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Eyes underground: Regression of visual protein networks in subterranean mammals

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ABSTRACT

Regressive evolution involves the degeneration of formerly useful structures in a lineage over time, and may be accompanied by the molecular decay of phenotype-specific genes. The mammalian eye has repeatedly undergone degeneration in taxa that occupy dim-light environments including subterranean habitats. Here we assess whether a decrease in the amount of light that reaches the retina is associated with increased regression of retinal genes, whether the phototransduction and visual cycle pathways degrade in a predictable pattern, and if the timing of retinal gene loss is associated with the entrance of mammalian lineages into subterranean environments. Sequence data were obtained from the publically available genomes of the Cape golden mole (*Chrysochloris asiatica*), naked mole-rat (*Heterocephalus glaber*) and star-nosed mole (*Condylura cristata*) for 65 genes associated with phototransduction, the visual cycle, and other retinal functions. Gene sequences were inspected for inactivating mutations and, when present, pseudogene sequences were compared to sequences from subaerial outgroup species. To test whether retinal degeneration is correlated with historical entrances into subterranean environments, estimated dates of retinal gene inactivation were compared to the fossil record and phylogenetic inferences of ancestral fossoriality. Our results show that (1) lower levels of light available to the retina correspond with an increase in the number of retinal pseudogenes, (2) retinal protein networks generally degrade in a predictable manner, although the extensive loss of cone phototransduction genes in *Heterocephalus* raises further questions regarding SWS1-cone monochromacy versus functional rod monochromacy in this species, and (3) inactivation dates of retinal genes usually post-date inferred entrances into subterranean habitats.

1. Introduction

Regressive evolution, or vestigialization, is the degradation of formerly useful anatomical structures, behaviors and/or genes in lineages over time (Fong et al., 1995). Regressive evolution may occur when an organism enters a novel niche and one or more characters degenerate either via relaxed selection, due to a lack of utility to the organism, or direct selection against these characters if they are maladaptive. Regressive evolution is widespread among the visual systems of vertebrates that have invaded dim-light niches, including caves (Wilkens, 2007), deep-oceans (Yokoyama et al., 1999; Meredith et al., 2013a), nocturnal environments (Jacobs, 2013), and subterranean habitats (Sweet, 1906, 1909; Sanyal et al., 1990; David-Gray et al., 2002; Mohun et al., 2010; Kim et al., 2011), and has previously been noted by prominent naturalists including Lamarck (de Monet, 2011), Darwin (1859) and even Aristotle (2004).

It is expected that morphological regression will be mirrored by the decay of underlying genes whose functions are solely dedicated to the specification of formerly useful morphological characters. This genetic decay results in the accumulation of deleterious mutations that convert a formerly functional gene into a nonfunctional unitary pseudogene (Meredith et al., 2009, 2011a, 2013b). Given that vision involves numerous retina-specific proteins, degradation of these genes should be positively correlated with the decay of the vertebrate eye in species that have secondarily lost one or more components of vision. The public release of three subterranean mammal genomes provides an opportunity to address questions relating to regression of the visual system by evaluating the patterns of inactivation among retina-specific genes. These species, *Chrysochloris asiatica* (Cape golden mole, Chrysochloridae), *Heterocephalus glaber* (naked mole-rat, Bathyergidae) and *Condylura cristata* (star-nosed mole, Talpidae) belong to three different superorders of mammals (Afrotheria, Euarchontoglires, and

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Laurasiatheria, respectively; Murphy et al., 2001), represent convergent forays into underground habitats, and provide independent natural experiments to assess the effects of subterranean life on vision.

Here, we address whether the degree of molecular regression mirrors the amount of light available to the retina. Only light that is capable of reaching the retina can be transduced into a signal to facilitate vision. Subterranean mammals are expected to have a reduced amount of light reaching their retinas compared to their subaerial counterparts, but even among the former there are differences in commitment to underground activity. Species that spend more time underground are predicted to have progressively more degraded eyes and a larger number of retinal pseudogenes. Additionally, some subterranean mammals can open their eyelids whereas others possess eyes that are completely subcutaneous. This difference is also predicted to influence the degree of ocular degradation with more extensive regression occurring in species with subcutaneous eyes. *Condylura cristata* has minute eyes with functional eyelids and spends time below ground, above ground, and in the water (Hamilton, 1931; Petersen and Yates, 1980). *Heterocephalus glaber* spends nearly all of its time underground, and typically opens its tiny eyes only under illumination (Jarvis and Sherman, 2002; Mills and Catania, 2004; Nikitina et al., 2004). *Chrysochloris asiatica* spends almost all of its time below ground and possess subcutaneous eyes (Sweet, 1909; Gubbay, 1956). Accordingly, we predict that the level of retinal genomic regression should increase from *Condylura* to *Heterocephalus* to *Chrysochloris*.

We also address whether retinal protein networks involved in vision degrade in a predictable pattern. We focus on two major processes: (1) phototransduction, the conversion of information encoded in photons to an electrical signal and (2) the visual cycle, the process that regenerates the vitamin-A-derived chromophores necessary for photoreception. Phototransduction occurs in the highly sensitive, dim-light photoreceptive retinal cells known as rods and the low-sensitivity, high-acuity cells that are more useful in bright light known as cones. This process is initiated by the absorption of photons by chromophores bound to opsin proteins. Rods and cones use separate but paralogous proteins for their respective phototransduction cascades, and share several proteins for regeneration, regulation and other processes (Fu, 2011; Invergo et al., 2013). For example, cones in most placental mammals contain SWS1 (short-wavelength sensitive 1) and/or LWS (long-wavelength sensitive) opsins, whereas rods contain RH1 (rod-specific) opsin. Rods and cones also possess a shared visual cycle that takes place in the retinal pigment epithelium, whereas a recently discovered cone-specific cycle appears to utilize many of the same proteins (Saari, 2012).

Given this information, we predict that inactivation of a gene that is crucial to the rod or cone pathway will be accompanied by the inactivation of all other rod- or cone-specific genes, respectively. Further, if only one pathway is disrupted, then all genes encoding shared pathway proteins should be retained owing to maintenance of the remaining phototransduction pathway. Finally, shared pathway proteins should only be lost if cone and rod phototransduction are both abrogated. The exceptions to these predictions involve any functionally redundant, or nearly redundant, proteins that occur in these pathways. Presumably these extra proteins are useful in conditions with bright light (e.g., higher rates of chromophore turnover), though in a subterranean habitat some, but not all copies, may become nonfunctional.

Ocular regression is not limited to subterranean mammals and also occurs among species that inhabit other dim-light niches (e.g., nocturnality; Jacobs, 2013; Shen et al., 2013). A final question, therefore, is whether subterranean lifestyles, as opposed to other selective pressures, have led to the regression of visual systems that are observed in subterranean species. To address this question we can compare the timing of ocular degradation to the origins of fossoriality in subterranean lineages. Soft tissues such as eyes are rarely preserved in the fossil record, but inactivation times of vision pseudogenes may be used as a proxy for eye degeneration. These estimates can then be compared to ancestral state reconstructions of fossoriality and fossil record-based estimates of the timing of deployment into subterranean habitats among different fossorial lineages. Inactivation dates of vision genes should be similar to or younger than inferred dates for fossoriality if life underground has led to the degradation of these genes. Conversely, inactivation dates that are older than the origins of fossoriality would suggest that visual regression commenced in response to other selective pressures in the earlier history of such lineages, e.g., nocturnality. In addition to *Condylura*, *Heterocephalus*, and *Chrysochloris*, we also estimate the timing of inactivation of published retinal pseudogenes from two other subterranean taxa: the marsupial mole (*Notoryctes typhlops*; Springer et al., 1997) and the Middle East blind mole rat (*Spalax ehrenbergi*; David-Gray et al., 2002).

To address these questions, we collected complete or nearly complete protein-coding regions of 65 retinal genes that are associated with phototransduction, the visual cycle, circadian photoentrainment, photoreceptor development, and/or retinal diseases (latter three collectively referred to as "other retinal proteins" below), performed phylogenetic analyses, and reviewed the literature on the fossil records of these taxa. Our results support the hypotheses that the amount of light reaching the retina is related to the proportion of retinal genomic regression, that degradation of retinal protein networks is largely predictable, and that the timing of retinal gene pseudogenization is broadly consonant with the entrance of different subterranean taxa into their underground niches.

2. Materials and methods

2.1. Gene sampling

Genes were selected based on (1) their known or inferred function in the retinal phototransduction cascade, visual cycle, circadian photoentrainment, or photoreceptor development (Fu, 2011; Saari, 2012; Invergo et al., 2013) and/or (2) their implication in retinal diseases (Wada et al., 2001; Grayson et al., 2002; Hattar et al., 2003; Liu et al., 2004; Zangerl et al., 2006; Boon et al., 2009; Bandah-Rozenfeld et al., 2010; Collin et al., 2010; Bujakowska et al., 2012; Di Gioia et al., 2012; Davidson et al., 2013). Reference sequences for mRNA transcripts were downloaded from GenBank.

DNA sequences were downloaded from the following sources: GenBank (GB), which includes NCBI's whole genome shotgun contig database (WGS); Ensembl (E); and in the case of *ABCA4*, OrthoMAM (OM; Ranwez et al., 2007) (Supplementary Table S1). Sequences for *Chrysochloris asiatica* (Cape golden mole; 66x genome coverage), *Heterocephalus glaber* (naked mole-rat; 90x [Broad, Kim et al., 2011]), and *Condylura cristata* (star-nosed mole; 113.1x) were all derived from WGS. For *H. glaber*, we gathered sequences from the Broad assembly and only included sequences from the Kim et al. (2011) assembly when the former returned negative BLAST results. All genes collected from the Broad assembly were BLASTed against the Kim et al. (2011) assembly for comparison. In cases where an inactivated copy of a gene was discovered in one or more subterranean taxa, gene sequences for representative outgroup taxa (Laurasiatheria, Euarchontoglires, or Afrotheria, respectively) were also downloaded to check for functionality versus inactivation and to perform pseudogene dating analyses (see Section 2.5). Laurasiatheria outgroups included *Bos taurus*