

BOOK REVIEW: EVOLUTION IN THE DARK: DARWIN'S LOSS WITHOUT SELECTION

Horst Wilkens and Ulrike Strecker 2017. Springer, Berlin. 217 p., 25 × 33 cm, hardbound, \$159, ISBN 9783662545102,

I give this new book my strongest possible endorsement. It is a tour de force and the best overview of evolution in cave organisms since David Culver's 1982 book. It is even better-written and argued than many of Wilkens's earlier publications of his pioneering 47 years of research. The sequence of chapters reads like a detective story with background, patterns, hypotheses, and tests.

Each of seven chapters has a great abstract, self-contained sub-sections, and hundreds of references. For every other page there is an average of one graph, diagram, table, photograph, map, or drawing, each with a self-contained legend. Many photos and photomicrographs are in color. Theorists may be disappointed not to find any equations, but Wilkens has covered both selection and neutrality theories in a critical review with David Culver (2000, p. 381–398, *Ecosystems of the World: Subterranean Ecosystems*).

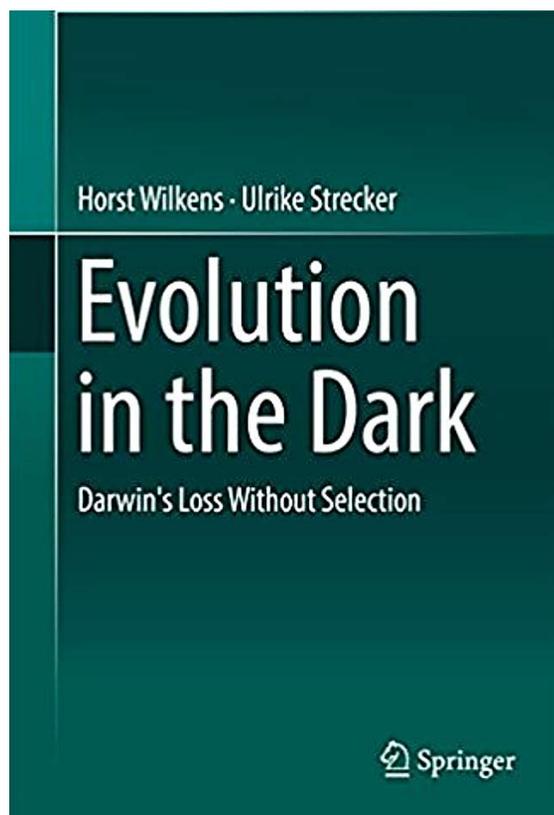
Despite many disagreements, Wilkens and all others agree that *Astyanax mexicanus* is an especially good model for study of evolution in caves. Everyone cites its short generation time, hundreds of offspring at each spawning, ease of husbandry in the lab, and that surface and cave populations are inter-fertile. Colonizations have been sudden, and many are recent and so different that populations have not diverged enough to become reproductively isolated. This is different from most cavefish, which have been isolated so long that their putative ancestors do not exist.

Wilkens explains how the surface-living *Astyanax* Mexican tetras are different from the ancestors of most cavefish, such as catfish, because they are only minimally pre-adapted to life in caves. As day-active schooling fish they strongly rely on vision. They do not have great elaboration of sense organs that are important for finding food in caves, like lateral line neuromasts, taste buds, and olfactory rosettes. But we know now that the surface fish do have taste buds and lateral-line sense organs and that they have standing genetic variation for these traits. Those surface fish individuals with the greatest lateral line, taste, and smell senses may have been the only ones to survive when first isolated in caves. They colonized successfully, whereas those colonizing later die of starvation.

Astyanax provides a huge advantage for reconstruction of the evolution of cave adaptation because it has had many independent colonizations of caves that range from old to recent. All populations are inter-fertile, and this has allowed a full range of classic genetic and molecular genetic studies in the laboratory. It has also allowed paleo-biogeographic reconstruction of past and ongoing colonizations. The dynamic evolution of caves and karst along with Pleistocene climatic change has resulted in continued extinctions and recolonizations, some with introgressive hybridization between surface and cave in at least the distant past.

Wilkens and all others also agree that there has been direct natural selection to elaborate sense organs and metabolic economy. But they disagree about mechanisms of regression or reducing of eyes and pigment. Wilkens shows that there have been many mutations in eye and pigment genes and that these have no disadvantage in caves. These mutants are not eliminated by purifying selection, and eyes become more regressed with time through still more mutations. Others argue that there has been both direct and indirect selection, by pleiotropy, against eyes and pigment.

Horst Wilkens, Richard Borowsky, and William Jeffery are the three titans of Mexican cavefish study. Each is interested in both regressive changes, especially of eyes and pigment, and adaptations of behavior and physiology to survive in caves. But their conclusions differ in many ways. Beginning in the late 1960s, Horst Wilkens and a few students and close colleagues did by far the earliest research on the biology, genetics, and history of *Astyanax* surface and cave populations. He is the only one who has consistently studied many surface populations and almost all the cave popula-



tions. He always emphasizes classic genetic crosses to study all aspects of cave and surface fish biology. Borowsky's expertise is with sophisticated ways of quantitative trait locus (QTL) mapping traits on chromosomes and searching for candidate genes. Jeffery emphasizes experimental developmental approaches. Wilkens and his colleagues and students have studied many other cavefish and crustacean species. Other groups, except for Borowsky's studies of Thai Balitorid fish and some recent work on cave isopods, only study the Mexican cavefish. The Wilkens group has used many approaches and studied both regressed and elaborated traits, but the other titans have narrower research niches. Wilkens and his colleagues give detailed attention both to adaptive elaborated traits and neutral regressed traits, and many of that group have pioneered the study of different traits. Some of Borowsky's colleagues have also pioneered new areas of study, particularly of sleep and hyperphagia in cavefish and possible standing genetic variation in surface fish. But most of Jeffrey's colleagues and students continue to push the same hypothesis of antagonistic pleiotropy between eye or pigment reduction and sense-organ elaboration, often uncritically, as Wilkens and Strecker document.

The other research groups give almost no attention to alternative hypotheses for regressed eyes and pigment in cavefish. They only cite a few of Wilkens' papers and never the critical review of theories with Culver, and rarely the historic luminaries who have contributed to recognizing the general importance and understanding of regressive evolution. Historically, Kosswig, Wilkens's hero and PhD advisor, expounded his neutral mutation theory. A modern version by Kimura and Nei is the neutral theory of molecular evolution. Central to both neutral theories, mutations on any trait are overwhelmingly neutral or negative. This was first recognized by Nobel Laureate Muller, but suggested by Darwin in a prescient paragraph quoted by Wilkens and Strecker (page 202). According to much recent research, this prevalence of deleterious mutations may result in what are called "pseudogenes" that have lost function owing to deletions and insertion mutations. Less extreme, but prevalent when looked for, is that function is compromised when non-synonymous substitution mutants hugely outnumber synonymous substitutions. Some non-synonymous mutations result in radical amino acid changes in protein. Without purifying, selection eye mutants are neutral and accumulate in cave populations.

Wilkens and Strecker are the only researchers who discuss the generality and mechanisms of regressive evolution, and they strongly support it for cavefish. As recognized by Darwin and emphasized by Kosswig, regressive evolution is almost universal in species and too little studied. Most regressive evolution is gradual over long periods of evolutionary time, so often we can only study fossils to see the ancestral conditions. Examples discussed by Wilkens and Strecker are teeth in humans, pelvic fins in whales, wings in Ratite birds, and eyes in naked mole rats and cavefish.

Research groups other than Wilkens's differ in the degree to which they espouse the importance of indirect selection against cavefish eyes and pigment via pleiotropy. To William Jeffery and also students and colleagues, antagonistic pleiotropy continues to be central (e.g., a 2005 review, *J. Heredity* 96). As functionless eyes are reduced, adaptive systems are enhanced. Two examples are eyes and taste buds and eyes and lateral line senses. Richard Borowsky is more even-minded. He even critiques the Jeffery et al. eye and lateral-line antagonistic pleiotropy. But he misses some of the same points he criticizes in his 2016 chapter in *Biology and Evolution of Mexican Cavefish*, "Regressive evolution: testing hypotheses of selection and drift." In fact, he tests three predictions that he believes falsify drift, but none that might falsify selection. One of his predictions is weakly correlative and two others are either open to other interpretations (Wilkens) or shown to be based on incorrect theory (Wilkens's citation of Lande). Sylvie Retaux (with Casane) claims that there is support for both selection and drift in a 2013 review paper (*EvoDevo* 4(1) entitled "Evolution of eye development in the darkness of caves: adaptation, drift, or both?") They uncritically evaluate antagonistic pleiotropy and cite many lines of evidence from cave and other animals that clearly support the accumulation of neutral mutations that result in different degrees of loss of function.

The strongest evidence Wilkens and Strecker adduce for accumulation of neutral mutations comes from various classic genetic crosses between surface and cave populations and quantitative trait locus mapping by Wilkens and by Borowsky. Wilkens and Strecker show that all studied traits are polygenic. For example, QTL mapping suggests at least 12 eye genes and 16 pigment genes. And F2 crosses (i.e., of the second filial generation) show that they are inherited independently. Wilkens and Strecker are the only workers to show that both eyes and pigment have two modules that control different aspects, i.e., lens and retina for eyes, and the number of melanophore cells and light-induced color change for pigment. In all cases, F2 crosses show all combinations of reducing and elaborating traits are not genetically linked, resulting in what Wilkens calls mosaic evolution. Mosaic evolution seems to be universal with polygenic traits, in which, as recognized only by Wilkens, there is epistasis with threshold effects. That can result in what appear to be sudden changes in traits, apparently including rapid declines in eye size.

Below I have summarized each of the book's chapters with a few notes that indicate where the authors' studies are pioneering:

1. Evolution in the dark: Introduction. 2 p. Constructive/elaborated traits are Darwin's gain and less-studied regressive/rudimented traits are Darwin's loss without selection.

2. The role of rudimentation in evolution. 8 p. Rudimentation is universal in all kinds of life, but too rarely studied. Some examples are loss of functional wings in Ratite birds, loss of hind limbs in whales, and extreme reduction in eyes in fossorial mammals and cave animals. Most cave animals' ancestors were pre-adapted to life in the permanent darkness of caves because they were nocturnal, with sensory systems that function without light.

3. Diversity and the phylogenetic age of cave species. 34 p. The authors concentrate on an aquatic hot spot around the Gulf of Mexico, where they have studied fish and crustacean cave species of very different biogeographic origins. These species became isolated in caves in different ways during past climatic change. With different time durations in caves, they have different degrees of evolutionary regression of eyes and pigment.

4. Surface and cave populations of Mexican *Astyanax*. 16 p. The authors distinguish "phylogenetically young" with variable eye and pigment reduction (VEP) and "phylogenetically old" with much less phenotypic and genetic variation and strong eye and pigment reduction (SEP). The SEP populations have both the most regressed and most elaborated traits. The most elaborated adaptations to restricted food supplies are metabolic economies, increased activity, decreased sleep, and increased appetite and fat storage when food is available. These go along with elaborated sensory systems, especially taste and lateral line, which are needed when sight is impossible in the dark with regressed eyes. The authors also discuss all other regressed traits related to blindness, including losses of aggressive interactions, phototactic behaviors, and schooling. Molecular markers used by Strecker (mitochondrial DNA and microsatellites) show multiple colonizations and extinctions. Cave colonization in VEP and SEP populations have taken place in parallel and resulted in multiple convergent evolutions.

5. Complexity of interrelationship between *Astyanax* cave and surface fish. 26 p. The authors compare divergence in nuclear DNA and mitochondrial DNA. The haplotype distributions show that four different VEP and three SEP populations have had a complex history of colonizations, bottlenecks, extinctions, and introgressive hybridization as the cave systems have come and gone, both connected and disconnected. The authors are the first to show that hybridization, which may add new variants to cave populations and so fuel new evolution, occurs only between SEP and VEP populations and between SEP and SEP populations. The authors suggest that all of the cave populations fit the criteria of being biological species.

6. Regressive and constructive traits in surface and cave fish. 112 p. The authors point out that with recent and ongoing colonization, the cave and surface populations are inter-fertile, and that this has allowed multiple kinds of studies of constructive/elaborated traits and regressed/rudimented traits. Of the 10 elaborated traits (27 p.) all but two—sleep and hyperphagia—have been studied only by Wilkens with his group of students and colleagues. Aside from pigment (7 p.) and eyes (25 p.), both studied by many researchers, there are seven regressive traits studied only by Wilkens and his group and only one studied by others (20 p.). The last ten pages of the chapter give a careful analysis of the genetics of complex regressive and constructive traits.

7. Mechanisms of regressive evolution. 26 p. Wilkens and Strecker evaluate hypotheses to support Darwin's concept of gain by selection, and to support Darwin's loss by accumulation of neutral mutations. They use both cave organisms and other animals like whales, flightless birds, and fossorial mammals. They give evidence that falsifies each hypothesis of selection against eyes or pigment in caves. Direct selection for regression based on energy conservation is not supported, since regression of eyes and pigment is universal in caves even with abundant food supplies. Each case of purported indirect selection based on antagonistic pleiotropy is convincingly flawed. Next, they review all the historic and current evidence that regression is based on accumulation of deleterious mutations in the absence of stabilizing selection in caves. This is Kosswig's neutral mutation theory and Nei and Kimura's neutral theory of molecular evolution. They show that there is always variability of regressive traits, always polygenic inheritance, and always a huge preponderance of deleterious mutations. For constructive/elaborated traits, deleterious mutants are eliminated by purifying selection and so these traits show low variability.

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