

Evolution in the dark: unifying our understanding of eye loss

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Synopsis

The evolution of eye loss in subterranean, deep sea and nocturnal habitats has fascinated biologists since Darwin wrestled with it in *On the Origin of Species*. This phenomenon appears consistently throughout the animal kingdom, in groups as diverse as crustaceans, salamanders, gastropods, spiders and the well-known Mexican cave fish, but the nature, extent and evolutionary processes behind eye loss remain elusive. With the advantage of new imaging, molecular, and developmental tools, eye loss has once again become the subject of intense research focus. To advance our understanding of eye loss as a taxonomically widespread and repeated evolutionary trajectory, we organized a cross-disciplinary group of researchers working on the historic question, 'how does eye loss evolve in the dark?'. The resulting set of papers showcase new progress made in understanding eye loss from the diverse fields of molecular biology, phylogenetics, development, comparative anatomy, paleontology, ecology and behaviour in a wide range of study organisms and habitats. Through the integration of these approaches, methods and results, common themes begin to emerge across the field. For the first time, we hope researchers can exploit this new synthesis to identify the broader challenges and key evolutionary questions surrounding eye evolution and so-called regressive evolution and collectively work to address them in future research.

Introduction

Light is one of the primary environmental cues used by animals to regulate circadian rhythms, orient, forage and navigate. But entire animal communities across the world

inhabit low-light or aphotic environments, including subterranean and submarine caves, burrows, the deep ocean, leaf litter and host organisms. Despite the diversity of these dark-living animals and their habitats, they often exhibit striking convergent morphological features. These can include the loss of pigmentation, elongation of limbs and antennae, the elaboration of non-visual sensory modalities, and the reduction and loss of eyes. The latter especially has inspired scientific curiosity for more than a century, and remains the focus of research and the centre of debate today. The advantages to vision in light-limited environments are clearly reduced, but the exact processes leading to their loss are still somewhat unclear. Initially, Darwin attributed the loss of eyes in cavernicolous species to “disuse”, with the release of selective pressures sustaining vision eventually resulting in their gradual degeneration and decline. Since then, several researchers have demonstrated that there are direct selective advantages to eye loss (Niven and Laughlin 2008; Moran, Softley, and Warrant 2015); given the costly maintenance of these energetically expensive organs, it is hardly surprising that the loss and reduction of eyes is so widespread and so consistent.

Yet, studies of eye loss continue to struggle with pinpointing the proximate mechanisms and the ultimate evolutionary forces leading to eye loss. Broader synthesis of these proximate and ultimate causes have been hindered, in part, by the lack of interaction among the scientific communities studying eye loss and reduction in different taxa, and especially in different habitats. Speleobiology in particular enjoys a close community dynamic, but even here eye loss has overwhelmingly been studied in fishes, crustaceans and insects in relative isolation from each other. One of the fundamental motivations for this symposium was to bring the diverse research

community studying eye loss together for the first time and to encourage collaboration and exchange of ideas between researchers working with different systems.

Core questions

Several key themes ran through the symposium and the complementary session, with some long-standing questions remaining after nearly two centuries, and several new ones emerging in the modern research landscape.

Q1. What are the evolutionary forces driving eye loss?

Although it was initially attributed solely to the relaxation of selective pressures, it is now clear that neutral evolution, selection and pleiotropic constraint may all play a role in the evolution of reduction and eventual loss of eyes. The relative balance and the interactions between these drivers remains subject to debate, and objectively quantifying their relative impact remains a significant challenge even in model systems.

Q2. How does eye reduction and loss arise?

The developmental and genetic bases of eye loss are only well-studied in the Mexican cave tetra system, *Astyanax mexicanus*, with the isopod system, *Asellus aquaticus*, described by Re et al. (2018) poised to make major contributions to understanding eye loss in an arthropod system. Beyond these two species, very little is understood about the proximate mechanisms causing eye reduction and loss in most species. Strikingly, even in the *A. mexicanus* system where the genetic and developmental bases for eye loss have been studied for well over 50 years, there are still significant discoveries

regarding the processes involved in eye loss published on a regular basis, such as the role of the neural crest as a key agent in cavefish eye regression, as demonstrated by Yoshizawa, Hixon, and Jeffery (2018). Studies of the proximate mechanisms of eye loss in additional organismal systems are now needed to begin to identify mechanistic commonalities across taxa.

Q3. Are losses similar in multiple cases, taxa, or habitats?

This theme arose across the board, whether researchers were working with model species and multiple dark-living populations (Re et al. 2018; Yoshizawa, Hixon, and Jeffery 2018) or with larger taxonomic groups and multiple lineages exhibiting eye reduction (e.g. Stern and Crandall 2018). Studies focused on which genes were affected by eye reduction and loss, either in terms of selective regimes or regulation of expression levels, and the developmental mechanisms leading to reduction and loss. Advances in sequencing technologies have made genomic- and transcriptomic-level data capture widely available, allowing researchers to begin to answer these questions within populations, and across populations (Pérez-Moreno, Balázs, and Bracken-Grissom 2018; Re et al. 2018; Yoshizawa, Hixon, and Jeffery 2018) and larger taxonomic groups of species (Emerling 2018; Stern and Crandall 2018; Tierney et al. 2018). Genome-level studies, such as those published here by Re et al. (2018) and Emerling (2018), are providing insights into whether certain regions of the genome are more susceptible to either relaxed selection, or the selection pressures related to lack of light.

Q4. How complete is eye loss?

Although eyes are dramatically degraded and reduced in many dark-living species, in many cases there are remnants of the visual system still present. However, the extent of this pattern is not well documented in most taxa, with most reports of eyelessness relying only on superficial examinations. The remaining structures may be in the evolutionary process of disappearing; this possibility has interesting implications for the impact of population ages, which can be between thousands and millions of years. They could result from some pleiotropic effect during development, or be sufficiently economised as to escape further directional pressure for reduction. This also sparks questions of functionality; does photoreceptor activity persist? Can degenerate eyes be 're-purposed' for other uses? Such questions were especially prominent in the symposium, particularly with the use of transcriptome sequencing enabling researchers to directly study gene expression and function (Valdez-Lopez et al. 2018; Stern and Crandall 2018; Pérez-Moreno, Balázs, and Bracken-Grissom 2018).

The role of model systems

The study of eye loss and its evolution has been transformed by one animal above all others: the Mexican cave tetra, *Astyanax mexicanus*. The suitability of *Astyanax* for laboratory experiments has been exploited with groundbreaking results by several groups (e.g. Yoshizawa, Hixon, and Jeffery 2018). We were fortunate to host Professor Bill Jeffery, one of the pioneers of developmental manipulation in the cave fish, to present some of his group's latest work on eye development in cave fishes. Here,

Yoshizawa, Hixon, and Jeffery (2018) demonstrate a likely pivotal role for neural crest cells in not only impaired eye development but also in pigmentation and the growth of melanophores. Previously, it has been suggested that the loss of pigmentation and the loss of eyes are driven by different evolutionary forces in *Astyanax* (Protas et al. 2007). The study by Yoshizawa, Hixon, and Jeffery (2018) demonstrates that there may be common players in both eye and pigment loss at the tissue level, and that there may be mechanistic differences depending on the level of investigation (genetics versus tissue organization).

Studies of *A. mexicanus* have been leading the field in understanding the evolutionary and developmental processes underlying eye loss for several decades. Extending these mechanistic hypotheses across the broad taxonomic groups exhibiting eye loss, however, has been difficult due to a lack of suitable species for comparable laboratory based studies. Re et al. (2018) detail the development of the first arthropod lab system, the isopod *Asellus aquaticus*, comparable to *A. mexicanus* in terms of suitability for understanding the genetic and developmental mechanisms leading to eye loss. The power of the *A. aquaticus* system parallels that of *A. mexicanus* for understanding loss within a phenotypically variable species, and the higher level comparisons across vertebrates and arthropods is likely to yield deeper insights into commonalities in mechanisms and evolutionary forces contributing to the repeated evolution of loss across animals. Such comparisons between constructive developmental mechanisms in vertebrates and invertebrates have been fruitful in the past in identifying functionally homologous morphogens (e.g. Krauss, Concordet, and Ingham 1993).

By contrast, increasing numbers of researchers are using more taxonomically diverse systems to study the reduction of eyes. Simon Tierney and colleagues have adopted the beetle family Dictysidae, which occurs in multiple networks of subterranean aquifers, for use in their study of eye loss on a much broader phylogenetic scale. Here, they have developed a bespoke set of RNA baits to target vision- and phototransduction-related genes in five species (sighted and eyeless) with the goal of deploying these across the family to gain a comprehensive view of visual transcriptomes in different photic environments (Tierney et al. 2018).

Transcriptomes

The past decade has seen the rise of transcriptomics, which has become an invaluable new tool for those studying eye loss. The increasing impact of transcriptomics was reflected in many of the invited and contributed talks, particularly those that addressed the loss of vision in non-model systems and wider comparisons, and certainly seems to be one of the dominant players in the recent expansion in comparative studies of eye loss. Using transcriptomes, entire genetic networks can be compared across taxa to determine expression patterns between eyed and eyeless populations or species, and to look for signatures of selection. Using this approach with eyed and eyeless species of crayfish, Stern and Crandall (2018) investigated genes involved in phototransduction and eye development and found that most were expressed at lower levels in blind species, with differences in selection strength depending on specific gene identity.

Strikingly, one of the patterns to emerge most clearly from these studies is that, even in highly reduced eyes, visual opsins are still expressed, functional, and in some

cases, multiple opsins have been detected (Pérez-Moreno, Balázs, and Bracken-Grissom 2018; Emerling 2018). Valdez-Lopez et al. (2018) even find that a burrowing mantis shrimp with monochromatic vision may still sustain multiple functions for visual opsins. It has also been demonstrated that multiple taxa exhibiting even dramatic eye reductions may still be photosensitive at some level (Friedrich et al. 2011; Fiser et al. 2016; Langille et al. 2018). The extent of functional persistence of course appears to be variable, with nocturnal mammals, for example, retaining greater opsin expression than their subterranean relatives (Emerling 2018). This could owe to numerous factors, but the difference in light environment and ecological niches between these two groups are likely to significantly impact the extent of degradation and gene loss (Sumner-Rooney 2018).

As the available transcriptomes from eyeless species continues to increase, researchers will be able to generate coverage of much larger study systems by customising and refining specific molecular tools to target transcripts of interest (e.g. Tierney et al. 2018). These datasets will not only enable quantitative analyses and objective comparisons between taxa, but they enable protein structure modelling that can reveal functional aspects of the proteins they encode (Valdez-Lopez et al. 2018).

Behavior and ecology

Although many studies of eye loss focus on changes in environmental light levels as drivers of eye reduction and loss, there are other evolutionary and ecological contexts in which eye loss can be observed regardless of light availability. One intriguing example is the sex-specific eye-loss observed in the ostracod *Euphilomedes carcharodonta*,

where females lack the image-forming compound eyes found in males. Arenz et al. (2018) use behavioral studies to tease out the role of ecological versus sexual selection in this system, finding the differences in exposure to predators lead to eye maintenance in males. Studies of these types of systems have much to add to the evolutionary context of eye loss, broadening our understanding of the selective forces and linked behaviors influencing the maintenance versus loss of visual systems.

Synthesis

The challenges moving forward in the field will be integrating all of these aspects into a comprehensive understanding of the evolution of eye loss. Sumner-Rooney (2018) provides a detailed primer for how to approach these challenges, providing a theoretical framework for incorporation of all of the data types (taxa, habitats, techniques) discussed as a part of the symposium. As more diverse taxa from a variety of habitats are studied, the ability to synthesize common patterns across large numbers of independent evolutionary events will continue to strengthen the inferences about the selective regimes leading to eye loss (patterns across habitats), and to testing hypotheses related to whether the large-scale convergence of eye loss observed is due to similar mechanisms (patterns across taxa).

Additionally, as model systems expand our understanding of the evolutionary forces (or lack thereof) and developmental mechanisms involved in eye reduction and loss, researchers in the field can begin to test specific mechanistic hypotheses in a broader range of non-model organisms. This is demonstrated by Emerling (2018), who used genes involved in human eye disease as a lens to approach eye reduction and

loss in subterranean mammals, and by Valdez-Lopez et al. (2018), who used protein modeling to look at functional hypotheses for the maintenance of visual genes in a monochromatic shrimp. As the *A. aquaticus* isopod system continues to be developed as a model system in understanding eye loss, comparisons of this system with what has been learned from *Astyanax* will provide a rich framework for generating these types of testable hypotheses.

It is exciting that these studies can be framed by an understanding of similar processes occurring over deep time. Multiple studies have described how examples of eye loss in the fossil record parallel those examples currently being studied in extant species, e.g. due to ecological shifts to deeper water habitats, endobenthic, or endoparasitic life styles (Schoenemann 2006; Clarkson, Levi-Setti, and Horváth 2006; Schoenemann and Clarkson 2013). By tracing the reduction and loss of eyes in well fossilized groups such as trilobites, the evolutionary time frame required for eye reduction and complete loss can be estimated across lineages and compared to extant systems. A look at the patterns of eye loss across taxonomic diversity through deep time may also elucidate whether certain groups or eye types are more susceptible to loss than others. Taphonomic biases mean that this same pattern was likely present in many other more poorly preserved lineages, too.

As elucidated in Sumner-Rooney (2018), the field of reductive evolution requires surveys at multiple taxonomic levels across animal diversity of the ecological, developmental, molecular, and morphological variations in the visual systems of animals living in the dark in order to move into a more integrative understanding of eye loss. Recent years have seen substantial progress in this area thanks to the increasing

use of transcriptomics, and integrated with other comparative techniques, we anticipate that this will rapidly enhance our understanding of the fundamental principles of regressive evolution across habitats and taxa. These studies should be pursued alongside continued studies of well-established (*Astyanax mexicanus*), currently emerging (*Asellus aquaticus*), and yet to be identified model systems. The diversity of research into this fascinating evolutionary pattern was demonstrated across the symposium, and we hope that by drawing together this community for the first time, we can begin to exploit this diversity to identify the fundamental trends and forces that underlie evolution in the dark.

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