The kingdom of the blind: disentangling fundamental drivers in the evolution of eye loss

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Running title: Fundamental drivers in the evolution of eye loss

Abstract

Light is a fundamentally important biological cue used by almost every animal on earth to maintain daily rhythms, navigate, forage, find mates and avoid predators. But an enormous number of species live in darkness: in subterranean caves, abyssal ocean depths, underground burrows, and within parasitic host bodies, and the loss of eyes appears consistently across these ecosystems. However, the evolutionary mechanisms that lead to the reduction of the visual system remain the subject of great interest and debate more than 150 years after Darwin first tackled it. Studies of model taxa have discovered significant roles for natural selection, neutral evolution and pleiotropy, but the interplay between them remains unclear. To nail down unifying concepts surrounding the evolution of eye loss, we must embrace the enormous range of affected animals and habitats. The fine developmental details of model systems such as the Mexican cave tetra *Astyanax mexicanus* have transformed and enriched the field, but these should be complemented by wider studies to identify truly overarching patterns that apply throughout animals. Here, the major evolutionary drivers are placed within a conceptual cost-benefit framework that incorporates the fundamental constraints and forces that influence evolution in the dark. Major physiological, ecological and environmental factors are considered within the context of this framework, which appears faithful to observed patterns in deep-sea and cavernicolous animals. To test evolutionary hypotheses, a comparative phylogenetic approach is recommended, with the goal of studying large groups exhibiting repeated reduction, and then comparing these across habitats, taxa, and lifestyles. Currently, developmental and physiological methods cannot feasibly be used on such large scales, but penetrative imaging techniques could provide detailed morphological data non-invasively and economically for large numbers of species. Comprehensive structural datasets can then be contextualised phylogenetically to examine recurrent trends and associations, and to reconstruct character histories through multiple independent transitions into darkness. By assessing these evolutionary trajectories within an energetic cost-benefit framework, the relationships between fundamental influences can be inferred and compared across different biological and physical parameters. However, substantial numbers of biological and

environmental factors affect the evolutionary trajectory of loss, and it is critical that researchers make fair and reasonable comparisons between objectively similar groups.

Introduction

Biologists have long been fascinated with the pale, eerie, creatures that inhabit the world of darkness. Animals that live in true darkness, rather than the dim light associated with nocturnality and the mesopelagic zone, are found in a multitude of different habitats, including the bathypelagic (ocean depths below around 1000 metres), subterranean and submarine caves, fossorial burrows and, to some extent, endoparasitic host bodies. Many dark-living animals share striking morphological features, including albinism, increased investment in chemo- and mechanoreceptors, and the reduction or loss of eyes (Poulson 2001). Eye loss in particular has captured attention for more than a century, and has been reported across Metazoa from burrowing amphibians (Figure 1; Mohun et al. 2010; Mohun and Wilkinson 2015) to deep-sea trilobites (Clarkson et al. 2006; Lerosey-Aubril 2006; Schoenemann in press).

We are now able to identify incremental molecular and developmental changes occurring in dark-living species, which have started to demystify this recurrent evolutionary pattern (e.g. Jeffery 2009; Stahl et al. 2015; Tierney et al. 2015). However, the overall evolutionary mechanisms behind eye loss remain controversial, more than 150 years after it was first explored by Darwin (1859; Rétaux and Casane 2013). Eye loss appears to be driven at least in part by direct selective pressure, but both pleiotropy and genetic drift are also key influencers (Rétaux and Casane 2013). Despite substantial recent progress, thanks to a combination of developmental and molecular techniques, it is not yet clear how these drivers interact, or, crucially, whether their relationships are similar across taxa and habitat types. Unifying concepts have been identified in the constructive evolution of eyes that apply across all eye types and animal groups (Nilsson 2013), but their regressive evolution remains a tangled matter. Here, a conceptual framework is proposed that could inform future studies of eye loss effectively across large and distant taxonomic groups, and several recommendations are made for such research directions.

This paper will not comprehensively review the substantial body of existing work on individual taxa, particularly the Mexican cave tetra *Astyanax mexicanus* by Jeffery, Wilkens, and colleagues (e.g. Wilkens and Strecker 2003; Protas et al. 2007; Jeffery 2009; Borowsky 2013). This species is an exceptional model for studying the developmental mechanisms behind eyelessness in numerous instances across a cave network, and the resulting work has transformed our understanding of eye loss (see Jeffery 2009; Wilkens and Strecker 2017 for reviews; and "The state of play" below). However, the current focus is on unifying concepts, cross-habitat comparisons and non-model groups, and not individual case studies. The majority of eyeless species have not yet been kept or bred in captivity (and some have never been seen live), for reasons of accessibility, availability or fragility; this paper mainly addresses how we can learn from these taxa in the absence of manipulative methods. The overarching aims are to explore the possibility of a generalised model for eye reduction, incorporating macroevolutionary trends that apply across taxonomic and ecological divides; and to promote a large-scale comparative morphological approach. This does not supplant the exceptionally important ongoing developmental, physiological, and transcriptomic work, but is intended to complement that work, and provide a taxonomic breadth and macroscopic evolutionary perspective that may be challenging to achieve using such methods.

If evolutionary biologists aim to identify unifying concepts in the reductive evolution of eyes (or any other system), we must embrace the enormous range of affected animals and habitats (Figure 1), and all the complications this entails. Broader taxonomic approaches have proved successful in other cases of repeated constructive evolution, including vision, which has been elegantly rationalised using trends that apparently apply to all animal groups (Nilsson 2013). Features including screening pigment, membrane folding, and focussing optics are causally linked to the increasing sophistication of visual tasks, and to the minimum light intensity mathematically required for these tasks. Such relationships are likely to be equally important in reductive evolution, as they affect the functionality and therefore the fitness value of visual structures in different light environments. By adopting this functional evolutionary approach to loss , we may more easily rationalise what we observe in nature. For example, high-resolution vision requires minimum light intensities roughly equivalent to those at ocean depths of 350–650 metres (Nilsson 2013). Unsurprisingly, this is the point beyond which dramatic alterations to eye structure, including loss (Syme and Oakley 2012; Sumner-Rooney et al. 2016; Gonzalez et al. 2018), begin to appear in multiple marine groups (Warrant et al. 2003; Warrant and Locket 2004; Johnsen et al. 2012). There must be fundamental relationships between the physiological, ecological and environmental characteristics of study systems and their evolutionary trajectory under darkness. However, unlike the constructive evolution of vision, a wealth of information about structure, function, development and ecology is largely lacking for reduced-eyed animals, and some of this information is more readily obtained than others.

The state of play: a (very) brief summary of eye loss research

The consistent loss of eyes in dark-living animals has intrigued evolutionary biologists for centuries. In *On the Origin of Species*, Darwin wrestled with a selective basis for eye loss, uncertain as to what disadvantage eyes might confer. He eventually attributed it to "disuse" of eyes in the dark, in what we would now consider to be neutral evolution (Darwin 1859). Neutral evolution theory in its modern form developed from the 1930's and 40's onwards, with its importance in regressive evolution being acknowledged decades before Kimura's seminal paper (1968). Though this view was not universally held, it dominated for another century (Culver and Pipan 2009). Following the new synthesis, and the accelerating discovery of more eyeless species, the evolution of loss became a subject of renewed interest in the second half of the 20th century. A new experimental approach, using cave fishes and crustaceans, produced evidence of convergent patterns in repeated cave invasions, and that natural selection favours the reduction and loss of eyes in energy-limited aphotic environments (Christiansen 1961; Poulson 1963; Sadoglu 1967; Barr 1968; Jones and Culver 1989). However, the dominant role of neutral evolution in eye loss was still championed by Wilkens and colleagues (e.g. Wilkens 1988; Wilkens and Strecker 2003, 2017: Chapter 7), who highlight the variation in regressive phenotypes and genotypes, pre-adaptation, and potential misconception of energy limitation in caves.

The past two decades have seen enormous growth in molecular, developmental and physiological studies, most notably using *Astyanax* (see Jeffery 2009; Wilkens and Strecker 2017 for reviews). These have generated substantial evidence to demonstrate that direct selection favours energetic economy via eye reduction (Culver et al. 1995; Niven and Laughlin 2008; Yoshizawa et al. 2012; Moran et al. 2015; *contra* Wilkens and Strecker 2017). Several also support a crucial role for pleiotropy in eye development through molecular players such as Shh (Yamamoto et al. 2009; Carlini et al. 2013).

In the last ten years, the capabilities of genetics and transcriptomics have improved dramatically, and transcriptome sequencing especially has been applied to a range of eyeless taxa (Friedrich et al. 2011; Carlini et al. 2013; Meng et al. 2013; Stahl et al. 2015; Tierney et al. 2015; Kim et al. 2017). Many of these found persistent expression of opsins and clock genes in (mainly cavernicolous) dark-living animals (Friedrich et al. 2011), often subject to downregulation or functional shifts (Gross et al. 2013; Hinaux et al. 2013; Tierney et al. 2015; Wong et al. 2015; Carlini and Fong 2017). It must also be noted that there are increasing examples of other roles for opsins beside photoreception (Leung and Montell 2017).

Knowledge gaps

Two key patterns have emerged that are potentially problematic to drawing broader conclusions about the reductive evolution of visual systems. Firstly, cave fauna has formed the focus of the majority of studies to date. As a crude measure, searches for "eyeless" or "blind" species on Zoobank return 65%

cavernicolous, 24% fossorial, 5.8% ground-living (leaf litter), 4.8% marine and <1% parasitic taxa (as of 15th May 2018). In studies specifically addressing the evolution of eye loss, the skew is far greater, with 78% focussing on cavernicolous species, 8% on fossorial species, 7% on marine species, and 7% nocturnal.

Secondly, certain taxonomic groups have received more attention than others. Fishes account for 51% of ZooBank search results returned for “eyeless” or “blind” species, yet there are fewer than 200 described teleosts of a predicted 21,000 global troglobionts (Juan et al. 2010; Soares and Niemiller 2013). Arthropods are, more understandably, the next largest group (36%), followed by snakes (12%), and others (including mammals, amphibians, and molluscs; <1%). Again, this trend is reflected in studies specifically examining eye loss, where target systems were 57% fish, 26% arthropods, 8% mammals, 5% amphibians, 3% molluscs and <1% reptiles.

Specialisation within one or two of these ecosystems or taxa has created artificial divisions between researchers working on cavernicolous, deep-sea, fossorial and parasitic species, and opportunities for synthesising information have largely been underexploited, aside from a handful of research groups. Of course, there are fundamental differences between habitat types and the transitions undergone by different lineages. An integrative approach exploiting these differences will facilitate more objective and informative comparisons between them. For instance, cave taxa may be the subject of greater research interest because eye loss appears to occur more consistently than in deep-sea taxa, but this also represents an opportunity to investigate why they differ.

What is ‘loss’?

As an added obstacle, many studies and species descriptions dedicate just a handful of words to eye structure, even when investigating trait evolution (Raupach et al. 2009; Williams et al. 2013; Gonzalez et al. 2018). Absence of retinal pigmentation alone may be taken to signify eye loss, with the implication that loss is total, and pigmented (therefore superficially visible) eyes are presumed to be unaffected. Absolutist attitudes towards loss are deeply inhibitory to fully understanding the complex evolutionary processes behind it. Many taxa have been found to have non-pigmented eyes, vestigial or intact, and eyes with retained pigmentation may be reduced in size, sunken beneath skin, affected by retinal disruption or lens degradation, or impacted by altered gene sequences or expression (Jeffery 2009; Bloom et al. 2014; Sumner-Rooney et al. 2016). These are all vitally important characters that could shed further light on the evolution of loss, but are going unrecorded.

Similarly, degradation of structure does not necessarily equate to complete loss of function. While pupil obstruction, lens degradation and loss of pigmentation may impede spatial resolution, there is substantial evidence for persistent photoreceptor function and light-responsive behaviour in species with dramatically reduced eyes (Friedrich et al. 2011; Fišer et al. 2016; Langille et al. 2018). This may include photoreceptor activity within the retina (David-Gray et al. 1998; Espinasa and Jeffery 2006; Zubidat et al. 2011) or elsewhere (Yoshizawa and Jeffery 2008), for phototaxis or circadian entrainment.

Moving forwards: the future of the field

The unifying questions in the evolution of eye loss remain; what are the evolutionary forces at play, what are their relative roles and how do animals move towards eye loss? (See Culver and Pipan 2009, p.109-130; and Rétaux and Casane 2013 for reviews) As outlined above, significant progress has been made to address these questions in various taxa, but there are still substantial gaps in both our knowledge and, to an extent, within the research community working in the field of eye reduction and loss. To provide a unifying framework, we must identify fundamental factors that influence the trajectory towards eye loss across all habitat types and taxa. Here, such a framework is outlined for the first time. Several specific considerations for future work are outlined; many have been proposed by other authors, but none, as far as the author is aware, have been addressed directly in empirical studies. In some cases, it may be possible to incorporate these considerations into experimental designs, but otherwise they should be accounted for in interpretation of results and the drawing of wider conclusions.

Fundamental drivers in the evolution of eye loss – a first attempt at conceptualisation

Eyes and corresponding brain activity are energetically expensive, which is fundamental to their loss; once their utility decreases, this energy could be reinvested elsewhere with greater results, and their high cost amplifies the potential benefits (Niven and Laughlin 2008; Moran et al. 2015). As with any costly trait, selection should favour the maximisation of fitness benefits against these energetic costs, consistently drawing animals towards a cost-benefit equilibrium (Figure 2, in green). The further an animal from equilibrium, the stronger the selective pressure, so we might imagine a fairly simple vector gradient of selective pressure towards equilibrium (Figure 2, background grey arrows).

If an animal, A, has evolved sophisticated eyes within a bright, stable, visually complex environment, we can assume that A approaches cost-benefit equilibrium; their energetic investment is adequately offset by the fitness benefits afforded by vision, through foraging, predator avoidance, or finding mates. If A moves to a darker habitat, their investment is the same, but the benefits may decrease (Δb), displacing A from equilibrium (Figure 2.1 A_1). Under stable conditions, directional selective pressure will draw A_1 along the shortest route back to equilibrium (Figure 2.1). If the available light is still sufficient to support vision, the shortest and most favourable route is often to decrease some cost and recover some benefit, perpendicular to the equilibrium gradient (Figure 2.1). The eye may adapt to exploit lower light levels by maximising sensitivity at the cost of resolution, as in nocturnal and mesopelagic animals (Warrant 1999; Tierney et al. 2017). This can include simplification through loss of colour vision, lower spatial resolution, neural summation, and slower photoreceptors, which may also reduce energetic cost (Warrant 1999; Johnson et al. 2000; Theobald et al. 2006; Stöckl et al. 2016b; Tierney et al. 2017; Emerling 2018; Valdez-Lopez et al. 2018). These savings (Δc) can be reinvested (r) to recoup potential fitness gains (g), in another sensory mode for example. A_1 may not fully reduce investment to match Δc; many animals living in dim light actually increase eye size, while simplifying other aspects, to maximise photon capture and boost sensitivity. If A_1 successfully survives this transition, it should eventually return to equilibrium (Figure 2.1).

Note that another animal, B, may inhabit the same original light environment as A but have simpler eyes (Figure 2.1). These may confer lesser cost and return less information, but still satisfy this equilibrium, perhaps due to a simpler lifestyle, lower predation pressure, or preferential investment in another sensory system. If B undergoes the same habitat shift as A, it will experience lesser displacement from equilibrium and be subject to weaker selective pressure.

The above model includes selection as the sole evolutionary driver, which is deeply biologically unrealistic. Neutral evolution also plays a crucial role in reduction and loss (Wilkens and Strecker 2017), so the path towards cost-benefit equilibrium is not usually direct (Figure 2.2). Their relative strengths depend on the magnitude of the deviation from equilibrium; as A approaches equilibrium, selective pressure decreases and the relative role of drift increases (Figure 2.2, inset). Neutral evolution also means that animals in stable environments will float within a tolerable zone surrounding equilibrium (Figures 2–4, pale green), rather than remaining static and optimised. The rate of neutral evolution varies taxonomically and is influenced by mutation and metabolic rates, population size, DNA repair efficiency, and generation time (Brirten 1984; Ohta 1992). However, rates are generally low, so the effects of drift may take many generations to become apparent – possibly leading to the apparent dominance of selection in 'young' dark-living groups (Fumey et al. 2018).

The third key evolutionary driver is pleiotropy. Many genes involved in the visual system also fulfil other functions, such as non-visual phototransduction or multiple developmental roles (Yamamoto et al. 2009; Carlini et al. 2013). These may be protected from degradation by selective pressure, resulting in pleiotropic obstructions on the evolutionary route back to equilibrium, valleys in the adaptive landscape, where certain genes or characteristics cannot be altered without deleterious consequences elsewhere (Figure 2.2). Combined with the influence of drift, even closely related species or populations may therefore take different evolutionary trajectories when subjected to the same displacement (Figure 2.2; Niemiller et al. 2013; Sumner-Rooney et al. 2016).

So far, our model does not include any constraints. Visual systems are physically constrained, in that they are subject to optical and biological laws that limit the amount and quality of information they can collect within certain means. Nilsson (2013) demonstrated that there are minimum light intensities for different visual tasks to be optically viable. This introduces a threshold that, once crossed, limits the potential to recover benefit via adaptation as seen in dim-light environments (Figure 2.3). This is the real crux of eye loss. If A moves into a truly dark habitat, beyond this threshold (Figure 2.3, A_2), the selective landscape will differ from that under dim (A_1) and bright (A) light. Instead of balancing pressures to reduce cost and improve sensitivity, the pressure to economise now dominates, and the visual system will be reduced. This may also increase the role of drift relative to animals in dimly lit environments, which remain optically constrained and subject to two directional selective pressures. If there is any benefit to basic photosensitivity, for circadian rhythms or phototaxis, this is likely to be retained in line with the cost-benefit equilibrium; otherwise, the eventual outcome may be complete loss. However, pleiotropy can also introduce a lower limit on energy expenditure in visual systems, where further reduction, such as genetic losses of function, would cause collateral damage in other areas. This may lead to the persistence of vestigial structures, which are degraded and have no functional benefit (Figure 2.3, A_3).

Several caveats must be mentioned: these schematics (Figures 2,3) are highly simplified, with absolute threshold limits, a linear cost-benefit relationship, assumptions of no fundamental change in eye type, *caeteris paribus* in other physiological, ecological and evolutionary drivers, and a simple vector gradient selective landscape. Although these assumptions may be flawed in their reflection of true biological systems, this could provide a starting framework to facilitate future developments.

Biological and environmental factors

Although these drivers are the three major influences on the general directions and trends we might expect to see in dark habitats, there are of course many additional factors at play. These may affect the nature and location of thresholds, the geometry of cost-benefit relationships, the extent of developmental constraints, and the nature of the selective pressure landscape both before and after transitions (Table 1). These will have significant impacts on the evolutionary trajectories of different taxa in different habitats (e.g. Figure 3), and are likely responsible for the substantial differences we observe between instances of eye loss in nature, but to date, few studies have attempted to characterise the relative effects of these parameters (Gonzalez et al. 2018).

Table 1. Biological and environmental factors affecting the evolution of eye loss.

Factor	More affected	Less affected	Effect	Additional references
Physiological				
Complexity of eye	Complex	Simple	Higher Δb, stronger selective pressure to reduce complex eyes.	
Possession of extraocular photoreceptors (EOPs)	EOPs	No EOPs	More potential benefit to maintaining reduced eyes, e.g. for circadian entrainment, if no other EOPs.	(Yoshizawa and Jeffery 2008)
Eye type	Apposition eyes, small camera eyes, rapid photoreceptors	Superposition eyes, large camera eyes, slower photoreceptors, eyespots	Systems better suited for high sensitivity have lower visual thresholds and sustain vision in darker habitats, may be more likely to adapt than reduce	(Land and Nilsson 2012; Nilsson 2013)
Life history	Direct developers, no photic phase; longer generation times	Larvae or juveniles in photic habitats; shorter generation times	Pressure to retain larval eye and development more constrained where a photic phase exists. Shorter generation times allow faster degradation	(Poulson 2001)
Rate of drift	Smaller populations; higher metabolism	Larger populations; lower metabolism	Higher relative influence of drift vs. selection	(Ohta 1992)

Pre-adaptation to darkness	Not pre-adapted	Pre-adapted (e.g. nocturnal, mesopelagic)	Pre-adapted animals have lower Δb	(Poulson 2001)
Ecological				
Pelagic/benthic	Benthic	Pelagic	Benthic species have increased availability of other cues and likely to be less active (below)	(Childress 1995)
Active predator/scavenger	Scavenger	Active predator	Greater selective pressure to sustain visual hunting where possible in active predators	(Gibert and Deharveng 2002)
Sedentary/active	Sedentary	Active	Lower selective pressure to sustain vision in sedentary species	(Warrant 1999; Warrant and Locket 2004)
Predation pressure	Caves, fossorial	Deep sea, parasites?	Weaker selective pressure to maintain vision where few top predators	(Gibert and Deharveng 2002)
Connectivity	Isolated populations (e.g. caves, fossorial, parasites, deep sea trenches and vents)	Mixing populations (e.g. open deep sea, nocturnal)	Restricted gene flow enables faster change	(Ohta 1972)
Age of transition	Older populations? e.g. deep sea	Younger populations? e.g. caves	Increased time may be needed for change to occur; however, this is variable.	(Culver and Pipan 2009; Derkarabetian et al. 2010)

Environmental				
Non-solar radiation	Most caves, fossorial, parasites?	Deep sea, glow worm caves, nocturnal	Where present, greater functionality and improved fitness (foraging/mate finding)	(Poulson 2001; Warrant and Locket 2004)
Stability in light level	Caves, deep sea, parasites	Nocturnal, fossorial	Interrupted darkness may maintain benefits of vision	(Culver and Pipan 2009)
Speed of transition	Sudden (some caves)	Gradual (deep sea, some caves, fossorial)	Gradual transitions may enable constant adaptation and less departure from cost-benefit equilibrium	(Danielopol et al. 1996)
Energy limitation	Most caves, deep sea	Some caves (e.g. guano caves), fossorial, parasites, nocturnal	Energy limitation as an upper cost threshold, with greater pressure to economise	(Poulson 2001; Niven and Laughlin 2008; Izutsu et al. 2016)

Combined, these evolutionary, physiological, ecological and environmental factors make disentangling eye loss a daunting task. However, they already go some way in explaining the existing variation we see in different taxa and habitats, and caution that extrapolations from model systems may not always be appropriately applied to alternative species or groups. Two examples are highlighted below, but the reader will appreciate that there are myriad combinations of similarities and differences in these factors that could apply to any pair of animals or habitats.

Ex. 1. Non-solar radiation. The availability of non-solar radiation is critical and affects multiple habitats. Indirect solar radiation and starlight promote investment in highly sensitive vision in nocturnal taxa, but non-solar radiation accounts for deviations in habitats where we usually expect to observe regression (Figure 3.1). The most obvious example is marine bioluminescence, with signals potentially indicating prey, predators, or mates and therefore exerting strong selective pressure in favour of retaining vision. This represents a crucial difference between the evolution of cavefishes and deep sea fishes, despite them sharing similar lifestyles, energy-limited habitats, and developmental constraints (Poulson 2001). This is further supported by findings that in glow worm caves, the only cave habitats to host bioluminescence, animals do not lose their eyes and maximise sensitivity instead (Meyer-Rochow and Liddle 1988).

Ex. 2. Lifestyle and activity. Although bioluminescence enables many deep sea fish, cephalopods, and even sea stars to sustain large, sensitive eyes (Johnsen et al. 2012; Garm et al. 2018), it does not 'rescue' vision in many other groups. Scallops, gastropods, ostracods, scale worms and isopods have all been shown to reduce and lose visual systems with increasing depth (Raupach et al. 2009; Syme and Oakley 2012; Malkowsky and Götze 2014; Sumner-Rooney et al. 2016; Gonzalez et al. 2018). The extent of investment in dim-light vision is often greatest in highly mobile predators, such as fish and cephalopods, which have the greatest potential to recover benefit from vision in low light, while the taxa that exhibit loss are often more sedentary. Interestingly, eye type and complexity may not be dominant factors, with camera (gastropods, fish) and compound (crustaceans, sea stars), simple (gastropods, sea stars) and complex (scallops, cephalopods) eyes being both lost and retained.

Strategies and recommendations

So, how can all these factors be recognised and evaluated on a large scale? First, comparisons between distant groups (geographically or phylogenetically) should incorporate variation within those groups, too. Despite superficially convergent eye losses, histological-level investigations consistently reveal more variation than anticipated within closely related groups (Malkowsky and Götze 2014; Sumner-Rooney et al. 2016). Phylogenetically contextualised comparative studies have been underused in the study of eye reduction, and have enormous potential to resolve evolutionary trajectories for comparison and hypothesis testing (Desuter-Grandcolas 1997; Klaus et al. 2013; Gonzalez et al. 2018).

Second, if caves are 'natural replicates' of evolutionary experiments, we can exploit the similarities and differences between dark ecosystems and taxa as experimental treatment groups. To incorporate the various factors outlined above, similar or identical methods should be deployed across different study groups (taxa, habitats, etc.) where possible to identify common trends. Molecular and physiological techniques are being used with great success to quantify the impacts of selection versus drift, and identify potential pleiotropic effects in agents such as Shh (Culver et al. 1995; Jeffery and Martasian 1998; Yamamoto et al. 2004, 2009; Espinasa and Jeffery 2006; Protas et al. 2008; Carlini et al. 2013). However, physiological techniques especially cannot currently be feasibly extended to multiple large groups of animals, yet such comparisons are critical to identifying truly unifying patterns in eye loss. Morphological studies can be deployed across large groups non-destructively and with relatively low costs. Coupled with continued transcriptomic, physiological, developmental and behavioural research in model systems, there is considerable unexplored potential in comparative morphological studies.

Comparative morphology and phylogenetic reconstruction

The detailed study and comparison of visual and central nervous system anatomy can provide a wealth of information on evolutionary trajectories, as well as indicating to some extent the underlying mechanisms. Examining multiple lineages that have independently lost or reduced eyes provide ‘natural replicates’ of the evolutionary process, and by applying this to multiple taxonomic groups in multiple habitat types, we can conduct a macroevolutionary experiment to evaluate the proposed conceptual framework, and test evolutionary hypotheses.

It is clear that presence/absence data does not capture the extent of structural diversity in visual systems (Malkowsky and Götze 2014; Sumner-Rooney et al. 2016; Garm et al. 2018), but phylogenetic reconstruction of eye loss is a highly effective tool (Raupach et al. 2009; Syme and Oakley 2012; Gonzalez et al. 2018). By combining detailed morphological studies with phylogenetic methods, the evolutionary trajectories of individual eye characters can be reconstructed across large groups (Figure 4.1). This approach revealed variable evolutionary trajectories during multiple deep-sea invasions within a family of ecologically similar and even sympatric snails (Sumner-Rooney et al. 2016). Resolution was not sufficient to draw concrete conclusions about evolutionary processes, but supported strong roles for both selection and neutral evolution in sedentary, benthic detritivores with simple ancestral eye structures, in line with the expectations presented here. If expanded to include more taxa and more characters, including transcriptomic data where possible, this technique could facilitate both within- and between-group comparisons by charting the progression of individual cases of eye loss and reduction phylogenetically.

Behaviour and physiology: are there robust morphological proxies?

Behavioural and physiological data are hugely desirable but not always obtainable, as animals may be too rare, inaccessible, or fragile. However, some basic indicators may be given by features such as neural investment (Banister 1984), numbers of ommatidia, focal length, aperture size, and the predicted functionality of photopigments if sequences are available. Blind mole rats, for example, are sensitive to circadian light regimes but cannot see, and this is elegantly reflected in their morphology: very reduced eyes and visual processing centres in the brain, but an intact suprachiasmatic nucleus (Zubidat et al. 2011). Similarly, differential investment in other areas of the central nervous system can indicate the importance of alternative sensory modalities, including olfaction (Cooper et al. 2001; Stöckl et al. 2016a), gustation, and mechanoreception (Franz-Odendaal and Hall 2006; Soares and Niemiller 2013).

Testing evolutionary hypotheses

Assembling large datasets of visual, central nervous system and other sensory characters will enable us to identify regressive features that appear more or less frequently, recurrent associations between specific characters, habitat specificity, and the extent of reinvestment in, and trade-offs with, other sensory modes. Ancestral state reconstructions would allow researchers to story-board the evolution of eye reduction and loss in multiple replicates, multiple habitat types and multiple ecological niches (Figure 4). Armed with such a powerful dataset, we could test evolutionary hypotheses on an enormous scale. Differential reduction and reinvestment under varying physical and biological conditions may provide clues as to which factors dominate in different taxa and environments. Recurring features may indicate cost-effective routes to reduction; conversely, the repeated or unexpected survival of other characters may betray pleiotropic limitations, persistence of functionality, or repurposing of retained structures. These features can be used to plot trajectories across adaptive landscapes and help visualise the strength of the various evolutionary drivers (Figure 4.2). Multiple lineages convergently following the same progressive changes have likely moved through the adaptive landscape in the same way and would suggest significant constraints on evolutionary trajectory. These patterns may be consistent across biological settings, but more likely the differences between them will reveal more about the most influential factors and the more variable subjects. Beyond eye loss, this approach could help us to better understand the nature of reductive evolution overall, applied to any number of traits.

Specific recommendations

Study material. Such large-scale comparative studies will require large amounts of specimen material and careful selection of focal groups (see below). While collecting dedicated fresh specimens is ideal, it is not always possible. Natural history collections offer an invaluable resource to supplement or substitute fresh material, covering a large range of taxa, geographical locations, and time periods (Sumner-Rooney and Sigwart 2017). As with any resource, there are limitations to using collection material; samples may be unsuitable for certain analyses due to fixation or preservation methods, age, or the need for destructive sampling. However, high-quality morphological data can sometimes be extracted from even very old ethanol-preserved samples, and spirit collections represent a major untapped resource for large comparative studies.

Methods. Rapid, non-destructive penetrative imaging tools, such as X-ray tomography (µ-CT and SXRT), are ideal for large-scale studies of precious specimens. Both have produced stunning results in very small visual and nervous systems (Sombke et al. 2015; Taylor et al. 2016), and improved scanners and visualisation software now allow the study of eye structure in very small, unstained, wet material (Sumner-Rooney et al., in prep).

Taxon selection. The selection of study groups requires careful consideration. For comparisons across habitats, researchers should ensure as much similarity between groups as possible in line with the considerations in Table 1. Criteria include:

a) *Multiple transitions into dark niches within a relatively low taxonomic rank*. All empirical studies should incorporate multiple independent replicates, i.e. multiple lineages independently transitioning into dark habitats or niches (Tierney et al. 2017). Taxonomic rank is also important: the lower the rank, the less likely that confounding phyletic factors are introduced. In cases such as *Cicurina* and *Astyanax*, multiple cave invasions have occurred within genera or species (Wilkens and Strecker 2003; Hedin 2015), but in others, family-level analyses are required to capture sufficient replicates (Syme and Oakley 2012; Sumner-Rooney et al. 2016).

b) *Existence of, or ability to produce, a robust phylogeny.* Resolved phylogenetic relationships are fundamental to any comparative study. If relationships between taxa are not clear, trait history reconstructions are ambiguous and character evolution cannot be reliably traced (Harvey and Pagel 1991).

c) *Inclusion of sighted taxa.* Sighted relatives must be included in studies of loss , especially when examining large groups of eyeless species; without them, assigning character polarity and the resolution of fine evolutionary changes between reductive lineages are problematic.

d) *Multiple types of habitat transition.* Comparisons between different habitat shifts are a major potential avenue for future research, but identifying suitable candidate study systems poses a major obstacle. Relatively few taxonomic groups have undergone transitions from, for example, both shallow to deep water and from surface to subterranean habitats in multiple lineages.

Conclusions

After more than 150 years of study, the biological conundrum of eye loss is firmly back in the research spotlight, and modern methodologies offer real possibilities of collecting large datasets across broad taxonomic ranges. Armed with these new tools, researchers can truly close in on the evolutionary patterns underlying loss. A basic evolutionary framework attempts to incorporate the fundamental evolutionary forces acting on species that transition into dark habitats, with the aim of facilitating future development and guiding future research. We urgently need to survey the morphological variety observed in dark-living animals on a broader scale, alongside continued high-resolution developmental and transcriptomic studies on specific species. Harnessing this variation using the comparative method across a large range of animals and habitats will help us identify the fundamental influences on the evolution of eye loss on a global scale.

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Figure 1. Eye loss affects a wide variety of animals living in many different dark habitats. Clockwise from top left, *Bathymophila diadema*, a marine gastropod found at depths of 650–1,000 metres; *Microcaecilia dermatophaga*, a burrowing caecilian (image courtesy of Mark Wilkinson); *Metagonia jamaica*, a cavernicolous spider (specimen SMNH 1408109); *Astyanax mexicanus*, cave population (image courtesy of Bill Jeffery).

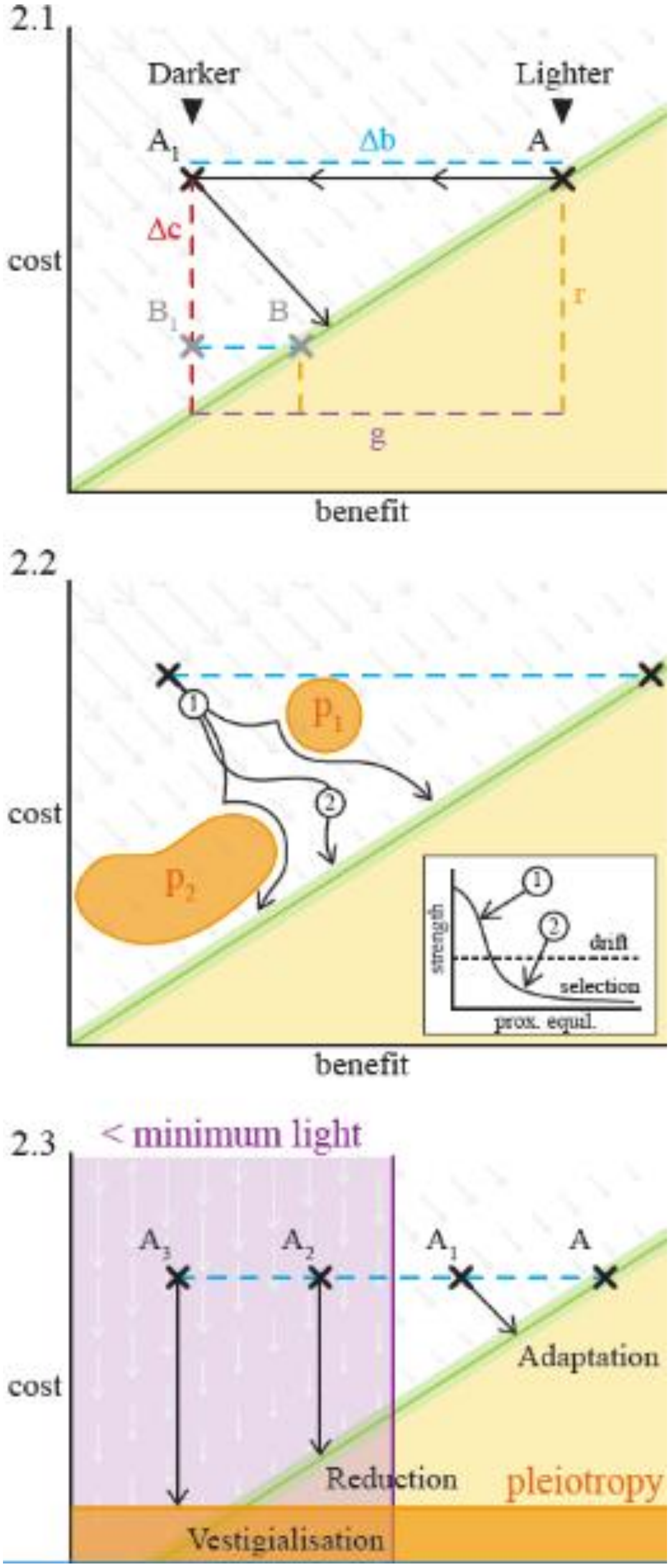

Figure 2. Schematic cost-benefit relationships for visual systems in changing photic environments, and the evolutionary drivers involved in loss.

2.1. Selection. Animal A inhabits a bright, visually complex and stable environment, and has evolved a visual system well-suited to its habitat and lifestyle. Overall energetic costs and fitness benefits from the visual system are at (green line) or near (pale green zone) equilibrium. If A moves to a darker habitat (A_1) the return on these costs decreases (Δb). Natural selection (background, grey arrows) draws A_1 back towards equilibrium, favouring the shortest route (black arrow) and with greater strength at greater distance from equilibrium. The fitness area beneath the line of equilibrium (in yellow) is inaccessible due to physical constraint; the eye cannot optically obtain more information from a stable environment without increased investment in size, complexity, innervation etc. However, A_1 can fully or partially recover its cost deficit (Δc) by reinvesting energy elsewhere (r) to maximise potential fitness gains (g) in other ways, such as an alternative sensory mode. Species with simpler visual systems and lower initial investment (B) undergo smaller displacements (B_1) and are likely subjected to weaker selective pressure.

2.2. Drift and pleiotropy. Evolutionary trajectory is simultaneously affected by two additional drivers: drift (departure from the direction of selection), and pleiotropic effects (orange obstacles). Selection is strongest after greater displacement (1) and decreases as equilibrium is approached (2). The relative importance of drift, and therefore trajectory variability, increases with proximity to equilibrium (inset). Pleiotropic effects on genes involved in vision may obstruct certain evolutionary routes and cause additional diversions (p_1 and p_2).

2.3. Constraints. Owing to optical constraints, there is a threshold light intensity value beneath which vision is no longer physically viable (purple). Beyond this threshold (A_2, A_3), the selective pressure landscape changes, with the animal no longer being able to recoup benefits from the visual system and with no alternative but to economise by reducing it. A second potential threshold is a lower limit to cost (orange), with pleiotropic effects preventing extreme reduction if it would cause collateral damage elsewhere. This may prevent optimisation and leave the animal with a deleterious vestigial structure.

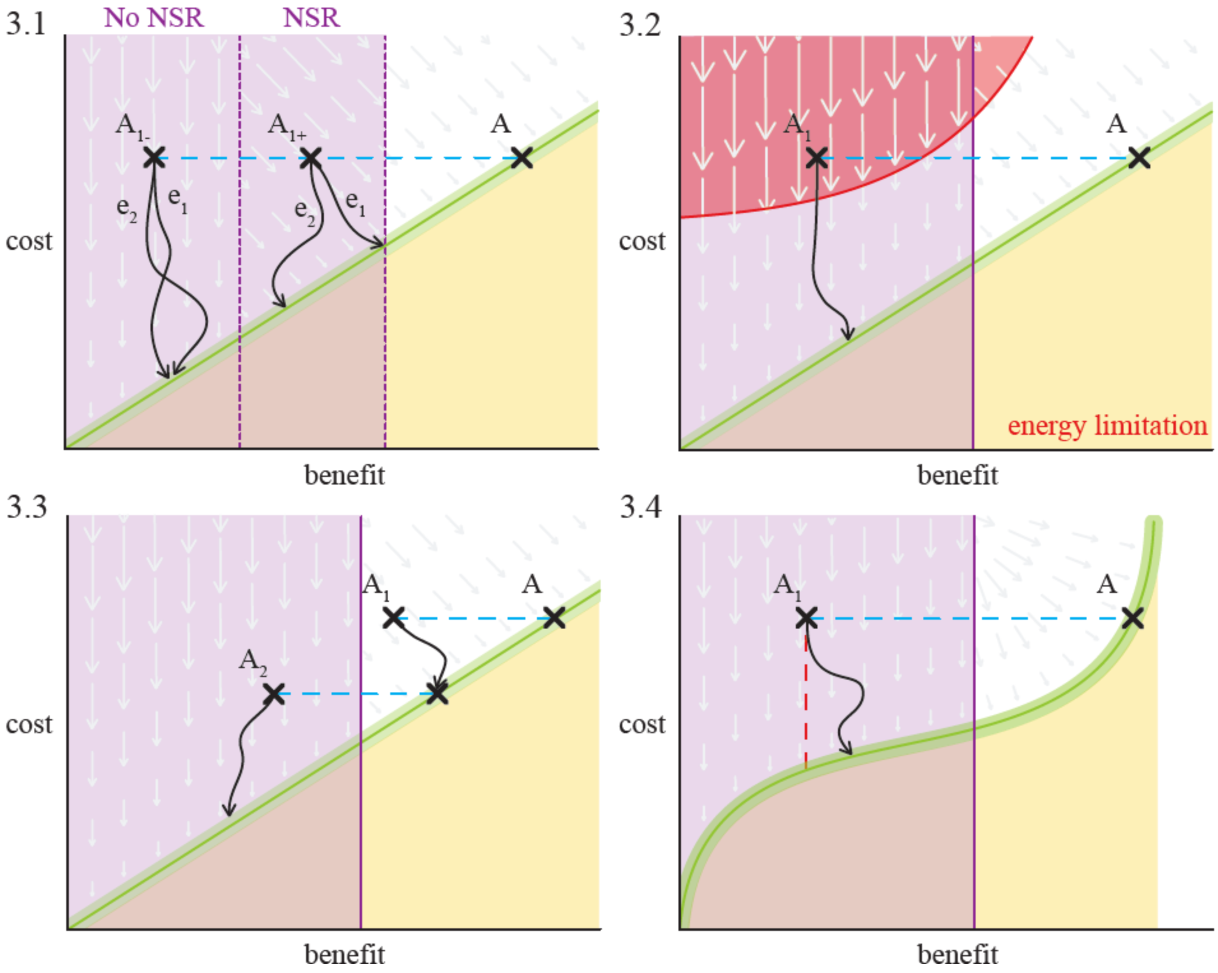

Figure 3. Physiological, ecological and environmental factors in the evolution of eye loss. 3.1. Non-solar radiation (NSR) recovers the viability of visual tasks below threshold values for solar radiation, so animals exposed to bioluminescence (A_{1+}) may respond as to dim light whereas those without bioluminescence (A_{1-}) are more likely to exhibit reduction. Ecological niche may also introduce additional selective pressures. Active species and predators (e_1) may be subject to stronger selective pressures to adapt to exploit dim light. Sedentary species (e_2) may economise more on visual systems to reinvest in chemo- or mechanoreceptors. In the absence of NSR, e_1 species, having higher overall energetic costs, are likely to be subject to stronger pressure reduce the visual system. 3.2. Energy limitation. Low-light and aphotic habitats are often energy-limited due to reduced photosynthetic capacity. This imposes an upper limit on energetic cost and enforces very strong directional selective pressure to reduce wastage in costly visual systems. 3.3. Gradual transitions from light to dark environments. 3.4. Different cost-benefit relationships will change the selective landscape and therefore the evolutionary dynamics of eye reduction and loss. For example, a sigmoidal curve may result in a shallower $\Delta b/\Delta c$ during habitat transitions (A_1), reducing the cost deficit and decreasing selective pressure.

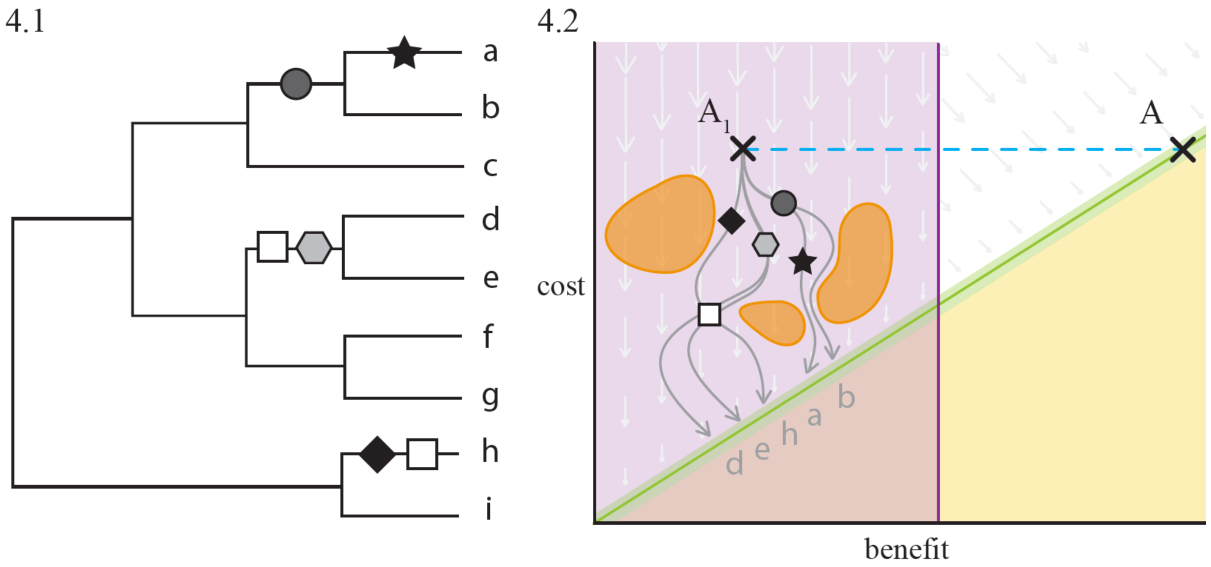

Figure 4. Using phylogenetic character reconstructions to chart evolutionary trajectories in the cost-benefit framework. Tracing character histories across phylogenetic trees (4.1) allows the 'story-boarding' of multiple independent eye loss events, and how these may relate and differ to each other. Lineages sharing reduced features in the same order have likely moved through the adaptive landscape in the same way. By charting this within our cost-benefit framework (4.2), we can begin to visualise the relative importance of evolutionary drivers of selection, neutral mutation and pleiotropy. Here, reduced eye characters (shape icons) have appeared in five out of nine species examined. Repeated evolution of the white square character in independent lineages h and d+e indicates that they occupied the same regions on the adaptive landscape, for example.