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The rise of *Astyanax* cavefish

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Abstract

Numerous animals have invaded subterranean caverns and evolved remarkably similar features. These features include loss of vision and pigmentation, and gains in non-visual sensation. This broad convergence echoes smaller-scale convergence, in which members of the same species repeatedly evolve the same cave-associated phenotypes. The blind Mexican tetra of the Sierra de El Abra region of northeastern Mexico has a complex origin, having recurrently colonized subterranean environments through numerous invasions of surface-dwelling fish. These colonizations likely occurred ~1–5 MYa. Despite evidence of historical and contemporary gene flow between cave and surface forms, the cave-associated phenotype appears to remain quite stable in nature. This model system has provided insight to the mechanisms of phenotypic regression, the genetic basis for constructive trait evolution, and the origin of behavioral novelties. Here, we document the rise of this model system from its discovery by a Mexican surveyor in 1936, to a powerful system for cave biology and contemporary genetic research. The recently sequenced genome provides exciting opportunities for future research, and will help resolve several long-standing biological problems.

Keywords

Regressive phenotypic evolution; troglomorphy; cave biology

Introduction

Blind Mexican cavefish are remarkably well adapted to life underground. For unknown reasons, numerous ancestral surface-dwelling stocks invaded a geographically delimited karst region in northeastern Mexico numerous times over the past several million years (Ornelas-García et al., 2008; Bradic et al., 2012). Population genetic studies revealed that different epigean (surface-dwelling) stocks likely colonized each of three discrete areas: the older El Abra region, and the younger Guatemala and Micos regions (Bradic et al., 2012; Bradic et al., 2013; Coghill et al., 2014). Irrespective of geographic and phylogenetic distance, independent cavefish populations seem to move in the same phenotypic direction towards eye and pigmentation loss (Jeffery, 2006; 2009) and extra-visual sensory expansion (Bensouilah & Denizot, 1991; Yoshizawa et al., 2013). Several decades ago, breeding experiments revealed that cave and surface forms were capable of producing viable hybrid offspring (Şadoğlu, 1957a; Şadoğlu, 1957b), demonstrating the utility of these animals for laboratory investigations. This early discovery led to classical genetic studies revealing the participation of both Mendelian (albinism and *brown*; Şadoğlu & McKee, 1969) and complex cave-associated phenotypes (Wilkens, 1988) evolving in cave lineages. Complementation studies showed that cave populations from the same geographic region (e.g., the El Abra caves) evolved similar regressive phenotypes (such as eye loss) through different genetic loci (Wilkens, 1971; Borowsky, 2008). Recently, quantitative trait locus (QTL) analyses further clarified the architecture of simple (Protas et al., 2006; Gross et al., 2009) and complex traits (Borowsky & Wilkens, 2002; Kowalko et al., 2013a; Kowalko et al., 2013b; O'Quin et al., 2013) participating in cave evolution. Most recently, transcriptome profiling has revealed that diverse patterns of gene expression underlie many cave-associated phenotypes (Gross et al., 2013; Hinaux et al., 2013).

Several natural systems have been implemented as models for understanding the genetic basis for evolutionary change. *Astyanax* cavefish, however, are unique in several respects. First, unlike many cave-dwelling animals, related surface-dwelling morphs survive in the rivers and streams surrounding the cave network. Extant surface- and cave-dwelling forms evolved from common eyed, surface-fish like ancestors that invaded this region over a million years ago. The presence of both morphotypes has enabled a powerful comparative approach which has led to a number of molecular (Avise and Selander, 1972), genetic (Behrens et al., 1998), behavioral (Duboué et al., 2011), developmental (Alunni et al., 2007) and physiological (Breder & Rasquin, 1947) studies. The dark, nutrient-poor environment of the cave has led to extreme phenotypic changes, many of which are mediated by fixed genetic differences between cave and surface forms (Borowsky & Wilkens, 2002). Second, unlike many natural systems, cavefish likely diverged from their ancestral forms over the course several millions (rather than thousands) of years (Ornelas-García et al., 2008; Bradic et al., 2012; Coghill et al., 2014). This period of separation, however, depends on which cave populations are being studied (Peters et al., 1975; Dowling et al., 2002). This is because multiple surface-dwelling populations colonized multiple cave environments over the past several millions of years. This complicated demographic history therefore enables us to determine if the same trait evolves through the same genetic pathways in “older” versus “younger” cave populations (Strecker et al., 2012; Kowalko et al., 2013a). Finally, both cave and surface forms are highly tractable laboratory systems (Hinaux et al., 2011), enabling high-resolution studies at multiple levels of analysis.

This intriguing evolutionary history mirrors an equally fascinating history as an experimental model system. Here, we chronicle use of the cavefish system from studies of taxonomic status and genus designation, to cutting-edge experimental observations and manipulation. A comprehensive survey of the scientific literature since their discovery demonstrates a clear growth of interest in this animal system – from humble origins in the commercial pet trade to a powerful genomic model for understanding evolutionary and phenotypic change (Fig.1A). The newly available physical genome opens further opportunities to provide unprecedented clarity to long-standing problems in the field of cave biology and beyond.

Cave animals as models for biological research

Cave-dwelling organisms have long held the fascination of scientists and the general public. This is due to the dramatic and seemingly maladaptive traits (lack of eye and pigmentation) demonstrated by these intriguing creatures (Protas et al., 2011). Extreme characteristics of cave animals have been appreciated for over a century (Eigenmann, 1909), and Charles Darwin famously commented on the "problem" of trait loss for his theory of natural selection, attributing phenotypic regression in cave organisms to "disuse" rather than through obvious selective forces (Darwin, 1859). Three principal theories seek to explain loss of phenotypes in cave animals: a) the accumulation of neutral mutations affecting traits that lose relevance in the darkness of a cave (Wilkens, 1988); b) negative selection driven by energy conservation in nutrient-poor subterranean environments (Protas et al., 2007); or, c) indirect selection through antagonistic pleiotropic interactions between constructive traits and expendable (regressive) traits evolving in the cave (Wright, 1964). Although much progress has been made in last several decades through diverse experimental approaches, a consensus view as to how and why traits regress in cave animals has not been reached (Gross, 2012). Indeed, a combination of different mechanisms may ultimately participate together in the evolution of regressive phenotypes.

A number of useful cave animal systems have emerged over the past century, including invertebrate systems such as *Gammarus minus* (Jones & Culver, 1989; Culver et al., 1995) and *Asellus aquaticus* (Kosswig & Kosswig, 1940; Protas et al., 2011). *Gammarus minus* is a freshwater, cave-adapted amphipod found in the eastern United States. Classical studies of multiple cave-adapted forms of *Gammarus* provided insight into natural selection and adaptation in cave-dwelling animals (Culver et al., 1995). More recently, this system has been adapted for genetic analyses. For instance, a recent report indicated no loss of functional constraint in *opsin* gene paralogs in cave-dwelling forms, but rather convergently reduced levels of gene expression. This loss of expression, without loss of gene function, may suggest previously pleiotropic roles for *opsins* (Carlini et al., 2013). *Asellus aquaticus*, a cave-adapted crustacean found in Slovenia, has emerged in recent years as a powerful invertebrate genetic model system. Using this system, Protas et al. (2011) demonstrated that, although significant morphological changes can be accomplished through one or a few genes, eye size variation within cave animals is likely mediated by multiple mechanisms even within the same population.

We propose that cave-dwelling organisms should be prioritized for further research. In addition to the question of how regressive changes occur, cave animals can also inform the genetic basis for constructive morphologies (Yoshizawa et al., 2010; Kowalko et al., 2013a; Yoshizawa et al., 2013). Cave-dwelling invertebrates evolve enhanced non-visual sensory systems such as longer antennae and limbs (Fong, 1989). Similarly, cave vertebrates evolve non-visual sensory expansions to their gustatory (Bensouilah & Denizot, 1991) and lateral line systems (Montgomery et al., 2001). Integrative studies of the genetic and developmental bases for regressive and constructive changes have the potential to inform how seemingly distinct phenotypes may be linked through shared genetic pathways. Further, closely related cave-dwelling animals frequently occupy the same geographic regions. Although similar phenotypes evolve across a localized landscape of cave-dwellers (Ornelas-García et al., 2008), they appear to do so through different genetic mechanisms (Borowsky, 2008; Gross et al., 2009). Thus, cave animals have potential to determine if certain genes or genetic pathways are favored in the evolution of particular traits evolving under similar cave conditions.

Cave systems can also be powerful models for human phenotypes of clinical relevance. For instance, the precise genetic basis for many visual system disorders in humans remains unknown (O'Quin et al., 2013). Evaluating analogous phenotypes in cave animals provides an opportunity for gene discovery in the context of a natural system subjected to intense environmental pressures (Albertson et al., 2009). Moreover, the tractability of this system permits unique functional genetic analyses wherein a derived (cave-associated) genetic variant can be directly tested in a surrogate "ancestral" (surface fish) organism (Elipot et al., 2014). Although many cave model systems have emerged over the years, one cave system is now poised to provide dramatic insights to many of these questions: the *Astyanax* cavefish of northeastern Mexico.

The rise of *Astyanax* cavefish in the scientific literature: 1936 - 2014

The first *Astyanax* cavefish were discovered at the Chica cave locality in the southern karst region of the Sierra de El Abra in 1936 (Hubbs & Innes, 1936). These fish were discovered and collected by Salvador Coronado, a Mexican surveyor. Coronado collected the specimens for a fish hobbyist, C. Basil Jordan, of the Texas Aquaria Fish Company in Dallas, Texas (Mitchell et al., 1977). This freshwater fish was assigned to the new genus *Anoptichthys* (lat. "bony fish with no eyes") by renowned University of Michigan ichthyologist, Dr. Carl Hubbs. About a decade after the discovery of Chica cavefish, two subsequent expeditions to the El Abra region identified cavefish from the Sabinos and Pachón cave localities (Alvarez, 1946; Alvarez, 1947). Early studies sought to determine if the three distinct cave populations were related, originating from a single colonization of the subterranean environment by surface dwelling forms (reviewed in Mitchell et al., 1977). Based in part on morphological evidence (dermal bone fragmentation affecting the facial skeleton) the first three cave populations were each assigned as a distinct species of *Anoptichthys* (Alvarez, 1946; Alvarez, 1947).

The emergent field of Mexican cavefish biology experienced a conceptual shift in nomenclature in the 1950s when it was discovered that eyed surface-dwelling fish, common in the rivers and streams surrounding the limestone cave complex, could hybridize with cave-dwelling forms. Hybrid offspring from these crosses were viable, and could be interbred to produce experimental pedigrees (Şadoğlu, 1957a; Şadoğlu, 1957b). The ability to produce these hybrid pedigrees launched two key directions in Mexican cavefish research. First, the use of *"Anoptichthys"* to describe these cavefish gradually transitioned to *"Astyanax"* as cave and surface dwelling forms became regarded as sub-morphotypes of a single species (Schemmel, 1967). This shift was likely based on the biological species concept, as well as shared molecular similarity of cave and surface morphs informed by allozyme studies (Avise & Selander, 1972). However, not all *Astyanax* researchers agree on a singular taxonomic nomenclature. For example, the names *Astyanax mexicanus* (Protas et al., 2007), *Astyanax fasciatus* (Van Trump & McHenry, 2013), *Astyanax hubbsi* (Ornelas-García et al., 2008), and *Astyanax jordani* (Boudriot & Reutter, 2001) have all appeared in the recent literature referring to cave-adapted forms (Fig.1B).

In a humorous exchange, Avise (2001) recounted an interaction with Carl Hubbs, who coined the term "*Anoptichthys*" in 1936. Avise shared his assertion with Hubbs that cavefish and surface forms of *Astyanax* should be regarded as members of the same species owing to their molecular similarity (Avise & Selander, 1971). Hubbs replied that anyone who thinks blind Mexican cavefish and surface-dwelling *Astyanax* are members of the same genus, let alone the same species, “must be as blind as the fish themselves” (Avise, 2001; p.51). Nonetheless, the ability to hybridize different cave populations with surface-dwelling populations (as well as with other geographically-distinct cave populations) launched *Astyanax* as a classical genetic model system (Şadoğlu & McKee, 1969).

From the 1940s through the 1970s, 27 additional cave populations were discovered and named (Wilkens & Burns, 1972; Mitchell et al., 1977), including an additional *Astyanax* cavefish population in the southern Mexican state of Guerrero in 2001 (Espinasa et al., 2001). These population discoveries began to illustrate the common convergence on cave-associated characters in this system, as well as its utility for understanding the convergent evolution of extreme phenotypes.

Astyanax as a powerful model system for understanding mechanisms of biological diversity

Following the first formal scientific description of Mexican cavefish in 1936, subsequent reports from this decade focused on taxonomic assignment and descriptions of the natural history of these remarkable organisms (Innes, 1937; Breder, 1942). The following decade saw the first uses for cavefish as an experimental system. For instance, in addition to descriptive studies, the first histological studies of the degenerating eye in cave forms were performed (Gresser & Breder, 1940). Additionally, several behavioral phenotypes were first examined, including studies characterizing differential schooling and shoaling behaviors (Breder, 1943a; Morrow, 1948), alongside increased aggression studies (Breder, 1943b) in eyeless cave forms.

The 1950s witnessed the first formal classical genetic studies in which surface and cave forms were hybridized to produce experimental F_2 pedigrees (Şadoğlu, 1956; Şadoğlu, 1957b). The first evaluated phenotype was albinism, which demonstrated a nearly perfect (1:3) Mendelian ratio (Şadoğlu, 1957a). This segregation pattern was later shown to occur roughly equally in both males and females (i.e., not sex-linked), for both albinism and another reduced pigmentation phenotype called *brown* (melanin reduction; Şadoğlu & McKee, 1969). The 1960s was marked by expanded research directions focusing on endocrine differences between cave and surface forms (Mattheij, 1968), as well as studies characterizing enhanced chemoreceptive and lateral line sensitivity (Humbach, 1960) in cave forms.

During the 1970s, several authors returned to the question of which evolutionary mechanism(s) explain the loss of phenotypic characters in cavefish (Wilkens, 1971; Peters & Peters, 1973). In addition, attention turned to the central nervous system (Voneida & Sligar, 1976), and early husbandry and breeding studies (Şadoğlu, 1979), which set the stage for future studies utilizing this model as a system for understanding evolution and development. The 1980s represented a period marked by growth in the number of investigations using *Astyanax* cavefish. Schemmel (1980) performed one of the first classical genetic analyses that advanced from a morphological trait to a behavior (feeding) in cavefish (Schemmel, 1980). Additional discoveries during this decade included the discovery of increased lipid storage (Rose & Mitchell, 1982), decreased oxygen consumption (Hüppop, 1986), putative alterations to the circadian clock (Erckens & Martin, 1982a; Erckens & Martin, 1982b), and acutely sensitive olfactory systems (Quinn, 1980) in cave-dwelling forms. Several studies advocated the importance of neutral mutation as a principal mechanism driving regressive phenotypic loss in cavefish (Wilkens, 1988). This set the stage for ongoing debates seeking to identify the mechanism(s) for trait loss as occurring through neutral forces, negative selection, indirect selection (pleiotropy) or a combination thereof (reviewed in Gross, 2012).

During the 1990s, a number of integrative studies focused on the intersection of behavior (Langecker et al., 1993), constructive phenotypic evolution (e.g., neuromast and taste bud structure; Bensouilah & Denizot, 1991) and cave adaptation (Langecker et al., 1993). Reports also began to focus on the mechanistic basis for increased sensitivity to the environment (Langecker et al., 1993). This decade also witnessed a dramatic growth in the application of molecular and genetic tools for studying cave evolution. These included genetic sequence analyses of *opsins* (Yokoyama & Yokoyama, 1990a; Yokoyama & Yokoyama, 1990b; Langecker et al., 1993), *crystallins* (Langecker et al., 1995; Behrens et al., 1998), and a key regulator of eye development, *pax6* (Behrens et al., 1997; Jeffery & Martasian, 1998).

Further genetic studies continued into the 2000s, including analysis of *Prox1*. This gene is a critical regulator of lens development, but is also expressed strongly in taste buds and neuromasts – two key constructive tissue types that are greatly expanded in cavefish (Jeffery et al., 2000). Genetic information was also used to clarify the relationships and origins of cave-dwelling forms (Espinasa & Borowsky, 2001; Strecker et al., 2003). Several population genetic studies were performed during this decade, using both mitochondrial (Dowling et al., 2002; Strecker et al., 2004) and nuclear markers (Ornelas-García et al., 2008), to understand the highly complex evolutionary origin of these remarkable creatures.

A landmark developmental study in 2000 demonstrated the inductive role of the lens in generating the adult structural eye in cave/surface heterospecific grafting experiments (Yamamoto & Jeffery, 2000). In 2006, another important study by Protas et al. discovered that the gene *Oca2* governs albinism in multiple independent cave populations. This study represented the first identification of a causative gene underlying a cave-associated QTL (Protas et al., 2006). This decade also marked a significant expansion in the study of *Astyanax* cavefish, with several labs around the world adopting this model system for studies of evolution (Jeffery, 2001; Wilkens & Strecker, 2003), development (Yamamoto et al., 2003; Menuet et al., 2007), genetics (Strickler et al., 2002; Hooven et al., 2004) and behavior (Yoshizawa & Jeffery, 2008; Sharma et al., 2009). Additionally, the first formal international *Astyanax* community meeting was held in Ciudad Valles, Mexico in the spring of 2009.

The field of *Astyanax* biology continues to grow. Over the past four years, a developmental staging table has been produced (Hinaux et al., 2011), two transcriptomic studies have been published (Hinaux et al., 2013; Gross et al., 2013), several QTL analyses of complex behavioral phenotypes have been performed (Yoshizawa et al., 2010; Kowalko et al., 2013a; O'Quin et al., 2013; Yoshizawa et al., 2013), and a first-draft physical genome has become available (McGaugh et al., 2014). In addition to new insights on classic problems (e.g., the evolutionary and developmental mechanism of vision loss; Ma et al., 2014), recent studies have forged new ground. For instance, *Astyanax* cavefish have provided a powerful natural example of the potential role for heat shock protein chaperones as a buffer for phenotypic variation in the natural world (Rohner et al., 2013). Further, this model system has provided new insights to the evolution of sleep (Duboué et al., 2011; Duboué et al., 2012), the potential selective value of albinism (Bilandžija et al., 2013), and the evolutionary underpinnings of aggression in cavefish (Elipot et al., 2013). Many advancements have been made since the discovery of *Astyanax* cavefish (Wiley & Mitchell, 1971), and many additional discoveries are waiting to be unveiled in light of these powerful new genomic resources.

Future directions for *Astyanax* biology

The expansion of research directions, in concert with the availability of a physical genome, suggests that we are poised for many great discoveries in the field of *Astyanax* biology. For instance, genomic tools provide a powerful template upon which genome scans for selection (Fumagalli et al., 2011) and genome-wide association studies (GWAS; Timmann et al., 2012) can now be implemented as tools for understanding the evolution of these unique organisms. QTL studies enabled us to explore the genetic architecture for trait evolution (Gross et al., 2014), however this approach is limited by natural recombination occurring within an experimental pedigree. GWAS will enable a more flexible approach (Clarke et al., 2013), with increased precision (Button et al., 2013), for detecting genetic associations. GWAS can also be expanded to multiple different cave populations to examine the genetic basis for traits evolving in geographically distinct populations (Dowling et al., 2002; Gross & Wilkens, 2013).

Contemporary genome editing tools, such as *Tol2-* and *I-SceI*-mediated transgenesis (Elipot et al., 2014) and TALENs (Ma et al., 2015), will accelerate functional validation of promising candidate genes. These tools will allow us to determine if certain genetic lesions recapitulate cave-associated phenotypes in surface-dwelling fish. Additionally, transcriptomic profiling will provide high-resolution information on changes in gene expression occurring as consequences of subterranean colonization. For example, are the same genes (as with albinism; Protas et al., 2006; Gross & Wilkens, 2013) targeted for expression level changes in distantly related cave populations evolving the same trait? Alternatively, are different genes (as is implied by complementation crosses for vision; Wilkens, 1971; Borowsky, 2008) recruited to mediate the evolution of cave-associated phenotypes? In addition to these discovery-based questions, a number of additional long-standing questions now have hope for increased clarity.

Have karyotypic changes accompanied recurrent adaptation to the cave environment?

Numerous small- and large-scale chromosomal alterations have likely accompanied the transition from a lighted environment to the darkness of a cave. *Astyanax* cavefish have undergone a series of natural replicate “experiments” of cave colonization (Mitchell et al., 1977). We can now understand these processes, using the surrogate “ancestral” surface fish genome for comparison, to understand chromosomal dynamics at the population level. For example, do closely related cave populations demonstrate higher similarity to one another compared to distantly related populations? Are certain regions of the genome consistent “targets” of cave evolution? How flexible is the *Astyanax* karyotype? Do “labile” regions of the *Astyanax* karyotype co-localize with genomic regions identified from earlier linkage mapping studies? Prior studies have characterized extensive synteny between the *Astyanax* and *Danio rerio* genomes (Gross et al., 2008; Gross et al., 2013; O'Quin et al., 2013; Carlson et al., 2015). Future approaches aimed at linking genomes from multiple cave and surface genomes will clarify how the karyotype has evolved in distinct populations, and identify the consequences of these changes.

How have the same phenotypes convergently evolved in numerous distinct cave populations?

One of the most intriguing discoveries in *Astyanax* biology over the past several decades is the finding that geographically and phylogenetically distinct populations have evolved eye loss through different genes. In 1971, Wilkens discovered that fish bred from two different eyeless populations had offspring with a larger structural eye than either of the parental populations (Wilkens, 1971). This complementation study indicates that distinct genetic mechanisms mediate trait loss, even when two cave populations arose from the same ancestral epigean stock. At present, however, the identity of these genes remains unknown. Genomic analyses provide the opportunity to identify candidate sequence variants impacting eye loss within lineages (McGaugh et al., 2014). Subsequent comparisons between populations will identify eye loss loci shared between distantly related cavefish, in addition to a subset of loci that may also be shared.

Transcriptional profiling carried out in different cave populations will identify the genetic pathways impacted by the evolution of trait gains and losses. For instance, even if distinct eye loss genes are altered in independent cave populations, do these genes converge on the same downstream genetic networks? To answer this question, it will be crucial to determine if certain gene expression patterns are *universally* associated with cave adaptation – irrespective of the time since colonization. Or, alternatively are entirely different genes recruited recurrently in geographically distinct populations? Evaluating the mRNA architecture of whole embryos, tissues or cells (using a comprehensive genomic template) promises extraordinary insight to this intriguing and unanswered question.

These experimental approaches, evaluated in the context of advanced population genetic studies (Ornelas-García et al., 2008; Hausdorf et al., 2011; Bradic et al., 2012; Strecker et al., 2012; Bradic et al., 2013; Coghill et al., 2014), will help inform the long-standing question of what mechanism(s) mediate the extinction of phenotypes from certain lineages.

Are regressive phenotypes evolving neutrally, through selection, or pleiotropy?

The question of why traits are lost within lineages is long-standing (Eigenmann, 1909). Darwin commented on the problem of trait loss for the theory of natural selection – a phenomenon he illustrated using cave animals (Darwin, 1859; Ha & Nehm, 2014). Over 150 years later, we still do not have a comprehensive understanding of how and why trait loss occurs (Gross, 2012). Some evidence suggests that traits, such as vision, can be lost through selection for constructive phenotypes. For instance, Yamamoto et al. (2004) first reported a role for *shh* signaling in cavefish eye degeneration. Specifically, an expanded *shh* signaling domain was found to negatively impact expression of critical vision genes. It was later shown that this expanded *shh* signaling domain is also developmentally linked to increased numbers of taste buds, which is likely under selection (Yamamoto et al., 2009). Since vision is expendable in the darkness of the cave, these two phenotypes are united through pleiotropic consequences of *shh* expression, a form of indirect selection (Jeffery, 2010).

By performing genomic scans for selection in the genomic intervals surrounding *shh* and other candidate genes (McGaugh et al., 2014), we can determine whether recent selection within populations is the causative mechanism leading to convergent trait loss. By combining these genomic approaches with sophisticated methods of genome editing using cavefish embryos, we will better understand if and how alternative phenotypic effects arise from targeted genetic changes. Finally, evaluating phenotypic consequences of gene editing experiments *in vivo* will help ascertain if certain genes may offer a (cryptic) selective advantage in the complete darkness of a cave through recapitulation of cave-associated phenotypes in surface fish.

Conclusions

Since the discovery of depigmented and eyeless fish at the Chica cave in 1936 (Hubbs & Innes, 1936), *Astyanax* has been an intriguing and powerful system for contemporary biology. Our historical analysis of the literature indicates that with each technical improvement, *Astyanax* has been used to catalyze important discoveries for the fields of evolution, development, genetics and behavior. The ability to breed cave and surface fish to create viable offspring (Şadoğlu, 1957b) led to the use of this model system for classical (Şadoğlu, 1956), and later quantitative (Protas et al., 2008), genetic studies. Experimental embryology procedures, such as lentectomy (Yamamoto et al., 2003), led to the discovery of the lens as a key inductive tissue. Knockdown analyses revealed a role for pleiotropy (Yamamoto et al., 2009) in driving the development of both regressive and constructive features. The application of QTL analyses led to the identification of genes that govern simple traits (Gross et al., 2009), and revealed the complex genetic basis for a variety of multifactorial traits (Kowalko et al., 2013a; Kowalko et al., 2013b). Transcriptomic studies have identified the sequence and expression of unique genetic features for the cave and surface forms (Gross et al., 2013; Hinaux et al., 2013). This historical pattern suggests that we are once again at an exciting stage in *Astyanax* biology. With the newly available sequenced genome (McGaugh et al., 2014), *Astyanax* is poised to make even further contributions to our understanding of traits with human relevance (Gross et al., 2014), the genomic basis for cave adaptation (Bradic et al., 2013), and help inform our understanding of why certain lineages discard phenotypes over evolutionary time (Cronk, 2009). While much has been done to this point, much more is waiting to be discovered, and this powerful natural system is on the cusp of providing exciting discoveries for many years to come.

Methods

We quantified primary literature, focused on *Astyanax* cavefish, through individual-year searches from 1936 through 2014 using the search terms "*Anoptichthys*" and "*Astyanax*". All literature searches were performed using Google Scholar (scholar.google.com) and titles were retained for each positive hit. Results were filtered, and irrelevant uses of each term were discarded. Search hits were screened to avoid redundant documentation of an earlier-published report. The results of this analysis are presented in Fig.1A.

Nomenclature usage in catalogued books was evaluated for several *Astyanax* and *Anoptichthys* species names using the Google NGram Viewer (books.google.com/ngrams). This open source search tool provides quantitative comparisons of term usage over delimited periods of time. We searched for species names associated with *Astyanax* cave and surface fish (e.g., *mexicanus, jordani, hubbsi, fasciatus,* and *jordani*) as well as various *Anoptichthys* species names (*jordani, hubbsi,* and *antrobius*). Although our search query ranged from 1936 through 2014, information through the Google NGram Viewer database is currently available only through the year 2008 (Fig.1B). All default settings were retained for this analysis (e.g., smoothing value = 3). Although several forms of *Astyanax* returned a positive hit, only one *Anoptichthys* species name (*jordani*) successfully returned a hit. The results of this analysis are presented in Fig.1B.

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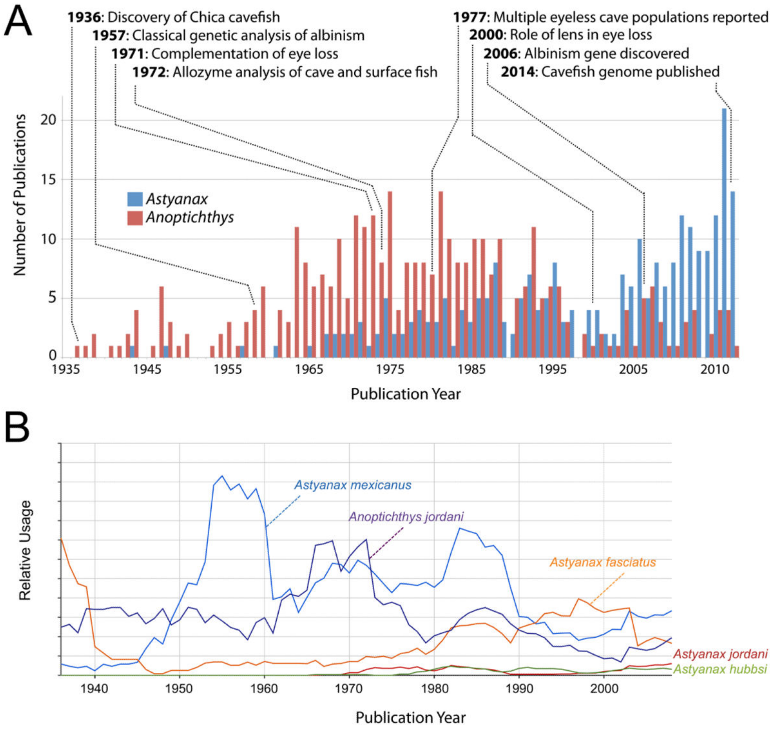
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1936: Discovery of Chica cavefish
1957: Classical genetic analysis of albinism
1971: Complementation of eye loss
1972: Allozyme analysis of cave and surface fish
1977: Multiple eyeless cave populations reported
2000: Role of lens in eye loss
2006: Albinism gene discovered
2014: Cavefish genome published
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Figure 1.