying neural tissues. According to Jeffery and Yamamoto (2000), changes in cavefish infraorbital bone structure are caused by (1) the expansion of dermal bone ossification into the empty orbital space and (2) the formation of additional ossification centers during craniofacial development. The formation of new ossification centers may be related to the fragmentation of cranial lateral line elements (Schemmel, 1967), the most likely seeding centers of dermal bone ossification. The relationship between infraorbital bone and eye development will be an interesting topic for further study in the *Astyanax* system.

Regressive Changes

Regressive changes have been the most extensively studied of the peculiar features of cave animals. Table 1 lists the

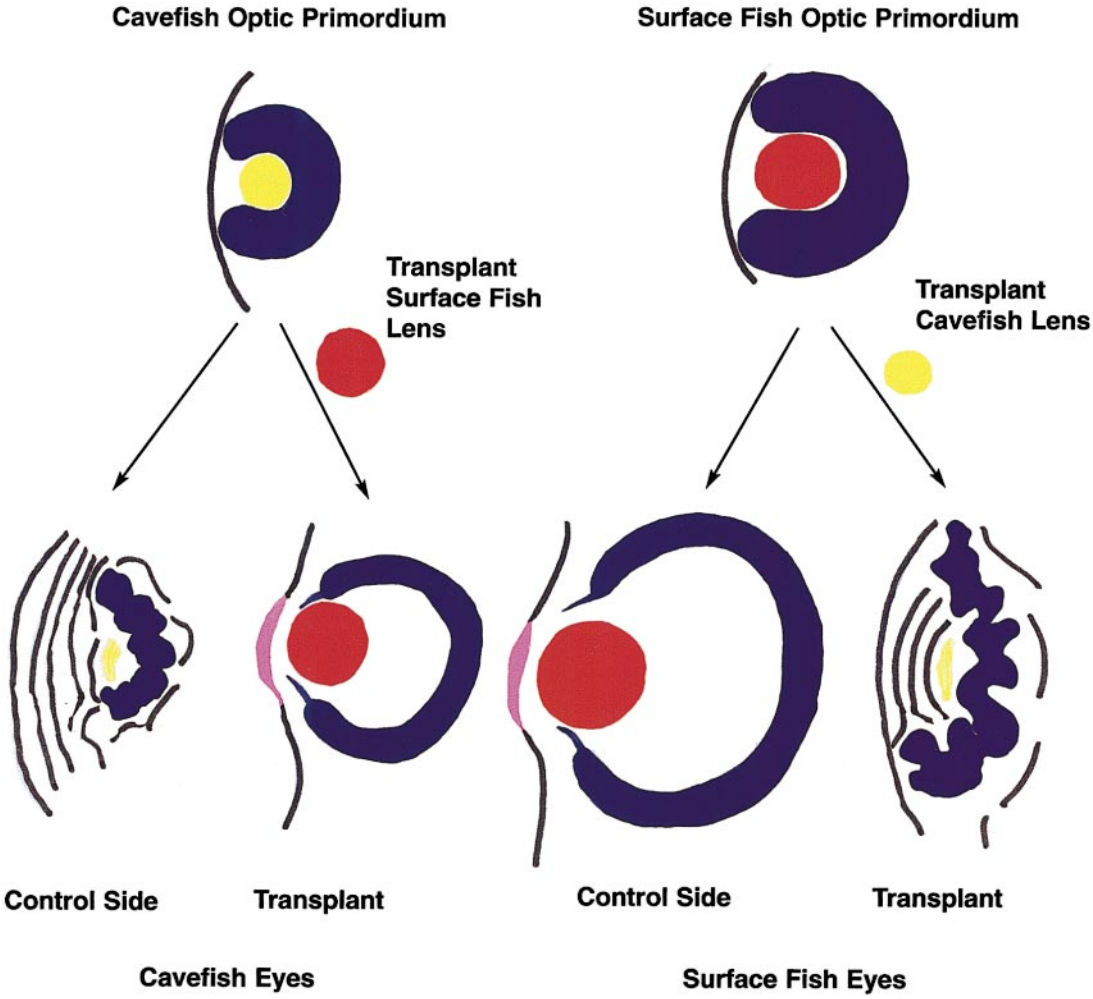

FIG. 4. The results of transplanting a surface fish lens vesicle into a cavefish optic cup and vice versa during embryonic development. Top: Optic primordia showing the lens vesicle in yellow (cavefish) or red (surface fish), the optic cup in blue, and the surface ectoderm in black. Bottom: Eyes that develop on the transplant and unoperated control sides of adult cavefish (left) and surface fish (right) hosts after transplantation of a surface fish (red) or cavefish (yellow) lens vesicle (middle). In adults: lens (red), region of degenerate lens (yellow), neural retina, and degenerate neural retina (blue), integument, including layers of skin surrounding degenerate eye (black) and cornea (pink). See Yamamoto and Jeffery (2000) for details.

major regressive changes in *Astyanax* cavefish. We will be concerned here with pigment and eye regression, although other regressed traits, such as the loss of thoracic vertebrae in some cavefish populations (Table 1), are also of considerable interest in evolutionary developmental biology.

Pigmentation. Body pigmentation in teleosts is due to three types of dermal chromatophores: the black, melanin-containing melanophores; the silver, purine-containing iridophores; and the yellow, pteridine-containing xanthophores. There is a dramatic decline in the total number of melanophores (Figs. 3A–3D) and a reduction or elimination of the capacity of these cells to synthesize melanin in cavefish. The loss of melanin is based on a recessive mutation in a single gene (Sadoglu, 1955). The iridophores and xanthophores have not been studied in detail, although some cave populations are reported to have a reduced amount of guanine in iridophores.

Genetic analysis indicates that multiple genes are responsible for the reduced numbers of melanophores in cavefish (Wilkens, 1970a). Melanophores and other pigment cells are derived from the melanogenic neural crest cells, which migrate through a dorsolateral pathway between the somites and the overlying epidermis to reach their major sites of differentiation in the yolk sac, dermis, and fins. Dye-tracing studies show that cells migrate into the yolk sac and fin buds from the neural keel, suggesting that melanogenic neural crest cells are present in cavefish embryos (McCauley and Jeffery, 2000). It is possible that the

melanogenic neural crest lineage may be diverted to other types of pigment cells or to a nonpigment cell fate. The *Astyanax* system offers an attractive opportunity to study evolutionary changes in development of the neural crest.

Eyes. Eye degeneration has a developmental basis in cavefish. Optic primordia are formed during cavefish embryogenesis, but they are smaller than their surface fish counterparts and eventually arrest in development, degenerate, sink into the orbit, and are covered by a flap of skin (Figs. 3E–3F, 3H–3I). The events and timing of eye development are similar in surface fish and cavefish. Eye formation is heralded by the protuberance of the optic vesicles. Eventually the lens placode, which has developed as a thickening of the surface ectoderm, buds into the optic cup, which invaginates from the optic vesicle.

Changes in the cavefish optic primordia begin during the next phase of eye formation, which involves differentiation of the lens, neural retina, and retinal pigment epithelium (RPE). The lens epithelial cells produce the primary fiber cells, which begin to elongate and synthesize crystallin mRNAs (Jeffery *et al*., 2000; also see Behrens *et al*., 1998), but they do not terminally differentiate into lens fiber cells. The secondary fiber cells are not formed in the cavefish lens, which undergoes extensive apoptosis (Jeffery and Martasian, 1998). Probably as a consequence of lens degeneration (see below), the cornea, pupil, and iris do not appear in the cavefish eye. At first, the neural retina develops normally, forming neuronal and glial cell layers from an active ciliary marginal zone (where new retinoblasts are formed) in a fashion similar to the surface fish. Although opsin gene expression begins, it terminates abruptly (Yokoyama and Yokoyama, 1990; Langecker *et al*., 1995) and photoreceptor cells do not differentiate. Later, local zones of extensive cell death also appear in the neural retina, which is retarded in growth during cavefish development (Langecker *et al*., 1993). The cavefish optic nerve is rudimentary, and the optic lobes are shrunken in the absence of visual input. Finally, although the RPE becomes pigmented, it appears to be abnormal in structure.

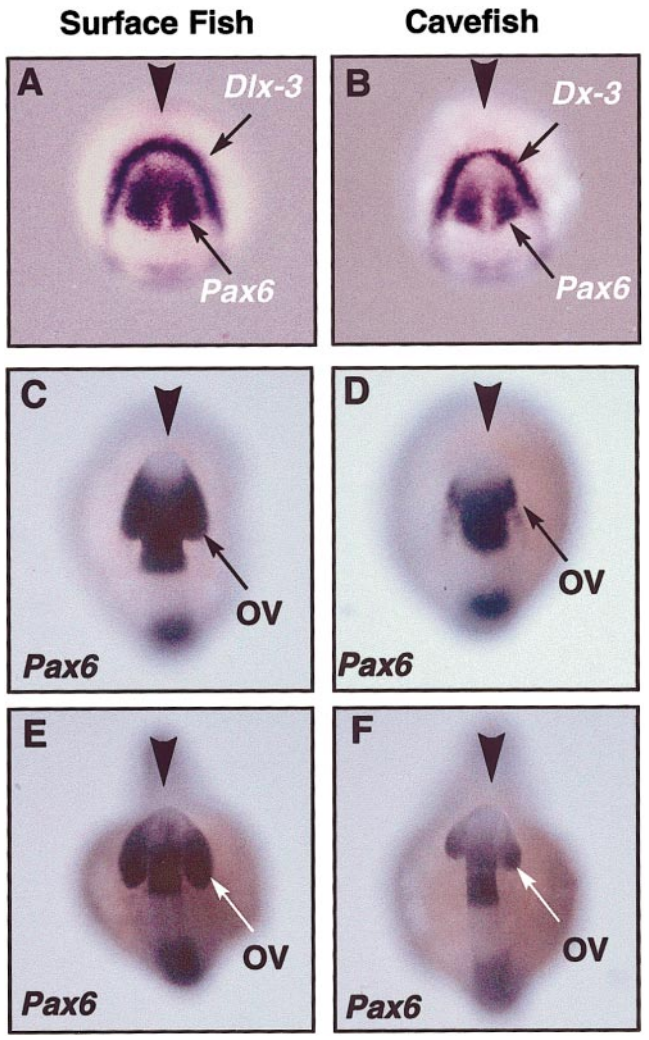

FIG. 5. *Pax6* expression is changed in early cavefish embryos. (A–B) The anterior *Pax6* expression domains corresponding to the eye primordia are small and lack a connection across the anterior midline of the neural plate in cavefish (B) relative to surface fish (A) embryos. The embryos are *in situ* hybridized with probes for *Pax6* and *Dlx-3*, which marks the anterior margin of the neural plate (Witlock and Westerfield, 2000). (C–F) After neurulation, cavefish and surface fish embryos differ in the size of the *Pax6*-expressing optic vesicles (OV). (A–B) Late tailbud stage. (C–F) Five- (C, D) and 18 (E, F) somite stages. From Strickler *et al*. (2001).

CONTROL OF EYE DEGENERATION

Role of the Lens Vesicle

The lens vesicle is a prime candidate for regulating eye regression because of its early degeneration in cavefish. Apoptosis could be controlled by processes within the lens or by signals emanating from tissues outside the lens, such as the optic cup. Yamamoto and Jeffery (2000) studied apoptosis after reciprocal transplantation of a surface fish lens vesicle into the cavefish optic cup and vice versa. The cavefish lens vesicle undergoes apoptosis on schedule after transplantation into the surface fish host. Conversely, the surface fish lens vesicle does not undergo apoptosis and is able to differentiate into a crystalline lens in the cavefish host. Thus, apoptosis is controlled autonomously within the lens vesicle. However, this result does not preclude the possibility that earlier interactions between the presumptive lens ectoderm, the optic vesicle, or other embryonic tissues may be responsible for specifying lens apoptosis.

The lens and other eye parts undergo reciprocal signaling during eye formation (Breitman *et al*., 1987; Landel *et al*., 1988; Quinn *et al*., 1996). Classic experiments have shown that the lens is important for differentiation of the anterior part of the eye and growth of the eyeball (Coulombre, 1965). The success of the lens transplantation experiments opened

the possibility of studying these interactions during cavefish development (Yamamoto and Jeffery, 2000). The experiments and results are summarized in Fig. 4. When a surface fish lens was transplanted into a cavefish optic cup, eye degeneration was prevented and an eye was restored in the host. The restored eye exhibited an anterior chamber, an iris, a cornea, and a retina containing differentiated photoreceptor cells: features that are missing in cavefish. Conversely, when a cavefish lens was transplanted into a surface fish optic cup, or the surface fish lens was extirpated, the cornea, anterior chamber, and iris failed to differentiate, retinal growth was retarded, and the eye sunk into the orbit. The results of the transplantation experiments indicate that surface fish lens can stimulate cavefish eye growth and differentiation and that cavefish have retained the ability to respond to a lens inductive signal. The persistence of retinal growth and differentiation, albeit at a much slower rate, in the surface fish host (Fig. 4) shows that the lens does not control all aspects of eye formation. Thus, evolutionary changes in the lens are a major (but not the only) cause of cavefish eye degeneration.

The lens transplantation experiments were carried out with a single cavefish population. The lens probably has the same role in other cavefish populations because they also undergo lens apoptosis (Jeffery and Martasian, 1998). However, convergence at the tissue level should not be taken to mean that the same genes are involved in eye regression because changes in many different genes can trigger cell death.

Genes Regulating Eye Regression

Genetic studies have shown that multiple genes control cavefish eye regression. The F1 backcross progeny of surface fish × cavefish matings show a broad distribution of eye sizes, suggesting regulation by at least six to seven genes (Wilkens, 1970b). Several groups are comparing the expression of known eye regulatory genes during surface fish and cavefish development.

The homeobox gene *Prox 1* is involved in the differentiation of lens fiber cells and several retinal cells types during vertebrate development (Tomarev, 1997; Wigle *et al*., 1999). No differences were found in Prox 1 expression during early eye development, suggesting that genetic changes occur either upstream or in a pathway running parallel to *Prox 1* during cavefish eye development (Jeffery *et al*., 2000).

Prox 1 expression is initiated after size differences are already apparent between the surface fish and cavefish optic primordia. In contrast, the *Pax6* gene, which encodes a transcription factor essential for eye development in vertebrates (Chow *et al*., 1999), is expressed before optic vesicle formation. Behrens *et al*. (1997) cloned the *Astyanax Pax6* gene and did not detect changes in *Pax6* mRNA levels in cavefish but the early stages of eye development were not investigated. More recently, Strickler *et al*. (2001) reported two differences in *Pax6* expression in early cavefish embryos (Figs. 5A–5B). First, the *Pax6* expression domains corresponding to eye primordia are smaller in cavefish embryos. Second, there is a gap in *Pax6* expression at the anterior margin of the neural plate in cavefish embryos, suggesting the existence of a more active midline signaling system. These differences precede the formation of smaller optic vesicles in cavefish (Figs. 5C–5F). It is known that midline-signaling genes, such *sonic hedgehog*, suppress *Pax6* and thereby influence the extent of eye development in vertebrates (Macdonald *et al*., 1995; Ekker *et al*., 1995). Although further studies are necessary, these results suggest that constructive (enhanced midline signaling activity) rather than regressive mechanisms may ultimately explain cavefish eye degeneration.

Developmental Steps in Eye Regression

According to current evidence, the steps involved in cavefish eye degeneration are as follows. First, *Pax6* expression is reduced at the anterior midline during neural plate specification. Second, a smaller lens and optic vesicle/cup are formed possibility as a result of earlier *Pax6* suppression. Third, the small cavefish lens undergoes apoptosis instead of differentiation. Fourth, in the absence of lens signaling, the cornea, iris, pupil, and retinal photoreceptor cells fail to develop. Fifth, the eye eventually collapses into the orbit and is covered by a flap of skin. Although further research is needed to fill in the gaps between these steps, it is clear that we now have a blueprint of how eye development has changed during cavefish evolution.

CONCLUSION

This review has shown that cavefish are a rich source of constructive and regressive changes for studying the evolution of developmental mechanisms. While comparative and molecular studies of surface fish and cavefish development are still in their infancy, several general conclusions can be made from the available data. First, apoptosis has been shown to play a key role in the evolution of cavefish eye regression. Apoptosis also plays a major role in vulval evolution and development in nematodes (Sommer and Sternberg, 1996) and in avian digit morphogenesis (Saunders, 1966; Zou and Niswander, 1996), and thus may be a fundamental way of generating rapid morphological diversity. Second, the central role of the lens in cavefish eye degeneration shows how an embryonic inductive activity may be amplified through development to have major consequences in adult morphology. Third, the cavefish studies bring to our attention the possible role of tradeoffs between constructive and regressive processes in evolutionary developmental biology. Further studies of the *Astyanax* system may shed light on the genetic and developmental mechanisms underlying the evolutionary transformation of a surface fish into a cavefish.

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