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ADAPTATION IN CAVE FISH: DARWINIAN OR BY DESIGN?

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Content

Cover image: Cav fish *Astyanax mexicanus*. (H. Zell, CC BY-SA 3.0)

Adaptations in cave fish – Darwinian or by design?

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Introduction

Cave-dwelling animals have long been a popular subject of study in evolutionary research. Blind and colourless cave-dwelling animals are found amongst fish, insects, crustaceans, millipedes, spiders and salamanders (Borowsky 2018, R61). Among cave-dwelling fish alone, approximately 230 species are known worldwide (state as of 2020), occurring on every continent except Europe and Antarctica (Krishnan & Rohner 2017, 1), with more being discovered every year (Maldonado et al. 2020, 464). This article will focus primarily on the best-studied blind cave characin, *Astyanax mexicanus*.

Astyanax mexicanus is a freshwater fish and is particularly well suited to studies of the differences between cave-dwelling and surface-dwelling forms (see Culver 1982, Borowsky 2018). The blind cave fish can be reared in the laboratory just as easily as surface-dwelling fish. As they can be readily crossed despite significant morphological differences – even when the crossing partners are geographically distant (Krishnan & Rohner 2017, 1) – they are classified as belonging to the same species. Originally, the cave form had been classified as a separate genus, Anoptichthys ('fish without eyes') (Hubbs & Innes 1936). At least 34 cave fish populations of *Astyanax* have been discovered, most of them in north-eastern Mexico (North et al. 2025; Shennard [2026] cites the number 35). Genetic studies show that at least five cave-dwelling fish groups have evolved independently from surface-dwelling fish[1] and thus represent excellent models for parallel and convergent evolution[2] (Jeffery 2020; Bradic et al. 2012, 1; Dowling et al. 2002; Strecker et al. 2003). The changes observed in cave populations compared with their surface-dwelling counterparts are remarkably similar across the various groups, even where they have evolved independently (by convergence) (Bradic et al. 2012, 1).[3]

The colonisation of caves represents one of the most extreme changes in habitat (Čupić et al. 2023, 821). Total darkness, a scarcity of food[4] and the presence of only a few predators are the key conditions of cave life. In fact, cave fish differ from surface-dwelling fish in numerous characteristics, as will be explained below. Nevertheless, the development of cave fish characteristics occurred surprisingly quickly (Herman et al. 2018; Fumey et al. 2018).

Cave fish exhibit a high degree of similarity amongst themselves in terms of their sensory, morphological, physiological and behavioural characteristics (**Figs. 1, 2**). An overview of common cave fish characteristics is provided, for example, by Casane & Rétaud (2016, 125f). Typical and striking characteristics of cave tetras are a reduction in eye size (**Fig.** f) and a reduction in pigmentation. Pigmentation serves to protect against sunlight, provide camouflage, enable mimicry (false warning colouration) and facilitate the identification of species and sex (Jeffery 2009b, 38). It is therefore superfluous in the dark caves. However, many physical characteristics have undergone non-degenerative changes: the organisation of the facial skull, differences in the number and shape of the skull bones around the eye and an unusual lateral deformation of the skull (Powers et al. 2020), a large mouth and a greater number of teeth, the enlargement of the jaw, the structure and reduction in size of the brain, and the structure and regenerative capacity of the heart (Moran et al. 2015, 3; Borowsky 2018, R 61; Jeffery 2020). Some cave fish popu-

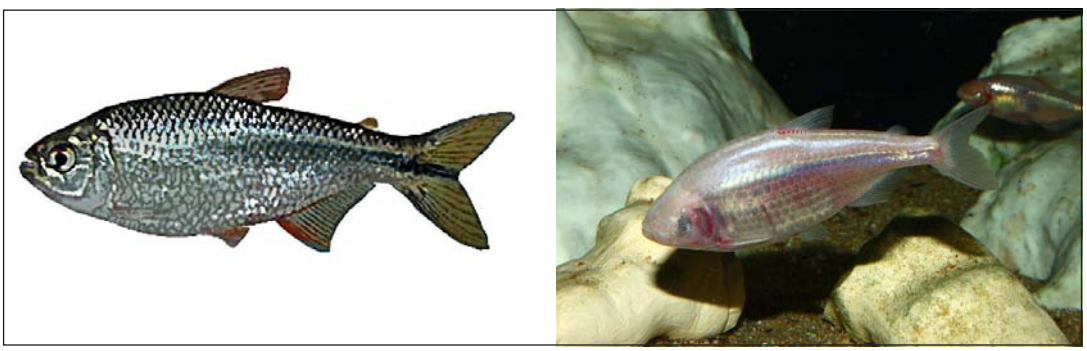

Fig. 1 Surface and cave specimens of *Astyanax mexicanus*. (Art-e-fakt, CC BY-SA 3.0; H. Zell, CC BY-SA 3.0)

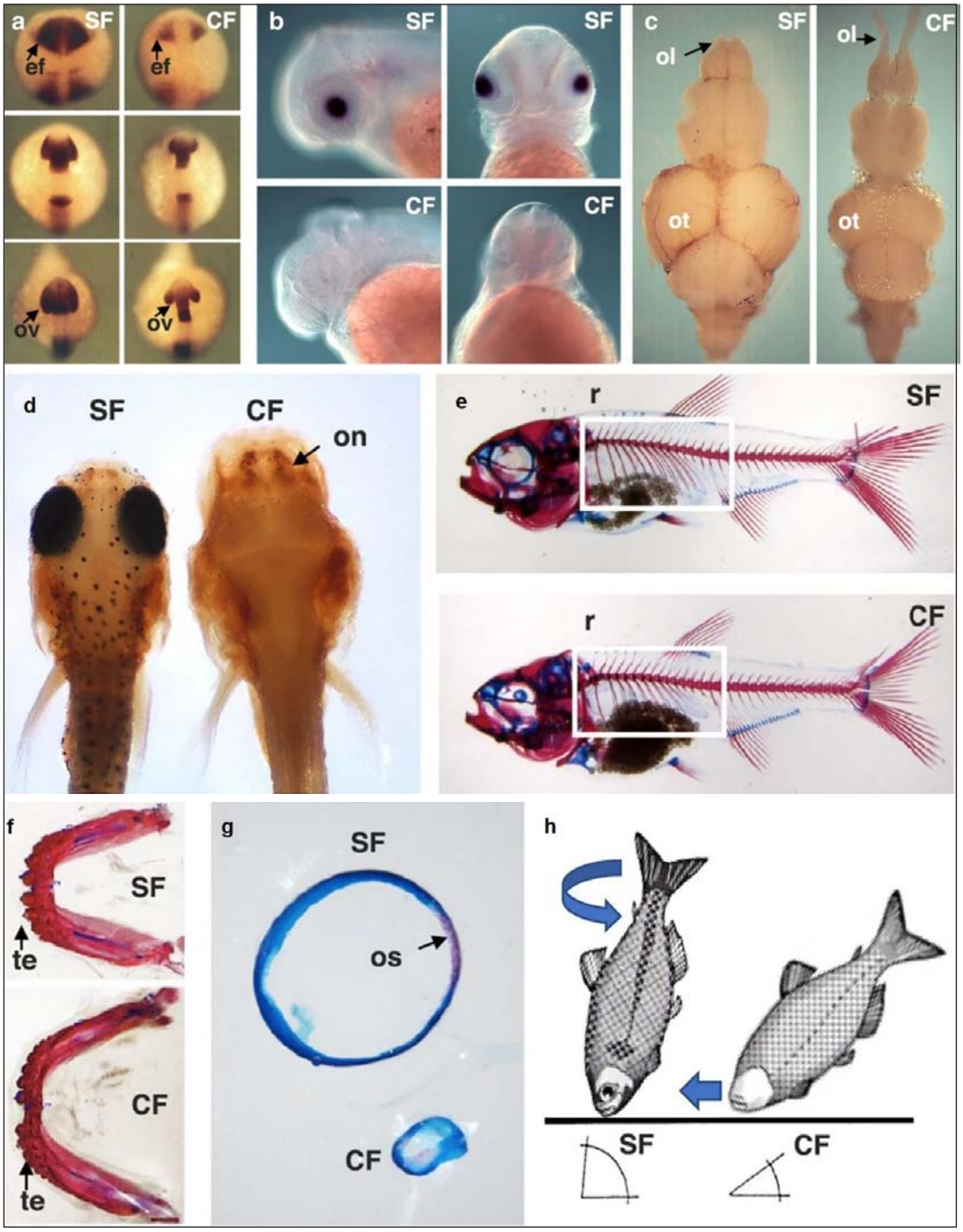

Abb. 2 Examples of morphological differences between surface-dwelling fish (SF) and cave-dwelling fish (CF).
a In situ hybridisation showing differences in *pax6* expression from the neural plate (top) to the late optic nerve stages (bottom). ef eye field, ov optic nerves.
b In situ hybridisation showing differences in αA-crystallin expression in the lens, viewed from the side and from above (40 hours after fertilisation).
c Differences in the morphology of the adult brain, viewed from above. ot midbrain roof (the centre of the brain where nerve impulses from the eyes are processed), ol olfactory lobes.
d Differences in the head morphology of fish larvae (six days after fertilisation). Anti-tyrosine hydroxylase staining reveals differences in the olfactory neuronal regions (on).
e Larval skeletons showing differences in the number of ribs (r and inset).
f Differences in jaw size and the number of teeth in adult animals.
g Isolated sclera, stained for cartilage (blue) and bone (red), showing differences in the size and formation of the ossicles (os).
h Differences in feeding posture in relation to the substrate. (From Jeffery 2020, CC BY 4.0, http://creativecommons.org/licenses/by/4.0)

lations also have fewer ribs than surface-dwelling fish (Jeffery 2020, 6). The density of neuromasts* is increased, but the patterning of the neuromasts is disrupted due to the loss of the eyes (Powers et al. 2020).

In addition, enhancements in sensory functions such as taste, smell or mechanosensory perception are regularly observed to varying degrees (Borowsky 2018, R61; Jeffery 2009a). Furthermore, there are changes in feeding behaviour, altered activity levels, a loss of the day-night rhythm, significantly increased wakefulness (ibid.; North et al. 2025) and reduced aggression (McGaugh et al. 2020, 144). The fish move about more, are better able to locate food through vibration, have feeding behaviour adapted to food scarcity, and feed on the bottom by positioning themselves at a 45° angle to the substrate (compared to a significantly steeper position in surface-feeding fish; Casane & Rétaud 2016, 129).

Cave fish are better able to store fat reserves and possess further metabolic adaptations that enable them to compensate for the limited availability of nutrients; their metabolism is correspondingly lower and more efficient (Čupić et al. 2023, 830; Aspiras et al. 2015; cf. Riddle et al. 2021; Xiong et al. 2018). They also have a longer lifespan, which can likewise be attributed to metabolic changes (Cobham et al. 2025).

Casane & Rétaud (2016, 126) emphasise that cave fish are by no means degenerate species; rather, the combination of numerous (often constructive) adaptations constitutes

Abstract

Cave fish, which live permanently in completely lightless caves, exhibit a number of changes in their body structure. The most striking of these are the regression of the eyes and the reduction or loss of pigmentation. However, there are also enhancements in sensory abilities (taste, smell and mechanosensory perception) as well as a number of anatomical changes (e.g. to the skull, brain and heart) and physiological changes.

Around 35 cave populations of the well-studied tetra *Astyanax mexicanus* are known to exist, mainly in north-eastern Mexico. Genetic analyses show that several populations have adapted to cave life independently of one another and that typical 'cave characteristics' have developed convergently. Despite some differences, these cave characteristics are strikingly similar overall. The causes of these changes are still the subject of controversy. There is some evidence to suggest that there is a mixture of causes. Classic (neo-)Darwinian causes include the selection of mutant forms that are advantageous in the cave environment and deleterious mutations in characteristics that have become superfluous, which spread through gene drift in the populations. More recently, however, there has been increasing evidence that variation already present in surface forms *(standing genetic variation)* plays a major role. There is also evidence of epigenetic control and plastic responses (changes triggered by environmental stimuli). There is also evidence for epigenetic control and plastic responses (changes triggered by environmental stimuli through the activation of variation programmes), e.g. through DNA methylation.

This article presents and discusses the findings that support these different causes. A number of findings support programming and argue against the Darwinian interplay of random mutation and natural selection as the main cause of the development of cave characteristics: the occurrence of convergences, including in the genetic basis of cave characteristics, the pleiotropic effect of some genes, which enable several changes suitable for cave life at the same time, epigenetic regulation and plastic responses. These processes enable coordination and rapid adaptations that would hardly be possible through Darwinian mechanisms. Random mutation and natural selection are therefore only of secondary importance.

what is known as the 'cave fish behavioural syndrome'.[5]

As mentioned, many cave fish characteristics occur repeatedly and independently (convergent evolution). In addition to the striking characteristics of the loss of eyes and pigmentation, examples include the fragmentation or reduction in the number of suborbital bones in *Astyanax* and the curvature of the skull, which has occurred repeatedly at various, mutually independent cave sites, albeit to varying degrees (Powers et al. 2020, 451).

However, not all of these traits are present in every species of cave fish (Borowsky 2018, R60), nor are they expressed in the same way or to the same degree in all populations (Jeffery 2019, 87). For example, there are varying degrees of eye reduction. Different cave fish populations exhibit varying degrees of reduced pigmentation, ranging up to the complete absence of melanophores (melanin-containing cells).[6] Furthermore, different melanin phenotypes are observed. All of this points to several independent origins of the reduced pigmentation (Jeffery 2009a, 208; Jeffery 2009b, 38). Differences are also evident, for example, in adaptations of fat metabolism (Xiong et al. 2018).

The situation is somewhat different for two cave-dwelling species of catfish, *Rhamdia zongolicensis* and *R. reddelli*. They exhibit an intermediate stage of reduction in their biologically non-functional eyes, melanin pigmentation and negative phototactic behaviour (avoidance of light). The surface-dwelling species *Rhamdia laticauda* (**Fig. 4**) is perfectly adapted to life in the dark, despite living in a well-lit environment.

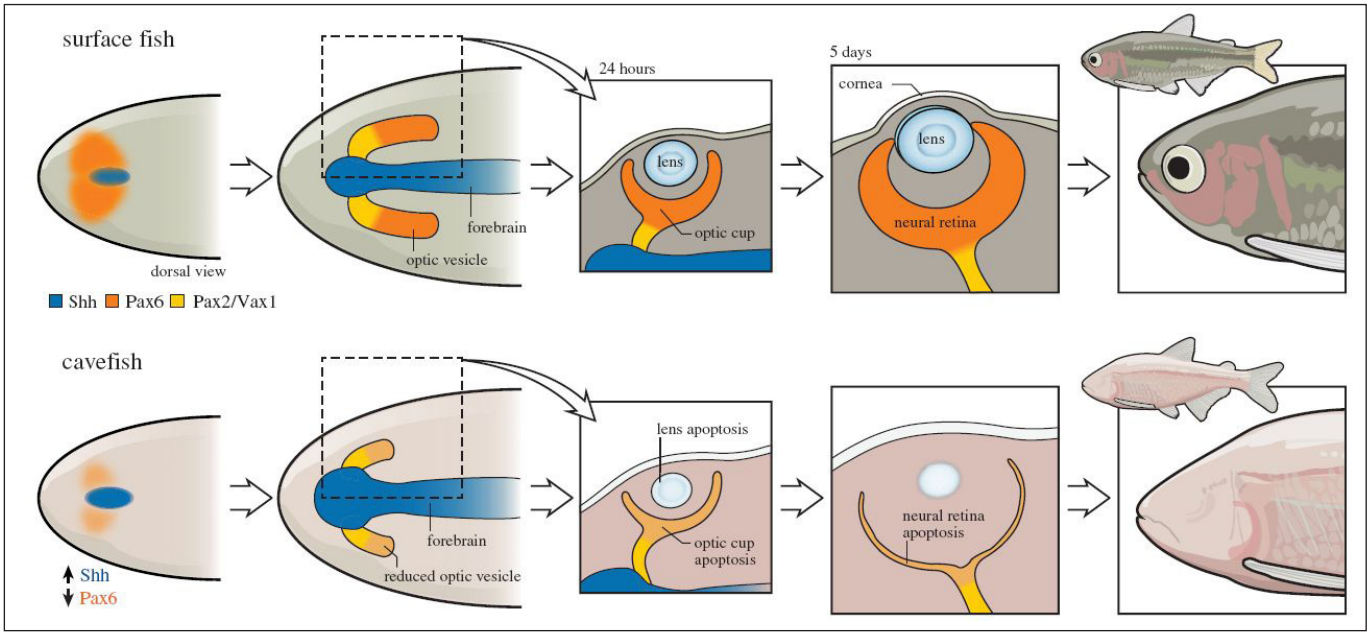

Fig. 3 Eye development in surface-dwelling fish and cave-dwelling fish. **Top:** surface-dwelling fish. The expression patterns of various key genes are colour-coded. **Bottom**: cave-dwelling fish. The concentration of *Pax6* (orange) decreases, whilst the concentration of *Shh* (purple) and *Pax2/Vax1* (yellow) increases. This results in smaller eye cups and longer eye stalks. *Apoptosis* (programmed cell death) of the lens begins around 24 hours after fertilisation, followed by apoptosis of the retina, which ultimately leads to the loss of the eye. (From Krishnan & Rohner 2017, with kind permission)

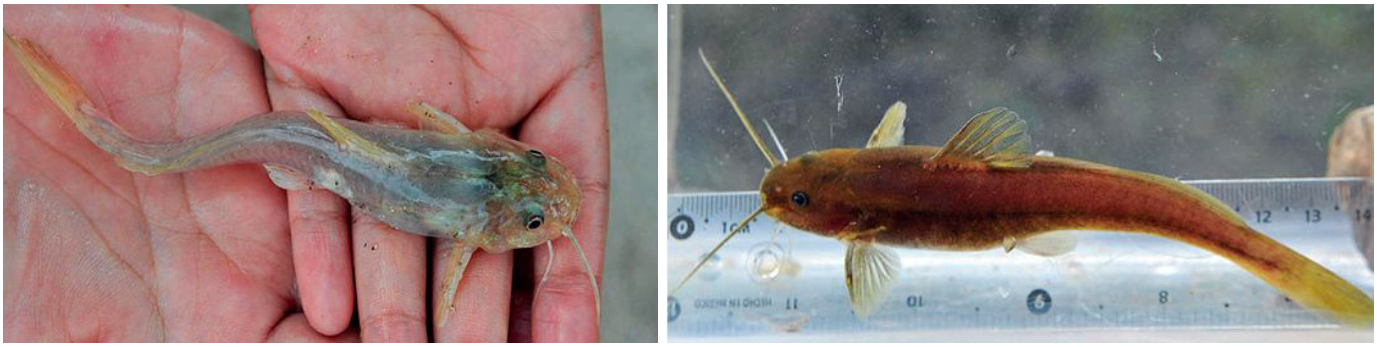

Fig. 4 *Rhamdia laticauda*, on the left in its albino form and on the right with normal colouring. (From Julián-Caballero et al. 2023, CC BY 4.0)

Accordingly, the cave-dwelling species exhibit only a few structural adaptations compared to the surface-dwelling species, which are related to the limited food supply in the caves: The barbels are elongated, which enhances the senses of taste and touch, and more fat can be stored in the tissue of the cave fish. Geological data suggest that the two cave fish species began their evolutionary changes at the end of the Pleistocene and are therefore relatively recent (Wilkens 2001). The changes in the *Rhamdia* cavefish "is not as complete as in other troglobites, but rather is in an intermediate interval" (Wilkens 2001, 258). This finding can be interpreted to mean that the cavefish are in the process of losing genetic traits.

Researchers have long been puzzled by the fact that the eyes of cave fish develop largely normally at the start of embryonic development (Gore et al. 2018, 1155; Culver 1982; see **Fig. 5**). However, later it was recognised that the early stages also fulfil essential morphogenetic functions in the region of the skull. More recent studies have shown that the expression of the same genes that are crucial for early eye development is also important for the formation of the forebrain. Degeneration occurring too early would apparently have an adverse effect on the forebrain. In blind cave-dwelling invertebrates, this function is absent; consequently, these animals can lose their eyes entirely (Borowsky 2018, R62). For further details on the loss of eyes, see Jeffery (2020, 4).

Developmental and genetic studies

The role of the lens and retina

Yamamoto & Jeffery (2000) demonstrated that eye development depends largely on the presence and correct formation of the lens (see also Pennisi 2000). If, during embryonic development, the lens of a surface-dwelling *Astyanax mexicanus* is transplanted into a cave-dwelling specimen, a largely normal eye develops. This suggests that epigenetic regulation is involved. Conversely, removal of the lens leads to the atrophy of the otherwise normally developing eye; the cornea and iris fail to form and the retina degenerates. Yamamoto & Jeffery (2000) concluded from this that the lenses emit signals that trigger eye development. The ability to respond to signals from the lens has therefore not been lost in cavefish. Apparently, all genes downstream of lens function are present in cavefish and can be

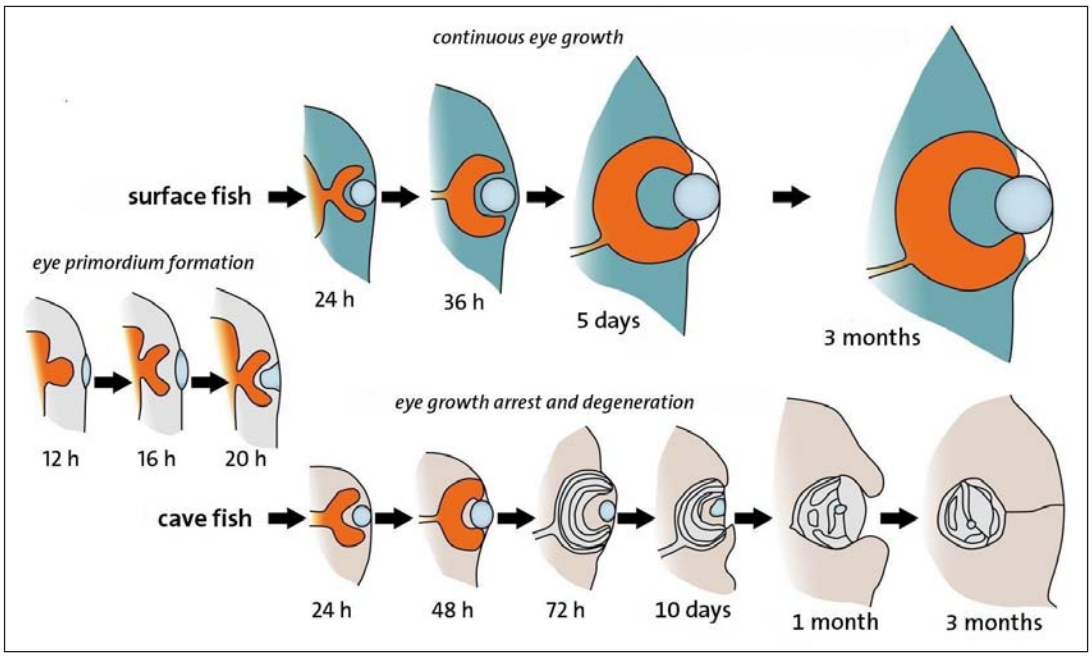

Fig. 5 Eye development and degeneration in *Astyanax*. Development of the eye primordium up to approximately 12 hours after fertilisation in cave-dwelling and surface-dwelling fish. The eye of surface-dwelling fish differentiates and increases rapidly in size from 1 day after fertilisation to 1 month (top), whilst the eye of cave-dwelling fish ceases to grow, degenerates and gradually sinks into the eye socket (bottom). (Adapted from Jeffery 2009a)

activated (Jeffery 2009a, 217).[7] Subsequent experiments by Soares et al. (2005) suggest that the lens has a specific influence on the development of the optic nerve and the tectum (roof of the midbrain) during eye growth in *Astyanax*.

Wilkens (2007) demonstrated that the retina and the lens are independent developmental units within the eyeball. It is thought that the genetic systems responsible for each are inherited independently of one another, a finding confirmed by hybrids from F2 crosses between cave and surface fish, in which the development of the lens and retina does not correlate. The lens induces the cornea, pupil and anterior chamber, whilst the formation of the retina correlates with the retinal pigment epithelium (RPE), the optic nerve and the ciliary muscle. The size of the eyeball is influenced by both structures (Wilkens 2007, 188). Strickler et al. (2007) also concluded from their experiments that the lens, together with another optical component—possibly the RPE—promotes the survival of retinal cells (dual signal model; **Fig. 6**). Accordingly, a deficiency in either of these optical structures can lead to degeneration of the eye in cave fish.

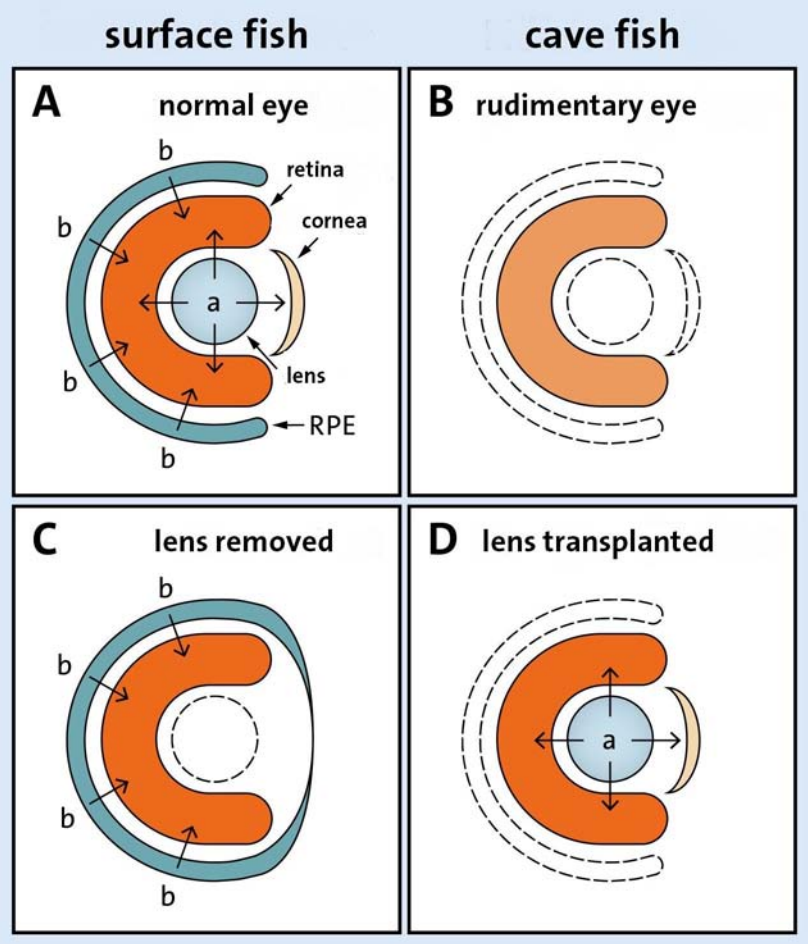

Fig. 6 The dual signal model for coordinated eye growth and eye development. Signal *a* is produced by the lens and signal *b* by another part of the eye, possibly the RPE (RPE = retinal pigment epithelium, the layer that nourishes the retina). **A** In the normal surface-dwelling fish eye, signals *a* and *b* are both present and functional. **B** In the rudimentary cave-dwelling fish eye, signals *a* and *b* are either absent or non-functional. **C** In the eye of the surface-dwelling fish without a lens, signal *a* is absent and signal *b* is present. **D** In the reconstructed cave fish eye, which contains a transplanted surface fish lens, signal *a* is present and signal *b* is absent. The lens is shown in blue. The eye structures shown as dotted lines are either absent or non-functional. The eyes of surface fish and cave fish are not drawn to scale. (Adapted from Strickler et al. 2007)

Genetic studies

Gene mapping studies have shown that several gene loci contribute to differences in eye size, pupil size and changes in the retina in cave fish compared with surface-dwelling fish (Shennard et al. 2025, 2). In both *Astyanax* and *Rhamdia*, all individual structures are present during the early ontogenetic phase. Wilkens (2001, 260) therefore considers it possible that the reduction in eye size is mainly due to mutations in developmental genes rather than in structural genes. A number of studies have confirmed this. Strickler et al. (2001) demonstrated that the pattern of expression of the eye master regulator gene *Pax6* is altered during early and late embryonic development. According to the researchers, the results suggest "that changes in *Pax6* expression are involved in the evolution of cavefish eye degeneration". The expression of *pax6* is also downregulated in the lens of cave fish (Jeffery & Martasian 1998; Jeffery 2019, 90). Many crystallin★ genes also show reduced expression in the cave fish lens; these, in turn, are under the control of the transcription factor★ *sox2*. The downregulation of SOX2 and the blocking of the SOX2-αA-CRYS signalling pathway could be one of the causes of lens atrophy (apoptosis) and eye degeneration in cave fish (Ma et al. 2014).[8] *Sox2*, in turn, is regulated by an lncRNA★ (Da Silva et al. 2023). The αA-*crystallin* gene, which encodes a protein that controls lens survival, is downregulated, whilst heat shock protein 90α (hsp90α), which encodes a protein that promotes cell death, is upregulated in the cavefish lens. It is thought that these two changes in gene expression together trigger cell death in the cavefish lens (Jeffery 2019, 90).[9]

Pax6, in turn, is under the control of other regulatory genes. For example, upregulation of the Sonic hedgehog *(Shh)* gene in the region of the embryonic midline leads to an increase in the transcriptional activity of the *pax2* and *vax1* genes, which in turn negatively regulate pax6 in the optic cup. *Shh* also induces cell death in the lens (Jeffery 2019, 90). Yamamoto et al. (2004, 847) speculate that *shh* and *twhh*, which are also upregulated, may be subject to upstream regulatory control, which would explain the coordinated changes in the expression of *shh* and *twhh*.

Ultimately, therefore, the increase in *Shh* production leads to fewer cells being made available for eye formation (Jeffery 2009a; 2009b). It is

Glossary

Allele: A variant of a gene.
cis-regulation: A process whereby DNA sequences located in close proximity on the same DNA molecule control the switching on and off of a gene, or the increase or decrease in its expression. They act as molecular switches via binding sites for -> transcription factors to regulate development and physiology.
Crystalline: water-soluble structural proteins which are responsible, amongst other things, for the refractive power and transparency of the eye's lens.
Deletion: loss of a piece of a gene.
Ectoderm: the outermost of the three germ layers during embryonic development. It gives rise to the skin, skin appendages, parts of the sensory organs, the nervous system and other parts of the body.
Endoderm: the innermost of the three germ layers that form during embryonic development. It primarily gives rise to the majority of the digestive system.
Epigenetics: processes by which genes are switched on and off without the genetic material itself being altered.
Genetic drift: random changes in the -> allelic composition of a population that are not influenced by selection.
Homeobox: a conserved DNA sequence that codes for specific transcription factors and plays a role in cell differentiation and the formation of specific body segments.
Candidate gene: a gene that may be associated with the occurrence of genetically influenced traits (or diseases).
Convergence: the independent evolution of structurally identical or similar structures in different organisms; that is, the compared similar structures cannot be traced back to a common ancestor.
lncRNA: long non-coding RNA; RNA fragments which, unlike mRNA, are not translated into a protein sequence but instead perform regulatory functions in the -> methylation of DNA and are over 200 nucleotides in length.
Melanophore: a cell containing melanin.
Methylation: the attachment of a methyl group to DNA as a kind of marker that switches certain genes on or off.
Neuromasts: secondary sensory cells in the lateral line organs of fish.
oral-pharyngeal: oral = relating to the mouth; pharyngeal = relating to the pharynx.
plasticity: changes in the anatomy, physiology or behaviour of an organism during its lifetime, triggered by environmental stimuli.
Pleiotropy: A gene is utilised in several contexts. This means that several characters are caused or co-determined by the same gene.
Polymorphism: the existence of several -> alleles within a population.
QTL: Quantitative Trait Locus, a genetic region influencing the phenotypic quantitative variation of a complex character, such as body size or body weight.
RNA-Seq: RNA-Seq analysis is a method for determining the nucleotide sequences of RNA.
SNP: Single nucleotide polymorphism, i.e. different alleles of a gene differ by a point mutation.
Standing genetic variation: the presence of multiple -> alleles within a population (-> polymorphism) before a new environmental situation arises, to which the population can thereby react rapidly.
Transcription factor: a genetic element that binds to specific regions of DNA and thereby regulates gene expression at those sites.
Transcriptome: the totality of all DNA sequence regions transcribed into RNA.
Transposon: a section of DNA in the genome that can change its position within the genome and thereby influence other genes (e.g. switch them on or off).

presumably for this reason that the lens cannot develop properly and disintegrates. The atrophy of the eye is also the cause of the changes in the skulls of cave fish; at the same time, however, the increased production of *Shh* also leads to the strengthening of the jaw and the taste buds (Jeffery 2009a). Hybrids between cave fish and surface fish showed an inverse relationship between eye size on the one hand and jaw size or the number of taste buds on the other, which demonstrates a genetic link between the constructive features of the oropharyngeal region and the degeneration of the eyes (Jeffery 2009a). Based on this coupling, Jeffery considers it possible that positive selection for structural oral-pharyngeal* traits is responsible for the loss of eyes during the evolution of cave fish via the pleiotropic* function of the *Shh* signalling pathway. However, this is speculative.

Shennard et al. (2025) demonstrated that eyes do not develop when the retinal homeobox gene *rx3* is absent. The *rx3* gene is expressed differently in surface-dwelling and cave-dwelling fish during early development, whilst the eye field is being specified (Shennard et al. 2025, 2). Furthermore, *rx3* mutants in surface-dwelling fish exhibited altered behaviour compared with wild-type surface-dwelling fish, suggesting that the loss of eyes influences sensory-driven behaviours. Hybrids between mutant surface-dwelling fish and cave-dwelling fish suggest that cis-regulatory variation at the *rx3* locus contributes to the evolution of eye size in cave-dwelling fish. Previously, analysis of the *rx3* sequence in cave-dwelling and surface-dwelling fish had revealed no coding variants within this gene (McGaugh et al. 2014, 3; cf. Sifuentes-Romero et al. 2020). The *rx3* expression domains were significantly smaller in the Molino cavefish than in surface-dwelling fish, but significantly larger than in the Pachón cavefish (Sifuentes-Romero et al. 2020, 433).

Da Silva et al. (2023, 2) summarise the findings of genetic studies: "[M]any genes are suggested to have a relevant role in eye development and degeneration, that includes, but not restricted to, the crystallin genes αA-*crys, cryaa, crybb1, crybb1c and crybgx*, transcription factor *sox2*, retinal homeobox *rx3*, cone-rod homeobox *crx*, *cbsa* and *dusp26*."

Studies into the role of *Shh*, *Pax6* and other genes in eye reduction can be interpreted to

suggest that changes in regulatory genes are an important factor in eye degeneration in *Astyanax*. Jeffery (2009a, 200) notes that most of the genes examined in a candidate gene analysis★ show no changes in expression in surface-dwelling and cave fish embryos; however, some genes are down- or up-regulated in cave fish (see his Table 8.1, p. 201ff).

It is interesting to note in this context that the upregulation of *Shh* in surface-dwelling fish leads to eye phenotypes that are strikingly similar to those seen in cave-dwelling fish. Conversely, inhibiting *Shh* in cave fish does not result in the eyes being fully restored; rather, it merely leads to slightly larger eyes; thus, other factors also play a role. These findings underscore the potentially important role of *Shh* in eye degeneration (Krishnan & Rohner 2017, 3).[10]

Sifuentes-Romero et al. (2020) compared the genetic changes in the independently evolved cave populations of the Pachón and Molino caves. They found that the loss of eyes in the two cave populations is partly underpinned by the same genetic and developmental changes or mechanisms.[11] However, there are also unique genetic changes in the two populations.

Genetics of pigmentation

According to research by Protas et al. (2006; summarised in Jeffery 2009a), a single gene, p/oca2, controls the loss of melanin pigment in cave fish. Crossbreeding between Pachón and Molino cave fish produces albino F_1 offspring, "suggesting that mutations in the same gene underlie albinism in many different *Astyanax* cavefish populations" (Jeffery 2009a, 212). However, the specific mutations differ between the Pachón and Molino cave fish, "suggesting that cavefish albinism evolved by independent changes in p/oca2" (Jeffery 2009a, 215). A third independent mutation in the oca2 gene, perhaps in a regulatory sequence, is responsible for the convergent albinism in the Japonés Cave (Protas et al. 2006, 109).

A similar phenotype is observed in the 'brown mutation' in *Astyanax*, whose individuals have reduced melanin levels, a reduced number of melanophores★ and brownish eyes. This phenotype has arisen independently on several occasions in geographically separate caves, through different mutations of the same gene, *Mc1r*. "This example of parallelism indicates that certain genes are frequent targets of mutation in the repeated evolution of regressive phenotypes in cave-adapted species" (Gross et al. 2009, 1).

Protas et al. (2006, 110) point out that other studies have also shown "that one gene is responsible for the same morphological change in multiple populations or species: Ectodysplasin *(Eda)* for body armor and *Pitx1* in pelvic reduction in the three-spine stickleback; *ovo* (shaven-baby) in trichome loss in different drosophilids; and *Mc1r* for pigmentation in pocket mice, jaguars and several avian species. Thus, the same morphology often evolves by mutation in the same gene, possibly because this is the most efficient or best way for a certain phenotype to evolve."

QTL analyses

Quantitative trait loci (QTLs) are genetic regions that influence the phenotypic quantitative variation of a complex trait, such as body size or body weight. QTLs can be identified through a statistical genetic analysis known as *QTL mapping*★.[12] Based on this, further genetic studies can be carried out to attempt to identify the underlying genes and their functions.

QTL analyses of hybrids between surface and cave forms have shown that loss of eyes is an additive character controlled by more than a dozen QTLs scattered across the genome; furthermore, several QTLs are also responsible for reduced pigmentation (Jeffery 2020, 4f; cf. Jeffery 2009b, 33). Studies on QTL mapping showed that each cave population examined harbours 10 to 12 QTLs responsible for a specific cave trait (Borowsky 2018, R62). Protas et al. (2007) identified 48 QTLs: 8 QTLs influencing eye size; 6 influencing lens size; 18 influencing pigmentation; 7 influencing the length of the jawbones; 6 influencing the number of upper jaw teeth; and 3 influencing the number of taste buds.

In light of this finding, the question arises as to how this relates to the previously described role of *Shh* and *Pax6*, the up-regulation and down-regulation of which, respectively, trigger a cascade of changes in cave-dwelling fish (see above in the section 'Genetic studies'). Are the regulatory genes the key triggers, whilst the genes identified in QTL analyses as also being involved merely serve as a kind of 'background noise' and are of secondary importance?

It is therefore unclear what these results mean. "As in all QTL studies, the challenge is to find the relevant gene(s) within the identified QTL" (Jeffery 2009b, 37).[13] Krishnan & Rohner (2017, 3), based on the experiments mentioned above involving increased *shh* levels in surface-dwelling fish and reduced *shh* levels in cave-dwelling fish, conclude that it is unlikely that mutations in or near *shh* are directly responsible for the cave phenotypes, as none of the QTLs for eye size overlap with the *shh* locus.[14] None of the approximately 12 known QTLs for eye size are located near *pax6*, *shhA*, *shhB* or other

known *hedgehog* gene loci on the *Astyanax* genetic map (Jeffery 2009b, 37). Jeffery therefore concludes that these genes, or their *cis-regulatory*★ regions, have not mutated in cave fish. Instead, they are likely to be downstream genes that act downstream of the mutated genes responsible for the reduction in eye size.[15]

A notable finding in this context is that populations of *Astyanax* cave fish generally exhibit only minimal genetic variation in the coding regions of their genes, both in comparison with surface-dwelling fish and amongst themselves (Jeffery 2009b, 28).[16] This can be interpreted as an indication that coding genes do not play the primary role in the expression of cave traits.

Shennard (2026, 131) also reaches this conclusion in his doctoral thesis on the genetic basis of eye development in the cave-dwelling forms of *Astyanax*. The results therefore suggest "that evolution acts on conserved pathways and genes, primarily through regulatory changes".[17]

Surprisingly, not all relevant regulatory genes could be assigned to a specific QTL. This applies to *Pax6*, *twhh* and *shh* (Casane & Rétaud 2016, 140f).[18] By contrast, *rx3* can be assigned to an eye QTL (Shennard et al. 2025, 2).

Causes for parallel and convergent changes

What factors and processes have driven the changes in cave fish? In the past, two hypotheses in particular have been discussed, both of which are based on the neo-Darwinian model of evolution. According to these, random mutations and natural selection play the main role (see Cartwright et al. 2017). The differences between the two hypotheses lie in the assessment of the respective importance of mutation and selection. According to the first hypothesis, neutral or deleterious random mutations play the main role. These were able to accumulate, it is argued, because certain selective pressures are absent in the caves. Charles Darwin (1859) attributed the reduction in eye size to disuse.[19] According to the other hypothesis, adaptation occurs as a result of positive selection (possibly indirect): as food is scarce in the caves, a selective pressure arises to eliminate unnecessary structures. Experimental findings contradict both hypotheses (for an older overview of the state of the debate at the time, see Junker 2002, 126). Numerous recent findings have complicated the picture. The causes of these regressions remain unclear to the present day (Moran et al. 2015[20]; Borowsky 2018, R61; Krishnan & Rohner 2017, 6; Jeffery 2009a, 217; Jeffery 2009b, 41[21]; Niven 2008, R28[22]; Cartwright et al. 2017; Shennard 2026, 11[23]).

However, a number of recent studies now suggest that the genetic changes in cave populations are not, or at least not solely, attributable to random mutations, but to rare alleles★ (gene variants) that are *already present* in surface-dwelling fish. The term *'standing genetic variation'* has become established to describe this phenomenon (hereinafter also referred to simply as *'existing variation'*), which may also be present in regulatory sequences. It has generally been shown that existing variation occurs more frequently than previously assumed (Krishnan & Rohner 2017, 5).[24] The origin of this variation is a separate question, which will be set aside for the time being. Existing variation enables rapid adaptations to changing environmental conditions, as there is no need to wait for rare beneficial mutations that would only become established in populations over the course of many generations.

Furthermore, according to recent studies, there are other sources of variation in cave fish populations: epigenetic regulation via DNA methylation★ (Gore et al. 2018; see the section 'Epigenetic changes as a source of cave traits') and, related to this, plasticity (see the section 'Plasticity as a source of variation').

For all these processes – summarised in Table 1 – which contribute to the establishing of cave-specific traits, findings from experimental research can be cited. However, the relative importance of the different factors is unclear or disputed: "The extent to which convergent or parallel changes draw on preexisting genetic variation in ancestral populations versus new mutations is still debated", and "The molecular and genetic changes that underly most convergences are still unknown" (Bradic et al. 2012, 1[25]). This will be discussed in more detail below.

The role of random mutations in the development of cave traits

Genetic studies have shown that some of the genes responsible for eye reduction are not the same across different populations, which could explain the differences in eye reduction (see above; cf. Junker 2002, 128). In hybrids of fish from different caves, different alleles can be combined, thereby partially restoring certain cave-associated traits—such as rudimentary eyes and other characteristics—although the extent of this restoration may vary from cave to cave (Borowski 2008; 2018, R62[26]; Niven 2008; Jeffery 2009b, 29f; Jeffery 2019, 90). It follows that "at least some of the genetic changes accounting for cave-associated traits are unique to each cave

Process	Duration
1a. Degeneration caused by **random mutations**, which do not result in a selective disadvantage in the lightless caves and are therefore not subject to natural selection	requires many generations for them to become fixed in the population
1b. Positive **selection** of random mutations that lead to adaptations to cave environments, which enable better survival	requires many generations to obtain several suitable beneficial mutations
2. **Standing genetic variation** in rare alleles in surface-dwelling fish	within a few generations
3. **Epigenetic** changes and **plasticity**	within a few generations

Table 1 Proposed sources of adaptation in cave fish. 1a and 1b are variants of the neo-Darwinian explanatory framework and are discussed together.

lineage" (McGaugh et al. 2014, 7[27]; Jeffery 2009b, 29[28]). Crossbreeding of this kind allows for what is known as a *complementation test*.[29] Genetic complementation shows that some of the genes responsible for eye degeneration are unique to different cavefish lineages (Jeffery 2019, 90). However, this does not apply to all genes; genes that control eye loss are either the same or unique in some populations. The *cbsa* gene is mutated at the same cis-regulatory position in six different cavefish populations; thus, it repeatedly plays a role in eye loss. In some cavefish populations (e.g., Pachón, Tinaja), *cbsa* exhibits the same mutation, while in others (e.g., Molino), a different mutation occurs at the same location, indicating a possible DNA hotspot for mutations—that is, a region where mutations occur with above-average frequency. The *mc1r* gene is also mutated at the same site in the coding region in several cavefish populations, and the *oca2* gene exhibits large deletions★—albeit at different sites—in the coding region in Pachón and Molino cavefish. These studies show that the same genes, albeit with different mutations, may be responsible (Jeffery 2020, 5f).[30]

With regard to pigmentation as well, complementation tests in F_1 hybrids[31] sometimes show more intense pigmentation, suggesting different genes are involved, while in other cases no complementation occurs, suggesting that the same genes are involved in those cases (Jeffery 2009b, 38).

A single mutation in the monoamine oxidase (MAO) gene reduces its catalytic activity and may be associated with changes in aggressive behavior. Mutations in the *oca* gene reduce melanin synthesis but also lead to a variety of changes in biochemical metabolic pathways and may be indirectly involved in changes in behavioral traits (Maldonado et al. 2020, 467). A specific mutation in the insulin receptor reduces its affinity for insulin and leads to hyperglycemia (high blood sugar), which causes an increase in body weight and insulin resistance (Riddle et al. 2018; see Maldonado et al. 2020, 469).

Pigmentation and melanophores. The loss of melanin pigmentation in cave fish can result from a reduction in the number of melanophores (pigment cells), a change in the structure of the melanin pigment, or the complete loss of melanin (while the precursors of melanophores are still present[32]). In *Astyanax*, the loss or alteration of melanin can be attributed to mutations in individual genes, whereas the reduction in the number of melanophores in each population involves ten or more genes (Borowski 2018, R62).

Loss-of-function mutations in the *oca2* gene, which is thought to be involved in melanin production, have been characterized in three independently evolved populations of cave fish, although the specific mutations differ (Borowski 2018, R62; Jeffery 2009b, 41; see above).

In both the Pachón and Molino cavefish, mutations in the same gene, *oca2*, are responsible for the loss of pigmentation; however, these mutations involve different deletions in the protein-coding sequence, suggesting that the albinism has an independent origin (Jeffery 2019, 92). The *mc1r* gene, which encodes the melanocortin-1 receptor—located on the surface of melanocytes and playing an important role in pigmentation[33]—has also undergone repeated and varied mutations, yet these mutations produce the same troglomorphic (cave-adapted) phenotype with brown eyes in different cave fish populations (Jeffery 2019, 92[34]; Jeffery [2009b, 42] refers to parallel or convergent evolution[35]). However, no sequence differences were found in the coding region of mc1r between the Piedras and Curva cave fish, "implying that the relevant mutations may be in gene regulatory regions" (Jeffery 2009b, 41).[36]

Jeffery (2009b, 42) summarizes: "Pigment regression has at least three causes in *Astyanax* cavefish. First, the predominantly albino Pachón and Molino cavefish have accumulated different mutations in the *oca2* gene, causing defective melanin synthesis. Second, some cavefish populations, including some of those with albinism, have also

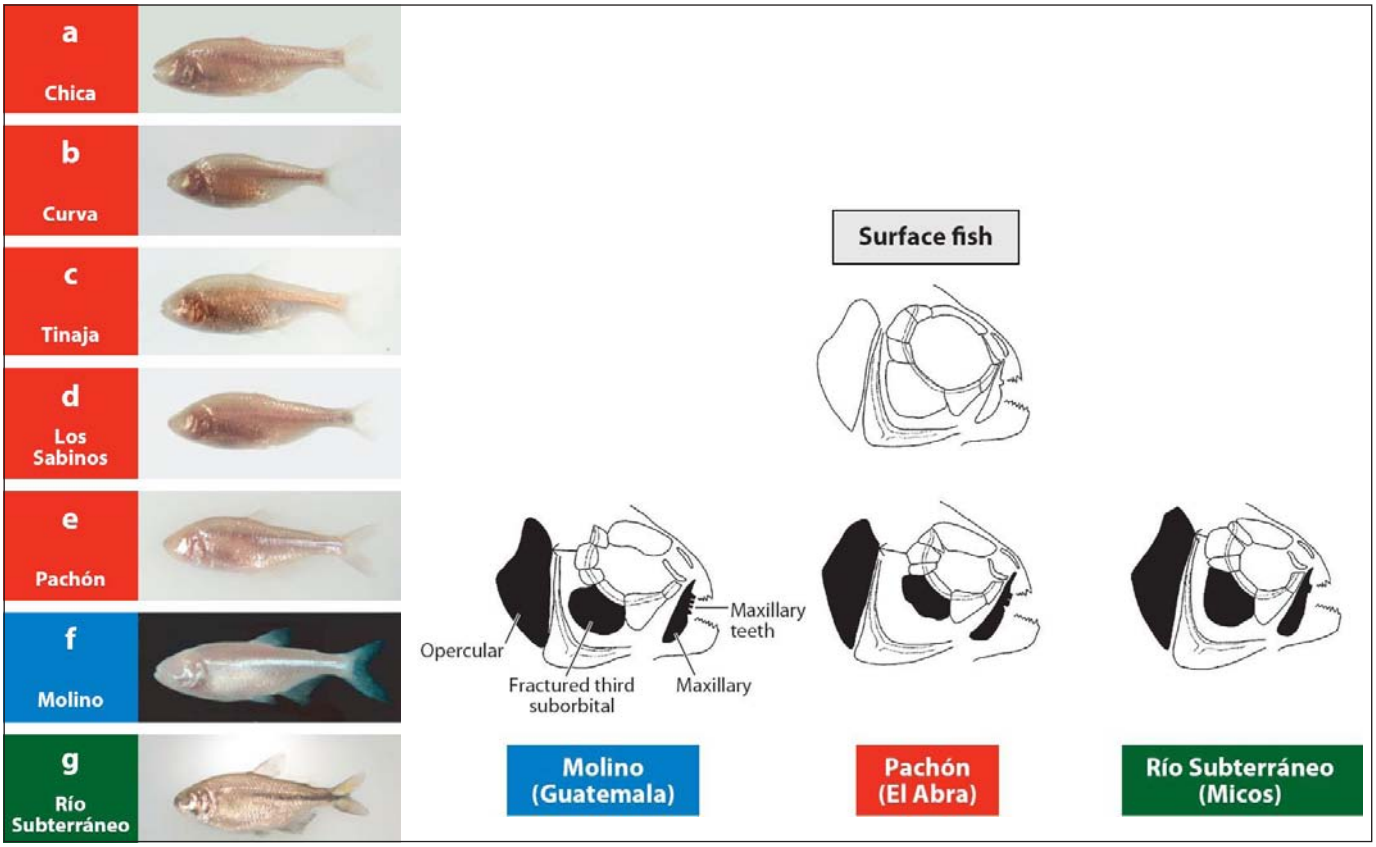

Fig. 7 **Left**: Examples of various cave fish. Adapted from Jeffery et al. (2009). **Right**: Surface-dwelling fish and cave-dwelling fish exhibit differences in skull morphology. The shaded bones indicate the greatest differences between surface-dwelling fish and cave-dwelling fish, as well as between the cave-dwelling fish groups from Guatemala, El Abra and Micos. (Adapted from Yamamoto et al., from Jeffery 2009b, with permission)

accumulated different mutations in the *Mc1r* gene causing reduced numbers of melanophores and melanin pigmentation due to defective MSHα signaling. … Third, mutations in additional genes that remain to be identified also contribute to the reduction of melanophores. Thus, certain genes are repeatedly targeted for mutations leading to evolutionary changes in cavefish pigmentation."

Sinocyclocheilus. In another cave-dwelling fish genus from the carp family, *Sinocyclocheilus*, the eyes are sunken, similar to those of *Astyanax*, but the overall structure of the eye remains intact. Since the lens of adult cave-dwelling fish of the species *Sinocyclocheilus anophthalmus* shows no obvious morphological changes, this suggests a different pathway of eye degeneration than that seen in Astyanax. In *S. anshuiensis*, however, severe lens defects were observed (as well as defects in the retina), suggesting that even within a single fish genus, there are multiple mechanisms for eye regression (Krishnan & Rohner 2017, 7). Comparisons of whole genomes and transcriptomes* of surface-dwelling and cave-dwelling species within this genus revealed numerous differences in both sequence and expression between the two forms. "The genes that differed most in expression profiles include those involved with eye development and vision, loss of pigmentation, and enhancement of taste in the cave form" (Borowsky 2018, R63).

Phreatichthys. The situation is different again for the Somali cave fish *Phreatichthys andruzzii*, which also belongs to the carp family and is estimated to be 2 million radiometric years old. Its eyes and pigmentation have completely disappeared, as have its scales. Unfortunately, there are no closely related surface-dwelling species, so hybridization studies—at least so far—are not possible. Early markers of eye development in embryos of these fish showed that the development of the eye region and the formation of the optic nerves—unlike in *Astyanax*—proceed without any apparent abnormalities. Furthermore, there were fewer signs of apoptosis in the lens tissue. Both of these findings contrast with the mechanisms underlying eye degeneration in *Astyanax* cave fish. In contrast, late differentiation events in the retina were affected, and retinal apoptosis was the driving force behind eye degeneration in these fish. Thus, despite a very similar phenotypic outcome, different developmental processes underlie the cave forms of *Astyanax* and *Phreatichthys* (Stemmer et al. 2015; Krishnan & Rohner 2017, 7).

On the role of natural selection

The changes in the development of cave fish eyes are consistently directed toward atrophy. All known QTLs associated with eye development are linked to atrophy. Within the framework of neo-Darwinism, this consistency is regarded

as evidence of the action of natural selection (Protas et al. 2007, 452; Jeffery 2009b, 42; Jeffery 2019, 92). It can also be argued here that, depending on the animals' age, the visual system requires up to 15% of their total energy budget, with the highest demand occurring during the juvenile stage (Moran et al. 2015). This energy savings could therefore be a positive selective factor.

If genetic drift were the primary factor, changes in various directions would be expected. This is indeed the case with changes in pigmentation, which is why these changes are more likely attributable to genetic drift rather than selection (Protas et al. 2007, 452; Jeffery 2009a, 218).[37]

Jeffery (2019, 92) suggests that the reduction in the size of the eyes and the brain's visual processing areas may create more space for the development of non-visual sensory systems. As mentioned, a connection can be demonstrated here with the increased expression of the pleiotropic* gene *Shh*, which, in addition to reducing eye size, also leads to the enlargement of the jaw and taste buds. Yamamoto et al. (2004, 847) also argue that, based on this connection, eye regression could be favored by natural selection for one or more adaptive traits.

The fact that most genes with altered expression patterns—such as those in the *Shh* signaling pathway and hsp90α—tend to increase rather than decrease their activity in cave fish also suggests, according to Jeffery (2009b, 42f), a role for natural selection, as does the finding that the genes involved in eye development that have been studied so far do not appear to have mutated to the point of losing their function (Jeffery 2009a, 217; see above).

Evidence suggesting existing variation as the source of cave traits

A number of recent findings suggest that a (larger?) portion of cave fish traits is drawn from a "pool of standing genetic variation for the caves" (McGaugh et al. 2014, 7). Gross & Wilkens (2013) investigated the role of the aforementioned *oca2* gene in pigmentation in *Astyanax* and discovered that albino Micos cave fish possess two copies of an *oca2* allele that had previously been identified in the geographically distant Pachón cave population. Given the geographical distance, this finding suggests that this *oca2* allele was inherited from the ancestors of the surface-dwelling animals and thus stems from existing genetic variation.[38] Alternatively, there could be programmed pathways for mutations that cause the same or similar mutations to arise independently; however, this hypothesis would need to be tested through further research.

Aspiras et al. (2015) identified coding mutations in conserved amino acids of the *melanocortin-4 receptor* gene *(MC4R)* in the Tinaja cave fish that contribute to the insatiable appetite and resistance to hunger observed in some populations of cave fish. The presence of the same allele in multiple caves could be attributed to selection from the genetic variation present in surface populations ("standing genetic variation").

Studies by Bradic et al. (2012, 6) on the proportions of shared alleles and so-called private alleles (which occur in only one of several populations) in surface and cave populations suggest "that the allelic contents of cave populations are largely subsets of alleles of the surface stock. Thus the observed variation in the caves is mostly the result of standing genetic variation from the ancestral surface stock as well as possible gene flow between the populations."[39]

Sifuentes-Romero et al. (2020, 434) also suggest that the genetic differences among various cave populations are attributable to genetic variation already present in the ancestral population.

Fumey et al. (2018, 14) view existing genetic variation as the reason why many phenotypic changes can accumulate in parallel and within a short period of time. Similarly, in their review article, Casane & Rétaud (2016, 151f) conclude that it is plausible to assume that the evolution of cave fish was a very rapid process that occurred only relatively recently, and that existing genetic variation played a particularly important role in this process.

In another review article, Borowsky (2018, R63) also notes that many *Astyanax* researchers "accept that a significant portion of the genetic variants that proved important for cave adaptation existed in ancestral surface populations as rare variants (standing genetic variation)."[40] This view is supported by the presence of identical mutations and many identical SNP* markers in two or more cave populations. Further research could also investigate whether identical mutations could have been acquired independently of one another due to a common mechanism of origin.

It is interesting to note in this context that, despite their relatively great geographical distance, the surface populations are very similar, while the cave populations are more distinct (Bradic et al. 2012, 1). These researchers were also able to document significant gene flow between surface and cave populations in both directions. The cave populations exhibited lower genetic diversity than the surface populations, which, according to Bradic et al. (2012, 1, 14), is

related to their smaller effective population size, which is likely due to food and space constraints.

"The presence of existing and rare, ancient polymorphisms* thus represents an additional mechanism for rapid evolution in cave populations," Gross & Wilkens (2013, 129) note, also with regard to albinism in cave-dwelling animals.[41] This demonstrates that standing genetic variation is a source of cave-specific changes. However, it remains unclear whether this reflects a general phenomenon. Cave-associated phenotypes, such as albino forms, can emerge remarkably quickly in captive-bred fish from cave populations that do not exhibit albinism in the wild. Here, too, it is unclear whether such rapid emergence is also possible for other complex cave-associated phenotypes (such as loss of eyes).

Finally, the previously mentioned finding that the genes identified so far as being involved in eye development—as noted earlier—have not mutated to the extent that they have lost their function is also significant. Otherwise, restoring the eyes through lens transplantation would hardly be possible. Apparently, genes downstream of lens function are present in cave fish and may be active (Jeffery 2009b, 42). This finding, too, could be explained by standing genetic variation.

Cryptic variation. Of particular interest in this context is what is known as cryptic variation. To understand this, we first need to take a step back.

Living organisms are, so to speak, buffered against genetic and environmentally induced variations and disturbances: This is referred to as "developmental capacitance," which could be translated as the "capacity" or "resilience" of development (Moczek 2008). This "developmental resilience" means that ontogenetic development can compensate for—and thus buffer and cope with—a certain degree of external disturbances (from the environment) and genetic errors through corrective mechanisms. These variations in the genome, which are disruptive in and of themselves, are thus protected from selection because their effects are, so to speak, filtered out internally and do not manifest themselves. They therefore do not become apparent in the phenotype. This effect is referred to as the "channelling" of ontogenetic development, because development proceeds in the correct direction despite certain disruptions. To the extent that these buffering systems shield genetic changes, corresponding changes in the genome can accumulate. This allows new cryptic (hidden) variation to emerge. However, if certain thresholds for tolerable environmental disturbances are exceeded and the error burden thereby becomes too high, the cryptic variation can "break through" phenotypically and become apparent, thereby also becoming subject to selection (which, after all, acts on the phenotype). If the "revealed" phenotype is adaptive (i.e., beneficial in a specific environmental situation), it will be selectively favored, and the phenotype may eventually become independent of the specific environmental change *(genetic assimilation)*.

The heat shock protein Hsp90, for example, provides such buffering; it is a chaperone, meaning it ensures the correct folding of proteins. Hsp90 buffers against environment-induced errors, such as when a temperature-dependent error in protein folding occurs (which is then corrected). It also ensures that folding occurs correctly despite genetic variation *(silent polymorphism)*. Accordingly, the loss of Hsp90 results in significant damage, as errors and disruptions are no longer corrected. This "reveals" the cryptic genetic variation. However, as long as Hsp90 remains intact, further cryptic variation can accumulate.[42]

There is evidence that Hsp90 is part of a program responsible for the suppression or release of the genetic diversity required for eye degeneration under various environmental conditions (Rohner et al. 2013; Pennisi 2013; Jeffery 2019, 90). Rohner et al. (2013) show that Hsp90 causes the existing phenotypic variation in eye size in surface populations of *Astyanax* to be masked. Inhibition of Hsp90 "unmasks" this cryptic variation, allowing it to be selected for, which ultimately leads to a reduced eye phenotype. Conversely, treating fish embryos with an Hsp90 inhibitor resulted in some fish developing larger or smaller eyes and eye sockets compared to untreated fish. Breeding the small-eyed fish produced offspring that also had small eyes, suggesting that eye size is genetically determined.

It is interesting to note that the Hsp90-dependent change resembles a natural adaptation. The authors speculate that the loss of Hsp90 promotes new genetic variations by releasing mobile DNA elements, known as transposons* (Pennisi 2013). Rohner et al. (2013, 1372) conclude: "Although de novo mutations may exist and contribute to phenotypic evolution, repeated use of standing variation has played an important role in the evolution in these fish." And since multiple variants can be activated simultaneously, this system offers the possibility of generating (more precisely: releasing) complex traits in a single step. Eye size in the cave form is influenced by at least 14 mapped QTLs (see above). Inhibition of Hsp90 allows the expression of cryptic variation in eye size within *Astyanax* populations (Rohner et al. 2013, 1373).[43] The Hsp90-dependent variation in eye size that exists in the surface population of *A. mexicanus* may

have contributed to enabling a rapid response to the cave environment (Rohner et al. 2013, 1375). This demonstrates that the alleles selected for during the evolution of cave fish are alleles that are, at least in part, Hsp90-dependent and responsive to it.

The researchers further found that the individuals that develop the smallest eyes in the presence of Hsp90 inhibition carry alleles that may contribute to the inheritance of small eye size in the absence of Hsp90 inhibition. "This demonstrates that a cave-specific environmental stress can elicit similar changes in morphological eye development as biochemical inhibition of HSP90" (Rohner et al. 2013, 1374).

Later, Jeffery (2019, 90) demonstrated that inhibiting lens cell death with the Hsp90 antagonist Radicicol can partially restore eye development, suggesting that the cell death program is reversible.

Unlike with the eyes, however, the researchers were unable to detect any cryptic variation in body size or in the number of neuromasts★.

Epigenetic changes as the source of cave traits

Gore et al. (2018) found that methylation-based epigenetic gene silencing is an adaptive mechanism for eye degeneration in the Pachón cave fish. In contrast, inactivating mutations were not found in developmental genes active during eye development.[44] Through parallel analyses of both blind cave fish and surface-dwelling fish, and using the zebrafish *(Danio rerio)* as a comparative model for sighted fish, these researchers discovered that DNA methylation of specific sites in the genome causes eye-specific gene silencing and also regulates early eye development.[45] The authors conclude that changes in gene silencing caused by DNA methylation can give rise to phenotypic diversity during both individual development and evolution.

Gore et al. (2018, 1155) found that eye regression is preceded by reduced expression of certain eye-specific genes, including the lens crystallins CRYBB1, CRYBB1C, and CRYAA. Their expression can be suppressed through epigenetic silencing via DNA methylation, which is performed by de novo DNA methyltransferases (DNMTs). RNA-Seq data★ also showed that a large number of different eye development genes exhibit reduced expression in the eyes of cave fish (Gore et al. 2018, 1155; cf. Koch 2018). These authors conclude that "small genetic changes that alter epigenetic regulation can play an important role in rapid adaptive evolution by triggering dramatic changes in the expression of large sets of genes" (Gore et al. 2018, 1158). The extent to which DNA methylation is responsible for eye degeneration would need to be clarified by further research. Jeffery (2019, 92) writes that current research shows that some of the numerous eye genes involved in the loss of eyes in cavefish may be under epigenetic control via DNA methylation.[46]

As early as the late 1980s, Ferdinand Schmidt had postulated an important role for epigenetic factors, which he referred to as active repression. In a discussion of a paper by Peters (1988), he presents the rather speculative argument that structural losses in cave organisms are not caused by defective mutations but are actively suppressed, and he posits a "synthesis of repressor genes due to feedback processes in the cybernetic system of cells and organisms" (Schmidt 1988, 172). Peters counters that under these circumstances, in fish that return to daylight, this repression should be reversed so that the eyes are reformed. However, this is not observed. In the meantime, however, such cases have in fact been documented (see the section "Mutations"). Schmidt argues that after too long a period, this reversal process may no longer be possible. Schmidt's idea appears to be entirely testable and should be tested in cave fish in statu nascendi.

Plasticity as a source of change

In recent years, another important factor has come into focus: plasticity. This refers to the ability of organisms to produce different phenotypes in response to environmental stimuli. The genome does not change in the process; rather, plastic capabilities are based on variation programs present within the genome. Remarkably, the review by Casane & Rétaud (2016) makes no mention of this, just as it makes no mention of epigenetic mechanisms. Plasticity has apparently only recently come into focus. The following section describes the results of several studies that examine plasticity in relation to cave fish traits.

Bilandzija et al. (2020) discuss phenotypic plasticity as a mechanism of colonization and adaptation in caves. The rapid rate at which cave traits emerge does not accord with the classical view of gradual adaptation. The researchers found that surface-dwelling forms that grow in complete darkness exhibit numerous altered traits at both the gene expression and phenotypic levels, most of which resembled the traits of cave-dwelling forms—all within a single generation. These changes include metabolic, neurological, morphological, and molecular alterations. If these plastic responses are adaptive, they could be selected for and further refined in future ge-

nerations. In subsequent generations, these plastic changes could be fixed by consecutive mutations (genetic assimilation according to Waddington 1953), so that later cave fish can no longer revert to the surface form, even if they live in water exposed to light (Bilandzija et al. 2020, 13f). This process is considered more convincing than the assumption that new mutations have gradually accumulated.[47] However, not all cave-adaptive traits are formed through a rapid plastic response (Bilandzija et al. 2020, 13f). Therefore, other factors—such as existing genetic variation, new mutations, or a combination of these processes—are likely to play a role as well. It is noteworthy that many (though not all) plastic changes are well-suited to cave life.

Experiments on *Poecillia mexicana*, another bony fish with lineages adapted to cave habitats, showed that darkness caused degenerative changes in the spine. However, it also promoted sexual isolation between neighboring surface and cave lineages (Bilandzija et al. 2020, 12).

Bilandzija et al. (2020, 15) conclude from their experiments: "Overall, plasticity has a critical role in producing variability (adaptive or not) on which selection can act (for or against) to produce individuals with optimal fitness in the new environment." Phenotypic plasticity could fill several gaps in the current model of cave colonization and the evolution of cave-dwelling organisms. Plasticity could also enable the maintenance of different phenotypes despite gene flow from the original surface-dwelling populations. Plasticity could be a general phenomenon in the colonization of and adaptation to cave environments (Bilandzija et al. 2020, 16).

Behrmann-Godel et al. (2024) report on similar experiments involving the brook loach *(Barbatula barbatula)*—a species belonging to the order Cypriniformes that is also found in caves. The fish were collected from the Danube-Aach river system in southern Germany. Surface-dwelling fish and hybrids of surface-dwelling and cave-dwelling forms, both of which were reared in complete darkness, were phenotypically similar to the cave-dwelling fish, whereas cave-dwelling fish reared under natural daylight conditions resembled the surface-dwelling form. According to Behrmann-Godel et al. (2024, 742), differences in the eye region are attributable to existing genetic variability, whereas traits related to chemo- and mechanoreception as well as body pigmentation are also heritable when reared in darkness but are highly influenced by phenotypic plasticity.[48]

The authors argue, as do Bilandzija et al. (2020), that subsequent selection could stabilize the cave traits (genetic assimilation, see above). It remains a challenge to clarify whether genetic differentiation occurs through selection on existing genetic variation or through phenotypically driven plasticity. The observations contradict slow, time-consuming processes (Behrmann-Godel et al. 2024, 742). The ancestors of the cave forms may have been predisposed to an underground habitat due to their nocturnal lifestyle (Behrmann-Godel et al. 2024, 743).

Despite the short period of colonization—20,000 [radiometric] years—cave-fish traits have become established, including structural features such as elongated barbels and an enlarged olfactory epithelium. Complete loss of eyesight has not occurred, and there are significant differences in eye size. This could indicate ongoing gene flow between surface and cave fish populations (Behrmann-Godel et al. 2024, 741).

A third study demonstrating the essential role of plasticity in cave colonization focuses on cave fish in the Dinaric Mountains of Croatia. It concerns the karstic dace *(Telestes karsticus)* of the family Leuciscidae (Čupić et al. 2023). Cave and surface forms differ in morphological and physiological traits as well as in gene expression patterns, but show no genetic differences.[49] When the fish were reared under laboratory conditions, all morphometric differences between the two forms were erased, suggesting that phenotypic plasticity drives the divergence in form and size among wild fish. In response to stress and exposure to new and challenging environments, cryptic genetic variation would be released, which could explain the increased variability in cave-dwelling fish (see the section "Cryptic Variation"). This could indicate "that the surface population is large enough and sufficiently diverse to contain necessary standing genetic variation for plasticity". Čupić et al. (2023, 822) point out that plasticity has also played an important role in how other animals with both surface and cave forms—such as salamanders, isopods (Isopoda), and amphipods (Amphipoda)—have responded to their new habitats.

Another study worth mentioning is that by Sears et al. (2020). These researchers demonstrated that rearing *Astyanax* cave fish in the dark (i.e., their natural habitat) results in significantly different gene expression patterns compared to rearing them under alternating light and dark conditions. These patterns are associated with known cave traits such as loss of pigmentation and loss of vision (Sears et al. 2020, 527). Thus, light-dependent plasticity is present.

McGaugh et al. (2020) also observed rapid changes in *Astyanax* fish that began living in the Honey Creek Cave in Texas only about 100 years ago. The researchers observed numerous differences in behavior and body structure among the recently established cave fish populations

compared to surface-dwelling fish, which correspond to those known in the long-established Mexican cave forms: e.g., body color, dorsal fin position, feeding behavior, swimming along walls, and the number of neuromasts. On the other hand, some characteristics of the Honey Creek cave fish also differed significantly from those observed in Mexican cave fish populations. Some trait shifts run in the opposite direction to those observed in Mexican cavefish populations, e.g., neuromast size, aggressiveness, and physical condition (McGaugh et al. 2020, 156).

Observations of mole rats

The findings on *Astyanax* do not allow conclusions to be drawn about the genetic basis of the adaptations in other cave-dwelling animals. Gore et al. (2018, 1155, 1158) report on studies by Kim et al. (2011) concerning cave-dwelling, almost blind mole rats (*Heterocephalus glaber*, Fig. 8), in which inactivating mutations were found in several critical eye genes,[50] unlike in *Astyanax*. Accordingly, of around 200 genes associated with visual perception in humans and mice, almost 10 per cent were inactivated or absent in the mole rat (Kim et al. 2011, 225).[51] It must be borne in mind, however, that the mole rat is the sole species in its own family and, from a creationist perspective, may constitute a distinct *monotypic* (consisting of a single species) basic type. In any case, cross-breeding between sighted and blind forms, as seen in *Astyanax*, is not possible, meaning that arguments can only be based on comparative biological data, not on experimental data. It is therefore not possible to determine with certainty whether a process of *regression* has taken place here – the situation is very different from that of the blind cave tetra.

If the vestigial eyes of naked mole rats did indeed arise through regression, it would be necessary to examine whether, despite these findings, the CET model described in the text box can be successfully applied to these cave-dwelling rodents.

Perhaps the inactivating mutations are merely one aspect of a larger overall picture and are under epigenetic control.

Fig. 8 Naked mole rat *(Heterocephalus glaber)*. (Roman Klementschitz, CC BY-SA 3.0)

Discussion

Numerous studies into the genetic causes of cave-dwelling traits in *Astyanax* indicate that the changes observed in cave-dwelling fish may be due to various factors: de novo mutations[53] (plus selection), loss mutations and genetic drift, standing genetic variation (plus selection), as well as epigenetic regulation and plasticity. Of particular interest is which of these factors play the leading role. An experimentally straightforward criterion for distinguishing between de novo mutations as a source on the one hand, and standing genetic variation as well as epigenetics or plasticity on the other, is the *speed* of the respective processes. New mutations leading to adapted phenotypes are rare and must become established within populations over the course of, as a rule, many generations (fixation); *this process is time-consuming.* By contrast, standing genetic variation and changes in epigenetic markers can be utilised within the course of just one generation or fewer; *this source is therefore readily available.* The research findings presented have shown that, alongside mutation and selection as understood in standard evolutionary theory, existing variation, epigenetics and plasticity also play a major role. There is no evidence to suggest that mutations lead to constructive changes. Adaptive, constructive or non-degenerative changes (e.g. enlargement of the jaw, heart structure, altered metabolism, improved sensory functions) appear to be available on demand, as they can be produced by just a few mutations in regulatory genes (see below).

Furthermore, standing genetic variation and variation arising from epigenetic regulation raise the question of their own origins. There is strong experimental evidence that random mutations play a certain role in cave fish. And there is also strong evidence for the diverse effects of selection. However, this does not apply to the *de novo* emergence of standing

A design-based model of the origin of cave animal characters

As outlined, a number of findings suggest that the typical cave fish traits in *Astyanax* are not best explained by the classical neo-Darwinian model, but rather by *standing genetic variation,* which is available at any time, as well as by epigenetic regulatory processes (in particular DNA methylation) and plasticity. These sources of cave-dwelling traits enable *rapid* variability and adaptability within generations (see Table 1). The corresponding changes do not need to arise first through the *time-consuming* process of random mutation, selection and *genetic fixation* (complete spread within the species or population in question). Scientists at the *Institute for Creation Research (ICR)* have developed a model according to which epigenetic regulation and pre-existing variation play a decisive role in adaptation processes. This model is based on a design-based approach known as *'continuous environmental tracking'* (CET) (Boyle et al. 2023). According to this model, organisms actively track the prevailing environmental conditions; they are therefore equipped with a corresponding 'sensory system' and adapt themselves to the prevailing environmental conditions on the basis of adaptive pathways encoded in their genome. Changes are therefore, as it were, pre-planned and, for this reason, repeatable rather than random, which may explain convergences and parallelisms. Furthermore, they are reversible.

However, this model does not appear to refer to the ability of an organism, or its somatic cells, to adapt individually to changing environmental conditions *(plasticity)* – as distinct from adaptations that can be inherited via germ cells through sexual reproduction. Plastic adaptations are based on programmes that are activated by environmental stimuli (possibly on the basis of the summation of various stimuli) and manifest themselves during the lifespan of an individual (often within hours or days, see above). Unfortunately, it is not clear from the explanations provided by its proponents which adaptive processes are specifically accounted for by the CET model. The experiments described below demonstrate individual plasticity rather than existing genetic variation.

Experiments on the CET approach in Astyanax

To test the CET approach, Boyle et al. (2023) conducted experiments on the pigmentation of cave-dwelling forms of *Astyanax*. In these experiments, they exposed low-pigment[52] specimens of the cave-dwelling form of *Astyanax mexicanus* to intense light. They also exposed cave-dwelling fish and surface-dwelling fish to water with a high CO_2 content (and consequently a low pH). The result was that the cave-dwelling fish increased the pigmentation across their entire bodies when exposed to strong light. The changes became apparent within a few weeks. After five months, the cave-dwelling fish were almost as pigmented as the surface-dwelling fish. The authors speculate that a latent melanin synthesis pathway is stimulated by biochemical induction. This suggests that colour changes are likely to be epigenetic rather than genetic in nature. Similarly, the cavefish exhibited behavioural and physiological adaptations to water with high CO_2 levels. Surface-dwelling fish reduce pigmentation across their entire bodies in water with high CO_2 levels, but otherwise show no obvious signs of adaptation (Boyle et al. 2023, 139).

The depigmentation observed in these fish can therefore hardly be interpreted as the result of a 'disrupted' pigmentation pathway caused by random mutations, as the melanin synthesis pathway in the low-pigment cave forms is evidently functional, responsive, reversible and therefore adaptable.

However, it is worth recalling the results of Jeffery's experiments (2009b, 38), according to which complementation tests sometimes show that hybrids of fish from different caves exhibit stronger pigmentation in some cases, suggesting that different genes play a role in the expression of pigmentation.

How can the CET model be validated?

The model predicts that organisms can alter the course of their individual development, that adaptive larval and adult characters are sometimes reversible, that epigenetic changes are often heritable across several generations, and that similar phenotypic characters can be observed in a wide variety of organisms living in similar environments. As regards epigenetic inheritance, it remains unclear whether, or to what extent, the typical characteristics of cave fish that persist across generations have in fact been specifically influenced epigenetically (as is the case with some other vertebrates), or whether they are the result of random mutational events (cf. Boyle et al. 2023, 123, 131–133). Further research must show whether the aforementioned predictions also hold true for other traits typical of cave characins. In doing so, attempts should also be made to elucidate the specific role of transposons in changes to gene expression and in possible epigenetic inheritance. In any case, the CET model raises questions about nature and encourages relevant research.

genetic variation and epigenetic regulatory processes. Within established evolutionary theories, it is simply tacitly assumed that existing variation *originally* stems from mutations. Yet this is merely an assumption motivated by evolutionary theory. For epigenetic regulatory processes and plastic changes, the question of origin arises in an even more pressing form. This is because these require a higher degree of integration of various factors: they can only be understood *top-down*, but would have had to have arisen *bottom-up* if they had developed through evolutionary processes.

If, when considering the possible answers to questions of origin, one does not limit oneself to naturalistic-evolutionary explanatory approaches but takes the possibility of creation into account, the question arises as to what arguments in favour of one view or the other can be derived from the experimental results – in short: is there any evidence of design? Or, more generally: Do the relevant indications fit better with natural evolution or with creation?

Mutations

Arguments in favor of a significant role played by newly arising *random (non-programmed)* mutations in the establishment of characters or traits in the *Astyanax* cave fish arise from the following findings, which have been summarised above:

1. Different cave fish populations carry, at least in part, *different* mutations associated with cave traits. The range of variation differs in some respects between different cave populations.

2. There are various forms of *transitional forms* between surface and cave fish (also in the genus *Rhamdia*). These could be interpreted, on the one hand, as *successive* stages in the loss of sight and pigmentation and, on the other hand, as optimisations of other sensory functions and characters. These could be attributed to mutations that occurred successively.

3. F_2-generation hybrids of cave and surface fish exhibit a wide variety of cave-fish characteristics; for example, their eyes range from almost normal to almost invisible to the naked eye (Kosswig 1963; Jeffery 2009b, 33; **Figs. 7, 9**).

4. Genetic complementation in hybrids between fish from different caves shows that some of the genes responsible for eye degeneration are unique to different cave fish lineages. When individuals from different populations are crossed, characters of surface-dwelling fish can thus be partially restored.

5. With regard to the overall size and development of the retina, hybrids of *Astyanax* surface fish and cave fish exhibited 'dramatic differences' (O'Quin et al. 2013; **Fig. 10**). Without reference to various mutations, these findings would be difficult to understand.

6. In the cave forms of *Astyanax* and *Phreatichthys*, despite very similar phenotypic outcomes, different developmental processes underlie them (Stemmer et al. 2015).

Alternative explanations would also need to account for these findings.

For these reasons, the conclusion drawn by Boyle et al. (2023, 138) – that it is a misconception to believe that random mutations provide the genetic variation required for adaptive changes – appears to be exaggerated. Boyle et al. (2023, 138) base this statement on the experiments described in the text box, in which fish with low pigment levels, when exposed to light, increase their pigmentation over a few weeks to a level almost equivalent to that of surface-dwelling fish. This, they argue, demonstrates that the pigmentation pathway has not been disrupted by random mutations. However, this conclusion would only be correct, firstly, if the cave fish did not synthesise *any* pigment at all. In fact, in most cases, only the *level of production* has been *reduced*, so it is clear in any case that the pigment synthesis pathway has not been disrupted. Secondly, this would also need to be demonstrated for the other typical cave fish characteristics. This has in fact been demonstrated for those genes that are downstream of lens function: they are present in cave fish and are potentially active (Jeffery 2009a, 217; see above).

It is also noteworthy in this context that *Astyanax* cave fish populations living in a karst

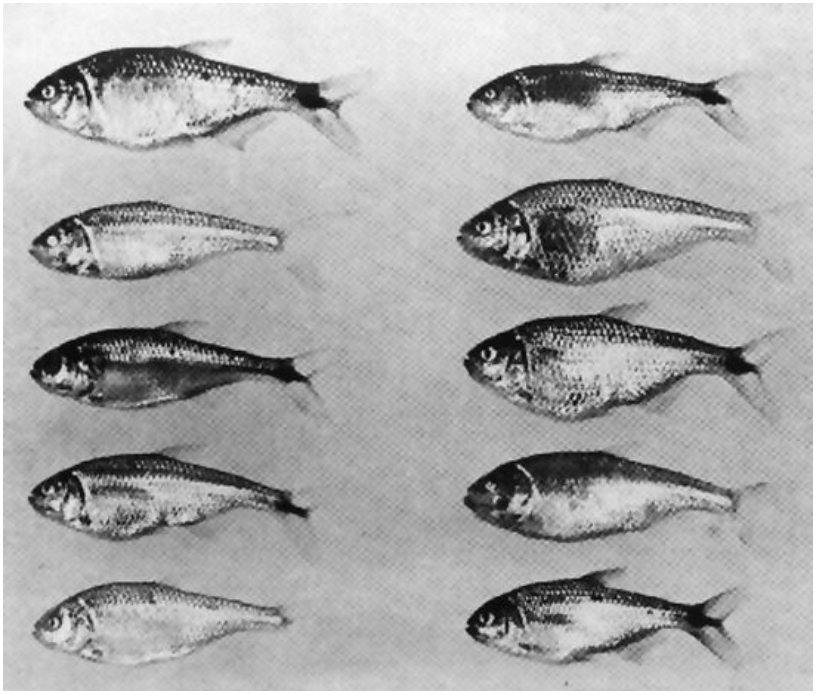

Fig. 9 A selection from the F_2 generation of crosses between cave and surface forms of *Astyanax mecianus*; on the left, fully pigmented individuals, and on the right, unpigmented individuals, with a spectrum ranging from blind to sighted. (From: Kosswig 1963, Zeitschrift für zoologische Systematik und Evolutionsforschung, Vol. 1, 1963, reproduced with the kind permission of the publisher; authorised for the book 'Ähnlichkeiten – Rudimente – Atavismen')

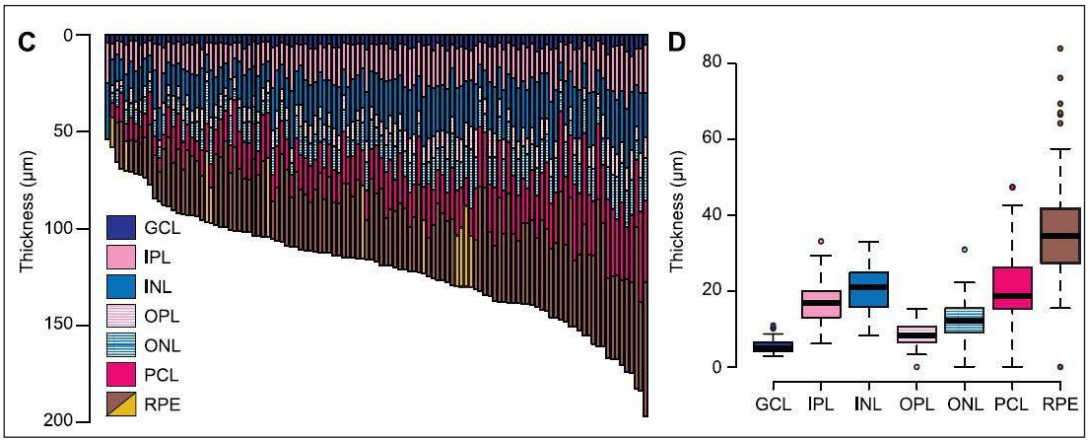

Fig. 10 **Left**: Retinal thickness in 115 *Astyanax* hybrids of cave and surface forms of *Astyanax* in the F_2 generation. Yellow bars indicate retinal pigment epithelia (RPE) that were underpigmented. **Right**: Box plots illustrate the variance in the thickness of the individual retinal layers. The retinal layers shown are: ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), photoreceptor cell layer (PCL) and retinal pigment epithelium (RPE). (From O'Quin et al. 2013, doi:10.1371/journal.pone.0057281.g001, Creative Commons CC0)

Tab. 2 Evidence for the role of regulatory mutations

- The ability to respond to signals from the lens has not been lost (Jeffery 2009a, 217).
- Changes in regulatory genes are an important factor in eye degeneration in *Astyanax* (Jeffery 2009a, 200, Table 8.1).
- Populations of *Astyanax* cave fish generally exhibit only minimal genetic variation in the coding regions of their genes compared with surface-dwelling fish and amongst themselves (Jeffery 2009b, 28). This could suggest that coding genes do not play the primary role in the expression of cave traits.

area within the geologically young Caballo Moro Cave in north-eastern Mexico possess eyes. Genetic analyses using RAPD markers showed that these fish are more closely related to the blind fish in the cave than to the eyed fish on the surface, suggesting that they have *regained* their sight after being exposed to light once again (Espinasa & Borowsky 2000; cf. Krishnan & Rohner 2017, 7). However, the researchers cannot rule out the possibility that the sighted fish are the direct descendants of surface-dwelling fish that have acquired markers from blind fish through hybridisation, although the clear distinguishability of the two subpopulations suggests that they have regained their sight. This suggests that the genes required for vision were merely 'switched off' in some way, which would support the CET model.

Further studies will be required to confirm these exciting observations – and, as mentioned, the CET model therefore provides a basis for specific research approaches.

Despite the findings suggesting that random mutations play a significant role (see 1–6), the changes observed in cave-dwelling populations compared with their surface-dwelling cousins are strikingly similar across the various groups, even assuming they arose independently (Bradic et al. 2012, 1). At the phenotypic level, therefore, there is a striking *convergence*. Even if the underlying mutations differ in some cases, this finding suggests that other causes play the primary role in explaining these similarities. Otherwise, such a high degree of similarity would hardly be expected. It should be borne in mind that a number of findings indicate that changes in *gene regulation* play an important, perhaps even decisive, role (**Table 2**).

So what is behind the convergences in the changes observed in cave fish? Why are the cave phenotypes that have been living in caves for some time relatively similar (albeit not identical), despite having, in some cases, different genetic backgrounds?[54] The key question here is: Are these morphological, anatomical and physiological convergences underpinned by *different genetic changes*? What are these, and what form do they take? If the cave traits were to be attributable, in their crucial aspects, to programming (with certain variables), one would expect similar genetic and/or epigenetic bases for key traits. The *crucial question for the CET model* (see text box) is therefore: Can such common causes for the convergent changes in cave traits be demonstrated?

Convergence and standing genetic variation

According to the classical neo-Darwinian approach, the marked convergences in cave fish traits can be explained by similar natural or sexual selection acting on bearers of newly arisen random mutations (Bradic et al. 2012[55]). However, the convergences could also be explained by *the same existing genetic variation (standing variation)* in the surface populations. In this case, the morphological/anatomical and physiological convergences would be attributable to *genetic homologies*, specifically to *the same alleles* of certain genes within the gene pool of existing variation.[56] These, in turn, could be interpreted as the basic equipment of the original surface populations. From a creationist perspective, this basic equipment could be regarded as having been created originally. The *Astyanax* fish—or living organisms in general—would therefore have been equipped from the outset with various options enabling them to adapt rapidly to different habitats. Which initial scenario is more likely: pre-existing variation or a 'clean slate'? Convergences are unlikely to arise from mere chance processes; they tend to point to a common cause.

A number of the findings summarised above suggest the existence of pre-existing genetic variation within the founding populations:

- The presence of identical alleles in geographically distant cave fish populations
- The allele sets of the cave populations are largely subsets of those of the surface population.
- The presence of identical mutations and many identical SNP markers in two or more cave populations
- Surface populations are very similar despite their relatively great geographical distance.
- Cave-associated phenotypes, such as albino forms, rapidly emerge in captive-bred fish from cave populations that do not exhibit albinism in the wild.
- Genes involved in eye development have not mutated to such an extent that they have lost their function – they could therefore form part of standing genetic variation.

- Albinism in cave fish arises from independent changes in the same gene, *p/oca2* (Jeffery 2009a, 215).
- Certain genes are frequently mutated during the repeated evolution of regressive phenotypes in cave-adapted species (Gross et al. 2009, 1).
- At least some of the genetic changes responsible for cave-associated characters are unique to each cave lineage (McGaugh et al. 2014, 7; Jeffery 2009b, 29).
- The *cbsa* gene is *mutated at the same cis-regulatory position* in six different cave fish populations, suggesting that it plays a role in the repeated loss of eyes. In some cave fish populations (e.g. Pachón, Tinaja), *cbsa* exhibits the same mutation, whilst in others (e.g. Molino) a different mutation occurs at the same site, indicating a possible DNA hotspot for mutational changes. The *mc1r* gene is also mutated at the same position in the coding region in several cavefish populations, and the *oca2* gene exhibits large deletions – albeit at different positions – in the coding region in Pachón and Molino cavefish. These studies show that *the same* genes, but with *different mutations*, may be responsible (Jeffery 2020, 5f).
- With regard to pigmentation, complementation tests on F_1 hybrids sometimes reveal stronger pigmentation, suggesting that different genes are responsible for the loss of pigmentation (Jeffery 2009b, 38; Jeffery 2019, 90).
- Certain genes are repeatedly targeted by mutations that lead to evolutionary changes in the pigmentation of cave fish (Jeffery 2009b, 42).
- In the cave-dwelling forms of *Astyanax* and *Phreatichthys*, different developmental processes underlie their phenotypes despite their very similar appearance (Stemmer et al. 2015; Krishnan & Rohner 2017, 7).
- There is ample evidence of standing genetic variation, including cryptic variation, as well as of epigenetic regulation.

Tab. 3 Chance or programming?

However, the extent to which these observations reflect phenomena that are generally present has not yet been clarified.

Another significant finding is that, in several cases, the same regulatory genes have mutated in different populations, although the mutations are often not identical. This is the case with *oca2* (relating to pigmentation), *mc1r* ('brown' eye mutation), *cbsa* (which plays a role in the loss of eyes) and possibly with *shh* (changes leading to different eye phenotypes).

Molecular convergence is also evident in the albinism phenotype and in melanin synthesis across various *Astyanax* cavefish populations, in unrelated albino cavefish species, and also in albino cave grasshoppers: In all cases, melanin synthesis is disrupted at its first step (Bilandžija et al. 2013, 2). Accordingly, in albino cave fish, melanin synthesis can be restored by the exogenous addition of L-DOPA[57], but not of L-tyrosine (Jeffery 2019, 91; see Fig. 4B therein).[58]

Table 3 summarises findings that may provide evidence as to whether random mutations or programmed changes play the primary role in the adaptations of cavefish.

Regulation and Epigenetics

Epigenetic regulation may also form part of the original basic machinery of *Astyanax* fish; this is a sophisticated capability that can only be understood in a top-down anner, which suggests that it was present from the very beginning. Epigenetic regulation via DNA methylation requires feedback loops. The entire system must be able to measure, compare and react to deviations between desired and actual values; furthermore, desired values must be *specified*. 'Measuring' here refers to a form of environmental monitoring (cf. the CET approach, see text box); 'comparing' refers to the difference between the actual state and the desired state suitable for the prevailing environmental conditions; and 'responding' means initiating changes to the phenotype by utilising available metabolic resources. The increase in pigmentation during the fish's lifetime in the experiments conducted by Boyle et al. (2023) is an example of such a process. Such processes demonstrate the *plasticity* of living organisms. Plastic responses usually occur rapidly – in some cases even within hours – and in any event within the same generation; this was also the case in the pigmentation experiments by Boyle et al. (2023).

The experimental results described above by Gore et al. (2018) also point in this direction: eye degeneration in the Pachón cave fish is (also) due to DNA methylation, which leads to the epigenetic silencing of genes, whilst no inactivating mutations were found in the genes themselves that are active during eye development. DNA methylation can be triggered by various signals, including certain environmental stimuli (Angers et al. 2010, 1286f).

The fact that *genetic* variability does not play a particularly significant role is also suggested by the following finding: whilst the surface and cave forms do exhibit a number of distinct anatomical, physiological and behavioural traits, the overall genetic variability between the surface and cave variants is limited, and their genomes are virtually identical (Boyle et al. 2023, 124). It is not without reason that the populations are classified as belonging to one and the same species, *Astyanax mexicanus* (Jeffery 2019). This is an argument in

favor of the view that the key differences are not attributable to changes in coding genes.

Hotspots? The finding that certain genes have mutated in the same or a similar way across different cave fish populations is also relevant in this context. Is there a higher-level regulatory mechanism at stake? As mentioned above, the *mc1r* gene, the *oca2* gene and the *cbsa* gene have all mutated multiple times at the same coding or cis-regulatory position; in some cases, the changes in *cbsa* involve the same mutation, whilst in others – as with *mc1r* and *oca2* – they involve different mutations at the same site. Jeffery (2020, 5f) interprets this finding as indicating the presence of a 'possible DNA hotspot for mutations'.

Plasticity

Significant changes in cave fish are also possible as a result of plasticity, that is, through the triggering of changes based on existing variation programmes. Insights into this have only recently been gained and have provided a completely new key to understanding the origin of cave fish characteristics. A large proportion of the cave-specific traits could be encoded in the genome of surface-dwelling fish and available on demand at any time. In this case, their expression does not require long evolutionary timescales, but can occur from one generation to the next (see the experiments by Boyle et al. 2023; Sears et al. 2020; Čupić et al. 2023; Behrmann-Godel et al. 2024 and Bilandzija et al. 2020; see the sections 'Plasticity as a source of change' and the text box 'A design-based model of the origin of cave-dwelling animal traits').

However, according to current understanding, plastic changes are usually not heritable; only the plastic options present in the genome are inherited. Adaptive changes must be genetically fixed in order to be permanently inherited. This could occur through subsequent mutations; this has long been discussed at the phenotypic level (genetic assimilation according to Waddington 1953; also described as the 'Baldwin effect'). To date, research into fixing mutations appears to have been scarcely investigated in cave fish.

Pleiotropy

An important aspect is the evidence that some specific cave-dwelling characters are linked through shared regulatory processes *(pleiotropy)*. Various changes in cave-dwelling fish may therefore be coordinated. For example, an increased level of the *shh* gene downregulates the expression of the *pax6* gene and upregulates the expression of *pax2* and *vax1* (Yamamoto et al. 2009; Krishnan & Rohner 2017, 3). Changes in the expression of the genes *pax6*, *pax2* and *vax1* under the influence of the hyperactive *shh* gene trigger the process of cell death in the lens and retina during embryonic development. At the same time, the increase in *shh* expression in the oral ectoderm★ and pharyngeal endoderm★ triggers enhanced development of taste buds and enlargement of the jaw (Yamamoto et al. 2009; Jeffery 2009b, 42; Jeffery 2020). When *shh* is experimentally overexpressed in surface-dwelling fish, strikingly similar eye phenotypes result, and there is a similar coupling of eye degeneration with improvements in the gustatory system (taste buds and jaw) as seen in cave-dwelling fish (Yamamoto et al. 2009; Krishnan & Rohner 2017, 3). Powers et al. (2020) demonstrate a link between cranial bone abnormalities and adaptive sensory enhancements. The senses of sight and smell are also genetically linked (Blin et al. 2018). Regressive and progressive changes therefore go hand in hand here, and all responses are appropriately adapted to the conditions within the caves (cf. Jeffery 2009a, 217[59]). This constellation presents a strong argument in favor of design.

The CAT signalling pathway could also promote adaptive physiological and behavioural characters in cave environments through varying levels of the catecholamine neurotransmitter (CAT) in the brain and kidneys, and thus represents a further example of pleiotropy in *Astyanax* (Bilandžija et al. 2013, Boyle et al. 2023, 134). Bilandžija et al. (2013) found that the suppression of *oca2* expression in embryos of surface-dwelling fish delays the development of pigmented melanophores, whilst simultaneously increasing the levels of L-tyrosine and dopamine in the brain and norepinephrine in the kidneys, due to a coupling of the synthesis pathways for melanin and catecholamines.[60] These authors conclude that a possible evolutionary advantage of albinism in *Astyanax* cave fish could lie in the fact that the suppression of *oca2* simultaneously leads to an excess of L-tyrosine as a precursor for the enhanced catecholamine synthesis pathway, which could be important for adaptation to the harsh cave environment. O'Gorman et al. (2021) found evidence that the *oca2* gene fulfils a pleiotropic function in the adaptive emergence of albinism and sleep deprivation.

Boyle et al. (2023, 134) note that the use of terms such as 'trait gains and losses', 'trade-offs', 'antagonism', 're-deployment' and 'sensory trait linkages' – as used by Jeffery (2020, 4) – suggest, from a technical perspective, highly integrated, intrinsic adaptations that enable *pre-programmed* responses by organisms to a changing environment.

The anatomy, morphology and function of cave fish hearts are also optimised: the heart is smaller, the heart rate slower, and the heart structure more spongy, with rounder ventricles and a lower ratio of wall to trabecular surface area. This allows for a larger surface area exposed to the blood (Tang et al. 2018). These traits develop during early development, "suggesting that such traits are genetically determined" (Tang et al. 2018, 237); they could also be attributable to pleiotropy.

Based on the findings described, Boyle et al. (2023, 135) conclude: "From all of the essential systems described above, we are told that *Astyanax* cavefish have evolved hypoxic [to oxygen deprivation] adaptations as a consequence of life in a low-oxygen environment. Clearly, such a complex series of highly-specified adjustments must be pre-planned and integrated on multiple scales in order to facilitate the stringent mechanisms of gas exchange and circulation in hypoxic environments." And further: "It is incredulous to think that a random, unguided mutation-selection model of evolution could cobble together all functional components with such precision to establish this irreducible suite of adaptive traits, …" (p. 135).

Questions regarding the CET model

According to the CET model ('continuous environmental tracking'; see box), organisms actively monitor the prevailing environmental conditions; they are therefore equipped with a corresponding 'sensory system' and adapt themselves on the basis of adaptive pathways encoded in their genome. Changes are therefore, as it were, preplanned and, for this reason, are repeatable rather than random. It follows that convergences and parallelisms can be explained by common genetic foundations that were already in place and did not have to arise gradually *de novo* through mutation and selection, as postulated by neodarwinistic model.

A review of the findings compiled in this article regarding the changes underlying the specific trait expressions of cave fish reveals that several factors are at play: random mutations, programmed mutations (possible hotspots and similar mutations that have arisen independently), particularly in regulatory genes, selection, genetic drift, standing genetic variation, epigenetic factors and plasticity. However, the role played by the various factors does not yet appear to have been sufficiently clarified. Even though all the factors mentioned can be demonstrated, it may be that some play only a minor role. For instance, random mutations and the findings from QTL mapping may be of secondary importance. Selection very likely plays a significant role, but – as in other cases – *not a creative one*. When assessing the significance of selection, it is irrelevant whether the genetic variation arose gradually through mutations or whether it consists of pre-existing genetic variation.

As described by Boyle et al. (2023), the CET model appears to focus primarily on the *plasticity* of individuals – that is, their ability to respond adaptively to environmental stimuli (or changes in their internal physiological states). The authors' experiments on pigmentation can be explained by plasticity, although the adapted phenotype only emerged after several weeks to months. It will be fascinating to see whether such plastic responses can also be detected in the numerous other specific traits and phenotypic expressions of cave-dwelling animals. A number of recent studies have demonstrated precisely this (see section 'Plasticity as a source of change'). Boyle et al. (2023, 136) write: "Common adaptations are the result of common engineering. Within all *Astyanax* cavefish there is an internal system of preprogrammed adjustments that actively deploy in response to distinct sets of environmental stimuli. In essence, these fish continuously track a range of environmental parameters, assess those parameters on all levels (e.g. molecule, gene, cell, organ system, physiology, anatomy), and adjust rapidly, and appropriately." According to the current state of knowledge, findings that can be interpreted as 'programming' have so far been demonstrated in some cases. However, a number of findings do not appear to be explainable by the CET model. For instance, the finding that there are different manifestations of transitional forms between surface-dwelling and cave-dwelling fish cannot be explained by plasticity alone. How can the CET model explain the fact that the mutations corresponding to cave-dwelling traits are *not* the same across different cave-dwelling populations? The findings summarised in the section on mutations (1–6) cannot be explained by the CET model.

Conclusion 1 – Regulation is crucial

The cave-adapted characters of the tetra *Astyanax* offer a unique opportunity to investigate the causes of their development, as cave and surface forms can interbreed with full fertility – despite numerous significant morphological, physiological, genetic and behavioural differences. It should be borne in mind that these

differences are so great that, after their discovery, the cave fish were initially even placed in a separate genus.

The differences are so great and the presumed period of divergence so short that, under the premises of evolutionary theory, it is unlikely that they arose through the Darwinian interplay of random mutation and natural selection. The majority of cave fish characters are based on adaptations to cave life and *cannot* be explained by defects. This also applies to the regression of the eyes, which is partly linked to constructive traits through regulatory processes.

Rather, the key lies in regulatory processes. As far as is known, the relevant genes show no significant changes; by contrast, the level of expression of a number of regulatory genes is altered. The differences are therefore due to regulatory changes in conserved signalling pathways and genes (Shennard 2026, 130f). Furthermore, epigenetic regulation (silencing of genes through DNA methylation) plays an important role.

Recent studies show that a significant proportion of cave fish characters may initially have arisen from plastic responses, which may subsequently have been fixed by mutations (genetic assimilation).

Conclusion 2 – Evidence of Design

According to the (neo-)Darwinian explanatory framework, all cave-dwelling animal traits and the underlying processes would have to have arisen without any purpose, solely through experimentally verifiable processes, including the particularly sophisticated capabilities of plasticity and epigenetic control. These *top-down* organised processes would have to have arisen in small steps through the Darwinian *bottom-up* mechanism. There is no meaningful experimental evidence for this, and it is also highly implausible from a theoretical perspective. Processes that can only function correctly through higher-level control constitute evidence of planning, as only an agent acting with foresight can implement top-down regulated processes.

The examples of pleiotropy mentioned also provide strong evidence of planning, as it is unlikely that processes—some of which are quite different—are co-ordinated simply by the fact that the same genes are utilised or up- or down-regulated. If, as mentioned, regressive and progressive changes go hand in hand and many reactions are finely tuned to the conditions inside the caves, this constitutes a strong argument for underlying co-ordination and, consequently, planning.

The ability to exhibit a wide range of plastic responses in the cave environment could also point to a programmed situation. Plasticity enables the continuous expansion of species that do not normally live in caves into this specialised cave habitat.

Further possible evidence of planning – which, however, needs to be tested by further research – includes mutation hotspots that support targeted adaptation to cave conditions, as well as the activity of transposons. Further research could investigate whether identical or similar mutations might also have arisen independently of one another due to a shared mechanism of origin.

Finally, *standing genetic variation* could also be interpreted as an inherent endowment of the original populations, which may contribute to rapid adaptation in altered environments.

Acknowledgements

I would like to express my sincere thanks to Dr Peter Borger and Benjamin Scholl for their critical review and helpful comments.

Endnotes

1 Shennard (2026), however, has recently written that there were at least [only] two independent cave colonisations.

2 Evolution is used here in the general sense of heritable changes resulting from a wide variety of genetic (in particular *mutations* and specialisations based on *existing variation*) or epigenetic causes. This does not include the phenomenon of plasticity, i.e. individual adaptability based on adaptive programmes triggered by environmental or internal stimuli.

3 "Cave-dwelling organisms provide the best known examples of convergences, sharing similar phenotypes such as loss of eyes and pigmentation across diverse taxonomic groups" (Bradic et al. 2012, 1).

4 "A hallmark of cave environments is scarcity of food. Cavefish populations rely almost entirely on sporadic food input from outside of the caves" (Aspiras et al. 2015, 9668).

5 "Far from supporting the long-held view that cavefish are degenerated species, this combination of adaptations more likely constitutes a so-called 'cavefish behavioral syndrome'" (Casane & Rétaud 2016, 126).

6 "Río Subterráneo cavefish show a modest reduction in melanophore pigmentation, Chica, Curva, Los Sabinos, and Tinaja cavefish show substantial decreases in melanophore pigmentation, and most Molino and Pachón cavefish show little if any melanophores" (Jeffery 2009a, 208).

7 "First, the genes involved in eye development that have been studied thus far do not appear to have mutated to a degree in which they have lost function. In addition, the restoration of eyes by lens transplantation suggests that all genes that act downstream of lens function are present and potentially active in cavefish" (Jeffery 2009a, 217).

[8] "The results reveal a genetic pathway leading from *sox2* to αA-*crys* that is required for survival of the lens in *Astyanax* surface fish. Defects in this pathway may be involved in lens apoptosis and thus a cause of cavefish eye degeneration" (Ma et al. 2014, 1).
"During lens development, the crystallin genes are regulated by a complex array of transcription factors, including *Pax6*, retinoic acid receptors, members of the *Sox*, *Maf*, and *CREB* families, *AP-1*, and *Prox1*" (Ma et al. 2014, 2).
"The expression of crystallin genes is regulated by the combinatorial activities of several different transcription factors, including Pax6 and Sox2" (Ma et al. 2014, 8).

[9] "Many crystallin genes show reduced expression in the cavefish lens. The αA-*crystallin* gene, which encodes a protein controlling lens survival (Ma et al., 2014), is downregulated, whereas the heat shock protein 90α (hsp90α) gene, which encodes a protein that promotes cell death, is upregulated in the cavefish lens. Together, these two changes in gene expression are thought to trigger cell death in the cavefish lens" (Jeffery 2019, 90).

[10] "Overexpression of *shh* in surface fish results in strikingly similar eye phenotypes as seen in cavefish. The reciprocal experiment of inhibiting *Shh* in cavefish does not fully restore eye development, but does lead to slightly bigger eyes, underscoring the potentially important role of *Shh* in eye degeneration" (Krishnan & Rohner 2017, 3).

[11] Examples: "An expansion of the expression of *shh* along the midline is observed in Pachón, Chica, and Los Sabinos cavefish (…) and a reduction of expression domain size of *pax6* is found in Pachón, Los Sabinos, and Curva populations (…). Further, the induction of apoptosis in the lens has been reported in Los Sabinos, Chica, and Pachón populations" (Sifuentes-Romero et al. 2020, 433f).

[12] QTL mapping: Homozygous (true-breeding) individuals are crossed for the character of interest to produce an F_1 generation. The F_1 generation is then crossed or backcrossed to produce the F_2 generation or subsequent generations. These generations contain a combination of the parents' genetic material. The phenotypic information from these generations is recorded and correlated with the genotypic information. This enables researchers to observe which regions of the genome segregate with the traits in question.

[13] Jeffery (2019, 92) writes: "It is difficult to pinpoint the precise identity of the mutated genes. However, a large number of candidate genes have been identified, which are currently being evaluated for the presence of mutations."

[14] "… it is unlikely that mutations in or nearby *shh* are directly responsible for the cave phenotypes as none of the eye size quantitative trait loci (…) overlap with the *shh* locus" (Krishnan & Rohner 2017, 3).

[15] "Although what shows up in QTL analysis may not reflect the entire spectrum of altered genes, these results suggest that these genes (or their cis-regulatory regions) are not mutated in cavefish. Instead, they are probably subsidiary genes that act downstream of genes that were mutated to initiate eye reduction" (Jeffery 2009b, 37).
Krishnan & Rohner (2017, 5) schreiben: "It came as a surprise that none of these QTL were near the earlier studied genes—*shh* or *pax6*—indicating that mutations in these genes were not directly responsible for the eye phenotype and that genes upstream were most probably involved; however, in a more recent study, a QTL for eye diameter was found to be near the *pax6* locus."

[16] "As a rule, however, *Astyanax* cavefish populations show minimal genetic variation in the coding regions of nuclear genes relative to surface fish and each other." Jeffery (2009b, 28) goes on to note that phylogenetic conclusions must therefore be drawn from other findings "Therefore, phylogenetic inferences have been based on more divergent randomly amplified polymorphic DNAs (RAPDs), microsatellite sequences, and rapidly evolving mitochondrial DNA."

[17] And further: "The work shown here in *A. mexicanus* supports the growing body of literature which suggests that evolution primarily acts through a large number of regulatory changes rather than protein coding changes" (p. 130).

[18] "…the *Pax6* gene itself is not found in regions encompassing QTLs for eye size or retinal layers" (Casane & Rétaud 2016, 140). However, once again neither *twhh* nor *shh* genes are found within eye QTL regions" (ibid. p. 141).

[19] "As it is difficult to imagine that eyes, though useless, could be in any way injurious to animals living in darkness, I attribute their loss wholly to disuse" (Darwin 1859).

[20] "… the evolutionary processes that led to the loss of the visual system are debated … " (Moran et al. 2015, 1).

[21] " It should be emphasized that the current data does not allow us to conclude unequivocally that either selection or neutral mutation is responsible for regressive evolution" (Jeffery 2009b, 41).

[22] "One key question, however, remains unresolved: is this eye loss due to selective pressure to reduce eye size in the dark or to genetic drift in the absence of selective pressure to maintain eye function?" (Niven 2008, R28).

[23] "the genetic underpinnings of eye degeneration and loss in cavefish is still largely unknown" (Shennard 2026, 11).

[24] "Standing genetic variations (or simply population polymorphisms) are more common than previously thought and there is an increasing acknowledgement of their importance in evolution and adaptation" (Krishnan & Rohner 2017, 5).

[25] The extent to which convergent or parallel changes draw on preexisting genetic variation in ancestral populations versus new mutations is still debated. The molecular and genetic changes that underly most convergences are still unknown" (Bradic et al. 2012, 1).

[26] "… the mutations responsible for pigmentation or eye loss, as examples, differ among the caves. When two blind and depigmented individuals from different cave populations are crossed, the hybrid offspring may look more like surface fish than their cave parents. This is because the non-functional alleles from one parent are complemented by the functional alleles of the other parent, and vice versa" (Borowsky 2018, R62).

[27] "Crosses between fish from different caves complement and restore certain cave-derived phenotypes (for example, rudimentary eyes); thus, at least some of the genetic changes accounting for cave-associated traits are unique to each cave lineage" (McGaugh et al. 2014, 7).

[28] " Different cavefish populations have distinct mor-

phologies (Figure 3a–f, Figure 4)" (Jeffery 2009b, 29).

[29] "Complementation refers to a genetic process when two strains of an organism with different homozygous recessive mutations that produce the same mutant phenotype (for example, a change in wing structure in flies) have offspring that express the wild-type phenotype when mated or crossed." (https://en.wikipedia.org/wiki/Complementation_(genetics))

[30] "Complementation crosses between different cavefish populations (Fig. 4b) have shown that genes controlling eye loss can be the same or unique in these populations. The *cbsa* gene is mutated at the same *cis*-regulatory position in six different cavefish populations, suggesting that it functions repeatedly in eye loss. In some cavefish populations (e.g., Pachón, Tinaja) *cbsa* shows the same mutation, while in others (e.g., Molino) a different mutation occurs at the same site, revealing a possible DNA hotspot for mutational change. Likewise, the *mc1r* gene is mutated at the same position in the coding region of multiple cavefish populations, and the *oca2* gene shows large deletions at different places in the coding region in Pachón and Molino cavefish. These studies show that the same genes harboring different mutations can be responsible" (Jeffery 2020, 5f).

[31] F_1 = the first daughter generation; F_2 = the second daughter generation, resulting from the cross-breeding of F_1 individuals.

[32] "In the most extreme cases, melanin pigment is absent in the albino Pachón and Molino cavefish. However, the precursors of melanophores are still present, as evidenced by their ability to convert L-DOPA to melanin" (Jeffery 2019, 87).

[33] https://medlineplus.gov/download/genetics/gene/mc1r.pdf

[34] "The *brown* gene encodes the melanocortin 1 receptor *(Mc1r)*, a component of the melanosome-stimulating hormone-α (MSH-α) signaling system, which regulates pigmentation in many different vertebrates. Different mutations in the *mc1r* gene, located in the coding or regulatory regions, are responsible for the brown eye phenotype in various cavefish populations. Thus, *mc1r* is another example of a gene that has been mutated repeatedly to generate the same troglomorphic phenotype in different cavefish populations" (Jeffery 2019a, 92).

[35] "Therefore, *Mc1r* is another example of a single gene that is repeatedly targeted by different mutations to change a complex phenotype during parallel or convergent evolution" (Jeffery 2009b, 42).

[36] "No sequence differences in the *Mc1r* coding region have been detected in Piedras or Curva cavefish, implying that the relevant mutations may be in gene regulatory regions" (Jeffery 2009b, 41).

[37] "The data suggest that eyes and pigmentation regressed through different mechanisms. Cave alleles at every eye or lens QTL we detected caused size reductions, consistent with evolution by natural selection but not with drift. QTL polarities for melanophore number were mixed, however, consistent with genetic drift" (Protas et al. 2007, 452). – Jeffery (2009a, 218): "Genetic analysis, in which individual QTL governing the extent of melanophore development have been shown to either increase or decrease melanophore abundance, supports the role of neutral mutation and genetic drift."

[38] "Rather, the same coding sequence alterations first characterized in Pachón cavefish were also present in albino Micos cavefish. This implies that the *Oca2* haplotype discovered in Pachón cavefish was part of the standing genetic variation in the epigean stock that seeded the phylogenetically young Micos cave population and introgressed into the phylogenetically old Pachón cave population" (Gross & Wilkens 2013, 123).

[39] "… four of the cave populations (N3, O8, N2 and O2) contained alleles from surface populations at several loci while the surface populations showed a smaller number of the alleles from the caves" (Bradic et al. 2012, 7).

[40] He continues: Thus, the evolution of cave forms did not have to await new mutations. The existence of identical mutations and many identical SNP markers in two or more cave populations has been documented and supports this view" (Borowsky 2018, R63).

[41] "... unpigmented (albino) individuals spontaneously arose within this laboratory stock after approximately six or seven generations. ..." (Gross & Wilkens 2013, 129).

[42] Some researchers associate such processes with the hope that the release of cryptic variation in certain environments (particularly under environmental stress) might occasionally be advantageous and thus adaptive. At the same time, this allows for a rapid response to changing selective conditions. After some time, changes can become independent of the Hsp90 mutation (genetic assimilation). As long as Hsp90 remains intact, cryptic variation can accumulate (the effects of which are suppressed by Hsp90 and which is therefore not subject to selection). Rutherford & Lindquist (1998, 336) regard this process as a plausible mechanism for driving evolutionary changes in otherwise deeply entrenched developmental processes. They even speculate – albeit without experimental evidence – that the sudden release of cryptic genetic variation enables the rapid morphological radiations recorded in the fossil record (p. 341). Similarly, Cowen & Lindquist (2005, 2185) put forward the view that Hsp90 could play a role in evolution by acting as a buffer to accumulate and release genetic variation. Quite apart from the question of how realistic this scenario actually is, there is a major drawback: homozygous mutations in Hsp90 are *lethal* (death-causing). Consequently, Hsp90 mutations could only become established in the population up to a certain percentage. This situation is reminiscent of that seen in sickle cell anaemia, where homozygous mutations are lethal, whilst heterozygous (mixed-allele) mutations are advantageous in malaria-endemic regions. Unlike in that case, however, any advantage conferred by Hsp90 mutants in the heterozygous state remains purely hypothetical at present. The idea that cryptic variation could enable evolution is discussed in greater detail by Junker (2009).

[43] "… loss of eyes in the cave morph, a trait that has been shown to be influenced by at least 14 mapped quantitative trait loci. … Thus, inhibition of HSP90 permits the expression of cryptic variation in eye size present in the Astyanax populations" (Rohner et al. 2013, 1373).

[44] However, numerous findings regarding mutations in genes relevant to cave fish have been compiled above; according to Gore et al. (2018), it is therefore unlikely that any inactivating mutations were among them.

[45] Another interesting finding is that the genes that are most heavily hypermethylated and downregulated in the cave-dwelling form are also associated with

human eye diseases. This suggests that the function of these genes is the same across all vertebrates (in evolutionary terms: 'conserved'; Gore et al. 2018).

[46] "An intriguing possibility supported by current research is that some of the multiple eye genes involved in cavefish eye loss may be under epigenetic control by DNA methylation" (Jeffery 2019, 92).

[47] This would be consistent with the neo-Darwinian model.

[48] "This suggests that variability in phenotypic traits associated with vision is primarily heritable and variation among forms is the result of standing genetic variability. In contrast, characters associated with chemo- and mechano-sensation and body pigmentation are heritable as well as highly affected by phenotypic plasticity" (Behrmann-Godel et al. 2024, 742).

[49] "… patterns of gene expression without any evidence of genetic divergence" (Čupić et al. 2023, 821).

[50] "In contrast, genome sequencing of another subterranean animal—the naked mole rat *Heterocephalus glaber*– showed combined functional loss of more than a dozen key eye genes due to inactivating mutations" (Gore et al. 2018, 1155).
"Our findings indicate that eye loss in Pachón cavefish occurs via distinct molecular mechanisms compared with naked mole rats, where inactivating mutations are found in multiple key eye genes" (Gore et al. 2018, 1158).

[51] "Of about 200 genes associated with visual perception (GO:0007601) in humans and mice, almost 10% were inactivated or missing in the NMR" (Kim et al. 2011, 225). "Thus, whilst some genes responsible for vision are preserved in the NMR, its poor visual function may be explained by the deterioration of genes encoding various critical components of the visual system" (Kim et al. 2011, 226).

[52] "The expression of melanin is notably low in these cavefish, and geographical locations for the cave systems of our commercial cavefish …" (Boyle et al. 2023, 131).

[53] Such changes were not yet present in the original population of surface-dwelling fish

[54] "The final phenotypes are similar (although not identical), suggesting that there are many different ways to produce the same phenotypes" (Jeffery 2009b, 43).

[55] "These phenotypic convergences have occurred in spite of gene flow from surface populations suggesting either strong natural or sexual selection for alleles responsible for the cave phenotype in the cave environment" (Bradic et al. 2012, 1).

[56] Hypothetically, there could also be a mechanism behind this that produces similar changes. This hypothesis is speculative for the time being, but could be tested.

[57] A precursor of dopamine, an important neurotransmitter in the brain

[58] "In albino cavefish, melanin synthesis can be rescued by exogenous addition of L-DOPA (Fig. 4B), but not L-tyrosine. Therefore, melanin synthesis is blocked because cavefish melanophores do not provide L-tyrosine to their melanosomes as a substrate to form L-DOPA and ultimately melanin. The same defect at the first step in the melanin synthesis pathway has evolved in both the albino Pachón and Molino cavefish" (Jeffery 2019, 91).

[59] "As we have seen, Hh overexpression has a negative effect on eye development, and it is known from studies on other vertebrates that Hh signaling has positive effects on many other developmental traits. Thus, selection for the positive traits would automatically affect the negative ones" (Jeffery 2009a, 217).

[60] "These experiments reveal a potential evolutionary benefit of albinism caused by oca2 loss of function in cavefish: enhancement of the alternative CAT pathway to promote multiple physiological and/or behavioral functions that are adaptive for survival in the extreme cave environment" (Bilandžija et al. 2013, 12).

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