2018

Regressed but Not Gone: Patterns of Vision Gene Loss and Retention in Subterranean Mammals

Christopher A. Emerling
cemerlin@whittier.edu

Recommended Citation

Emerling, C. A. (2018). Regressed but not gone: patterns of vision gene loss and retention in subterranean mammals. Integrative and Comparative Biology, 58(3), 441-451. https://doi.org/10.1093/icb/icy004

Integrative and Comparative Biology

Integrative and Comparative Biology, volume 58, number 3, pp. 441–451
doi:10.1093/icb/icy004

Society for Integrative and Comparative Biology

SYMPOSIUM

Regressed but Not Gone: Patterns of Vision Gene Loss and Retention in Subterranean Mammals

Christopher A. Emerling[1,*,†]

*Museum of Vertebrate Zoology, University of California Berkeley, Berkeley, CA 94720, USA; †Institut des Sciences de l'Evolution de Montpellier (ISEM), Université de Montpellier, CNRS, IRD, EPHE, 34090 Montpellier, France

From the symposium "Evolution in the Dark: Unifying Understanding of Eye Loss" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2018 at San Francisco, California.

[1]E-mail: christopher.emerling@umontpellier.fr

Synopsis Regressive evolution involves the degradation of formerly useful traits as organisms invade novel ecological niches. In animals, committing to a strict subterranean habit can lead to regression of the eyes, likely due to a limited exposure to light. Several lineages of subterranean mammals show evidence of such degeneration, which can include decreased organization of the retina, malformation of the lens, and subcutaneous positioning of the eye. Advances in DNA sequencing have revealed that this regression co-occurs with a degradation of genomic loci encoding visual functions, including protein-coding genes. Other dim light-adapted vertebrates with normal ocular anatomy, such as nocturnal and aquatic species, also demonstrate evidence of visual gene loss, but the absence of comparative studies has led to the untested assumption that subterranean mammals are special in the degree of this genomic regression. Additionally, previous studies have shown that not all vision genes have been lost in subterranean mammals, but it is unclear whether they are under relaxed selection and will ultimately be lost, are maintained due to pleiotropy or if natural selection is favoring the retention of the eye and certain critical underlying loci. Here I report that vision gene loss in subterranean mammals tends to be more extensive in quantity and differs in distribution from other dim light-adapted mammals, although some committed subterranean mammals demonstrate significant overlap with nocturnal microphthalmic species. In addition, blind subterranean mammals retain functional orthologs of non-pleiotropic visual genes that are evolving at rates consistent with purifying selection. Together, these results suggest that although living underground tends to lead to major losses of visual functions, natural selection is maintaining genes that support the eye, perhaps as an organ for circadian and/or circannual entrainment.

Introduction

Animals that live in complete darkness represent some of the quintessential examples of the repeatability of regressive evolution (Fong et al. 1995). As various lineages have adapted to the lightless habitats of the deep sea (Sumner-Rooney et al. 2016), caves (Jeffery 2009; Protas et al. 2011; Pérez-Moreno et al. 2017; Stern et al. 2017) and subterranean ecosystems (Mohun et al. 2010; Tierney et al. 2015), many traits associated with light perception have been lost. Within mammals, this is best illustrated by repeated forays underground, where various lineages have become accomplished diggers with a reduced dependency on light.

A common evolutionary theme in such subterranean mammals is the regression of the eye. While this is not a universal pattern among fossorial mammals (Peichl et al. 2005; Nemec et al. 2007; Schleich et al. 2010; Kott et al. 2016), multiple lineages collectively show irregular morphology in nearly every anatomical trait involved in vision (Sweet 1906, 1909; Sanyal et al. 1990; Cooper et al. 1993a, 1993b; Mills and Catania 2004; Nikitina et al. 2004; Hetling et al. 2005; Nemec et al. 2007). Genomic analyses of these mammals have also revealed evidence of regression via the inactivation and deletion of multiple genes involved in visual photoreception (Kim et al. 2011; Emerling and Springer 2014; Fang et al. 2014a, 2014b).

Advance Access publication April 25, 2018

Despite the accumulating evidence for this evolutionary phenomenon, there remain important questions regarding the evolution of a regressed ocular phenotype. One problem involves the relative degree of eye gene loss compared with non-subterranean mammals. Although protein coding genes involved in vision are known to become inactivated in subterranean taxa, this also occurs in mammals adapted to less extreme dim-light niches, such as nocturnal and aquatic species (Jacobs 2013; Meredith et al. 2013; Shen et al. 2013; Emerling and Springer 2015; Springer et al. 2016). Studies that have reported vision gene loss in subterranean species have generally been limited in comparisons with other taxa, meaning that it remains unclear if such mammals are exceptional in their degree and distribution of vision gene loss.

Another important question concerns the fate of the vision genes that appear intact in subterranean species. Although some subterranean mammals have eyes that are degenerate to the point of being truly blind, even being covered by skin and fur (Sweet 1906, 1909; Haim et al. 1983; Sanyal et al. 1990), mammals have never reached the point of becoming completely eyeless. Despite this, the existence of congenitally eyeless humans (Verma and FitzPatrick 2007) and mouse strains (Chase and Chase 1941), and the repeated evolution of eyelessness in natural populations of Astyanax cavefish (Jeffery 2009), demonstrate that such a phenotype is possible. As such, this raises the question of whether the apparently intact loci undergirding the eyes of blind subterranean mammals are under relaxed selection and are trending toward eventual loss or are being maintained due to pleiotropy or non-visual ocular functions.

To test these hypotheses, I examined patterns of genomic regression in genes associated with visual perception in five subterranean mammals, including 2 blind species, and 25 additional mammals encompassing aquatic, nocturnal, and diurnal habits. I compared patterns of overall vision gene loss with genes that have eye-enriched expression, and analyzed the evolutionary rates of eye proteins that appear functional in blind mammals to test for evolutionary constraint. The results suggest that subterranean mammals do indeed tend to lose more visual perception genes than other dim-light adapted mammals, and their particular distribution of gene loss is distinct. However, even blind species show signals of natural selection maintaining the functionality of numerous eye-specific visual perception genes. This suggests a retained role of the eye in a lightless habitat, potentially associated with circadian photoentrainment.

Materials and methods

I recorded the presence/absence of 213 vision-related genes for 30 placental mammals (Supplementary Tables S1 and S2), including five subterranean taxa: Cape golden mole (*Chrysochloris asiatica*—Order: Afrosoricida, Family: Chrysochloridae), star-nosed mole (*Condylura cristata*—Order: Eulipotyphla, Family: Talpidae), Upper Galilee Mountains blind mole-rat (*Nannospalax galili*—Order: Rodentia, Family: Spalacidae), naked mole-rat (*Heterocephalus glaber*—Order: Rodentia, Family: Bathyergidae), and Damaraland mole-rat (*Fukomys damarensis*—Order: Rodentia, Family: Bathyergidae). The genes of interest were derived from the gene ontology (GO) database (Gene Ontology Consortium 2004) using the GO term "Visual Perception", restricting the list to genes present in humans.

I obtained human curated mRNA reference sequences (accession prefix "NM_") from NCBI's nucleotide collection (Supplementary Table S1), and BLASTed (discontiguous megablast) the entire set of genes against the nucleotide collection for each of the remaining 29 mammals. For every species, I obtained gene models derived from NCBI's Eukaryotic Genome Annotation (EGA) pipeline (accession prefix "XM_") and/or curated mRNAs. EGA utilizes RNA, DNA, and protein reference sequences to annotate genome assemblies deposited into the International Nucleotide Sequence Database Collection (https://www.ncbi.nlm.nih.gov/genome/annotation_euk/process/). Gene identification was almost always determined by the annotation, however in some instances strong BLAST hits were recorded for gene models without the appropriate annotation. In such cases, the gene models were BLASTed (discontiguous megablast) against the nucleotide collection to determine if their closest hits were the gene of interest, indicating that the gene was inappropriately annotated. In the process of obtaining gene models, I eliminated any genes with a phylogenetic distribution of absent BLAST hits suggesting that orthologs were not present in the last common ancestor of placental mammals. For example, based on its phylogenetic distribution, *OCLM* is likely unique to anthropoid Primates and was therefore not included in the analyses.

Curated mRNAs were assumed to encode functional protein products, as were gene models, unless there was an annotation indicating the gene likely encodes a "low quality protein". Such a designation is provided for gene models with frameshift insertions, frameshift deletions, and/or premature stop codons corrected from the reference genome assembly. This predicts a unitary pseudogene, which would indicate that the gene is nonfunctional. Absent BLAST hits (i.e., no curated mRNA or gene model available) were likewise treated as providing evidence

of gene loss, either through whole gene deletion or degradation to the point of insufficient recognizable homology.

However, a lack of BLAST hits and predicted pseudogenes can also be the result of sequencing, assembly, and/or gene model construction errors or unfixed variants. As such, I analyzed two different datasets described as "possible gene losses" and "probable pseudogenes", respectively. The former assumes all predicted pseudogenes ("low quality proteins") and absent BLAST results are representations of lost/inactivated genes. For the latter dataset, I only considered predicted pseudogenes with two or more corrected frameshifts and/or premature stop codons to be "probable pseudogenes", under the assumption that a gene with multiple putative inactivating mutations is less likely to result from unfixed variants or sequencing, assembly or gene model construction errors.

Although all 213 genes have been associated with visual perception in some capacity, they are not all restricted to, or have their highest expression in, the eye. As such, selection may retain them for non-visual functions. To separate eye-enriched genes from more pleiotropic loci, I obtained the protein and gene expression profiles for all 213 genes in the Human Protein Atlas (Uhlen et al. 2015; www.proteinatlas.org). Protein expression is characterized as high, medium, low, or absent, and I considered a gene to be eye-enriched if there is any protein expression in the lens and/or retina but not in other tissues, or if there is high expression in the lens and/or retina with at most low expression in other tissues. If no eye protein expression data were available and the highest tissue expression was deemed low, then I examined gene expression data from the FANTOM5 database (Forrest et al. 2014) as reported in the Human Protein Atlas. In such cases, I considered a gene to be eye-enriched if expression (tags per million) in the retina was greater than twice the expression level of the next highest tissue.

To test for differences in patterns of vision gene loss in mammals, I analyzed the data in a logistic principal components analysis (PCA) framework using the logisticPCA package in R (Landgraf and Lee 2015). Logistic PCA allows for the dimensional reduction of correlated binary traits into principle components, allowing for visualization of gene loss patterns common to multiple species. If certain sets of genes are lost repeatedly in subterranean mammals more frequently than in other mammals, then the principal components summarizing this variation should group subterranean mammals in a distinct portion of PCA space. I coded the genes as binary traits (0 = deleted/pseudogene, 1 = functional) and performed a logistic PCA analysis on the entire dataset, then performed a separate analysis with eye-enriched genes only.

Species were categorized as one of the following: nocturnal, diurnal, aquatic/semi-aquatic, microphthalmic, rod monochromat, and subterranean. While the microphthalmic and rod monochromat species are technically nocturnal/cathemeral or aquatic, their regressed eye anatomy warrants separate designations to test for differences from subterranean mammals. Activity pattern data (nocturnal, diurnal) are derived from EltonTraits 1.0 (Wilman et al. 2014), microphthalmic species are based on the definition following Nevo (2007), and rod monochromat designations derived from Meredith et al. (2013), Emerling and Springer (2015), and Springer et al. (2016).

I compared the relative rates (RERs) of protein evolution for the eye-enriched visual perception proteins in the blind subterranean species, *C. asiatica* and *N. galili*. These data, derived from Partha et al. (2017), are estimates of lineage-specific relative shifts in protein evolution rate, i.e., increases or decreases in protein evolutionary rates relative to the average evolutionary rate of the proteome of a specific lineage (Chikina et al. 2016; Partha et al. 2017). Increased rates potentially indicate positive or relaxed selection, whereas decreased rates likely indicate purifying selection. RERs were grouped into two categories: functional and pseudogenes. Functional genes had no evidence of inactivating mutations in the present analysis, whereas those coded as pseudogenes had no BLAST matches or were predicted to encode a low quality protein with more than one corrected inactivating mutation. Although the former may appear to be an impossibility (i.e., having an RER but lacking BLAST results), it stems from the different sources of the datasets: the RER analyses are based on the UCSC 100-species alignment, whereas the dataset for the present analyses is derived from NCBI's EGA. As such, UCSC's alignment may have aligned pseudogenes that were not annotated by EGA, allowing RER values to be derived. Instances where a gene model was predicted to encode a low quality protein with only a single inactivating mutation were not included in the RER analyses.

Results

Quantity of visual gene losses

Of the 29 non-human mammals examined, all showed evidence of at least four visual perception

gene losses (mean = 18.5; median = 19; Fig. 1A). Subterranean mammals had the first (49; *C. asiatica*), second (42; *N. galili*), fourth (35; *C. cristata*), fifth (28; *F. damarensis*), and ninth (tied at 22; *H. glaber*) positions in terms of possible gene losses. When only considering probable pseudogenes (mean = 6.6; median 5), subterranean mammals occupied the first (27; *C. asiatica*), second (25; *N. galili*), third (14; *C. cristata*), fifth (12; *F. damarensis*), and sixth (10; *H. glaber*) positions (Supplementary Fig. S1).

It is possible that the high number of predicted pseudogenes in subterranean mammal genomes is due to poor gene model annotations and/or low quality assemblies, which would lead to an increase in predicted pseudogenes across all gene categories. For instance, the Chinese tree shrew (*Tupaia chinensis*) is tied for the sixth highest number of possible vision gene losses (25; Fig. 1A), having three more than the subterranean *H. glaber*; an unexpected result given the bright light conditions experienced by this diurnal mammal. However, EGA predicts that *T. chinensis* has 2119 pseudogenes genome-wide, the third highest number among the species examined (mean = 1166; median = 1037; Supplementary Table S2), which suggests that the high number of predicted vision gene losses in this taxon may be an artifact of poor assembly quality and/or gene models. After correcting for this potential bias (i.e., visual perception predicted pseudogenes/total predicted pseudogenes), *T. chinensis* was re-ranked at 16th with 0.99% of its predicted pseudogenes being associated with visual perception, which is below the mean (1.39%) and median (1.06%) values (Supplementary Fig. S2). By contrast, predicted pseudogenes in subterranean mammals consist of higher proportions of visual perception genes, occupying the first (4.26%; *C. asiatica*), second (3.28%; *Condylura condylura*), third (2.65%; *N. galili*), sixth (1.8%; *F. damarensis*), and ninth (1.47%; *H. glaber*) positions in the rankings (Supplementary Fig. S2).

Of the 213 visual perception genes, *C. asiatica* had the highest number of total losses (49; Fig. 1A), suggesting that as many as 77% are retained as functional in this blind mammal. After examining protein and gene expression data, I redefined 91 visual perception genes as eye-enriched to test whether the putatively functional genes are more pleiotropic in expression. Of these, subterranean mammals again had among the highest numbers of possible gene losses (mean = 11; median = 9.5), occupying the first (two tied at 35; *C. asiatica*, *N. galili*), third (26; *C. cristata*), fifth (21; *F. damarensis*), and tenth (13; *H. glaber*) positions (Fig. 1B). When reanalyzing the eye-enriched loci with only probable pseudogenes (mean = 4.3; median = 2), subterranean mammals occupy the first (24; *N. galili*), second (22; *C. asiatica*), third (12; *C. cristata*), fourth (10; *F. damarensis*), and sixth (6 [tied]; *H. glaber*) positions (Supplementary Fig. S3).

Distribution of visual gene losses

Although subterranean mammals tend to have the highest numbers of visual perception gene losses compared with mammals occupying other photic niches, I also explored the distribution of gene losses using logistic PCA analyses. This allows for the visualization of the distribution of gene losses shared between different species, to determine if subterranean mammals are distinct in the sets of vision genes they have lost, rather than simply the quantity. PCA plots of principal components 1 and 2 from the complete (Fig. 1C) and eye-enriched (Fig. 1D) datasets yielded highly similar patterns. Diurnal species and three aquatic/semi-aquatic taxa occupied a very similar, restricted PCA space (Fig. 1C,D), likely associated with the relative rarity of visual gene loss in these photic niches (Fig. 1A,B and Supplementary Figs. S1–S3). Nocturnal species show much more variation, but overlap strongly with diurnal and aquatic/semi-aquatic species. This suggests that mammals adapt to nocturnal niches in divergent ways, with some trending toward regression, like rod monochromats and subterranean mammals, and others retaining gene sets similar to diurnal taxa.

Subterranean mammals, by contrast, generally occupy a distinct portion of PCA space in both analyses, though there is substantial variation (Fig. 1C,D). For instance, despite the clear separation between *C. cristata*, *N. galili*, and *C. asiatica* from their above-ground counterparts, *H. glaber* overlaps strongly with certain epigean mammals. These include nocturnal species (Fig. 1C,D), such as microphthalmic echolocating bats and the lesser hedgehog tenrec (*Echinops telfairi*), and the microphthalmic common shrew (*Sorex araneus*).

Also included in the plots are three rod monochromat mammals (Fig. 1C,D), which lack cone photoreceptors but otherwise appear to have intact eyes (Meredith et al. 2013; Emerling and Springer 2015; Springer et al. 2016). Although these include aquatic (giant sperm whale [*Physeter microcephalus*], minke whale [*Balaenoptera acutorostrata*]) and nocturnal (nine-banded armadillo [*Dasypus novemcinctus*]) species, rod monochromats occupy a portion of PCA space distinct from other aquatic and nocturnal species. Notably, some subterranean mammals, such as *C. asiatica*, *H. glaber*, *N. galili*, and *F. damarensis*,

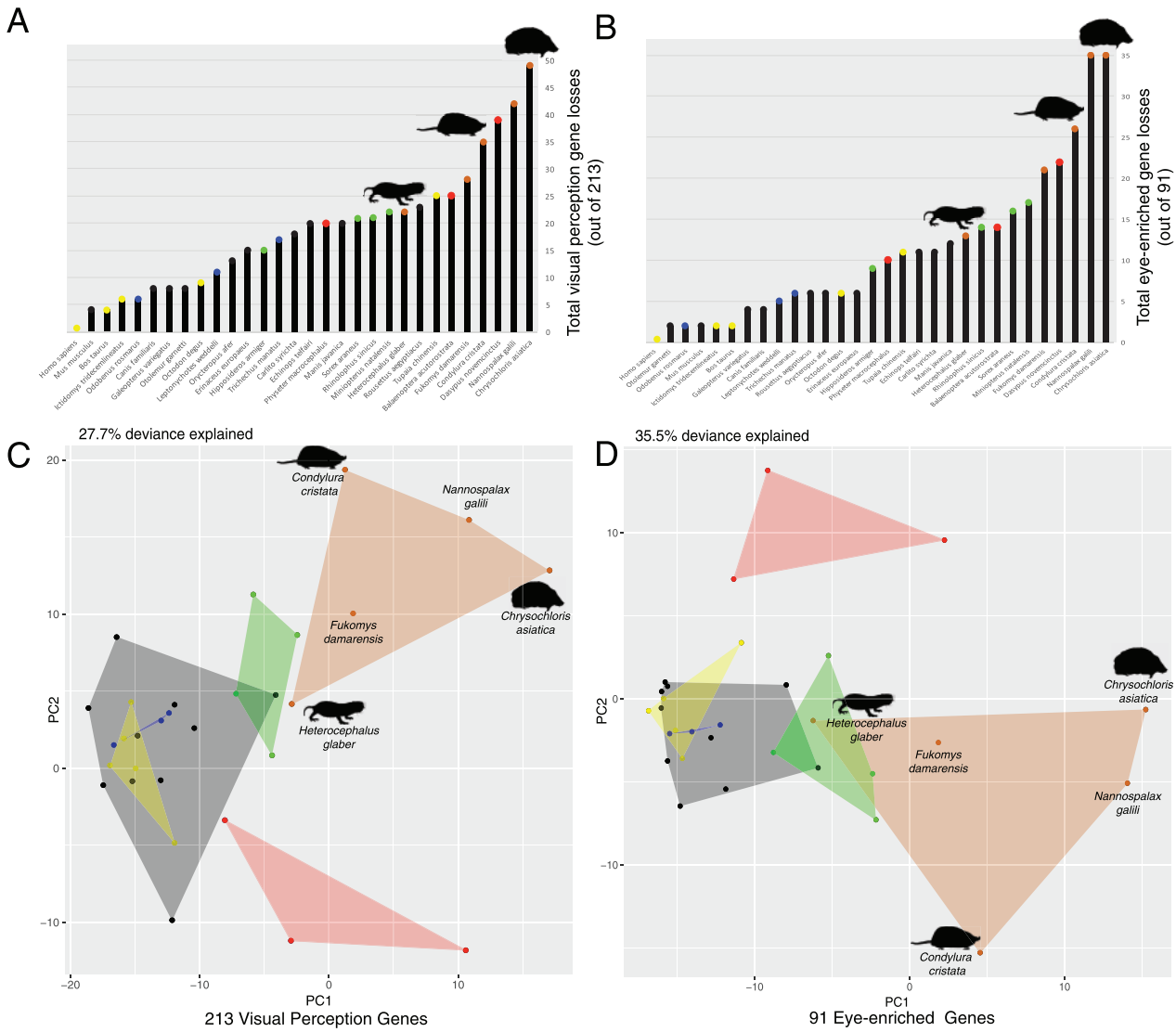

Fig. 1 Quantity and variation in visual perception gene losses. (**A**) Total number of possible visual perception gene losses. (**B**) Total number of possible eye-enriched visual perception gene losses. (**C**) Logistic PCA plot of possible visual perception gene losses. (**D**) Logistic PCA plot of possible eye-enriched visual perception gene losses. For C and D, % deviance explained is analogous to % variance explained in standard PCA analyses. The % deviance explained provides a measure for how good of a fit the principal components are for reconstructing the data, with 100% deviance indicating a perfect fit. Color coding: yellow, diurnal; black, nocturnal; blue, aquatic/semi-aquatic; green, microphthalmic; red, rod monochromat; brown, subterranean. Silhouettes and associated licenses from phylopic.org.

show evidence of rod monochromacy (Emerling and Springer 2014; Emerling et al. 2017), which involves inactivation of commons sets of genes underpinning cone phototransduction (Emerling et al. 2017). Yet, despite the shared gene losses associated with rod monochromacy, subterranean rod monochromats do not overlap with these taxa in PCA space, further pointing to the unique sets of genes lost in mammals living underground.

Among the subterranean mammals, the two most closely allied in PCA space are the two blind species, *C. asiatica* and *N. galili* (Fig. 1C,D). Indeed, of their 49 and 42 possible gene losses, respectively, 36 are shared by both species, suggesting highly similar sets of visual perception genes are lost in mammals with highly regressed, subcutaneous eyes.

Relative rates analyses of eye-enriched proteins in blind subterranean mammals

Both *N. galili* and *C. asiatica* demonstrate evidence of inactivation in 35 of 91 (38.5%) of the eye-enriched visual perception genes, possibly suggesting retention of function for the remaining 56 genes. However, the method used here to determine gene functionality is limited to whole gene deletions,

frameshift indels, and nonsense mutations, and is unable to provide information on potentially inactivating splice site mutations, missense mutations, or mutations in non-coding regulatory DNA. Nonetheless, if any putatively functional genes are inactivated, they are expected to be evolving with a loss of evolutionary constraint at an accelerated rate (Chikina et al. 2016; Partha et al. 2017). Alternatively, if any genes show evidence of deceleration on a branch, their history has likely been dominated by purifying selection.

Given that *C. asiatica* and *N. galili* have among the most regressed visual systems of mammals, along with the highest number of visual perception gene losses, I examined the RERs of their putatively functional eye-enriched visual perception proteins for evidence of evolutionary constraint. Between these two species, Partha et al. (2017) calculated RERs for 103 proteins with putatively functional eye-enriched visual perception genes (Supplementary Table S1). Sixty-nine of these proteins have RERs that are accelerated compared with the proteome-wide average for these two species, whereas 34 (33%) were estimated to be decelerating (Fig. 2 and Supplementary Fig. S4). By contrast, among the 46 RERs associated with predicted pseudogenes, only 6 (13%) show evidence of deceleration (Supplementary Fig. S4). Given that most, if not all, decelerating proteins are likely under purifying selection, and some accelerating proteins that do not deviate significantly from the proteome average also are plausibly under purifying selection, it indicates that a number of the eye enriched visual perception genes retained in *C. asiatica* and *N. galili* likely remain under evolutionary constraint.

Discussion

Comparative morphologists have long remarked that the eyes of subterranean mammals appear degraded compared with their above-ground counterparts, a fact that has long been attributed to regressive evolution (Darwin 1859; Fong et al. 1995). With the advent of DNA sequencing, the results from anatomical studies appeared to be reinforced at the molecular level, demonstrating a pattern that mirrors the regression of ocular morphology. Springer et al. (1997) reported that the marsupial mole (*Notoryctes typhlops*) has an inactivated interphotoreceptor retinoid-binding protein gene (*RBP3/IRBP*), which participates in the visual cycle to regenerate the opsin-bound retinal chromophore. David-Gray et al. (2002) found that *OPN1SW/SWS1*, which encodes a visual opsin, is likewise inactivated in a blind mole rat (*Nannospalax ehrenbergi*). Analysis of the whole genome of *H. glaber* revealed as many as 19 inactivated or deleted genes associated with visual functions (Kim et al. 2011), suggesting that the degradation of visual loci can occur *en masse* during evolution. Emerling and Springer (2014) examined 65 genes with retinal functions in the genomes of *C. asiatica*, *H. glaber*, and *C. cristata* and found evidence of 18, 12, and 6 gene losses, respectively. Furthermore, using a molecular dating method, they found evidence that nearly all of the gene losses post-dated fossil and ancestral state reconstructions of fossoriality, providing temporal evidence that life underground has led to the dispensing of some retinal genes. Subsequent genome assemblies of *N. galili* and *F. damarensis* found 22 and 14 vision-related pseudogenes, respectively (Fang et al. 2014a, 2014b), underscoring the fact that multiple lineages of subterranean mammals show evidence of visual regression at the genomic level.

While it may seem intuitive that these mammals have lost visual gene functions due to their specialized adaptations to a nearly lightless habitat, studies have demonstrated that regression of visual loci occurs in nocturnal and aquatic species as well. The visual opsin gene *OPN1SW* has been lost numerous times in nocturnal and aquatic mammals (Levenson and Dizon 2003; Tan et al. 2005; Zhao et al. 2009; Jacobs 2013; Meredith et al. 2013; Emerling et al. 2015). Echolocating bats have repeatedly inactivated *GJA10* (gap junction protein, alpha 10; Shen et al., 2013), a gene associated with retinal horizontal cell receptive fields, and *RBP3* has been pseudogenized in a number of nocturnal and aquatic species (Shen et al. 2013; Emerling and Springer 2014; Hudson et al. 2014). Numerous other genes associated with visual functions, such as *ARR3* (cone arrestin), *CRB1* (crumbs homolog 1 [*Drosophila*]), *GRK7* (G protein-coupled receptor kinase 7), *GUCA1B* (guanylate cyclase activator 1B), and *GUCY2F* (guanylate cyclase 2F, retinal) have been inactivated in various mammals (Emerling and Springer 2014; Hudson et al. 2014), particularly nocturnal species, and multiple genes involved in cone phototransduction have been pseudogenized in several whale lineages (Meredith et al., 2013; Emerling and Springer, 2015; Springer et al., 2016). Together, these data point to the possibility that there is nothing distinct about vision gene loss in subterranean mammals, but rather any type of dim-light adaptation may lead to gene inactivation. Instead, perhaps subterranean mammals have evolved their particularly regressed vision phenotypes primarily through relaxed selection on regulatory DNA elements (Berger et al. 2017; Partha et al. 2017; Roscito et al. 2017).

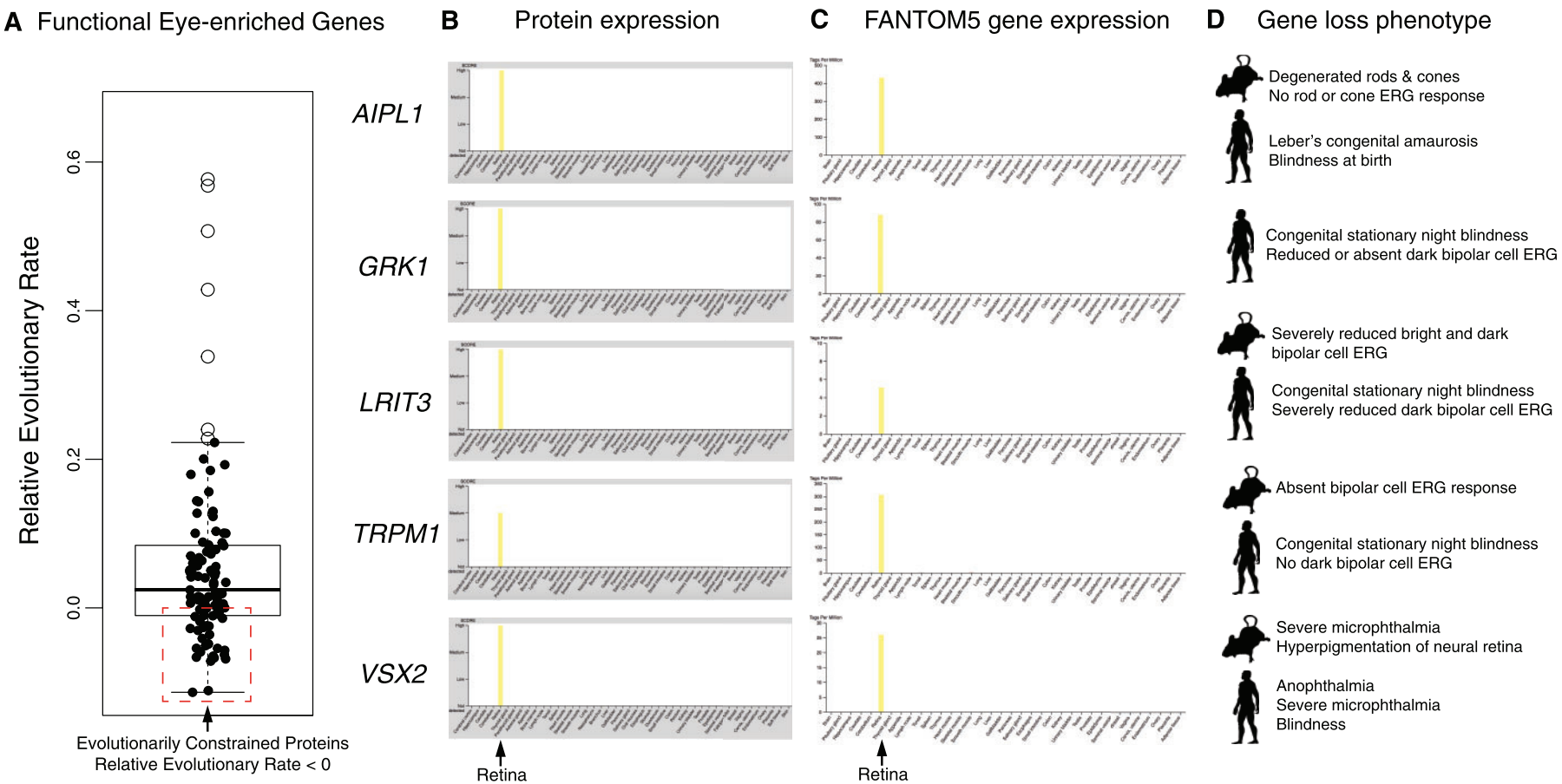

Fig. 2 Phenotypic consequences of the loss of five eye-enriched genes showing evidence of evolutionary constraint in *Nannospalax galili* and/or *Chrysochloris asiatica*. (**A**) Box and whisker plot overlayed by jitterplot of Relative Evolutionary Rates (RERs) of eye-enriched proteins predicted to be functional in *N. galili* and *C. asiatica*. Dashed box indicates evolutionarily constrained proteins with RERs less than zero. (**B**) Protein expression profiles of five evolutionarily constrained proteins. (**C**) Gene expression profiles of proteins in B. (**D**) Phenotypic consequences of the loss of proteins listed in B in mouse (gene knockout) and human (genetic association study). Fig. 2B,C is from www.proteinatlas.org/humanproteome. Citations for D in main text. ERG, electroretinogram. Silhouettes and associated licenses from phylopic.org.

Although loss of regulatory elements has likely been important in the acquisition of a regressed eye phenotype, the results reported here suggest that subterranean mammals are indeed generally distinct from other mammals in both the number and distribution of visual gene losses. Whether looking at possible or probable gene losses, after correcting for the total number of predicted pseudogenes in their genomes, and when focusing only on eye-enriched genes, subterranean mammals consistently rank in three to four of the top five spots in terms of visual gene loss. *Chrysochloris asiatica* and *N. galili* almost always occupy the first and second positions, often showing evidence of substantially more regression than other species, consistent with their particularly degraded subcutaneous eyes.

Furthermore, logistic PCA analyses demonstrate that subterranean mammals largely cluster separately from other mammals, pointing to their distinctive patterns of visual gene loss. Indeed, these analyses picked up on the high degree of overlap in gene loss between *C. asiatica* and *N. galili* (36 out of 49 and 42 genes, respectively), a remarkable pattern of convergent molecular evolution given that their most recent common ancestor dates to over 90 million years ago (Emerling et al. 2015). Whether similar patterns of visual gene loss occur in even more distantly related species with highly regressed eyes, such as the marsupial mole, scolecophidian snakes, or burrowing caecilians, should be investigated.

At the same time, not all subterranean mammals contrast with their subaerial counterparts in terms of vision gene loss, particularly the naked mole-rat (*H. glaber*). Despite the fact that this species is completely devoted to a life underground, has eyelids that typically remain closed, and shows evidence of some ocular regression (Mills and Catania 2004; Nikitina et al. 2004; Hetling et al. 2005), it often ranks behind other dim-light adapted mammals, such as bats and whales, in terms of quantity of gene loss (Fig. 1A,B and Supplementary Fig. S2). In the logistic PCA plots, *H. glaber* occupied PCA space particularly near to nocturnal echolocating bats and the common shrew. These mammals have particularly tiny eyes that are considered microphthalmic (Peichl et al. 2000; Nevo 2007), which leads to a reduced image size on the retina and therefore minimal visual acuity. This suggests that for at least some characteristics, *H. glaber*'s eyes may be largely indistinct from those of aboveground mammals with minute eyes.

One above-ground mammal that typically ranked in the top five in terms of gene loss is the nine-banded armadillo (*D. novemcinctus*; Fig. 1A,B and Supplementary Figs. S1 and S3). While most armadillos burrow to some degree, only the fairy armadillos (Chlamyphorinae) are considered to be wholly committed to a life underground. However, analyses of vision genes in armadillos and their sloth kin, paired with anatomical comparisons of extant and extinct species of their superorder (Xenarthra), has led to the suggestion that *D. novemcinctus* descended from ancestors that were committed to a highly fossorial, possibly even subterranean, lifestyle (Emerling and Springer, 2015). The relatively high number of vision pseudogenes in this species, ranking this animal along with committed subterranean mammals, appears to provide further evidence for this hypothesis.

Notably, not all eye-enriched visual perception genes showed evidence of degradation, even in the blind *C. asiatica* and *N. galili*, with some putatively functional genes showing evidence of evolutionary constraint. While some of this is likely due to pleiotropy, there exist multiple genes with decelerating RERs and no apparent expression outside of the eye. Among these are genes that are critical for minimal retinal function and eye formation (Fig. 2) (Sohocki et al. 2000; Ramamurthy et al. 2004; Zhang et al. 2005; Oishi et al. 2007; Morgans et al. 2009; van Genderen et al. 2009; Iseri et al. 2010; Zou and Levine 2012; Zeitz et al. 2013; Neuillé et al. 2014), suggesting that natural selection is maintaining eyes in these otherwise blind species, consistent with the retention of a well-organized retina in another species of blind mole rat (*Spalax ehrenbergi*) (Esquiva et al. 2016). One hypothesis posits that the eye is being maintained for photoperiodic functions (Pevet et al. 1984; Cooper et al. 1993a; David-Gray et al. 1998; Hannibal et al. 2002), which predicts that the retained genes may be essential for maintaining a "circadian eye". With further explorations of the functions of these genes, and determining whether they are critical for photoentrainment or other non-visual functions, we will come closer to understanding just how far the regressed eyes of subterranean mammals have degenerated, and why they may have stopped just short of complete loss.

Acknowledgments

I thank two anonymous reviewers and the associated editors for constructive feedback on an earlier manuscript, and Lauren H. Sumner-Rooney and Megan L. Porter for organizing the SICB "Evolution in the Dark: Unifying Understanding of Eye Loss" symposium. This symposium was generously sponsored by the Company of Biologists (http://www.biologists.

com); the Palaeontological Association (PA-GA201707); the American Microscopical Society; the Crustacean Society; and the SICB divisions DEDB, DEE, DIZ, DNB, and DPCB. The RER data were kindly provided by Raghavendran Partha and Nathan Clark. This is contribution ISEM-2018-056 of the Institut des Sciences de l'Evolution de Montpellier.

Funding

This work was supported by a National Science Foundation Postdoctoral Research Fellowship in Biology [Award No. 1523943]; a National Science Foundation Postdoctoral Fellow Research Opportunities in Europe Award; and the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme (FP7/2007-2013) under REA grant agreement no. PCOFUND-GA-2013-609102, through the PRESTIGE programme coordinated by Campus France.

Supplementary data

Supplementary data available at *ICB* online.

References

Berger MJ, Wenger AM, Guturu H, Bejerano G. 2017. Independent erosion of conserved transcription factor binding sites points to shared hindlimb, vision, and scrotum loss in different mammals. bioRxiv 197756.

Chase HB, Chase EB. 1941. Studies on an anophthalmic strain of mice I. Embryology of the eye region. J Morphol 68:279–301.

Chikina M, Robinson JD, Clark NL. 2016. Hundreds of genes experienced convergent shifts in selective pressure in marine mammals. Mol Biol Evol 33:2182–92.

Cooper HM, Herbin M, Nevo E. 1993a. Ocular regression conceals adaptive progression of the visual system in a blind subterranean mammal. Nature 361:156–9.

Cooper HM, Herbin M, Nevo E. 1993b. Visual system of a naturally microphthalmic mammal: the blind mole rat, *Spalax ehrenbergi*. J Comp Neurol 328:313–50.

Darwin C. 1859. On the origin of species by means of natural selection. London: Murray.

David-Gray ZK, Bellingham J, Munoz M, Avivi A, Nevo E, Foster RG. 2002. Adaptive loss of ultraviolet-sensitive/violet-sensitive (UVS/VS) cone opsin in the blind mole rat (*Spalax ehrenbergi*). Eur J Neurosci 16:1186–94.

David-Gray ZK, Janssen JW, DeGrip WJ, Nevo E, Foster RG. 1998. Light detection in a "blind" mammal. Nat Neurosci 1:655–6.

Emerling CA, Huynh HT, Nguyen MA, Meredith RW, Springer MS. 2015. Spectral shifts of mammalian ultraviolet-sensitive pigments (short wavelength-sensitive opsin 1) are associated with eye length and photic niche evolution. Proc R Soc Lond B Biol Sci 282:20151817.

Emerling CA, Springer MS. 2014. Eyes underground: regression of visual protein networks in subterranean mammals. Mol Phylogenet Evol 78:260–70.

Emerling CA, Springer MS. 2015. Genomic evidence for rod monochromacy in sloths and armadillos suggests early subterranean history for Xenarthra. Proc R Soc B 282:20142192.

Emerling CA, Widjaja AD, Nguyen NN, Springer MS. 2017. Their loss is our gain: regressive evolution in vertebrates provides genomic models for uncovering human disease loci. J Med Genet 54:787–94.

Esquiva G, Avivi A, Hannibal J. 2016. Non-image forming light detection by melanopsin, rhodopsin, and long-middlewave (L/W) cone opsin in the subterranean blind mole rat, *Spalax ehrenbergi*: immunohistochemical characterization, distribution, and connectivity. Front Neuroanat 10:1–15.

Fang X, Nevo E, Han L, Levanon EY, Zhao J, Avivi A, Larkin D, Jiang X, Feranchuk S, Zhu Y, et al. 2014a. Genome-wide adaptive complexes to underground stresses in blind mole rats *Spalax*. Nat Commun 5:3966.

Fang X, Seim I, Huang Z, Gerashchenko MV, Xiong Z, Turanov AA, Zhu Y, Lobanov AV, Fan D, Yim SH, et al. 2014b. Adaptations to a subterranean environment and longevity revealed by the analysis of mole rat genomes. Cell Rep 8:1354–11.

Fong D, Kane T, Culver D. 1995. Vestigialization and loss of nonfunctional characters. Annu Rev Ecol Syst 26:249–68.

Forrest ARR, Kawaji H, Rehli M, Baillie JK, De Hoon MJL, Haberle V, Lassmann T, Kulakovskiy IV, Lizio M, Itoh M, et al. 2014. A promoter-level mammalian expression atlas. Nature 507:462–70.

Gene Ontology Consortium. 2004. The Gene Ontology (GO) database and informatics resource. Nucleic Acids Res 32:D258–61.

Haim A, Heth G, Pratt H, Nevo E. 1983. Photoperiodic effects on thermoregulation in a "blind" subterranean mammal. J Exp Biol 107:59–64.

Hannibal J, Hindersson P, Nevo E, Fahrenkrug J. 2002. The circadian photopigment melanopsin is expressed in the blind subterranean mole rat, *Spalax*. Neuroreport 13:1411–44.

Hetling JR, Baig-Silva MS, Comer CM, Pardue MT, Samaan DY, Qtaishat NM, Pepperberg DR, Park TJ. 2005. Features of visual function in the naked mole-rat *Heterocephalus glaber*. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 191:317–30.

Hudson NJ, Baker ML, Hart NS, Wynne JW, Gu Q, Huang Z, Zhang G, Ingham AB, Wang L, Reverter A. 2014. Sensory rewiring in an echolocator: genome-wide modification of retinogenic and auditory genes in the bat *Myotis davidii*. G3 (Bethesda) 4:1825–35.

Iseri SU, Wyatt AW, Nürnberg G, Kluck C, Nürnberg P, Holder GE, Blair E, Salt A, Ragge NK. 2010. Use of genome-wide SNP homozygosity mapping in small pedigrees to identify new mutations in *VSX2* causing recessive microphthalmia and a semidominant inner retinal dystrophy. Hum Genet 128:51–60.

Jacobs GH. 2013. Losses of functional opsin genes, short-wavelength cone photopigments, and color vision—a significant trend in the evolution of mammalian vision. Vis Neurosci 30:39–53.

Jeffery WR. 2009. Regressive evolution in *Astyanax* cavefish. Annu Rev Genet 43:25–47.

Kim EB, Fang X, Fushan AA, Huang Z, Lobanov AV, Han L, Marino SM, Sun X, Turanov AA, Yang P, et al. 2011. Genome sequencing reveals insights into physiology and longevity of the naked mole rat. Nature 479:223–7.

Kott O, Němec P, Fremlová A, Mazoch V, Šumbera R. 2016. Behavioural tests reveal severe visual deficits in the strictly subterranean African mole-rats (Bathyergidae) but efficient vision in the fossorial rodent coruro (*Spalacopus cyanus*, Octodontidae). Ethology 122:682–94.

Landgraf AJ, Lee Y. 2015. Dimensionality reduction for binary data through the projection of natural parameters. arXiv preprint arXiv: 1510.06112.

Levenson DH, Dizon A. 2003. Genetic evidence for the ancestral loss of short-wavelength-sensitive cone pigments in mysticete and odontocete cetaceans. Proc R Soc B 270:673–9.

Meredith RW, Gatesy J, Emerling CA, York VM, Springer MS. 2013. Rod monochromacy and the coevolution of cetacean retinal opsins. PLoS Genet 9:e1003432.

Mills SL, Catania KC. 2004. Identification of retinal neurons in a regressive rodent eye (the naked mole-rat). Vis Neurosci 21:107–17.

Mohun SM, Davies WL, Bowmaker JK, Pisani D, Himstedt W, Gower DJ, Hunt DM, Wilkinson M. 2010. Identification and characterization of visual pigments in caecilians (Amphibia: gymnophiona), an order of limbless vertebrates with rudimentary eyes. J Exp Biol 213:3586–92.

Morgans CW, Zhang J, Jeffrey BG, Nelson SM, Burke NS, Duvoisin RM, Brown RL. 2009. TRPM1 is required for the depolarizing light response in retinal ON-bipolar cells. Proc Natl Acad Sci U S A 106:19174–8.

Nemec P, Cveková P, Burda H, Benada O, Peichl L. 2007. Visual systems and the role of vision in subterranean rodents. In: Begall S, Burda H, Schleich CE, editors. Life underground: the biology of subterranean rodents. Berlin: Springer-Verlag. p. 129–60.

Neuillé M, El Shamieh S, Orhan E, Michiels C, Antonio A, Lancelot ME, Condroyer C, Bujakowska K, Poch O, Sahel JA, et al. 2014. *Lrit3* deficient mouse (nob6): a novel model of complete congenital stationary night blindness (cCSNB). PLoS One 9:e90342–32.

Nevo E. 2007. Mosaic evolution of subterranean mammals: tinkering, regression, progression, and global convergence. In: Begall S, Burda H, Schleich CE, editors. Subterranean rodents: news from underground. Berlin, Heidelberg: Springer. p. 375–88.

Nikitina NV, Maughan-Brown B, O'Riain MJ, Kidson SH. 2004. Postnatal development of the eye in the naked mole rat (*Heterocephalus glaber*). Anat Rec A Discov Mol Cell Evol Biol 277A:317–37.

Oishi A, Akimoto M, Kawagoe N, Mandai M, Takahashi M, Yoshimura N. 2007. Novel Mutations in the *GRK1* Gene. Oncology 144:475–7.

Partha R, Chauhan BK, Ferreira Z, Robinson JD, Lathrop K, Nischal KK, Chikina M, Clark NL. 2017. Subterranean mammals show convergent regression in ocular genes and enhancers, along with adaptation to tunneling. Elife 6:1–26.

Peichl L, Chavez AE, Ocampo A, Mena W, Bozinovic F, Palacios AG. 2005. Eye and vision in the subterranean rodent cururo (*Spalacopus cyanus*, Octodontidae). J Comp Neurol 486:197–208.

Peichl L, Künzle H, Vogel P. 2000. Photoreceptor types and distributions in the retinae of insectivores. Vis Neurosci 17:937–48.

Pérez-Moreno JL, Balázs G, Wilkins B, Herczeg G, Bracken-Grissom HD. 2017. The role of isolation on contrasting phylogeographic patterns in two cave crustaceans. BMC Evol Biol 17:247.

Pevet P, Heth G, Hiam A, Nevo E. 1984. Photoperiod perception in the blind mole rat (*Spalax ehrenbergi*, Nehring): involvement of the Harderian gland, atrophied eyes, and melatonin. J Exp Zool A Ecol Genet Physiol 232:41–50.

Protas ME, Trontelj P, Patel NH. 2011. Genetic basis of eye and pigment loss in the cave crustacean, *Asellus aquaticus*. Proc Natl Acad Sci U S A 108:5702–7.

Ramamurthy V, Niemi GA, Reh TA, Hurley JB. 2004. Leber congenital amaurosis linked to *AIPL1*: a mouse model reveals destabilization of cGMP phosphodiesterase. Proc Natl Acad Sci U S A 101:13897–902.

Roscito JG, Sameith K, Parra G, Langer B, Petzold A, Rodrigues MT, Hiller M. 2017. Phenotype loss is associated with widespread divergence of the gene regulatory landscape in evolution. bioRxiv 238634.

Sanyal S, Jansen HG, Grip WJD, Nevo E, Jong WWD. 1990. The eye of the blind mole rat, *Spalax ehrenbergi*. Invest Ophthalmol Vis Sci 31:1398–404.

Schleich CE, Vielma A, Glösmann M, Palacios AG, Peichl L. 2010. Retinal photoreceptors of two subterranean tucotuco species (Rodentia, *Ctenomys*): morphology, topography, and spectral sensitivity. J Comp Neurol 518:4001–15.

Shen B, Fang T, Dai M, Jones G, Zhang S. 2013. Independent losses of visual perception genes *Gja10* and *Rbp3* in echolocating bats (Order: Chiroptera). PLoS One 8:e68867.

Sohocki MM, Perrault I, Leroy BP, Payne AM, Dharmaraj S, Bhattacharya SS, Kaplan J, Maumenee IH, Koenekoop R, Meire FM, et al. 2000. Prevalence of *AIPL1* mutations in inherited retinal degenerative disease. Mol Genet Metab 70:142–50.

Springer MS, Burk A, Kavanagh JR, Waddell VG, Stanhope MJ. 1997. The interphotoreceptor retinoid binding protein gene in therian mammals: implications for higher level relationships and evidence for loss of function in the marsupial mole. Proc Natl Acad Sci U S A 94:13754–9.

Springer MS, Emerling CA, Fugate N, Patel R, Starrett J, Morin PA, Hayashi C, Gatesy J. 2016. Inactivation of cone-specific phototransduction genes in rod monochromatic cetaceans. Front Ecol Evol 4:61.

Stern DB, Breinholt J, Pedraza-Lara C, López-Mejía M, Owen CL, Bracken-Grissom H, Fetzner JW, Crandall KA. 2017. Phylogenetic evidence from freshwater crayfishes that cave adaptation is not an evolutionary dead-end. Evolution 71:2522–32.

Sumner-Rooney L, Sigwart JD, McAfee J, Smith L, Williams ST. 2016. Repeated eye reduction events reveal multiple pathways to degeneration in a family of marine snails. Evolution 70:2268–95.

Sweet G. 1906. Memoirs: contributions to our knowledge of the anatomy of *Notoryctes typhlops*, Stirling. Q J Microsc Sci 2:547–72.

Sweet G. 1909. The eyes of *Chrysochloris hottentota* and *C. asiatica*. Q J Microsc Sci 2:327–38.

Tan Y, Yoder AD, Yamashita N, Li W-H. 2005. Evidence from opsin genes rejects nocturnality in ancestral primates. Proc Natl Acad Sci U S A 102:14712–6.

Tierney SM, Cooper SJB, Saint KM, Bertozzi T, Hyde J, Humphreys WF, Austin AD. 2015. Opsin transcripts of predatory diving beetles: a comparison of surface and subterranean photic niches. R Soc Open Sci 2:140386.

Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson A, Kampf C, Sjostedt E, Asplund A, et al. 2015. Tissue-based map of the human proteome. Science 347:1260419.

van Genderen MM, Bijveld MMC, Claassen YB, Florijn RJ, Pearring JN, Meire FM, McCall MA, Riemslag FCC, Gregg RG, Bergen AAB, et al. 2009. Mutations in *TRPM1* are a common cause of complete congenital stationary night blindness. Am J Hum Genet 85:730–36.

Verma AS, FitzPatrick DR. 2007. Anophthalmia and microphthalmia. Orphanet J Rare Dis 2:47–8.

Wilman H, Belmaker J, Simpson J, de la Rosa C, Rivadeneira MM, Jetz W. 2014. EltonTraits 1.0: species-level foraging attributes of the world's birds and mammals. Ecology 95:2027.

Zeitz C, Jacobson SG, Hamel CP, Bujakowska K, Neuillé M, Orhan E, Zanlonghi X, Lancelot ME, Michiels C, Schwartz SB, et al. 2013. Whole-exome sequencing identifies *LRIT3* mutations as a cause of autosomal-recessive complete congenital stationary night blindness. Am J Hum Genet 92:67–75.

Zhang Q, Zulfiqar F, Riazuddin SA, Xiao X, Yasmeen A, Rogan PK, Caruso R, Sieving PA, Riazuddin S, Hejtmancik JF. 2005. A variant form of Oguchi disease mapped to 13q34 associated with partial deletion of *GRK1* gene. Mol Vis 11:977–85.

Zhao H, Rossiter SJ, Teeling EC, Li C, Cotton JA, Zhang S. 2009. The evolution of color vision in nocturnal mammals. Proc Natl Acad Sci U S A 106:8980–85.

Zou C, Levine EM. 2012. *Vsx2* controls eye organogenesis and retinal progenitor identity via homeodomain and non-homeodomain residues required for high affinity DNA binding. PLoS Genet 8:e1002924.