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Convergent evolution of albinism in cave animals is driven by shared mechanisms and adaptive advantages

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Loss of pigmentation is a hallmark of cave-dwelling animals, yet the mechanisms and evolutionary forces driving this convergence remain unclear. Here, we investigate the cellular and biochemical basis of albinism across phylogenetically diverse cave species, including annelids, mollusks, and vertebrates. Across all taxa, melanin loss consistently results from disruption of the first step in the biosynthetic pathway. Despite this metabolic block, cells capable of melanin synthesis are retained showing distributions similar to pigmented surface relatives and contributing to innate immune responses during tissue repair. These conserved features raise the question of what evolutionary forces drive pigmentation loss. To test whether albinism reflects neutral drift or adaptive evolution, we use the model cavefish *Astyanax mexicanus* and find that melanin synthesis imposes a significant energetic cost, and that independently evolved albino populations show elevated dopamine levels. Thus, the convergent disruption of melanin biosynthesis may confer both direct benefits by energy savings under resource-limited cave conditions, and indirect advantages through a trade-off between melanin and catecholamine synthesis from their shared precursor, L-tyrosine. Our findings support an adaptive hypothesis for the repeated evolution of albinism in cave animals.

Convergent evolution is widespread, occurring not only between phyla or kingdoms but even across domains[1,2]. Classic examples include the evolution of bioluminescence, carnivory and multicellularity across kingdoms, camera-type eyes in vertebrates and cephalopods, and antifreeze proteins in Arctic and Antarctic fishes. It is well established that convergence arises when divergent lineages adapt to similar environmental pressures and ecological niches. However, it remains less clear whether convergent traits result from shared or distinct developmental, molecular, and evolutionary pathways. Uncovering the mechanisms underlying convergence may provide critical insights into the constraints and predictability of evolution[3]. At a time of accelerating environmental change, the ability to predict evolutionary outcomes is essential for conservation and managing future biodiversity[4].

The loss of pigmentation resulting in albinism is one of the hallmarks of cave-adapted animals and has evolved in diverse organisms ranging from planaria to vertebrates, regardless of their type of pigment. As such, albinism in cave animals is one of the best examples of convergent evolution in nature. In one of the cave forms of the teleost *Astyanax mexicanus* and two species of cave planthoppers, melanin biosynthesis is blocked at the first step of the pathway[5,6]. However, the mechanisms responsible for albinism in other cave-adapted species remain unknown. Are the underlying causes for convergent loss of melanin synthesis the same or different in divergent cave animals?

Melanin is one of the most common dark pigments found in plants, fungi, and different animal phyla[7]. Melanin biosynthesis begins with the amino acid L-tyrosine, which is obtained from the diet or synthesized by hydroxylation of L-phenylalanine. L-tyrosine is first converted into L-3,4-dihydroxyphenylalanine (L-DOPA), which is the rate-limiting step in the pathway. L-DOPA is then oxidized to dopaquinone, which is a critical branch point in the pathway. If dopaquinone undergoes further enzymatic reactions, this leads to the production of eumelanin. This pathway involves several enzymes and intermediate molecules, such as 5,6-dihydroxyindole-2-carboxylic acid (DHICA) and 5,6-dihydroxyindole (DHI). In vertebrates, melanin is synthesized in specialized lysosome-derived melanosomes, which are located in melanin-containing pigment cells called melanophores[8].

The specific enzymes, intermediates, and regulatory mechanisms involved in melanin synthesis vary among different animal species. Insects convert L-tyrosine to L-DOPA using tyrosine hydroxylase, whereas vertebrates and many other organisms catalyze this reaction using tyrosinase[9,10].

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Conversely, tyrosine hydroxylase catalyzes synthesis of the catecholamine (CAT) dopamine by utilizing L-tyrosine as a substrate in vertebrates. CATs are important for various physiological and neurological processes, including serving as neurotransmitters[11].

The evolutionary drivers of regressed traits including pigmentation in cave animals have been the subject of persistent debate[12]. Obviously, the functions of pigmentation, including visual processes, protection from UV light, behavioral displays, mimicry, and camouflage[13,14] are useless in the absolute darkness of caves. Charles Darwin suggested that regression in cave animals could be a consequence of disuse[15]. Indeed, negative selection against spontaneous mutations that result in albinism would not be expected in dark caves, whereas it is reasonable that albinos would be targets of predation or less desirable sexual partners on the surface. Thus, one hypothesis for pigmentation loss in albino cave animals has been neutral mutation and genetic drift in which purifying selection for mutations in the pigmentation pathways is presumed to be lacking[16,17]. However, except in extremely small populations, it is difficult to reconcile the slow and stochastic progress of genetic drift with relatively rapid evolution in some cave animals[18–22]. Accordingly, it has also been postulated that pigmentation loss may be adaptive either based on indirect selection dependent on pleiotropy[23] or on positive selection for energy conservation[12,24,25].

Numerous pleiotropic functions of melanogenesis have been discovered, including behaviors, thermoregulation, life history and immunity[26–28], and indirect selection for albinism via pleiotropy has been suggested in *Astyanax* cavefish[29–32]. The mutated *oca2* gene[33] controls albinism by functioning in L-tyrosine processing in the melanosome during the first step in melanin biosynthesis[34,35]. The *oca2* gene has been shown to have pleiotropic effects on CAT levels and related physiology and behaviors and to be under positive selection in three cavefish populations[29–32]. Additionally, trade-offs between melanogenesis and metabolic adaptations to a low nutrient environment have also been suggested in *A. mexicanus* cavefish via cis-regulatory changes in a gene involved in tyrosine metabolism[36]. The adaptive hypothesis for the evolution of albinism requires targets in which natural selection can act to increase fitness in cave-adapted species. Thus, another objective of this study was to identify potential benefits of albinism in cave animals.

Our results show that albinism has evolved convergently by disruption of the first step in melanin synthesis in diverse cave animals. Remarkably, despite the loss of melanin in albino cave animals, cells capable of melanin synthesis are conserved in patterns resembling their close surface relatives. Furthermore, we find that cells capable of synthesizing melanin are recruited to the sites of injuries, suggesting important roles in innate immunity. In addition, we provide experimental evidence consistent with the hypothesis that albinism could represent an energy-saving adaptation. Our results suggest that melanin synthesis has an energetic cost in the teleost *Astyanax mexicanus*, a single species consisting of pigmented surface fish and multiple populations of depigmented and albino cavefish[37]. Lastly, we find that the disruption of melanin synthesis at its first step enhances dopamine (DA) in multiple albino and depigmented cavefish populations, supporting a widespread tradeoff between the melanin and CAT synthesis pathways in *A. mexicanus*. The identification of multiple advantages for the loss of pigmentation suggests a role of natural selection in the convergent evolution of albinism in cave animals.

Results

Melanin pigmentation in surface relatives of albino cave species

Dark pigment in vertebrates is based on melanin, but invertebrates use melanin and/or other pigment types for dark coloration, most commonly ommochromes and porphyrins[38]. Therefore, to select cave species for this study, we first identified those with surface dwelling relatives showing melanin pigmentation. Assays were developed in which surface species were extracted with solvents that dissolve different types of pigment: acetone for pigments soluble in organic solvents such as carotenes, formic acid for ommochromes and porphyrins, and a bleach treatment (melanin bleach) for melanin. We tested the specificity of these solvents in *A. mexicanus* surface fish, which contain brown to black melanin-containing melanophores and orange carotene-containing xanthophores (Fig. 1A)[31]. Acetone removed xanthophores, but not melanophores (Fig. 1B), identifying carotene as the xanthophore pigment, formic acid did not affect xanthophores or melanophores (Fig. 1C), consistent with the absence of ommochromes and porphyrins, and melanin bleach extracted melanophores but not xanthophores (Fig. 1D). These results confirm melanin as the dark pigment in *A. mexicanus* and illustrate the specificity of the pigment solvent assays.

As additional controls, the solvent assays were done in surface invertebrate species known to contain ommochrome or porphyrin pigmentation in addition to melanin. In the isopod *Asellus aquaticus*, which contains only ommochrome pigmentation[39], dark body coloration was completely removed by formic acid (Fig. 1N). In arthropods, melanin is synthesized at wound sites as a response to injury[40]. Thus, in the amphipod *Gammarus minus*, body pigment, but not melanized wound pigment, was removed with formic acid (Fig. 1O). In the surface planarian *Polycelis sp*., porphyrin body pigmentation[41] was extracted with formic acid (Fig. 1P), but dark pigmentation in the multiple eyes was not affected (Fig. 1Q). This is consistent with planarian eyes containing melanin[42]. Lastly, in a surface-dwelling cixiid insect species, formic acid did not extract body or wound pigment, suggesting a melanin basis for dark pigmentation (Fig. 1Q and inset). However, eye pigment was extracted, suggesting it is likely to be ommochrome, a common arthropod eye pigment[43,44]. These results show that pigment solvent assays can distinguish between melanin and other dark pigments in the same animals.

Using the assays developed above, we next surveyed pigment types in different surface-dwelling invertebrate species with dark pigmentation. We found that dark pigmentation in body segments of the polychaete annelid *Ficopomatus enigmaticus*, a close surface relative of cave-dwelling *Marifugia cavatica*[45], was extracted with melanin bleach, but not formic acid or acetone, indicative of melanin pigmentation (Fig. 1 E–I). In the surface bivalve mollusk *Dreissena polymorpha*, a close relative of the cave-dwelling *Congeria kusceri*[46], dark pigmentation at the edge of the mantle and gills was also extracted with melanin bleach, but not acetone or formic acid, confirming melanin pigmentation (Fig. 1 J–M). In contrast, dark coloration in *F. enigmaticus* tentacles (Fig. 1 H, I) and in the shell and other body parts of *D. polymorpha* (Fig. 1 L) was resistant to extraction with melanin beach, and thus not confirmed as melanin. In *D. polymorpha*, extraction of dark pigment stripes in the shell by formic acid (Fig. 1M) suggests that they are composed of either ommochromes or porphyrins.

Together, these results underscore the necessity of testing dark pigmented species for the presence of melanin before related albino cave species are concluded to be deficient in melanin synthesis. We conclude that in addition to vertebrates, melanin is a body pigment in some annelid and mollusk species, permitting them to be coupled with closely related albino cave species in the pigmentation studies described below.

The first step of melanin synthesis is disrupted in diverse albino cave species

It was previously shown that melanin biosynthesis is blocked at the first step of the pathway, the conversion of L-tyrosine to L-DOPA, in the albino Pachón form of *A. mexicanus* cavefish[5] and in two albino cave cixiid species[6], but it is unknown whether disruption at this step could occur in other cave animals. To address this possibility, the melanogenic substrate assay[5,6], in which albino species are challenged to produce melanin when supplied with exogenous L-tyrosine or L-DOPA substrates, was applied to additional albino cave animals whose surface relatives contain melanin or are embedded in surface lineages already known to have melanin-based pigmentation (teleosts and amphibians). Treatment with L-DOPA, the second substrate in the melanin pigment pathway, restored pigmentation in the cave mollusks *Melledela werneri* (Fig. 2B) and C. *kusceri* (Fig. 2D), the cave polychaete *M. cavatica* (Fig. 2F), the cavefishes *Typhlichthys subterraneus* (Fig. 2H) and *Amblyopsis spelaea* (Fig. 2J), and the cave salamander *Proteus anguinus* (Fig. 2L). In contrast, the provision of exogenous L-tyrosine, the

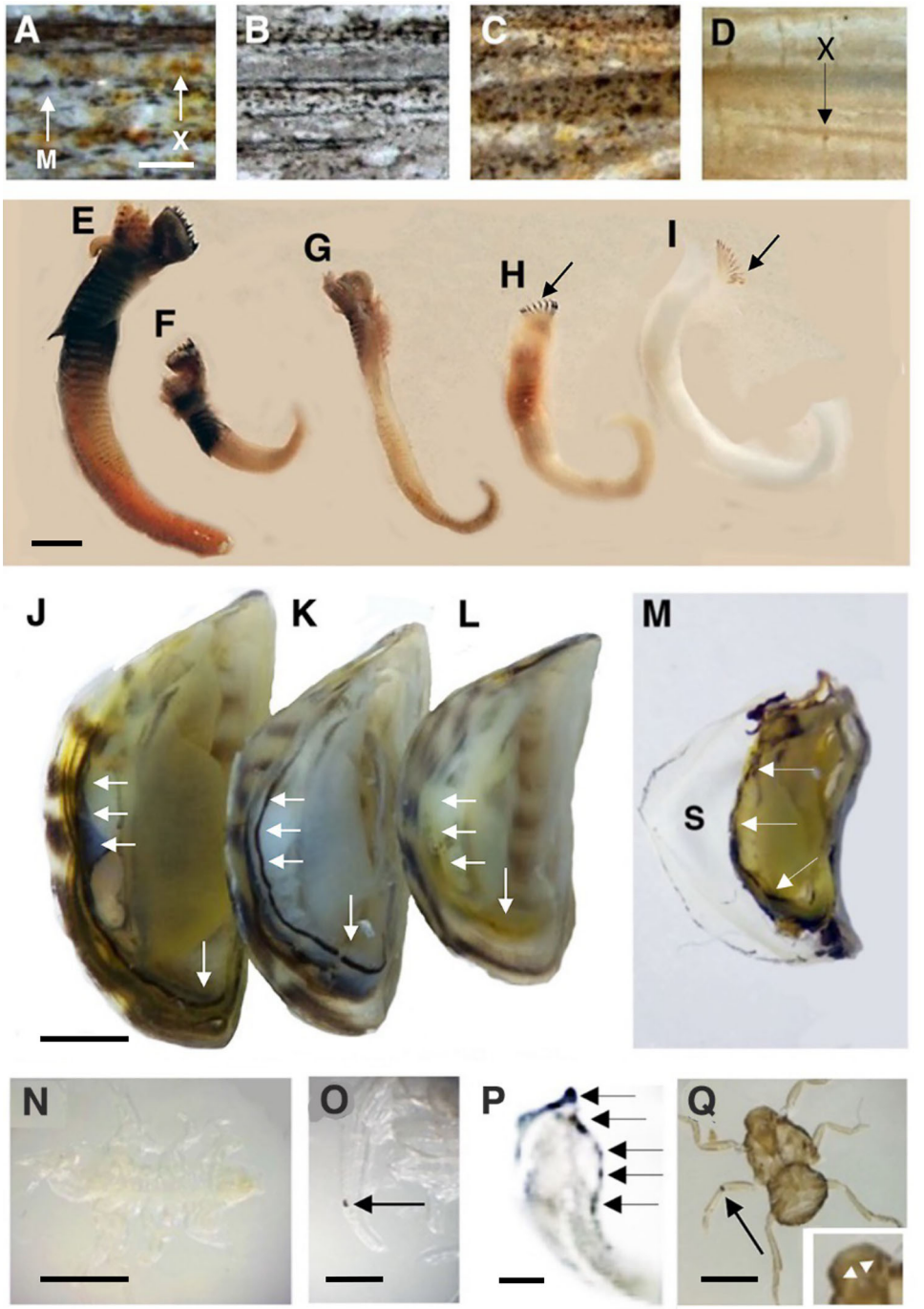

Fig. 1 | Pigment solvent assays distinguish between dark pigmentation types in vertebrate and invertebrate species. A–D *Astyanax mexicanus* surface fish tailfins. **A** Untreated control showing melanophores (M) and xanthophores (X). **B** Acetone removes xanthophores. **C** Formic acid has no effects on melanophores or xanthophores. **D** Melanin bleach removes melanophores but not xanthophores (X). **E–I**. *Ficopomatus enigmaticus*. **E** Untreated control showing dark pigmentation in tentacles and segments. Acetone (**F**) or formic acid (**G**) do not extract dark pigmentation. Melanin bleach decreases (30 min treatment, **H**) or removes (12 h treatment, **I**) dark body pigmentation but not tentacle pigmentation (arrows). **J–M** *Dreissena polymorpha*. **J** Untreated control showing dark pigmentation in the shell and body, including a pigment band along the edge of the mantle (arrows). **K** Acetone does not affect dark shell pigmentation or mantle pigmentation (arrows). **L** Melanin bleach removes the mantle band pigmentation (arrows) but not shell pigmentation. **M** Formic acid dissolves the shell (S) but does not affect dark mantle pigmentation (arrows). **N** *Asellus aquaticus*: formic acid removes ommochrome body pigmentation. **O** *Gammarus minus:* formic acid removes body pigment but not melanin in a wound on the antenna (arrow). **P** *Polycelis sp.*: formic acid removes body pigmentation but not dark pigment in multiple eyes (arrows). **Q** Unknown cixiid insect species: formic acid does not remove body pigment, eye pigment (inset), or melanized appendage wound (arrow). Scale bars: 25 μm in A, same magnifications in **A–D**; 2 mm in **E–I**; 5 mm in **J**, same magnifications in **J–M**; 5 mm in **N**; 2 mm in **O**, Q, 1 mm in **P**. **Q** insert 1.5X.

first substrate in the melanin pigment pathway, did not restore melanin biosynthesis in any of these albino cave species (Fig. 2A, C, E, G, I, K). Restoration of pigmentation by L-DOPA, but not L-tyrosine, indicates that a block in melanin synthesis occurs at the first step of the pathway in diverse albino cave species and all the downstream steps of the pathway are intact and functional in these species.

The pattern of restored melanin pigmentation in diverse cave species resembles natural dark pigmentation in surface relatives

To determine the patterns of pigment restoration in albino cave species, the distribution of L-DOPA rescued pigment cells in albino cave animals was compared to the natural pigmentation patterns in their surface relatives. In Pachón cavefish larvae (Fig. 3E) and adult tailfins (Fig. 3F), L-DOPA rescued pigment cells were distributed similarly to melanophores in surface fish larvae (Fig. 3G) and tailfins (Fig. 3H), confirming and extending an earlier report on the presence of cells with capacity for melanin synthesis in *A. mexicanus*[5]. In the cave polychaete *M. cavatica* (Fig. 3A), L-DOPA rescued pigmentation in a segmental pattern resembling the natural pattern of body pigmentation in surface *F. enigmaticus* (Fig. 3B). In the cave bivalve *C. kusceri*, L-DOPA rescued melanin in cells located at the edge of the mantle and gill where melanin pigment is normally located in surface *D. polymorpha* (Fig. 3C, D). Thus, despite the loss of melanin pigmentation, cells capable of melanin synthesis are conserved in diverse albino cave animals where they are distributed in patterns resembling melanin pigment cells in closely related surface forms. These results suggest that in cave animals from different phyla albinism is due to disruption of the capacity for melanin synthesis and not the loss of cells originally responsible for producing pigmentation.

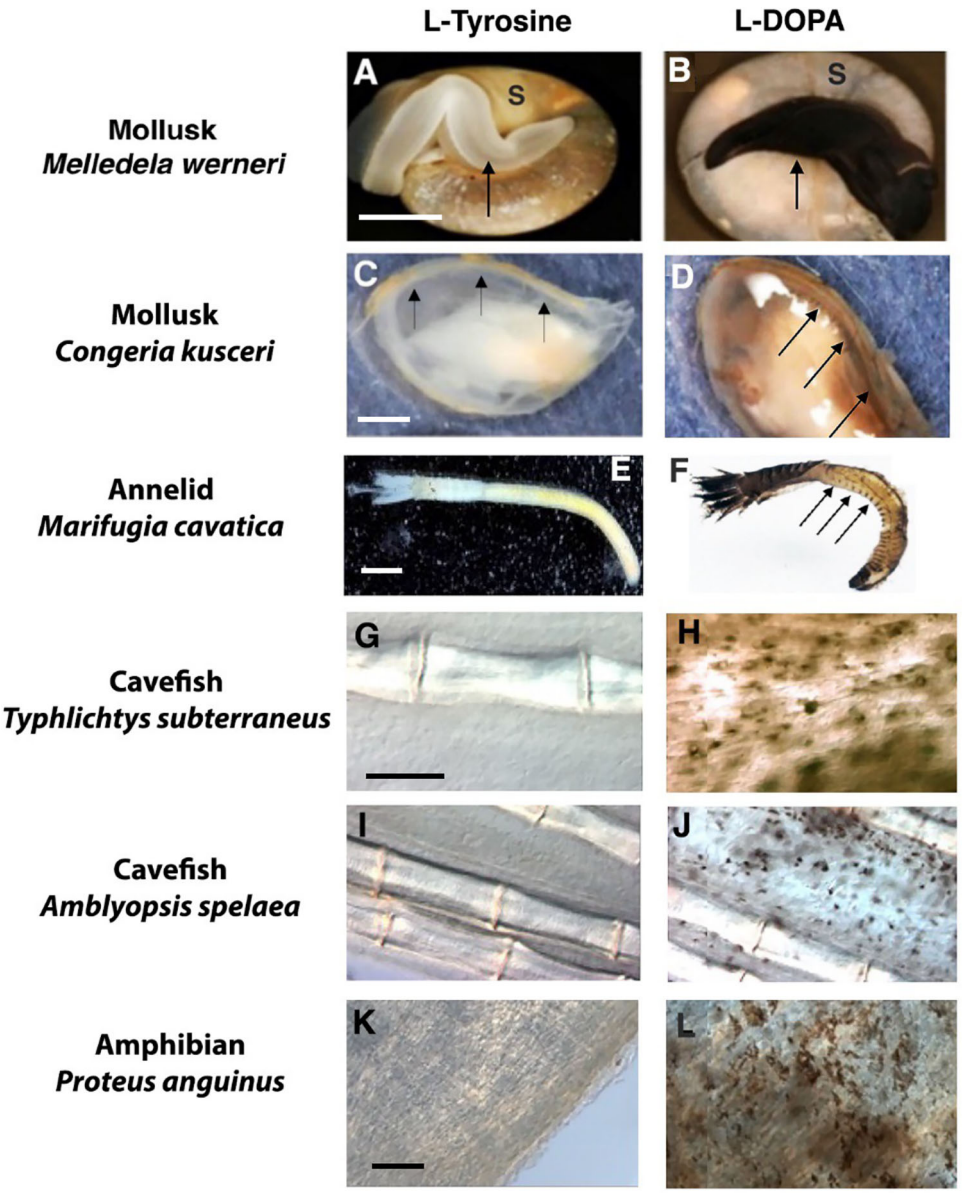

Fig. 2 | Diverse albino cave-adapted species are blocked at the first step of melanin synthesis. **A**, **C**, **E**, **G**, **I**, **K** The addition of L-tyrosine, the first substrate in the melanin synthesis pathway, does not rescue melanin pigmentation in (**A**) the albino cave gastropod *Meledella werneri*, (**C**) the albino cave bivalve *Congeria kusceri*, (**E**) the albino cave polychaete *Marifugia cavatica*, (**G**, **I**) the albino cavefish (**G**) *Ambylopsis spelaea* and (**I**) *Typhlichthys subterraneus*, and (**K**) the albino cave salamander *Proteus anguinus*. **B**, **D**, **F**, **H**, **J**, **L**. The addition of L-DOPA, the second substrate in the melanin synthesis pathway, rescued melanin pigmentation in each albino cave species. S in **A**, **B**: shell. Arrows in **A**, **B**: Protruding foot. Arrows in **C**, **D**: rescued pigmentation along the edges of the mantle. Arrows in **F**: rescued pigmentation stripes in the segmented posterior body. Scale bars: 10 mm in **A**; same magnifications in **A**, **B**; 2 mm in **C**; same magnifications in **C**, **D**; 1 mm in E; same magnifications in **E**, **F**; 15 µm in **G**; same magnifications in **G**–**J**; 25 µm in **K**; same magnifications in **K**, **L**.

Cells capable of synthesizing melanin pigment may function in innate immunity in albino cave animals

The conservation of cells capable of synthesizing melanin suggests that they have indispensable roles in albino cave species. In arthropods and other invertebrates, melanin is an important mediator of innate immunity[13,40,47–49], but this function is not well known in vertebrates, which in addition to innate immunity have evolved an adaptive immune response. To address whether the cells capable of synthesizing melanin in albino cave species function in innate immunity, we inflicted injuries to surface and cave *A. mexicanus*, surface *F. enigmaticus* and cave *M. cavatica*, and surface *D. polymorpha* and cave *C. kusceri*, and then followed the appearance of melanin-containing pigment cells and L-DOPA-rescued pigment cells, respectively (Fig. 4).

Superficial wounds were inflicted in the trunks of 7-day post-fertilization (dpf) *A. mexicanus* larvae and adult tailfins and the subsequent distribution of melanophores in surface fish and L-DOPA-rescued cells in Pachón cavefish were followed by microscopy (Fig. 4A–H). In surface fish, melanophores accumulated at wound sites in both larvae and adults (Fig. 4A–C, E, F). L-DOPA-rescued pigment cells also appeared at wound sites in Pachón cavefish larvae and adults (Fig. 4D, G, H). Notably, melanophores or L-DOPA rescued cells at the wound sites were joined by neutral red-stained macrophages (Fig. 4F), which serve as effectors in the innate immune system by eliminating cells damaged by programmed cell death[50]. These results show that wounding mobilizes macrophages and melanin-containing or L-DOPA rescued pigment cells around the wound sites in surface fish and cavefish and suggest that the retention of cells with a capacity for melanin synthesis in cavefish may be explained by their function in innate immunity.

Wounds were inflicted along the segmented bodies of *F. enigmaticus* and *M. cavatica* (Fig. 4I-L) and in the mantles, gills and/or foots of *D. polymorpha* and *C. kusceri* (Fig. 4M, N) and subsequently examined by direct imaging or following L-DOPA rescue. In *F. enigmaticus*, black pigment cells appeared around the wounds, breaking the normal segmentation pattern of the melanin pigmented bands (Fig. 4I), and L-DOPA-rescued pigment cells were detected surrounding injuries in cave *M. cavatica* (Fig. 4J-L). Although black pigment cells were not detected around the wounds in *D. polymorpha*, in the latter and *C. kusceri*, L-DOPA rescued pigment cells did appear at wound sites (Fig. 4M, N). These results suggest that cells capable of melanin synthesis in albino cave annelid and mollusk species may be involved in injury repair.

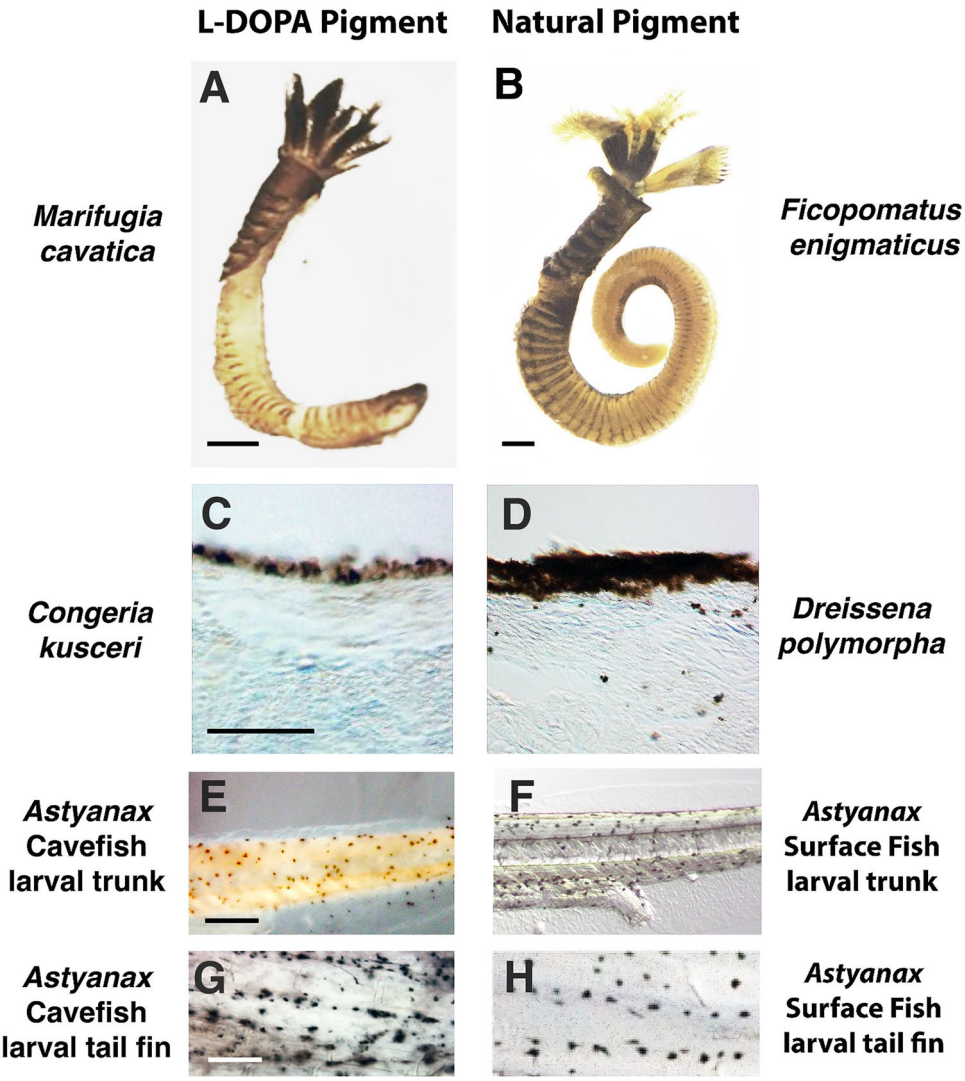

Fig. 3 | The pattern of L-DOPA-rescued melanin pigmentation in albino cave animals resembles natural melanin pigmentation in closely-related surface relatives. L-DOPA rescued body pigmentation in albino cave *M. cavatica* (**A**) and natural body pigmentation in surface *F. enigmaticus* (**B**). **C**, **D** L-DOPA rescued pigment cells at the edge of the mantle in albino cave *C. kusceri* (**A**) and natural pigmentation at the edge of the mantle in surface *D. polymorpha* (**B**). *A. mexicanus* Pachón cavefish larval body (**E**) and tailfin (**F**) with L-DOPA-rescued pigment cells in surface fish larval body (**G**) and tail fin (**H**) with melanophores. Scale bars: 1 mm in **A** and **B**; 10 µm in **C**; same magnifications in **C**, **D**; 50 µm in **E**, same magnifications in **E**, **F**; 25 µm in **G**; same magnifications in **G**, **H**.

In summary, our results suggest that cells capable of producing melanin may be conserved in diverse albino cave-adapted species because of important roles in innate immunity during tissue repair.

Blocking melanin synthesis at its first step may conserve energy in *A. mexicanus* embryos

Although energy conservation has been proposed as a driver of trait regression, including albinism in cave animals, this hypothesis has not been experimentally verified. Albinism in *A. mexicanus* Pachón cavefish is caused by a 130 base pair deletion in the *oca2* gene[33], encoding a melanosome transmembrane protein that may function in L-tyrosine transport into the melanosome or tyrosinase processing during melanin biosynthesis[34,35]. As described previously[29], injection of an *oca2* morpholino (MO) into fertilized eggs results in the development of morphant larvae lacking or with substantially reduced pigmentation. Therefore, to explore the possible role of energy conservation in pigment loss, surface fish embryos were injected with *oca2* MO, and the morphant larvae were carefully inspected under a stereomicroscope for pigmentation. They were then divided into an albino group with no visible pigmentation and a group showing reduced pigmentation. Oxygen consumption, as a measure of energetic cost, was compared between the two *oca2* morphant groups and normally pigmented larvae injected with a control MO (Fig. 5). The results showed that albino *oca2* morphants consumed less O_2 than pigmented control morphants ($p = 2.694e^{-7}$). Furthermore, *oca2* morphants with reduced pigmentation showed O_2 consumption levels intermediate between albino *oca2* morphants and fully pigmented controls ($p = 9.109e^{-4}$ for control MO versus *oca2* MO pigmented; $p = 0.09$ for *oca2* MO albino versus *oca2* MO pigmented). These results suggest that pigment loss via *oca2* knockdown decreased O_2 consumption during development of *A. mexicanus* surface fish. Therefore, the disruption of melanin synthesis at its first step could be an advantage for energy conservation and a target of natural selection in the cave environment.

Melanin pigment reduction and albinism increases dopamine synthesis in *A. mexicanus*

It was previously shown that L-tyrosine and DA are increased in whole larvae and adult brains of a single cavefish population (Pachón) compared to surface fish[29]. Furthermore, silencing of *oca2* in surface *A. mexicanus* caused an increase of L-tyrosine and DA levels suggesting a tradeoff between the melanin and CAT synthesis pathways[29]. To further address this tradeoff, we compared DA levels in multiple lineages of *A. mexicanus*, including cave populations with various degrees of depigmentation as well as albino hybrids produced by artificial selection (Fig. 6).

We measured DA and DOPAC concentrations and calculated DOPAC/DA ratios, the latter as metabolic indices of dopaminergic activity, in two HPLC experiments. Several groups (Pachón and Tinaja cavefish and surface fish) were included in both runs, and since there was no statistically significant difference in results between the two runs based on *T*-tests, we

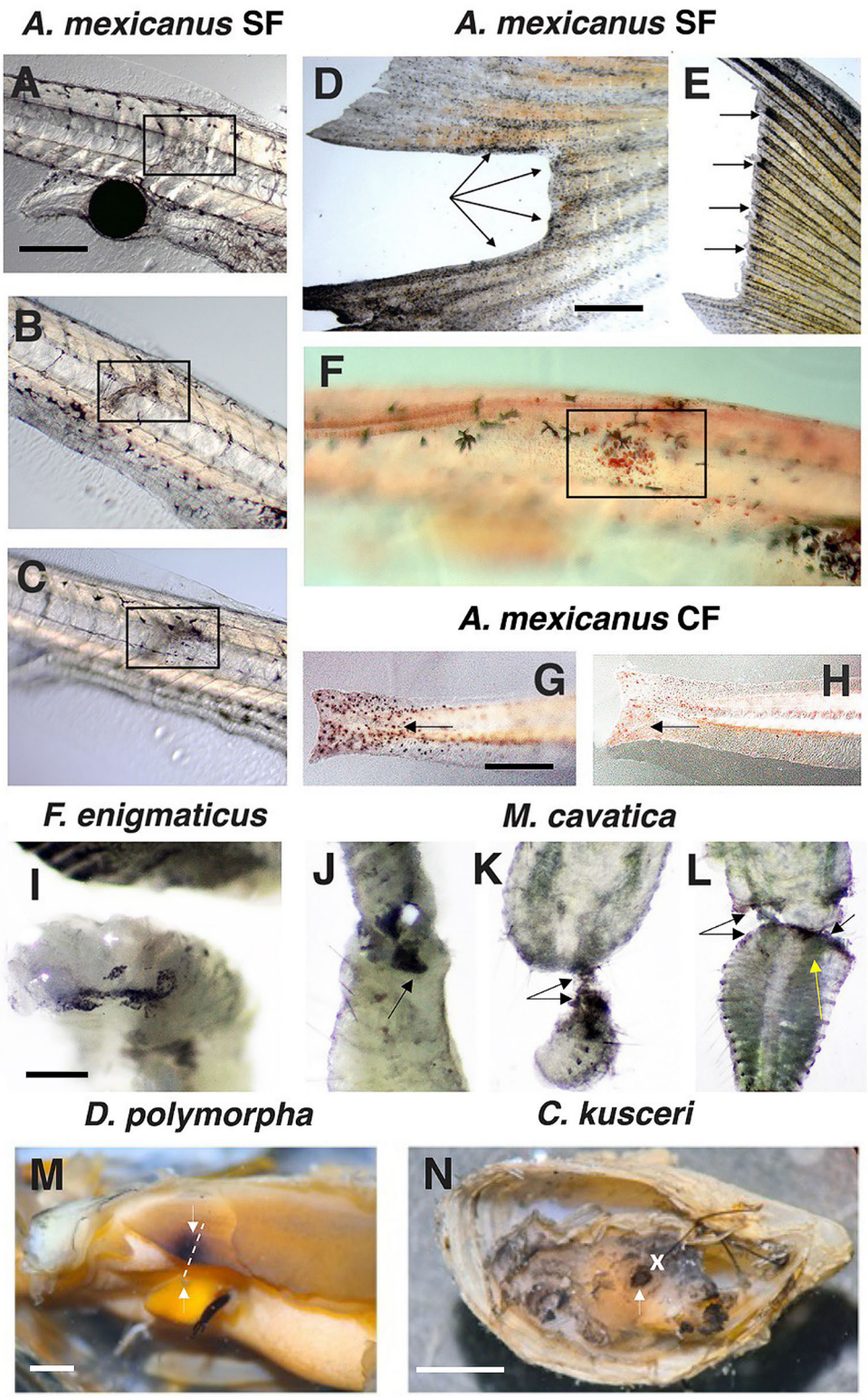

Fig. 4 | Melanin pigment and L-DOPA rescued cells are involved in injury repair in diverse cave albino species. A–C The accumulation of melanophores at wound sites (boxes) in a 7-day post-fertilization (dpf) *A. mexicanus* surface fish larva shortly after injury (**A**), at 24 h. post injury (**B**), and at 48 h. post injury (**C**). **D**, **E**. L-DOPA-rescued pigment cells or melanophores appear at the edge of wounds (arrows) in adult *A. mexicanus* Pachón cavefish (**D**) and surface fish (**E**), respectively. **F** Melanophores and neutral-red-stained macrophages appear at a wound site (boxed area) in 7 dpf surface fish. L-DOPA-rescued pigment cells (**G**) and neutral red-stained macrophages (**H**) at the severed edges of tailfin wounds in 7 dpf Pachón cavefish. Black pigment cells in surface *F. enigmaticus* (**I**, arrow) and L-DOPA rescued pigment cells in cave *M. cavatica* (**J–L**, arrows) are present about 24 h. after being wounded. Yellow arrow in L shows L-DOPA rescued cells in a segmented band. **M**, **N** L-DOPA rescued pigmentation in *D. polymorpha* and *C. kusceri* (white arrows). Dashed line in **M** and X in **N**: wound sites. Scale bars: 50 μm in **A**, same magnifications in **A–C**, F; 100 μm in **D**, **G**; same magnifications in **D**, **E** and **G**, **H**; 500 μm in **I**, same magnifications in **I–L**; 2 mm in **M** and **N**.

show combined results in the Figure (Fig. 6A–C) and statistics for each run separately in Supplementary Table 1. In the first experiment, DA and DOPAC/DA were determined in brains isolated from the Tinaja, Los Sabinos, and Molino forms of *A. mexicanus* cavefish, which show different levels of melanin pigmentation (medium levels, low levels, and albinism, respectively), and compared to brains of albino Pachón cavefish, fully-pigmented surface fish, and pigmented surface fish x Pachón F1 hybrids. The results showed increased levels of DA in brains from depigmented Pachón, Tinaja, and Los Sabinos cavefish relative to pigmented surface fish and F1 hybrids. Although albino Molino cavefish brains showed no differences in DA levels (Fig. 6A), increased DOPAC/DA ratios suggested that dopaminergic activity is higher in the brains of Molino cavefish (Fig. 6B). In the second HPLC experiment, increased DA was confirmed in Tinaja and Pachón cavefish brains, and increased DA was also observed in brains of the *A. mexicanus* albino eyed (AE) strain, which was produced by crossing Pachón cavefish by surface fish followed by multiple rounds of artificial

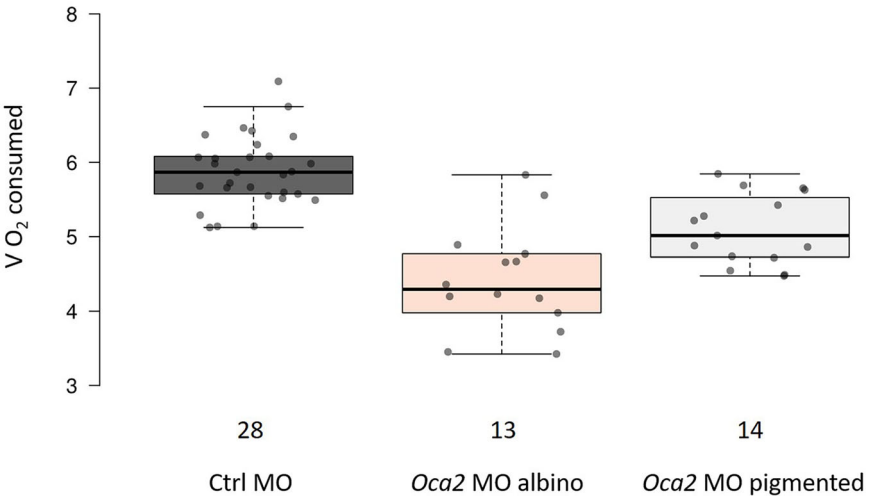

Fig. 5 | The effects of *oca2* knockdown on oxygen consumption in *A. mexicanus* larvae. Box and whisker plots showing 4-day post-fertilization larvae injected with *oca2* morpholino separated into albino (albino Oca2 MO) and pigmented (pigmented Oca2 MO) groups and compared with pigmented control morpholino (control MO). Two biological replicates of the experiment are combined and the total number of samples (*n*) is shown at the bottom of each box. Both Oca2 MO groups show significant differences compared to control MO at $p < 0{,}001$ according to Kruskal-Wallis followed by Dunn's test. Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by BoxPlotR[70]; whiskers extend 1.5 times the inter-quartile range from the 25th and 75th percentiles, outliers are represented by dots; data points are plotted as open circles.

selection for albino progeny with fully developed eyes[51]. These results show that DA or dopaminergic metabolism is increased across multiple depigmented or albino cavefish populations and artificially-selected albino hybrids, suggesting a widespread tradeoff between the melanin synthesis and CAT pathways in *A. mexicanus*.

Discussion

This study shows that convergent evolution of albinism is controlled by the same mechanism in diverse cave animals, supporting the hypothesis that the evolution of albinism could be explained as an adaptation involving natural selection[29,30,32]. The evidence stems from the broad convergence of a block at the first step of the melanin pathway and the discovery of multiple potential advantages in disrupting melanin biosynthesis at this step, which could serve as targets of natural selection.

As biological subjects in this study, we chose the cave polycheate *M. cavatica*, the cave mollusks *C. kusceri* and *M. werneri*, the cave salamander *P. anguinus*, and three different cavefish species, including the classic cavefish model *A. mexicanus*[37]. Based on assays designed to distinguish darkly pigmented species containing melanin from those containing porphyrins or ommochromes, we determined that the albino cave species in this study are closely related to surface-dwelling species with melanin pigment, and thus albinism in these species should be a consequence of disrupting melanin synthesis. Accordingly, we employed the melanin substrate assay[5], in which albino species are challenged to synthesize melanin following the provision of L-tyrosine, the first substrate, or L-DOPA, the second substrate in the pathway, to identify the step of melanin synthesis blockage. In each of the diverse albino cave species examined, we found that L-DOPA, but not L-tyrosine, rescued melanin synthesis. In addition, previous studies showed that *A. mexicanus* Pachón cavefish and two independently evolved cave cixiid species from Croatia and Hawaii, are also defective in the first step of melanin synthesis[5,6]. Thus, melanin synthesis is disrupted at its first step in a wide range of albino cave-adapted animals. Since most albino cave-adapted animals fit into one of the taxonomic groups covered in our studies, the results suggest that a block at the first step of melanin synthesis could be ubiquitous among albino cave animals.

According to the genetic drift hypothesis[16,17], stochastic mutations would be expected to cause albinism by disrupting any step in the melanin synthesis pathway. However, this scenario was not supported by our results. Instead, only one step, the first step in the pathway, was always disrupted, supporting this single step as a frequent target of natural selection. Moreover, the ability to rescue pigmentation by the provision of L-DOPA substrate suggests that all the downstream steps and their effectors are present and functional in albino species. A potential caveat could be that the first step of the pathway might be the only step where disruption is possible. However, this constraint can be rejected by evidence showing that spontaneous or laboratory induced *Drosophila* mutations disrupt the melanin synthesis pathway at several early and late steps[52]. Likewise, human albinism is caused by mutations in the early (OCA1 and OCA2 albinisms) or late (OCA3 and OCA4 albinisms) steps in melanin synthesis[53]. Thus, we conclude that blocking melanin synthesis at its first step, rather than at any other place in the pathway, may be a conserved feature of diverse albino cave animals.

Our discovery of cells with the capacity for melanin synthesis after L-DOPA provision in divergent albino cave animals suggests important functions other than body pigmentation. To explain these results, we tested the hypothesis that melanin pigment cells have anti-microbial properties[48]. This hypothesis is supported by melanophore recruitment to the sites of tissue damage in zebrafish[54] but has not been addressed in albino cave animals. When *A. mexicanus* surface fish and cavefish larval bodies or adult tailfins were wounded, melanophores or cells capable of melanin synthesis after supplying L-DOPA substrate, respectively, were recruited to the sites of injury along with macrophages, which are known to be attracted to damaged tissues during the innate immune response[50]. L-DOPA rescued pigment cells were also detected at wounds in cave *M. cavatica* and cave *C. kusceri*, suggesting that despite their albino phenotypes, cells with a cryptic capacity for melanin synthesis may be involved in innate immunity during wound healing in these species.

Energetic savings is another hypothesis that has been proposed to explain the regressive evolution of cave animals[12,23–25]. This hypothesis has received experimental confirmation only in the case of eye regression, where eye loss in *A. mexicanus* has been shown to reduce the overall metabolic expenditure[55]. However, it has not been tested for pigmentation loss. If there is a cost for pigment production, then it would be most effective in conserving energy to block melanin synthesis at the first step of the pathway. For example, a block at the first step is expected to prevent the transport of L-tyrosine into the melanosome, which is a potential role of the OCA2 protein in vertebrates[56]. Although energy conservation is an appealing explanation for blocking melanin synthesis at the first step, until now this hypothesis has not been tested in any cave animal. Our studies, in which oxygen consumption as a proxy for energetic cost, was measured in albino and depigmented *A. mexicanus oca2* morphants, show that melanin synthesis is associated with a metabolic cost in this system. Costs of melanin synthesis have been demonstrated in many taxa[57,58] but here we provide experimental evidence supporting the role of energy conservation in the evolution of albinism in a cave animal. Although *oca2* morphant larvae did not display obvious morphological abnormalities or gross behavioral differences relative to controls, we cannot exclude that differences in locomotor activity or other behaviors contribute to the reduction in oxygen

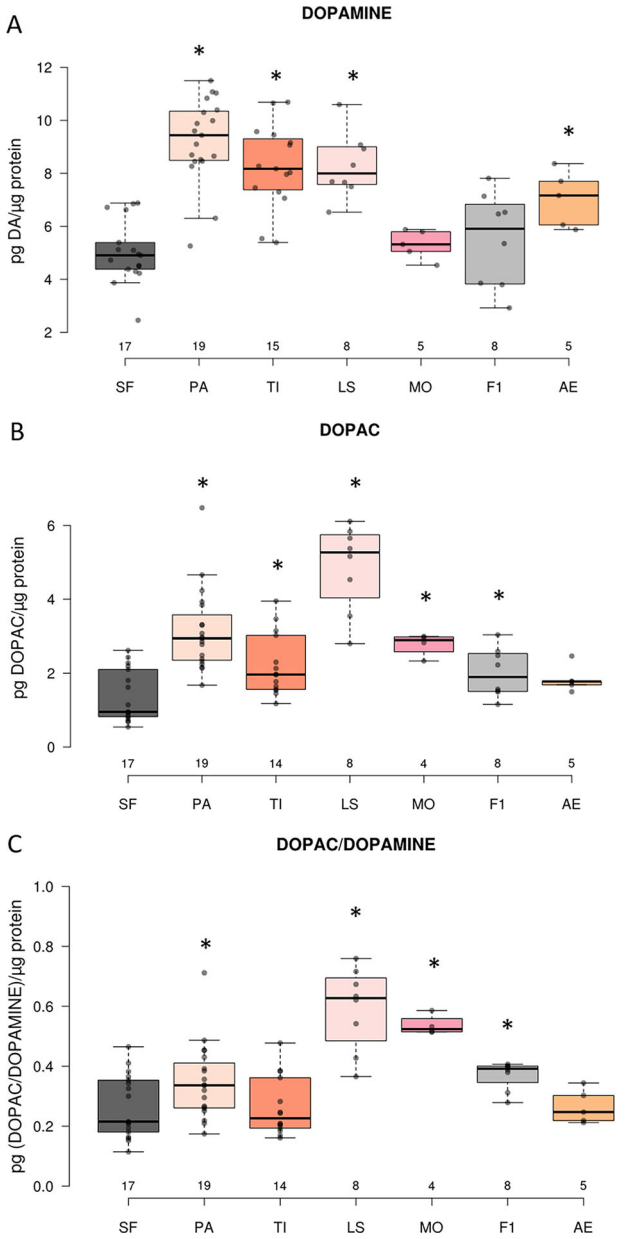

Fig. 6 | Dopamine levels and dopaminergic activity in the brains of different *A. mexicanus* cavefish populations and hybrids. Box-whisker plots showing dopamine (DA) (**A**), DOPAC (**B**), and dopaminergic activity (DOAC/DA) (**C**) in control surface fish (SF), Pachón (PA), Tinaja (TI), Los Sabinos (LS), and Molino (MO) cavefish, surface fish X Pachón cavefish F1 hybrids (F1), and the albino eyed strain (AE). One AE fish was excluded from the dataset as a significant outlier ($P < 0.05$) according to the Grubbs test. The final number of analyzed individuals (n) is indicated below each group on the x-axis. Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by BoxPlotR[70]; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by dots; data points are plotted as open circles. Boxes represent the combined dataset based on two separate HPLC runs. Asterisks show significant differences of each group compared to SF at $p < 0{,}001$ according to One-way ANOVA with Tukey HSD corrections. Separate statistics for each run can be found in Table S1.

consumption. Nevertheless, reduced energy expenditure, whether arising directly from impaired melanin synthesis or indirectly via downstream effects of melanin-pathway disruption, would be expected to confer a selective advantage to cavefish larvae inhabiting nutrient-limited environments. Together with studies showing that the mutated *oca2* gene is under positive selection[32] and that *oca2* CRISPR mutations may confer a feeding advantage[59], our studies involving O_2 consumption support the adaptive hypothesis for the evolution of albinism in *Astyanax* cavefish. These studies could not be extended to invertebrate cave species because the key gene(s) mutated to disrupt melanin synthesis have not been identified. Identifying the genetic changes underlying the disruption of the first step of melanin synthesis in invertebrate taxa remains an important direction for future work.

Altered behaviors and physiological functions in *A. mexicanus oca2* morphants and CRISPR mutants may be dependent on CAT functions in the brain and other parts of the body[29,30,32]. Because the CAT and melanin synthesis pathways begin with the same L-tyrosine substrate, a tradeoff has been proposed in which surplus L-tyrosine available from blocking melanin synthesis at its first step is used to increase CAT synthesis[29], which could be a target of natural selection. Accordingly, it has been shown that knockdown of *oca2* increases L-tyrosine and DA levels in *A. mexicanus* surface fish larvae and that naturally elevated levels of DA are present in the brains of Pachón albino cavefish[29]. The adaptive value of increased DA in cave evolution of *Astyanax* is not entirely clear. However, we reasoned that, if adaptive, increased DA would evolve in multiple independent cavefish lineages[60], similar to higher starvation resistance and triglyceride content[61]. Indeed, we found that increased DA or dopaminergic activity is present in four different cavefish populations. Our results documenting increases in DA levels and/or dopaminergic activity in the brains of depigmented and albino cavefish populations and albino hybrids produced through artificial selection expand and are consistent with a previous study of the melanin-CAT tradeoff in *A. mexicanus*[29]. Therefore, we conclude that another advantage of blocking melanin synthesis at its first step, at least in *A. mexicanus*, is the enhancement of DA levels, which could provide better fitness in the cave environment.

In summary, we have discovered multiple advantages for blocking melanin synthesis at its first step. Some of these advantages could be widespread in all albino cave animals, whereas others could be important in only some cave adapted species. For example, cave-adapted planthopper species feeding on roots in lava tube caves may not be subject to the same energy-cost saving strategies as other cave animals[62]. Nevertheless, these results support an adaptive hypothesis based on natural selection for the evolution of albinism in cave animals. The adaptive hypothesis is consistent with the rapid evolution of *A. mexicanus* cavefish[21,22] and specialized cave traits in other cave-adapted animals[18–20]. The adaptive hypothesis is also supported by historical field studies in Pachón Cave[63]. Researchers initially encountered *Astyanax* fish in Pachón Cave ranging from fully dark to albino individuals with all the different intermediate levels of pigmentation[64], suggesting that introgression had occurred with surface fish. More recently, all the fish observed in Pachón Cave were albino[63]. Genetic drift is unlikely to explain the rapid fixation of albinism in this case, suggesting that other evolutionary forces are involved, possibly based on the multiple advantages of the loss of pigmentation discovered in this study.

Our findings support an adaptive hypothesis driven by natural selection acting on the first step of melanin synthesis for the evolution of albinism in diverse cave animals. This convergence is accompanied by the retention of pigment-producing cells, which are conserved in their spatial distribution. Their preservation suggests functional roles beyond coloration. The combination of a highly specific metabolic block, cryptic pigment cell conservation, and multiple physiological advantages, suggest albinism may be a predictable, selectively favored outcome of life in caves. More broadly, our study shows that evolution can act not only through trait loss but also by repurposing existing cellular and metabolic systems, revealing how tradeoffs and constraints shape convergent evolution across deep phylogenetic divides. The repeated loss of a seemingly dispensable trait, pigmentation, reflects an evolutionary solution for the energetic and ecological pressures of cave life.

Materials and methods

Biological materials

A. mexicanus surface fish (SF) and cavefish were obtained from the Jeffery Laboratory Colony at the University of Maryland, College Park. Cavefish

populations used in this study were Pachón (PA), Tinaja (TI), Los Sabinos (LS), and Molino (MO). Fertilized eggs and larvae were obtained by spawning as described previously[65]. F1 hybrids were produced by crossing surface fish X Pachón cavefish. The albino eyed (AE) strain was prepared by artificial selection in a four-generation surface fish x Pachón cavefish intercross[51]. Methods for fish handling and experimental procedures were approved by the University of Maryland IACUC and conformed to USA National Institutes of Health guidelines. We have complied with all relevant ethical regulations for animal use.

The polychaetes *Marifugia cavatica* and *Ficopomatus enigmaticus* were collected in Tounjčica Cave, Tounj, Croatia and in the Krka River estuary, Skradin, Croatia, respectively. The mollusks *Congeria kusceri*, *Dreissena polymorpha*, and *Melledela werneri* were collected in Pukotina u tunelu polje jezero, Peračko Blato, Croatia; Jarun Lake, Zagreb, Croatia, and Ostaševica Cave, Mljet, Croatia, respectively. The crustaceans *Asellus aquaticus* and *Gammarus sp*. were collected in the Sava River, Zagreb, Croatia. The planarian *Polycelis sp*. was collected in Majerovo vrilo, Otočac, Croatia, and an uncharacterized species of cixiid planthopper was collected at Sedrena špilja, Oklaj, Croatia. Fin clips of the amphibian *Proteus anguinus* were collected in Oko Cave, Bosnia and Herzegovina. Permits for these collections were UP/I-612-07/20-48/83, 517-05-1-1-20-5 (Ministry of Environmental Protection and Energy); UP/I-352-04/22-08/96, 517-10-1-1-22-5 (Ministry of Economy and Sustainable Development); from the Department for Nature Protection of the Republic of Croatia, and 04-23-880/18 (Federal Ministry of Environment and Tourism, Federation of Bosnia and Herzegovina). *Ambylopsis spelaea* and *Typhlichthys subterraneus* fin clips were collected under scientific permits issued by the states of Tennessee (no. 1605) and Kentucky (no. SC1211135), USA and kindly provided by Professors Daphne Soares and Matthew L. Niemiller.

Pigment identification assays

To determine whether albinism in our target species was a result of disruption of melanogenesis and not some other pigment type, we developed simple pigment solvent assays. Specimens were fixed in 5% formalin for 1 h, rinsed in PBS, and subsequently incubated in solvents, and the effects on dark pigmentation were determined by microscopy. We used acetone, which extracts carotene[66], formic acid, which dissolves ommochrome and porphyrin but not melanin pigment[66,67], and the melanin bleach kit (Sigma, St. Louis, USA) to extract melanin, but not carotene or ommochrome pigment, in our test species. As controls, we incubated tailfin clips of *A. mexicanus* surface fish, which have xanthophores containing carotene pigment and melanophores containing melanin pigment. Additionally, we used *A. aquaticus* with ommochrome pigmentation, *G. minus*, which produces melanin at wound sites, cixiid insects with melanin pigmentation, and the planarian (*Polycelis sp*.) with porphyrin pigmentation in the body and melanin pigment in the eyes. Each experiment was performed on at least 3 biological replicates or individuals.

Melanogenic substrate assay

To identify the step of melanin synthesis that was disrupted in albino cave animals we performed the melanogenic substrate assay, as described previously[6]. Albino cave species and closely related pigmented surface species were fixed in 5% formalin in PBS for 1 h. After several cycles of washing in PBS, they were incubated in a 0.1% (1 mg/mL) buffered solution of L-DOPA or L-tyrosine for 12 h, washed into PBS, and photographed. The incubation time for L-DOPA and L-tyrosine was the same in each species. Some of the fixed L-DOPA positive albino cave species and closely related surface species were embedded in Paraplast and sectioned at 10 μm using a Microtome. Whole specimens were viewed using a Zeiss Axioskop compound microscope. The melanogenic substrate assay was performed on multiple occasions and on biological replicates. The N sizes were between 10 and 20 for invertebrate species and laboratory raised *A. mexicanus*, and up to 5 for other vertebrate species.

Wounding procedures

In *A. mexicanus* surface fish and Pachón cavefish larvae, wounds were inflicted in the dorso-lateral musculature just posterior to the yolk sac at 5–10 days post fertilization (dpf) using sharp microcapillaries ($N > 30$ for each group). In adults, wounds were produced by severing a distal part of the tail fin ($N > 5$ for each group). Injuries were inflicted following anesthesia with MS222. The wound sites of surface fish were observed under a stereomicroscope for several days. The cavefish were observed by stereomicroscopy and then fixed for 1 h at 4 °C with 5% formalin in PBS at several consecutive time points during wound healing. Following fixation, the tissue was subjected to the melanin substrate assay as described above, and the L-DOPA rescued cells were observed by stereomicroscopy. Macrophage distribution around the wound sites of surface fish and cavefish was followed by neutral red staining as described previously[68]. The polychaetes *F. enigmaticus* and *M. cavatica* and the bivalves *D. polymorpha* and *C. kusceri* were wounded by making a lesion in different body parts using microcapillaries (> 10 for each group). The wound sites of *F. enigmaticus* were observed under a stereomicroscope for 24–72 h. The wound sites of *M. cavatica, D. polymorpha, and C. kusceri* were observed at 24 and 48 h after wounding, fixed for 1 h at 4 °C with 5% formalin, and subjected to the melanogenic substrate assay. A single wound was generated per animal. Animals were randomly selected for each experiment, and wounding was performed in randomized order. Each wound was followed longitudinally within the same individual; therefore, blinding was not implemented.

Knockdown of the *oca2* gene

The *A. mexicanus oca2* gene was knocked down by injecting a translation-blocking morpholino (MO) (5'-CTTGTTCTCCAAATACATCACACCT-3') corresponding to base pair -7 to +18 of the gene, as described previously[29,30]. The *oca2* MO and a control MO (5'-CCTCTTACCT-CAGTTACAATTTATA-3') were designed and provided by Gene Tools (Summerton, OR, USA). We injected 400 pg of each MO into 1-2 cell embryos, which provided maximal inhibition of melanophore development with minimal effects on morphology[29].

Oxygen consumption measurements. We tested whether melanin synthesis requires energy in *A. mexicanus* by comparing oxygen consumption in *oca2* ($N = 27$) and control morphant larvae ($N = 28$). The morphant embryos were individually placed in 2 ml air-tight vials filled with autoclaved culture system water and incubated at room temperature for about 30 h. During that time, they were not disturbed. Multiple blank vials were also used, and their variance was below 3%. The vials were incubated in a water bath at same place in the laboratory to ensure larvae developed at the same temperature. The amount of oxygen left in vials was measured using a Membrane Inlet Mass Spectrometer (Bay Instruments, Easton, MD, USA) in random order. Vials without larvae were used as blanks. The value of oxygen consumption of each larva was calculated by subtracting the amount of oxygen measured in its vial from the mean of oxygen level of 5 blank vials. Because MO knockdown is a transient method, some pigmentation eventually breaks through the block[29]. Therefore, following the oxygen measurements, individual *oca2* morphant larvae were carefully inspected under a stereomicroscope for the presence or absence of pigmentation and separated into *oca2* albino ($N = 14$) and *oca2* pigmented ($N = 13$) morphant groups. Because data was not normally distributed, the Kruskal-Wallis followed by post-hoc Dunn's test were used to test for differences between the groups. Raw data is provided in Supplementary Data 1.

High-performance liquid chromatography

Whole brains were dissected from adult fish ($N = 75$: 17 SF, 19 PA, 14 TI, 8 LS, 4 MO, 8 F1, and 5 AE) and collected for high-performance liquid chromatography (HPLC). Fish of both sexes and similar sizes and ages were used, and they were housed under identical lighting and feeding regimes. They were anesthetized with MS222 before sampling. Dopamine (DA) and

3,4-dihydroxyphenylacetic acid (DOPAC) levels were quantified by HPLC with electrochemical detection, following previously described methods[30,69] with minor modifications. Brain samples were homogenized in 100 µL acetate buffer (pH 5.0) containing methyl DA as an internal standard (Merck & Co., Kenilworth, NJ), briefly sonicated, and stored at −80 °C until analysis. Prior to analysis, samples were treated with 4 µL ascorbate oxidase (1 mg/mL, Sigma-Aldrich) and subsequently centrifuged at 17,000 × *g* for 15 min. An aliquot (50 µL) of the resulting supernatant was injected using a Waters Alliance e2695 system onto a C18 Nova-Pak column (4 mm; Waters Associates, Milford, MA) maintained at 30 °C. Electrochemical detection was performed with an LC-4 potentiostat equipped with a glassy carbon electrode (Bioanalytical Systems, West Lafayette, IN), operated at 0.5 nA/V and an applied potential of 0.7 V relative to an Ag/AgCl reference electrode. The remaining pellet was dissolved in 400 µL of 0.4 N NaOH, and total protein concentration was determined using the Bradford assay. Monoamine levels were quantified using the ACSW32 software (DataApex, Czech Republic) in internal standard mode, with peak heights calculated from calibration standards. DA and DOPAC concentrations were corrected for injection versus preparation volumes and divided by micrograms protein to yield picogram monoamine per microgram protein. Data was tested for differences using a One Way Analysis of Variance because it passed normality and equal variance tests (SIGMASTAT version 3.5, Systat Software, San Jose, CA). In analyses that revealed a significant effect between groups, the Tukey HSD method was used to conduct pairwise comparisons. Application of Grubb's test to detect outliers resulted in the deletion of data from one AE brain from the dataset. Significance levels for all statistical tests were set at $p = 0.05$. Raw data is provided in Supplementary Data 2.

Statistics and reproducibility

Samples were selected at random either from large laboratory populations of *Astyanax mexicanus* or collected randomly from caves, depending on availability, colleague contributions, and permit limitations. For the quantitative data presented in Figs. 5 and 6, biological replicates were obtained under controlled laboratory conditions, and all experiments were independently repeated with consistent results. For qualitative data presented in Figs. 1–4, field collections were conducted at multiple time points between 2012 and 2025, and the observed patterns were consistent and reproducible across independent sampling events.

Statistical analyses were performed using SigmaStat version 3.5 (Systat Software, San Jose, CA) and BoxPlotR -[70]. Sample sizes (n) for each experimental group are indicated in the corresponding Materials and Methods section and figure panels. Replicates are defined as independent biological samples (distinct individuals).

Quantitative data were assessed for normality and homogeneity of variance prior to hypothesis testing. Appropriate statistical tests were applied as described in the corresponding sections of Materials and Methods, and adjustments for multiple comparisons were performed.

Descriptive statistics, including measures of central tendency and dispersion, are presented in box plot figures along with all individual data points. Exact test statistics are provided in the Supplementary Data. *P* values are reported in the Results, figure legends, and Supplementary Tables and Data.

Data availability

All raw data supporting the quantitative analyses in this study are provided in the Supplementary Data files. Additional information is available from the corresponding author upon reasonable request.

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References

1. Kaiser, H. J. et al. Molecular convergence of bacterial and eukaryotic surface order. *J. Biol. Chem.* **286**, 40631–40637 (2011).
2. Suzuki, T. & Numata, T. Convergent evolution of AUA decoding in bacteria and archaea. *RNA Biol.* **11**, 1586–1596 (2014).
3. Mas, A., Lagadeuc, Y. & Vandenkoornhuyse, P. Reflections on the predictability of evolution: Toward a conceptual framework. *iScience* **23**, 101736 (2020).
4. Nosil, P., Flaxman, S. M., Feder, J. L. & Gompert, Z. Increasing our ability to predict contemporary evolution. *Nat. Commun.* **11**, 5592 (2020).
5. McCauley, D. W., Hixon, E. & Jeffery, W. R. Evolution of pigment cell regression in the cavefish *Astyanax*: A late step in melanogenesis. *Evol. Dev.* **6**, 209–218 (2004).
6. Bilandžija, H., Ćetković, H. & Jeffery, W. R. Evolution of albinism in cave planthoppers by a convergent defect in the first step of the melanin biosynthesis pathway. *Evol. Dev.* **14**, 196–203 (2012).
7. Riley, P. A. Melanin. *Int. J. Biochem. Cell Biol.* **11**, 1235–1239 (1997).
8. Slominski, A., Zmijewski, M. A. & Pawelek, J. L-tyrosine and L-dihydroxyphenylalanine as hormone-like regulators of melanocyte functions. *Pigment Cell Melanoma Res.* **25**, 14–27 (2012).
9. Sugumaran, M. & Barek, H. Critical analysis of the melanogenic pathway in insects and higher animals. *Int. J. Mol. Sci.* **17**, 1753 (2016).
10. Vavricka, C. et al. Tyrosine metabolic enzymes from insects and mammals: A comparative perspective. *Insect Sci.* **21**, 13–19 (2014).
11. Fernstrom, J. D. & Fernstrom, M. N. Tyrosine, phenylalanine, and catecholamine synthesis and function in the brain. *J. Nutr.* **137**, 1539S–1547S (2007).
12. Culver, D. C., Kowalko, J. E. & Pipan, T. Natural selection versus neutral mutation in the evolution of subterranean life: A false dichotomy? *Front. Ecol. Evol.* **11**, 1080503 (2023).
13. Sugumaran, M. Comparative biochemistry of eumelanogenesis and the protective roles of phenoloxidase and melanin in insects. *Pigment Cell Res.* **15**, 2–9 (2002).
14. Protas, M. E. & Patel, N. H. Evolution of coloration patterns. *Annu. Rev. Cell Dev. Biol.* **24**, 425–446 (2008).
15. Darwin, C. *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*. 6th ed. (John Murray, 1872).
16. Juan, C., Guzik, M. T., Jaume, D. & Cooper, S. J. B. Evolution in caves: Darwin's 'wrecks of ancient life' in the molecular era. *Mol. Ecol.* **19**, 3865–3880 (2010).
17. Wilkens, H., Strecker, U. *Evolution in the dark: Darwin's loss without selection*. (Springer, 2017)
18. Niemiller, M. L., Fitzpatrick, B. M. & Miller, B. T. Recent divergence with gene flow in Tennessee cave salamanders (Plethodontidae: Gyrinophilus) inferred from gene genealogies. *Mol. Ecol.* **17**, 2258–2275 (2008).
19. Klaus, S. et al. Rapid evolution of troglomorphic characters suggests selection rather than neutral mutation as a driver of eye reduction in cave crabs. *Biol. Lett.* **9**, 20121098 (2013).
20. Zhang, Y. & Li, S. Ancient lineage, young troglobites: Recent colonization of caves by *Nesticella* spiders. *BMC Evol. Biol.* **13**, 183 (2013).
21. Fumey, J. et al. Evidence for late Pleistocene origin of *Astyanax mexicanus* cavefish. *BMC Evol. Biol.* **18**, 43 (2018).
22. Herman, A. et al. The role of gene flow in rapid and repeated evolution of cave related traits in Mexican tetra, *Astyanax mexicanus*. *Mol. Ecol.* **27**, 4397–4416 (2018).
23. Jeffery, W. R. Regressive evolution in *Astyanax* cavefish. *Annu. Rev. Genet.* **43**, 25–47 (2009).
24. Barr, T. C. Cave ecology and the evolution of troglobites. In: *Evolutionary Biology*. (ed. Dobzhansky, T., Hecht, M. K., Steere, W. C.) 35-102 (Springer, 1968).
25. Gross, J. B., Powers, A. K., Davis, E. M. & Kaplan, S. A. A pleiotropic interaction between vision loss and hypermelanism in *Astyanax mexicanus* cave × surface hybrids. *BMC Evol. Biol.* **16**, 145 (2016).

26. True, J. R. Insect melanism: The molecules matter. *Trends Ecol. Evol.* **18**, 640–647 (2003).
27. Ducrest, A. L., Keller, L. & Roulin, A. Pleiotropy in the melanocortin system, coloration and behavioural syndromes. *Trends Ecol. Evol.* **23**, 502–510 (2008).
28. Wittkopp, P. J. & Beldade, P. Development and evolution of insect pigmentation: Genetic mechanisms and the potential consequences of pleiotropy. *Semin. Cell Dev. Biol.* **20**, 65–71 (2009).
29. Bilandžija, H., Ma, L., Parkhurst, A. & Jeffery, W. R. A potential benefit of albinism in *Astyanax* cavefish: Downregulation of the *oca2* gene increases L-tyrosine and catecholamine levels as an alternative to melanin synthesis. *PLoS ONE* **8**, e80823 (2013).
30. Bilandžija, H., Abraham, L., Ma, L., Renner, K. & Jeffery, W. R. Behavioral changes controlled by catecholaminergic systems explain recurrent loss of pigmentation in cavefish. *Proc. Biol. Sci.* **285**, 20180243 (2018).
31. Jeffery, W. R., Ma, L., Parkhurst, A., Bilandžija, H. Pigment regression and albinism in *Astyanax* cavefish. In: *Biology and Evolution of Mexican Cavefish*. (ed: Keene, A., Yoshizawa, M., McGaugh, S.) 155-173 (Elsevier, 2016)
32. O'Gorman, M. et al. Pleiotropic function of the *oca2* gene underlies the evolution of sleep loss and albinism in cavefish. *Curr. Biol.* **31**, 3694–3701 (2021).
33. Protas, M. E. et al. Genetic analysis of cavefish reveals molecular convergence in the evolution of albinism. *Nat. Genet.* **38**, 107–111 (2006).
34. Manga, P., Boissy, R. E., Pifko-Hirst, S., Zhou, B. K. & Orlow, S. J. Mislocalization of melanosomal proteins in melanosomes from mice with oculocutaneous albinism type 2. *Exp. Eye Res.* **72**, 695–710 (2001).
35. Chen, K., Manga, P. & Orlow, S. J. Pink-eyed protein controls the processing of tyrosinase. *Mol Biol. Cell* **13**, 1953–1954 (2002).
36. Krishnan, J. et al. Genome-wide analysis of cis-regulatory changes underlying metabolic adaptation of cavefish. *Nat. Genet.* **54**, 684–693 (2022).
37. Jeffery, W. R. *Astyanax* surface and cave fish morphs. *Evodevo* https://doi.org/10.1186/s13227-020-00159-6 (2020).
38. Bandaranayake, W. M. The nature and role of pigments of marine invertebrates. *Nat. Prod. Rep.* **23**, 223–255 (2006).
39. Needham, A. E. & Brunet, P. C. The integumental pigment of *Asellus*. *Experientia* **13**, 207–209 (1957).
40. Bilandžija, H., Laslo, M., Porter, M. L. & Fong, D. W. Melanization in response to wounding is ancestral in arthropods and conserved in albino cave species. *Sci. Rep.* **7**, 17148 (2017).
41. Needham, A. E. Body-pigment of Polycelis. *Nature* **206**, 209–210 (1965).
42. Hase, S. et al. Characterization of the pigment produced by the planarian, *Dugesia ryukyuensis*. *Pigment Cell Res.* **19**, 248–249 (2006).
43. Osanai-Futahashi, M. et al. Identification of the *Bombyx* red egg gene reveals involvement of a novel transporter family gene in late steps of the insect ommochrome biosynthesis pathway. *J. Biol. Chem.* **287**, 17706–17714 (2012).
44. Dontsov, A. E., Ostrovsky, A. M. Ommochromes of the compound eye of Arthropods from the Insects and Crustacean classes: Physicochemical properties and antioxidant activity. In: *Arthropods-New Advances and Perspectives*. (IntechOpen, 2023). https://doi.org/10.5772/intechopen.107058.
45. Kupriyanova, E. K. et al. Evolution of the unique freshwater cave-dwelling tube worm *Marafugia cavatica* (Annelida: Serpulidae). *Syst. Biodivers.* **7**, 389–402 (2022).
46. Bilandžija, H., Morton, B., Podnar, M. & Ćetković, H. Evolutionary history of relict *Congeria* (Bivalvia: Dreissenidae): unearthing the subterranean biodiversity of the Dinaric Karst. *Front. Zool.* **10**, 5 (2013).
47. Gimaldi, A. et al. Amyloid/melanin distinctive mark in invertebrate immunity. *Invert. Surv. J.* **9**, 153–162 (2012).
48. Mackintosh, J. A. The antimicrobial properties of melanocytes, melanosomes and melanin and the evolution of black skin. *J. Theor. Biol.* **211**, 101–113 (2001).
49. Christensen, B. M., Li, J., Chen, C. C. & Nappi, A. J. Melanization immune responses in mosquito vectors. *Trends Parasitol.* **21**, 192–199 (2005).
50. Hirayama, D., Iida, T. & Nakase, H. The phagocytic function of macrophage-enforcing innate immunity and tissue homeostasis. *Int. J. Mol. Sci.* **19**, 92 (2017).
51. Ma, L., Ng, M., van der Weele, C. M., Yoshizawa, M. & Jeffery, W. R. Dual roles of the retinal pigment epithelium and lens in cavefish eye degeneration. *J. Exp. Zool. B Mol. Dev. Evol.* https://doi.org/10.1002/jez.b.22923 (2021).
52. Minakhina, S. & Steward, R. Melanotic mutants in *Drosophila*: Pathways and phenotypes. *Genetics* **174**, 253–263 (2006).
53. Oetting, W. S. & King, R. A. Molecular basis of albinism: Mutations and polymorphisms of pigmentation genes associated with albinism. *Hum. Mutat.* **13**, 99–115 (1999).
54. Lévesque, M., Feng, Y., Jones, R. A. & Martin, P. Inflammation drives wound hyperpigmentation in zebrafish by recruiting pigment cells to sites of tissue damage. *Dis. Model Mech.* **6**, 508–515 (2013).
55. Moran, D., Softley, R. & Warrant, E. J. The energetic cost of vision and the evolution of eyeless Mexican cavefish. *Sci. Adv.* **1**, e1500363 (2015).
56. Toyofuku, K. et al. The etiology of oculocutaneous albinism (OCA) type II: The pink protein modulates the processing and transport of tyrosinase. *Pigment Cell Res.* **15**, 217–224 (2002).
57. Stoehr, A. M. Costly melanin ornaments: the importance of taxon? *Funct. Ecol.* **20**, 276–281 (2006).
58. Britton, S. & Davidowitz, G. The effect of diet on melanin pigmentation in animals. *Funct. Ecol.* **37**, 206–217 (2023).
59. Choy, S. et al. Mutations in the albinism gene *oca2* alter vision-dependent prey capture behavior in the Mexican tetra. *J. Exp. Biol.* **228**, jeb249881 (2025).
60. Bilandžija, H. et al. Phenotypic plasticity as a mechanism of cave colonization and adaptation. *Elife* **9**, e51830 (2020).
61. Aspiras, A. C., Rohner, N., Martineau, B., Borowsky, R. L. & Tabin, C. J. Melanocortin 4 receptor mutations contribute to the adaptation of cavefish to nutrient-poor conditions. *Proc. Natl. Acad. Sci. USA* **112**, 9668–9673 (2015).
62. Howarth, F. G., James, S. A., McDowell, W., Preston, D. J. & Imada, C. T. Identification of roots in lava tube caves using molecular techniques: implications for conservation of cave arthropod faunas. *J, Insect Conserv.* **11**, 251–261 (2007).
63. Borowsky, R. Selection maintains the phenotypic divergence of cave and surface fish. *Am. Nat.* **202**, 55–63 (2023).
64. Mitchell, R. W., Russell, W. H. & Elliott, W. R. Mexican eyeless characin fishes, genus *Astyanax*: Environment, distribution, and evolution. *Spec. Publ. Mus. Tex. Tech. Univ.* **12**, 1–89 (1977).
65. Ma, L., Dessiatoun, R., Shi, J. & Jeffery, W. R. Incremental temperature changes for maximal breeding and spawning in *Astyanax mexicanus*. *J. Vis. Exp.* https://doi.org/10.3791/61708 (2021).
66. Ghidalia, W. Structural and biological aspects of pigments. (ed. Bliss, D. E., Mantel, L. H.) *The Biology of the Crustacean*. Vol. 9. 301-394 (Academic Press, 1985).
67. Needham, A. E. *The significance of zoochromes*. Zoophysiology and Ecology, Vol. 3. (Springer, 1974).
68. Ma, L. et al. A hypomorphic cystathionine β-synthase gene contributes to cavefish eye loss by disrupting optic vasculature. *Nat. Commun.* **11**, 2772 (2020).
69. Renner, K. & Luine, V. Analysis of temporal and dose-dependent effects of estrogen on monoamines in brain nuclei. *Brain Res.* **366**, 64–71 (1986).

70. Spitzer, M., Wildenhain, J., Rappsilber, J. & Tyers, M. BoxPlotR: a web tool for generation of box plots. *Nat. Methods* **11**, 121–122 (2014).

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Experimental design: H.B., W.R.J. Field sampling: H.B., W.R.J. Animal experiments: H.B., W.R.J. H.Ć. HPLC quantifications: K.J.R. Statistical analyses: H.B., K.J.R. Drafting of manuscript: HB with inputs from W.R.J. and K.J.R. All authors edited and approved the last version.

Competing interests

The authors declare no competing interests.

Additional information

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