Mechanisms of Regressive Evolution

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Abstract

In the face of the neodarwinian paradigm of selection as an agent exclusively claimed to explain evolutionary processes in recent days, almost countless efforts have been made to prove that selection plays an important role in rudimentation processes in cave animals. Efforts mostly focus on the eye and very often pleiotropy is looked upon as being responsible. For example, eye reduction is claimed to antagonistically drive the improvement of taste or the lateral line sense by pleiotropy. However, this could not be confirmed by crossing analysis or by quantitative trait loci mapping. Energy savings have been suggested as another selection factor. This hypothesis implies that all cave species would have to suffer from food limitation. This attempt ignores the fact that the majority of tropical caves, and even some subtropical ones, abound in energy supply. Nonetheless, the traits, having become functionless in the respective cave species occurring in these habitats, regress. Thus, energy limitation is not able to explain regressive evolution of biologically functionless traits in general, or in particular that of the eye in cave species. In fact, independent inheritance of traits suggests that *Astyanax* cave fish are subjected to mosaic evolution.

Besides, selection variability plays a central role in Darwin's concept of evolution. Even in the 1930s, the German evolutionary geneticist Curt Kosswig recognized the importance of the variability exhibited by biologically functionless regressive traits for the interpretation of regressive evolution. According to him, in such traits high phenotypic variability arises and is continuously exhibited for a longer time because a biological function no longer exists. He proposed that this variability was due to the absence of stabilizing selection, because regressive mutations are no longer eliminated and traits become reduced over time merely by their accumulation. Thus, variability and loss are correlated. This so-called "Neutral Mutation Theory" is in accordance with Nei's "Neutral Theory of Molecular Evolution" which applies to molecular evolution. However, Darwin's loss without selection is one of two sides of the same coin, the

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other being “Darwin’s gain”, in which variability is the basis of constructive evolution. However, usually variability does not exhibit an extent as conspicuous as in the case of functionless cave animal traits. There are only a few examples in which variability, for a relatively short period of time, becomes obvious during evolution. This occurs during the initial phase of processes of adaptive radiation, as can be observed in a series of fish species flocks.

7.1 Deleterious Risk

As the most self-evident explanation of eye regression, it was hypothesized that an exposed eye would be a deleterious risk in darkness (Barr 1968; Strickler et al. 2007). This hypothesis is rather improbable, though, because as a rule species colonizing caves are nocturnal. Such so-called troglophile species are already preadapted to a partial life in darkness, are mostly negatively phototactic, and exhibit only small eyes for detecting light. They have already developed improved senses such as taste, olfaction, or orientation, which enable them to orientate and live in complete darkness, too. The best examples of this are provided by catfish (Wilkens 2001). In surface *Astyanax*, a comparatively well-developed lateral line sense facilitated orientation in darkness (see Sect. 6.5).

7.2 Food Limitation and Energy Economy

Energy economy is often looked upon as a driving force of regressive evolutionary processes, because in general caves are considered food poor and energy limited (Hüppop 2000, 2012). However, this does not generally correspond to reality and is misleading. Tropical and some subtropical caves may abound with bat or bird guano and flood detritus, providing rich, stable food resources, and are inhabited by large numbers of eye- and pigment-reduced species (Deharveng and Bedos 2000; Fernandes et al. 2016; Gnaspini and Trajano 2000). For example, such conditions were described for a food-rich sulphidic Mexican cave, in which the live-bearing toothcarp *Poecilia mexicana* exhibits highly variable eye sizes and all degrees of pigmentation (Fig. 3.14) (Langecker et al. 1996; Palacios et al. 2016; Peters and Peters 1973, 1968). Direct evidence for the uncoupling of energy input and regressive evolutionary processes is provided by cave-living plant hoppers. Although they suck on the roots of trees growing outside caves, and thus are directly dependent on primary production for their energy, they exhibit reduced eyes and dark pigmentation (Hoch 2000). Whereas a lack of light is an exclusive characteristic of caves, a small energy supply is not. Therefore, food limitation is a potential secondary independent environmental factor influencing the evolution of cave animals.

In particular, eye reduction was the focus of many studies and used as the prime example when trying to prove the role of energy economy as a driving selective force. It has never been considered that several behavioural traits such as schooling, visually released aggressive behaviour, or the dorsal light reaction are no longer being performed in darkness by the surface ancestor because of the missing releaser, but nonetheless are reduced in the cave populations (see Sects. 6.12, 6.13, and 6.18). As concerns the eye, several authors claim that the high energetic costs of neural cell metabolism could be a strong selective force (Borowsky and Cohen 2013; Moran et al. 2015; Niven et al. 2007; Protas et al. 2007). However, all these studies were performed with light-bred specimens, which does not compare to a cave environment. Comparison of eyes of fish living in permanent darkness and those kept under normal daylight conditions revealed different degrees of retinal histological differentiation. For example, in the phylogenetically young cave fish *P. mexicana* (Poeciliidae), which still exhibits externally visible eyes with variable size, specimens collected in the cave showed considerable malformations of the retina, whereas their offspring hatched and kept at 12-h light/12-h dark conditions developed completely intact and functional retinas (Parzefall 2001; Peters and Peters 1968; Peters et al. 1975). The same was revealed for the cave salamander *Typhlotriton spelaeus* (Besharse and Brandon 1974). Also, adult *Astyanax* surface specimens bred and kept in continuous darkness in a laboratory exhibit retinas that are about one third thinner than those reared in light, and the number of visual cell nuclei building the outer nuclear layer is reduced by 20% (Wilkens 1988). This is corroborated by the differing eye sizes of wild Micos cave fish and their laboratory offspring (Wilkens 1976). Thus, experiments performed with fish bred in light do not consider that in caves their entire ontogenetic development takes place in complete darkness, without any light stimulus. It is known that light stimulus is important for the formation of the visual apparatus (Zeutzius et al. 1984; Yang et al. 1988a, b). Such results are not comparable with those from fish existing under the exclusion of light. Most importantly, the phenotypic variability of eye size in cave species brings into question the lack of directional selection in eye loss (see Sect. 7.8). The combination of all these facts challenges whether the energy gain of eye reduction is important.

7.3 Quantitative Trait Loci Polarity Test for Selection (Orr's Sign Test)

Applying the quantitative trait loci (QTL) test developed by Orr (1998), it was claimed that cave alleles at every eye QTL caused size reduction and therefore argued that they were submitted to directional selection (Borowsky 2015; Krishnan and Rohner 2017; Protas et al. 2007). In contrast, QTL polarities for melanophores were mixed and thus consistent with neutral evolution. In fact, Orr's sign test itself is unable to prove selection (be it positive selection, relaxed selection, or whatever) by looking at QTL allele patterns between populations. Russell Lande states:

> As I understand the model and assumptions of this method, it only deals with the divergent aspect of selection between two populations descended from a common ancestor, and is ambiguous about selection within them. In this case it is obvious that selection maintains functional eyes in surface-dwelling fish, and it is safe to assume that most mutations in functional eyes, especially mutations of substantial effect that are likely to be detected in QTL experiments, will tend to reduce them (consistent with the empirical conclusion of Muller 1950) and are deleterious in surface populations. On colonizing a cave environment, even if selection there is merely absent and not actually against eyes, the mutational bias toward eye reduction (in both originally existing and new mutations), by itself or particularly when combined with genetic drift, will tend to fix eye reduction alleles. With a sufficient number of QTL the sign test will conclude that divergent selection has caused the difference. This is true in the sense that selection favors relatively large eyes in surface populations in comparison to cave populations. One can conclude that a significant result in Orr's sign test says nothing about whether within cave populations selection favors reduced eyes or is absent (Lande, personal communication).

7.4 Pleiotropy

Pleiotropy in its classical sense is defined as a genetic action that occurs where one gene is responsible for two or more unrelated traits. The classical example is provided by the adaptive advantage of individuals heterozygous for the sickle cell disease with respect to malaria. Recently theories of eye regression, as originally suggested by Barr (1968), have come to the fore again. According to this theory, regressive evolutionary processes were driven by the same genes, which improve cave adaptive traits such as taste.

7.4.1 Pleiotropy of Sonic Hedgehog (*Shh*) Genes

In *Astyanax* cave fish embryos, eye primordia were found to degenerate under the influence of hyperactive *sonic hedgehog* (*shh*) signalling (Yamamoto et al. 2004). As *shh* also plays an important role in oral jaw and taste bud development, it was suggested that *shh* signalling might increase traits like oral jaw size and taste organ number in cave fish and that by antagonistic pleiotropy could be responsible for eye loss (Jeffery 2005; Yamamoto et al. 2009). Attempts were made to prove this by studying F3-crossing hybrids, the third generation between surface and cave fish. F3-hybrid specimens were found in which small eyes were combined with many taste buds and vice versa. However, F3 crossings are not an appropriate tool to detect pleiotropy, because such crossings are the offspring of F2 specimens, the genetic background of which is undefined and unknown. F2 specimens only represent a selected part of the whole genetic variability. The scientific standard for proving pleiotropy would have been in an F2 crossing, which derives from F1 hybrids that possess a complete haplome from each parental form. This hypothesis is finally rejected by QTL analysis, which revealed that none of the multiple QTL underlying eye regression is located near a known *shh* locus (Protas et al. 2007).

In a similar pleiotropic approach, attempts were made to show that, due to hyperactive *shh* signalling, not only would the cave fish eye anlage become smaller, but the forebrain including the hypothalamus, where crucial neural centres controlling aggressive behaviour are located, would increase in size (Menuet et al. 2007). A link was claimed to exist between a modified specification of the hypothalamus and the differential control of aggressive behaviour, which was assumed to be missing in the cave fish (Rétaux et al. 2008). This interpretation does not take into account that the cave fish still exhibit aggressive interaction (see Sect. 6.12). Furthermore, the existence of an increase of forebrain size is controversial. Whereas Peters et al. (1993) found that its superior area size was enlarged, calculation of the total volumes of the forebrain and the hypothalamus did not state an enlargement in cave fish compared with the surface fish (Rodriguez 2013) (see Sect. 6.22).

Above all, these conclusions do not sufficiently consider the differential gene expression and the cell-fate-determining activity of *shh* as a morphogen, which depends on its concentration and is time dependent. Thus, *shh* is definitely involved in trait morphogenesis, but in diverging circumstances. Additionally, F2 crossings between surface and cave fish revealed that the above-mentioned traits inherit independently (see Sects. 6.21.5, 6.21.6, 7.5) (Wilkens 2010). Thus, the alleged pleiotropic links between regressive eye and constructive traits like oral and taste bud number, or hypothalamus size and aggressive behaviour, are questionable (Wilkens 1988, 2010, 2016).

7.4.2 Pleiotropy of Neuromodulation (Melanin-Catecholamine Trade-Off Hypothesis)

Neuromodulation has received growing interest in being indirectly responsible for cave regressive and constructive processes of behavioural traits (Katz and Lillvis 2014). Neuromodulators like dopamine, serotonin, acetylcholine or histamine secreted by a small group of neurons regulate diverse populations of neurons. The neuromodulators diffuse through large areas of the nervous system affecting multiple neurons. The broad neuromodulatory control exerted by monoamines (MOA) in vertebrate brains explains why they have been implicated in a variety of functions and processes: attention and arousal (noradrenaline, NA), defence and anxiety (dopamine, DA; serotonin, 5-HT), stress and emotions (DA, NA, 5-HT), reward (DA), mood (5-HT), appetite and energy expenditure, sleep/wakefulness (5-HT, NA, melatonin), and motor control (DA, 5-HT).

Neuromodulatory systems provide sites at which natural selection can act to alter the output of neural circuits and thereby play a role in the evolution of behaviour (Katz 2011; Katz and Lillvis 2014). By changes in receptor expression levels or release of neuromodulatory substances affecting the expression of genes via mutations in regulatory regions, a mechanism is provided to alter neuromodulatory signalling. It is suggested that parallel evolution of neuromodulatory signalling systems is responsible for repeated evolution of specific behaviours of different

Astyanax cave fish populations (Hinaux et al. 2015). For example, it is proposed that parallel evolution of certain behavioural traits like feeding posture in different *Astyanax* cave fish populations causes slightly different angles of feeding posture between the strongly eye-and pigment-reduced (SEP) and the variably eye-and pigment-reduced (VEP) cave fish (Katz and Lillvis 2014).

Neuromodulation was thought to be responsible for changes in a whole range of different behaviours (Elipot et al. 2013). In cave fish brains, high levels of content and neurotransmission indexes for serotonin, dopamine, and noradrenaline, but low MAO activity was detected. By analyzing the MAO coding sequence a mutation was identified, which is assumed to be responsible for this. The same mutated allele was found in the SEP Pachón, Sabinos, and Tinaja cave fish. Low MAO activity was claimed to be advantageous for cave life, although in humans such condition would be pathological. It was proposed to provide the genetic basis for several aspects of the so-called "*Astyanax* cave fish behavioural syndrome", which includes loss of aggression, schooling, and sleep, and increased food searching. Curiously, the MAO mutation was not found in the VEP Micos and Molino cave fish, although they exhibit similar behavioural traits.

In another approach, it was found that L-tyrosine, dopamine, and norepinephrine levels were enhanced in larval pre-feeding stages and adult brains of the Pachón cave fish (Bilandzija et al. 2013; Jeffery et al. 2015). This was attributed to the *oca2* loss-of-function albino mutation, which therefore was suggested to be an evolutionary benefit, because the enhancement of catecholamine levels was made possible by surplus L-tyrosine deriving from the block in melanin synthesis as its precursor. It would be a possible target of natural selection due to the importance of these compounds as neurotransmitters and paracrine factors. The inverse relationship between the block in melanin synthesis and the enhancement in catecholamine synthesis is hypothesized to be an example of "secondary pleiotropy", a type of pleiotropy in which a single mutated gene in a defined biochemical pathway has multiple phenotypic consequences. The resultant increases in serotonin and catecholamine signalling were hypothesized to play a role in modulating behaviours like reduced schooling, aggression, or sleep that could "render cavefish more successful in their natural environment" (Bilandzija et al. 2013).

Both hypotheses described before assume that the cave fish have elevated brain levels of serotonin and dopamine (and secondarily noradrenaline) indirectly caused by two independent mutations in MAO and *oca2*. However, potential gains of low MAO activity or surplus L-tyrosine are hampered by the fact that traits like schooling and aggressive behaviour are visually released and therefore not performed in darkness by the surface fish. Thus, after the surface fish started to evolve to a cave fish, selection cannot have acted on a "hidden" trait and been responsible for the spreading of these mutations in the various cave fish gene pools. Furthermore, the traits involved in the "behavioural syndrome" like schooling, feeding posture, or sleep have a polygenic basis and do not inherit in a monogenic way, as must be expected when suggesting that their change relies on just one mutation in *oca2* or MAO, respectively. In addition the albino gene inherits independently from those traits "rendering the Pachón cave fish more successful in the cave environment". The hypothetic role of the albino gene is all the more probable given the existence of only three albinotic *Astyanax* cave fish populations among 27 without the albino gene, all of which nonetheless show the same cave behavioural traits mentioned before (Gross et al. 2009; Mitchell et al. 1977; Wilkens 1988, 2010; Wilkens and Strecker 2003).

In summary, neuromodulatory systems provide sites at which natural selection can act and it can be assumed that behavioural traits of *Astyanax* cave fish like feeding posture and sleep have constructively been adapted to cave life under the influence of selection. It is important, however, to differentiate between constructive and regressive traits. In contrast to constructive evolutionary processes, in regressive ones degenerative mutations will strike both the genetic neuromodulatory basis and the one responsible for the performance of the pattern of a certain behavioural trait. It was shown by crossing experiments that regressive mutations have accumulated in visually released traits like schooling and aggressive behaviour or dorsal light reaction (see Sects. 6.18, 6.12, and 6.13). These traits cannot be performed by the surface fish in the dark, because they depend on vision, nor are they performed by the cave fish, because the polygenic basis is deteriorated (Kowalko et al. 2013b; Langecker 1993; Parzefall 1993; Wilkens 1988). It seems much more parsimonious to explain the reduction of behavioural traits not being performed in darkness by the accumulation of regressive mutations due to loss of stabilizing selection than by a single mutation in MAO or *oca2,* respectively, simultaneously influencing neuromodulation of a large number of behaviours. Thus, it is questionable whether melanin-reducing genes like *oca2* (Bilandzija et al. 2013) or the MAO mutation (Elipot et al. 2013) are responsible for the regression of some specific behavioural traits in cave fish.

7.4.3 Pleiotropy of Vibration Attraction Behaviour (VAB) and Superficial Neuromasts in the Cave Fish Orbit

Using quantitative genetic QTL analysis, it was hypothesized that the adaptive vibration attraction behaviour (VAB) and a small group of the sensory receptors, superficial neuromasts, occurring within a small area of the cave fish eye orbit (EO) (Figs. 6.2 and 6.3), were genetically correlated with reduced eye size. From this was concluded that natural selection for the enhancement of VAB and EO neuromasts would indirectly promote eye regression in the Pachón cave fish population through an antagonistic relationship involving genetic linkage or pleiotropy among the genetic factors underlying these traits (Yoshizawa et al. 2012). However, this genetic analysis ignores the numerous other QTL that map to, and potentially interact, in the same chromosomal regions and thus fails to establish pleiotropy (Borowsky and Cohen 2013). Furthermore, it was found that neither eye regression itself nor experimental *shh* overexpression induces EO neuromast formation (Yoshizawa et al. 2015). Thus, as VAB is not exhibited in a series of SEP cave populations, which nonetheless carry strongly reduced eyes, a selective advantage of eye loss via improved VAB and increased number of neuromasts within a restricted area of the eye orbit as hypothesized by Yoshizawa et al. (2012) is unproven. This is all the more so since all free neuromasts developed on the cave fish head are inherited as one single unit and obviously rely on the same genetic basis. This is corroborated by the correlation between the numbers of superficial neuromasts on the cave fish head and those in a remote separate position along the maxillary canals underneath the jaw (Fig. 6.2b) (Wilkens 2010).

7.5 Independent Inheritance

The crossing analyses of F2 hybrids between surface and the different cave fish populations provide an excellent tool to analyze whether the most diverse constructive or regressive traits exhibit independent inheritance (Fig. 7.1). These results as well as those of QTL studies did not reveal phenotypic correlations between the size of the eye and cave adaptive traits such as the number of taste buds (Protas et al. 2007; Schemmel 1967), amount of fat storage (Hüppop, 1989), feeding posture (Kowalko et al. 2013b; Schemmel 1980), aggressive behaviour (Hofmann and Hausberg 1993), schooling (Kowalko et al. 2013a), or the number of teeth (Protas et al. 2007). There was also no correlation between the eye and jaw size, naris size, or mouth width, which are each larger in the cave fish (Wilkens 2010). All traits studied, including the diverse colour mutations, are inherited independently. Even within single traits like eye or melanophore system, the two associated units (lens and retina or number of melanophores and morphological colour change, respectively) that they consist of are inherited independently (see Sect. 6.23) (Wilkens 1988, 2010, 2016). The hypothesis that increased *shh*-signalling reduces eye size and synchronously amplifies taste bud number and jaw size does not consider the subtle equilibrium that exists in space and time between the different signalling systems orchestrating the development of the different traits (Hinaux et al. 2016). Manipulation of early signalling systems usually results in abnormal phenotypes. Thus, it is doubtful that morphological evolution is due to modifications at this early ontogenetic level.

The observations shown above fit into the concept of mosaic evolution, namely that every species is a mosaic of traits that persist unchanged, whereas some traits change when submitted to selection pressure, exhibiting different intensities or even being relaxed and missing (Wilkens 2010). Mosaic evolution was also found for overall brain structure in mammals and the central visual system of the naked mole rat (*Heterocephalus glaber*), which has selectively lost structures that mediate form vision while those needed for the entrainment of the circadian rhythm are retained (Barton and Harvey 2000; Crish et al. 2006). In African cichlids, there is a close relationship between the relative sizes of various brain structures and the utilization of habitat and prey (Huber et al. 1997). Deep sea fish provide evidence for a great variety of diverse specializations of lens and retina in adaptation to light-poor environments (Ellis 1996). For example, the fish *Ipnops murrayi* (Ipnopidae) has modified its eyes with the retina covering most of the upper surface of the head in order to perceive faint bioluminescent light signals, but a lens no longer exists.

Fig. 7.1 F2 hybrid specimen between SEP Piedras cave and surface fish exhibiting a mixture of surface and cave traits like loss of guanine in the scales, low melanophore density but no reduced melanin content, and intermediate eye size. *SEP* strongly eye- and pigment-reduced

Other deep sea fishes like the telescope fish *Gigantura chuni* (Giganturidae) possess heads dominated by large, forward-pointing, telescoping eyes with voluminous lenses. Disadvantages would arise from linkages if, for example, the lens could not be enlarged without consequences for the retina. This would impede the evolutionary process of optimal adaptation. From an evolutionary point of view, independent inheritance of traits is suggested to be mostly advantageous.

7.6 Evolutionary Rates of Regressive and Constructive Traits

Recent cave-colonizing species like the VEP Micos cave fish offer excellent opportunity to study the evolutionary rates of cave-specific traits. In contrast to the SEP cave fish populations, in which the process of cave evolution may have come to an end an unknown time ago, the VEP cave fish have not yet finished. It was revealed for the Micos cave fish that mean and variability of some of the constructive traits like egg yolk content, fat storage ability, or nose pit size have already achieved the degree characteristic of SEP cave fish (Aspiras et al. 2015; Bibliowicz et al. 2013; Hüppop and Wilkens 1991; Wilkens 1988). However, other constructive traits like number of taste organs and a newly evolving aggressive behaviour elicited by the lateral line sense have not reached a state comparable to that of the SEP cave fish, but are intermediate between surface and SEP cave fish. Regressive traits like eyes, dorsal light reaction, or the visually triggered aggressive behaviour exhibited are also at an intermediate stage. The number of melanophores, as another regressive trait, phenotypically equals that of the surface fish. Nonetheless, its genetic basis has already been submitted to regressive mutations. However, this defect does not yet manifest due to epistatic gene effect (see Sect. 6.23.3). In summary, it is suggested that the evolution of the specific traits in cave species, independently from being regressive or constructive, proceeds at different rates. It is therefore questionable to conclude from the degree of the reduction of a trait like the eye in comparison with that of another one (melanophore pigmentation) whether they are reduced with or without selection as claimed for balitorid cave loaches (Borowsky 2015). Such calculations do not take into consideration that discontinuous epistatic gene effects might falsify the time estimates based on trait manifestation. This calculation is all the more questionable as long as nothing is known about the genetic expression pattern of the two traitsstudied in the balitorid cave loaches. For example, eye size is reduced by a threshold-like 25% amount in the Micos cave fish at the beginning of reduction, from which strong selection pressure and rapid evolution might be concluded. However, further reduction proceeds in many smaller steps, implying a much slower advance of regression (see Sect. 6.23). The evolution of constructive and regressive traits can best be explained by mosaic evolution.

7.7 Reversibility of Regressive Evolution

Dollo's law of irreversibility states that evolution is not reversible (Dollo 1893). However, eyes and pigmentation of cave animals have often been claimed as providing examples to rebut this hypothesis (e.g. Dillman et al. 2010). Such a misleading assumption may have arisen from the observation that several pale cave species, when exposed to daylight, become completely black or at least darker. The amphibian *Proteus anguinus* from the Dinarian karst offers one of the most spectacular examples for this. It was also found in the cave fishes *Rhamdia zongolicensis* and *R. reddelli* (Fig. 2.7) (Wilkens 2001) as well as in the Yucatan cave swamp eel *Ophisternon infernale* (Fig. 3.18). In *Astyanax*, phylogenetically younger VEP cave populations like Micos deviate from the phylogenetically older SEP ones by the observation that at light they may get darker again. Also, cave crayfish like *Procambarus reddelli oaxacae* become brownish when kept at daylight. The phenomenon of darkening can be explained by the production of melanin and ommochrome pigments in part still being undisturbed by mutations in these cave species. It is just downregulated in continuous darkness. Therefore, the more recent cave-invading, phylogenetically young populations are still able to synthesize melanin. Independently they can still increase the number of melanophores by morphological colour change when kept at daylight. This by no means counts as an example of reversible evolution. In contrast, however, the majority of cave species, which are represented by a phylogenetically older age, can no longer produce dark pigments and higher numbers of melanophores under such conditions, because the ability has been genetically lost. They have become irreversibly colourless.

Size and histological differentiation of the eye rudiments of phylogenetically old SEP *Astyanax* cave fish from the cave habitat are not different from their offspring bred and kept under daylight. However, the eyes of phylogenetically younger *Astyanax* cave populations like the VEP Micos fish are slightly better developed when hatched at daylight. This was explained by light environmental influence on the still existing visual cell outer segments (Peters et al. 1975; Peters and Peters 1968). It had only minor effect on eye size as a whole, though. However, the "reappearance" of truly large eyes in cave animals was presented for the amphipod *Gammarus minus* (Culver et al. 1995) and the phylogenetically young VEP *Astyanax* Caballo Moro cave fish (see Sect. 5.3.3) (Espinasa and Borowsky 2000). Both species occur below a lighted karst window, by which cave animals get access to daylight.

In order to explain the occurrence of these larger eyes, it was hypothesized that the eyed *Astyanax* cave fish below the karst window in the Caballo Moro Cave were direct descendants of the blind cave fish population inhabiting the dark parts of this cave that had secondarily genetically "re-acquired" eyes. However, the more probable solution for these observations is provided by the results of crossing analyses between *Astyanax* surface and cave fish. They showed that due to an epistatic genetic threshold, the eye size is increased by a discontinuous step after a minimal number of the remaining not mutated eye genes are recombined (Wilkens 1988, 2010, see Sect. 6.23). In nature, a similar phenomenon has occurred in the variable phylogenetically young VEP Micos cave fish. However, whereas in the dark Micos cave such specimens with larger eyes do not have any selective advantage and are rare, this is different in the lighted karst window of the Caballo Moro cave. Here the eyed specimens are favoured by the lighter condition and concentrate to a population, which can be separated from the rest of the stronger eye-reduced cave population by molecular analysis (Espinasa and Borowsky 2000; Strecker et al. 2012). A comparable explanation is provided for the "re-appearance" of the large eyed *G. minus* amphipodes, which obviously exhibit a similar genetic pattern of eye manifestation (Culver 1987) as the fish *Astyanax*.

In summary, the "re-appearance" of both eyes and melanin pigmentation is exclusively observed in phylogenetically younger cave populations, in which it is characteristic that the genetic regression of these traits has not entirely been completed. Thus, this phenomenon is a consequence of the manifestation of still existing intact eye or melanophore/melanin pigment alleles and not restoration of degenerate ones by "back-mutations". In the case of the eye, the re-appearance is much more improbable because it would require back mutations of a large number of genes. It is furthermore generally not considered that cave species are not exclusively characterized by the reduction of eyes and melanin pigmentation, but that many more traits have been altered by regressive or constructive evolution. Thus, the reversibility to the former ancestor, as claimed by Dollo's law, would also require the "restoration" of many more traits and become even more unrealistic.

7.8 Variability and Loss: Neutral Mutation Theory

Besides selection variability plays a central role in Darwin's concept of evolution. It was the idea of the evolutionary geneticist Curt Kosswig (1903-1982) to study the genetic basis of regressive evolution by investigating cave animals. He reiterated that Darwin's ideas were in concert with the current well known facts of genetics: "*The majority of mutations is responsible for damages in phenotype, in particular in case a trait is no longer controlled by selection. Within the bounds of constraints provided by the developmental and genetic backgrounds of an organism regressive mutations are accumulating step by step, by which the ultimate result mentioned by Darwin will finally originate. It is the loss of purifying selection, which is looked* *upon as being the cause of rudimentation, because deteriorating mutations are no longer eliminated now*" (Kosswig 1935, 1949, 1960a, b, 1976; Kosswig and Kosswig 1940).

Like Kosswig, Nobel Laureate H.J. Muller (1949) embedded the ideas of Charles Darwin into the knowledge of modern genetics: *"But even though organisms may have reached a virtual stopping point in evolution, the mere maintenance of their present structure requires, as we realize today, the persistent operation of natural selection. Darwin, to be sure, stated that a relaxation of selection leads to an increased accumulation of individual variants. But in his time the knowledge was apparently lacking that the relaxation of selection with regard to any character would lead to decay down to the level at which selection does operate, and that an actual cessation of selection for a character would in time lead to its complete disappearance. In fact, the entire organization would deteriorate similarly, if selection in all directions were relaxed. Before the advent of modern mutation study, it was not known that genetic changes in the down-hill direction are in general far more frequent than those which increase or intensify an organ or character. True, Darwin was so astute as to point out that such a principle, if true, would be of great service in explaining the reason for the decline, and more especially for the total disappearance of features which had lost their usefulness, like the eyes and the pigmentation of cave animals"*.

The Neutral Mutation Theory (Kosswig 1960a; Wilkens 1988, 2010) incorporates the forward-looking principles and ideas of Darwin with the insights of modern genetics showing that constructive and regressive evolution are not contradictive but basically rely on identical mechanisms: mutation-driven phenotypic variability combined with absent or present selection. In case of the absence of selection, the variability is not eliminated and traits get reduced over time merely by the accumulation of regressive mutations. In contrast, in the presence of selection, mutations negatively influencing a specific trait are eliminated and no variability occurs. This genetic interpretation refines Darwin's explanation of rudimentation, when he proposed that "If, for instance, it could be proven that every part of the organisation tends to vary in a greater degree towards diminution than towards augmentation of size then we should be able to understand how an organ which has become useless would be rendered, independently of the effects of disuse, rudimentary and would at last be wholly suppressed; for the variations towards diminished size would no longer be checked by natural selection". A rising number of studies are corroborating Darwin's assumptions. For example, there has been repeated loss of functional constraint of rhodopsin in amblyopsid cave fishes, as at least three cave lineages have independently accumulated unique loss-of-function mutations. Although several cave lineages still possess functional rhodopsin, they exhibit increased rates of nonsynonymous mutations that have greater effect on the structure and function of rhodopsin compared with those in surface lineages (Niemiller et al. 2012). Kowalko et al. (2013a, b) argue that once vision was impaired by the lack of light, schooling was no longer under selection, and alterations in genes affecting this behaviour would be neutral in consequence. Transcriptome sequencing of embryonic and larval stages of the *Astyanax* surface fish and the Pachón cave fish show a high number of mutations in cave fish putative eye genes may be explained by relaxed selection for vision during evolution in the absence of light (Hinaux et al. 2013).

The Neutral Mutation Theory relies on principles similar to Kimura's "Neutral Theory of Molecular Evolution". This latter theory holds that at the molecular level most evolutionary changes and most of the variation within and between species is not caused by natural selection but by random drift of mutant alleles that are neutral and do not affect the ability of an organism to survive and reproduce (Freese and Yoshida 1965; Kimura 1968; King and Jukes 1969; Nei et al. 2010; Nei 2013). The phenotypic variability of regressive traits, for which cave animals in particular provide many examples, would be one of the rare cases in which random neutral mutations can phenotypically manifest without being eliminated by natural selection normally acting to preserve the functional capability of a feature.

7.8.1 Variability of Regressive Traits

A generally observable and outstanding feature of functionless regressive traits is their long-lasting genetic and phenotypic variability in size and form. It occurs in all taxonomic orders and is shown, for example, by the reduced hind wings of flightless carabid ground beetles (Fig. 7.2) as well as by the rudimentary hip bones of whales (Fig. 2.2). However, it is particularly obvious in cave species. The important specific characteristic of variability is generally not adequately considered. One of the first to call special attention to it was Eigenmann (1909), who observed it while studying the eyes of the Cuban bythidid cave cusk eels, genus *Lucifuga* (Fig. 6.38). Kosswig and Kosswig (1940) recognized its general relevance for the interpretation of regressive evolutionary processes and were the first to expound the problems of the variability of functionless traits and to focus on the implication for evolution (Culver et al. 1995). They started studying the variable eyes and pigmentation in cave populations of the cave wood louse *Asellus kosswigi* (Verovnik et al. 2009; Konec et al. 2016), performing morphological as well as crossing experiments for genetic analysis (Kosswig and Kosswig 1940). The genetic basis of the variability of eyes and pigmentation of this cave isopod was later stated by QTL analyses (Protas et al. 2011).

Further examples of variability are provided by cave species of all taxonomic orders. For example, the eye rudiments of the diverse Caribbean cave shrimps genus *Typhlatya* (Fig. 3.12) (Juberthie-Jupeau 1976) and those of the cave mysid *Heteromysoides cotti* (Meyer-Rochow and Juberthie-Jupeau 1987) as well as those of the cave catfish genus *Rhamdia* (Wilkens 2001), the amblyopsid cave fish (Poulson and White 1969), and the Bahamian bythidid cave cusk eels are variable in structure and size (Wilkens et al. 1989) (Figs. 3.17 and 6.38). The live-bearing cave-living tooth carp *Poecilia mexicana* provides another interesting example (Peters and Peters 1973). Its eyes exhibit a gradient of decreasing size and increasing variability within the same cave from outside, where the ancestral well-eyed surface fish lives, into deeper cave regions (Fig. 3.14).

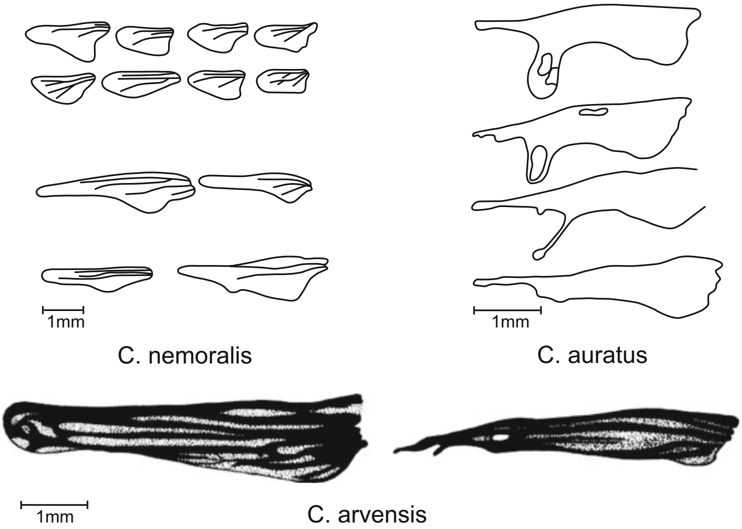

Fig. 7.2 Variability of the reduced hind wings of different flightless *Carabus* ground beetle species (Carabidae, Coleoptera) in size, structure, and form (adapted from Wilkens et al. 1979)

In the *Astyanax* cave fish, variability of regressive features is manifold, too. It exists within populations as well as between them (Fig. 6.39). Eye variability is particularly obvious in the phylogenetically young VEP cave fish populations like Micos, which exhibit externally visible eyes, the sizes of which encompass almost half of those developed in F2 crossings between surface fish and the phylogenetically old SEP cave fish (Figs. 5.4, 5.7 and 6.55a). In all cave fish, individual left-right asymmetry of size and histological differentiation of the eyes occurs (Fig. 6.48). However, variability of regressive features is not restricted to morphological traits like eyes and pigmentation but may also develop in behavioural ones. For example, the rudimentary visually released aggressive behaviour as well as the dorsal light reaction is submitted to it in the phylogenetically young VEP Micos population (Langecker et al. 1995, see Sects. 6.12 and 6.13).

Unlike in cave species, rudimentation mostly starts with an evolutionary process of change during which the function of a specific trait is gradually substituted by another one. During this phase, phenotypic variability will not appear, because stabilizing selection is still in play. Only at the end of this development, when the original function of a trait has completely been transferred to the new one, may variability arise for the one being reduced. For example, during phylogeny the hind legs of the ancestral whales were for a long time still functionally involved in promotion and could only completely regress after the tail fin had taken over that function. Only at that moment genotypic and phenotypic variability arose (Fig. 2.2). In contrast, it is an extraordinary characteristic that when species become permanently cave living, traits that depend on light to function abruptly lose their biological function without substitution.

In accordance with the recapitulation theory, which claims that during ontogeny phases of phylogeny are recapitulated, this may also be observed during the ontogeny of regressive traits; for example, variability does not exist during early ontogeny of the cave fish eye, but arises later. This could be exemplified in cave *Astyanax*. Whereas at early stages eye variability of the cave fish does not surpass that of the surface fish, it develops during later ontogeny at the adult stage. It is suggested that the eye anlage is developmentally involved in head and brain formation and therefore is still subjected to stabilizing selection. Only after this developmental constraint has been accomplished does variability arise and become obvious (see Sect. 6.21.7) (Figs. 6.43 and 6.48).

7.8.2 Loss of Behavioural Traits Not Performed in Darkness

Behavioural traits that depend on vision or light perception provide further strong support for the Neutral Mutation Theory. Such traits are not performed by the surface fish in darkness and their loss does not provide any advantage. Therefore, they are not subjected to selection. Nonetheless, their genetic basis is reduced in the cave fish. This was shown for both the visually triggered aggressive as well as the negative phototactic behaviour (see Sect. 6.12 and 6.14) (Hausberg 1995, Hofmann and Hausberg 1993, Langecker 1989, 2000), the dorsal light reaction (see Sect. 6.13) (Langecker 1993), and schooling (see Sect. 6.18) (Kowalko et al. 2013a, b; Parzefall 1993).

7.8.3 Genetic Studies

In the naked mole rat (*Heterocephalus glaber*), which is no true cave animal but lives underground in the absence of light, out of 200 genes categorized with the GO (gene ontology) term “visual perception”, 10% are pseudogenes showing insertion or deletion events (Rétaux and Casane 2013). These include two crystallins (cryBA4 and cryBB3), two out the four vertebrate opsins, and other genes involved in phototransduction and photoreceptor function. In addition, cryGS carries a point mutation. For several of these genes, including the two cited crystallins, a relaxation of functional constraints was noted, as seen through the ratio of non-synonymous substitutions, which surpasses that of synonymous ones. Thus, it seems that genes involved in visual function have been particularly targeted by loss-of-function mutations during the evolution of the naked mole rat genome, suggesting neutral evolution (Fig. 2.6) (Emerling and Springer 2014). A large-scale survey of polymorphism and fixed mutations in the transcriptome of a surface and a cave population of *Astyanax* revealed that a high proportion of the genes carrying mutations responsible for radical amino-acid changes in the cave fish lineage correspond to “eye genes”, as deduced from their strong and specific expression in the developing

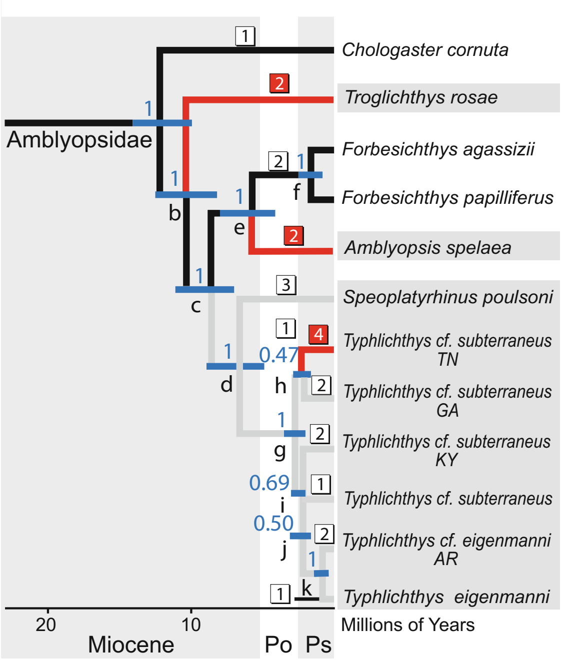

Fig. 7.3 Chronogram inferred from a multilocus divergence time analysis in amblyopsid cave and surface fish. *Blue bars* at nodes represent 95% highest posterior density intervals of age estimates. Clade posterior probabilities are indicated by *blue numbers* next to nodes. Cave lineages are indicated by *dark gray* tip labels. Branches in *black* are reconstructed as surface and *gray* branches are reconstructed as cave. Nonsynonymous substitutions in *rhodopsin* (*white* or *red square*) are mapped above branches. Lineages with loss-of-function mutations in *rhodopsin* are indicated by *red* branches and *red squares*. *AR* Arkansas, *GA* Georgia, *KY* Kentucky, *Po* to *Ps* Pleistocene to present, *TN* Tennessee (adapted from Niemiller et al. 2012)

visual system in zebrafish (Hinaux et al. 2013). This confirms that eye-related genes are also under relaxed selection in *Astyanax* cavefish.

Further evidence for repeated loss of functional constraint of rhodopsin is also present in amblyopsid cave fishes where, in at least in three cave lineages, loss-of-function mutations have independently accumulated and variability of regressive mutations is extant (Fig. 7.3) (Niemiller et al. 2012). Furthermore, intra- and inter-species analyses suggest that the “blind” clock in *Phreatichthys andruzzii* evolved because of the loss of selective constraints on a trait that was no longer adaptive. Based on this change in selective regimen, it was estimated that the functional constraint on cave fish melanopsin photo pigment (*opn4m2*) was relaxed at ~5.3 million years ago. The visual photoreceptor rhodopsin, expressed in the brain and implicated in the photophobic behaviour of *P. andruzzi*, shows similar evolutionary patterns (Calderoni et al. 2016). Also, in the subterranean bathynellean crustaceans, a reduced opsin repertoire was detected (Kim et al. 2017).

In *Astyanax* cave fish, it was shown that the genetic basis of eye reduction partially diverges in the different cave populations. The same could be shown for the albino and the brown gene (Gross et al. 2009; Protas et al. 2007; Wilkens 1971; Wilkens and Strecker 2003). All this corroborates that most of the regressive mutations originate after cave entry, occur at random, and accumulate after a species has colonized the cave habitat.

Besides such de novo mutations, which characteristically appear after cave colonization, cryptic or standing variation is hypothesized to play a role in the regression of *Astyanax* cave fish traits, also. It is assumed that standing variation already pre-exists in the ancestral surface fish. For example, the melanocortin 4 receptor (*MC4R*) gene contributing to the insatiable appetite of cave fish appears in them due to selection from standing genetic variation already present in surface populations (Aspiras et al. 2015). Also, standing variation for eye size was revealed to exist in *Astyanax* surface fish. Its phenotypic manifestation is usually masked by heat shock protein 90 (HSP90). Blocking HSP90 by the chemical inhibitor radicicol elicits large variation in eye size of larval surface fish. HSP90 is hypothesized to provide a molecular mechanism for buffering genetic variation and release it in response to environmental stress (Rohner et al. 2013).

However, independently from whether the phenotypic variability of regressive traits originates from de novo mutations and/or from standing variation, the alleles are neutral in absolute darkness and can only persist because of the loss of purifying selection. Assuming selection for regressive traits would play a role, variability should not persist for long because selection acts strongly to eliminate it.

7.9 Variability and Constructive (Darwin's) Gain

Irrespective of the presence or absence of selection, variability plays the central role in Darwin's concept of evolution. It is generally accepted that this phenomenon is not restricted to regressive processes, as might be the impression now, but is the source of origin and the starting point of constructive evolution too. However, phenotypic variability normally does not arise, because in order to preserve the optimal functional capability of a trait, variability is eliminated by selection.

Nonetheless, examples demonstrating the importance of variability for the evolutionary progress can be found in some rare cases. They are, for example, provided by the intralacustrine radiation of endemic species flocks, as is described for fish like the cichlids in the Great Lakes of East Africa (Barlow 2000; Schön and Martens 2004), the cyprinid genus *Puntius* from the Philippine Lake Lanao, the sculpins from Lake Baikal, the Arctic char (*Salvelinus alpinus*) from Lake Thingvallavatn in Iceland (Jónasson 1992, 1993, 1998), the lake whitefish genus *Coregonus* from lakes in the northern hemisphere (Schluter 1996; Turgeon et al. 1999) or the egg-laying toothcarps (Cyprinodontidae) genus *Orestias* from the Andean Lake Titicaca (Villwock 1986) and the genus *Cyprinodon* from Laguna Chichancanab in the Yucatán in Mexico (Humphries and Miller 1981). Such lakes are either characterized by geographical isolation or extreme abiotic conditions resulting from chemistries or low water temperature leading to depauperate faunas with open niches. Only limited spectrums of one or rarely more fish species are primarily able to colonize such habitats. They encounter an environment that is characterized by the absence of all those species normally occupying the various niches offered by the lake ecosystem. Generally each species is so well adapted to its specific niche that according to Gause's law no other species using the same niche can compete. However, species invading an "empty" ecosystem are no longer restricted to the original niche that they are morphologically and ecologically adapted to. Due to the lack of competing species, interspecific selection is missing and variability may arise. Now disruptive selection can act and generate ecotypic adaptive divergence, for example, in trophic differentiation to exploit different food resources. Such resource-based polymorphism can lead to different morphotypes, which might be the initial step in a speciation process and finally result in different species.

Many examples of intralacustrine speciation in fishes occur in large and geologically old lakes containing complex ecosystems. Due to their age, the process of radiation and speciation is widely advanced or already finished. In contrast, however, the endemic *Cyprinodon* species flock of Laguna Chichancanab, situated in the centre of the Yucatan Peninsula (Mexico), has recently evolved. The lake was probably totally desiccated about 8000 years ago, after which the water level was higher than today for about 5000 years. From 2000 years ago to the present day, lake levels have remained more or less stable (Covich and Stuiver 1974; Hodell et al. 1995, 2001). The Laguna Chichancanab is a small and shallow tropical lake with nearly constant water temperatures throughout the year. It provides an excellent system to study mechanisms of adaptive radiation and speciation because of the simplicity of the lake's ecosystem, its relatively small size, and its young geological age. The water is brackish and saturated with calcium sulphate. Therefore, it is intolerable to most freshwater species (Humphries and Miller 1981; Strecker 2006; Strecker et al. 1996). The invertebrate fauna is low in diversity, and the fish community originally solely comprises an endemic *Cyprinodon* species flock and only one other species, the live-bearing toothcarp *Gambusia sexradiata* (Poeciliidae) (Humphries 1984; Humphries and Miller 1981; Strecker 2006). Mitochondrial DNA data indicate that the species flock is monophyletic and evolutionarily young and has probably evolved after the lake filled up about 8000 years ago. The most likely ancestor, C. *artifrons*, still occurs in coastal lagoons today (Strecker 2006).

The *Cyprinodon* species flock consists of seven species, and a group of specimens amounting to 45–60% of the whole assembly that cannot be assigned unequivocally to one of these species (Strecker 2006). All species co-occur throughout the whole lake. Within the flock, the most common species, C. *beltrani,* is morphologically and ecologically similar to the presumed sister species of the flock, C. *artifrons*. The other members differ strikingly from C. *beltrani,* as well as from each other, mainly by head morphology (Fig. 7.4). These differences suggest trophic divergence and exploitation of different feeding niches by each species. *C. beltrani* is a substrate feeder with a diet consisting

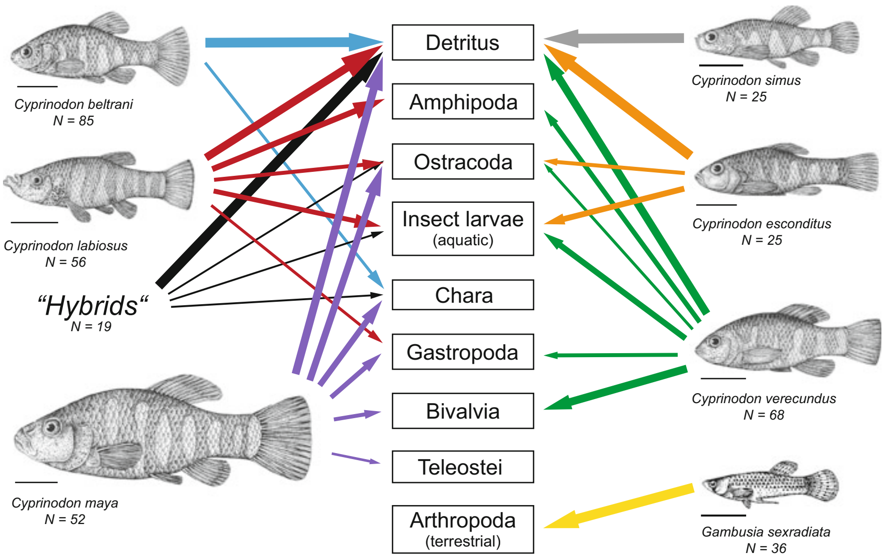

Fig. 7.4 Prey items of members of the *Cyprinodon* species flock (Cyprinodontidae) and of *Gambusia sexradiata* (Poeciliidae). *Thickness of arrows* corresponds to the mean relative dry weight, values below 1% not shown (adapted from Horstkotte and Strecker 2005)

primarily of detritus. The other six species, C. *maya,* C. *labiosus,* C. *verecundus,* C. *simus,* C. *esconditus,* and C. *suavium,* have a significantly shorter gut indicating a more carnivorous diet (Humphries 1984; Humphries and Miller 1981; Stevenson 1992; Strecker 2002, 2006, Horstkotte and Strecker 2005). Studies of gut content have revealed that all species, like C. *beltrani,* still rely on detritus as an important food item but, in contrast to it, feed to varying degrees on different animal prey. For example, the gut of C. *labiosus* contains a relatively higher amount of amphipods, that of C. *verecundus* of bivalves, and that of C. *maya* of ostracods and gastropods. The group of specimens that cannot be assigned to one of the described species of the flock has a short gut similar to the more carnivorous species (Fig. 7.4), yet, in contrast, there was no apparent preference for any particular food item. Most interestingly, no *Cyprinodon* ecotype has taken advantage of the water surface feeding niche, which was originally occupied by *P. sexradiata* (Horstkotte and Strecker 2005).

Based on mtDNA and microsatellite data, as well as behavioural studies, only the largest species, *C. maya,* shows evidence of reproductive isolation (Strecker 1996, 2006; Strecker et al. 1996; Strecker and Kodric-Brown 2000). For the other endemic species, it is unclear whether they are in the initial phases of speciation and/or if hybridization occurs among them. Female choice experiments examining chemical and visual cues in three of the species, *C. beltrani, C. labiosus*, and *C. maya,* indicate that different levels of reproductive isolation have been reached (Fig. 7.5). These differences are supported by mate choice trials allowing females to spawn with conspecific or heterospecific males (Strecker and Kodric-Brown 2000). From these behavioural experiments it is concluded that species-specific male traits, in combination with female preferences for them, differ among the three species. In *C. maya* there has been sufficient divergence to establish a premating reproductive barrier based on visual and chemical cues alone. The mate choice behaviour between *C. beltrani* and *C. labiosus* is asymmetrical (i.e. *C. beltrani* females do not discriminate between conspecific and heterospecific males whereas *C. labiosus* females prefer their conspecific males). It is likely that the *Cyprinodon* species of Laguna Chichancanab are at different stages of differentiation. The group of *Cyprinodon* that cannot be assigned unequivocally to one of the species may represent morphological plasticity, hybrids, or include as-yet undetected species (Kodric-Brown and Strecker 2001, Strecker et al. 1996).

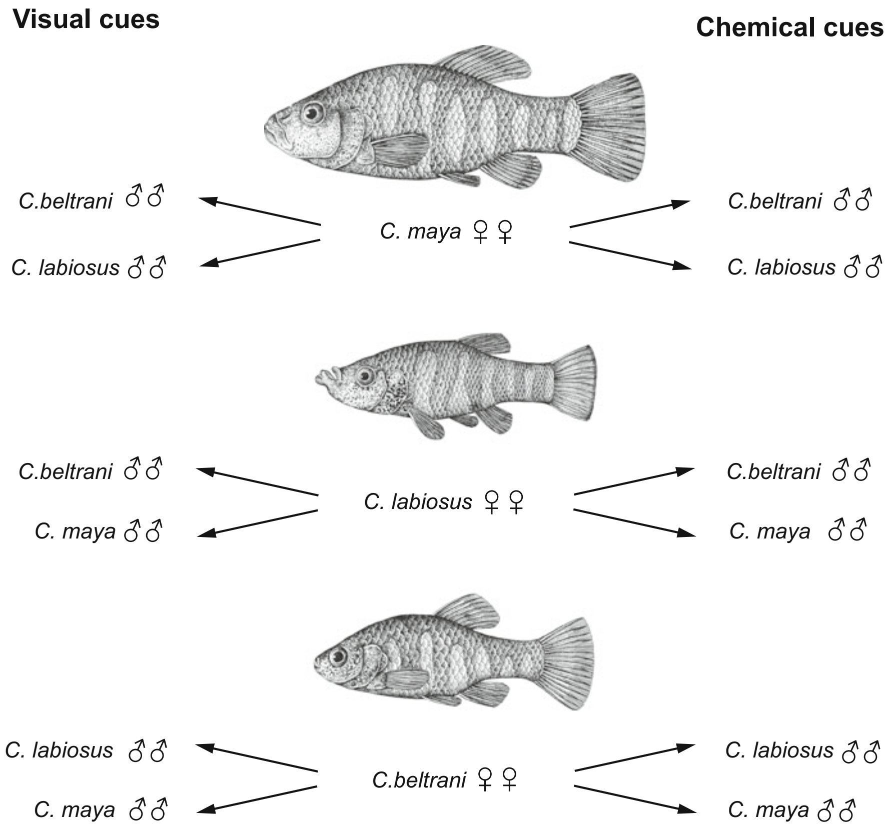

Fig. 7.5 Summary of responses of *Cyprinodon beltrani, C. labiosus,* and C. *maya* females (Cyprinodontidae) to visual and chemical cues of conspecific and heterospecific males (adapted from Kodric-Brown and Strecker 2001)

7.10 Concluding Remarks

The bizarre appearance of the blind and pale phenotype of cave animals might insinuate that the basic principles of rudimentation are exclusive to them. This understanding of a special position is reinforced by the regressive processes in cave animals being interpreted above all as dominated by limited energy supply or by the central role of eye reduction providing a pleiotropic spin-off of constructive evolution of compensatory traits like lateral line and taste senses. Anthropocentrically, cave animals are often even looked upon as suffering from the same pathological phenomena that occur in humans (Pennisi 2016). For example, "sleep loss" (Duboué et al. 2011), "food addiction" (Elipot et al. 2013), "obesity" (Aspiras et al. 2015), or the deleterious risk of the eye being hurt in darkness (Barr 1968) have all been proposed. However, caves in general are not food poor, and nor could the before-mentioned spin offs be proven. Cave animals are no outliers but demonstrate that rudimentation is a universal evolutionary phenomenon, which may in principle concern every trait of all members of each systematic group. For example, eye reduction has also evolved in fossorial mammals, flightless birds have reduced their wings, and the taste for umami got lost when the Giant Panda changed from a carnivorous to a vegetarian diet. Principles found here can also be detected in the human species (Gross and Perkins 2008). Regressive mutants causing eye defects no longer had a selective disadvantage after the transition from hunting to agriculture. To a certain degree, the same happened to human dentition after the development of cooking, barbecuing, and knives. In both traits, the existing high variability indicates relaxed selection. The ambiguous character of some mutational events, that, for example, are found in traits like changed activity patterns in cave animals, was termed adaptive gene loss in the case of the evolution of photosensitivity in vertebrates (Davies 2011). It is also demonstrated by human skin pigmentation in which it was found that light skin colour is the derived state and, like the regressive eye in different *Astyanax* cave populations, is independent in origin in Europeans and Asians, because these mutations occurred at random, whereas dark skin colour seems to reflect the ancestral state (Lao et al. 2007). Regressive mutations of dark pigmentation are neutral in northern latitudes and genetically independently facilitated vitamin D synthesis. In contrast, they were deleterious close to the equator, where they were eliminated by stabilizing selection (Beleza et al. 2012). Cave animals, in particular, when closely related sister surface forms are still available, play a prime role in the study of evolutionary processes.

References

Aspiras AC, Rohner N, Martineau B et al (2015) Melanocortin 4 receptor mutations contribute to the adaptation of cavefish to nutrient-poor conditions. Proc Natl Acad Sci U S A 112: 9668–9673

Barlow GW (2000) The cichlid fishes. Nature's grand experiment in evolution. Perseus Publishing, Cambridge

Barr TC (1968) Cave ecology and the evolution of troglobites. In: Dobzhansky T, Hecht MK, Steere WC (eds) Evolutionary biology. Plenum Press, New York, pp 35–101

Barton RA, Harvey PH (2000) Mosaic evolution of brain structure in mammals. Nature 405: 1955–1058

Beleza JC, Santos AM, McEvoy B et al (2012) The timing of pigmentation lightening in Europeans. Mol Biol Evol 30(1):24–35. doi:10.1093/molbev/mss207

Besharse JC, Brandon RA (1974) Effects of continuous light and darkness on the eyes of the troglobitic salamander *Typhlotriton spelaeus*. J Morphol 149:527–546

Bibliowicz J, Alié A, Père S et al (2013) Differences in chemosensory response between eyed and eyeless *Astyanax mexicanus* of the Rio Subterráneo cave. EvoDevo 4:25–31

Bilandžija H, Parkhurst A, Jeffery WR (2013) A potential benefit of albinism in *Astyanax* cavefish: downregulation of the *oca2* gene increases tyrosine and catecholamine levels as an alternative to melanin synthesis. PLoS One 8:1–14

Borowsky RL (2015) Regressive evolution: testing hypotheses of selection and drift. In: Keene AC, Yoshizawa M, McGaugh SE (eds) Biologiy and evolution of the Mexican cavefish. Elsevier, Amsterdam, pp 93–107

Borowsky RL, Cohen D (2013) Genomic consequences of ecological speciation in *Astyanax* cavefish. PLoS One 8(11):e79903. doi:10.1371/journal.pone.0079903

Calderoni L, Rota-Stabelli O, Frigato E et al (2016) Relaxed selective constraints drove functional modifications in peripheral photoreception of the cavefish *P. andruzzii* and provide insight into the time of cave colonization. Heredity 117:383–392

Covich A, Stuiver M (1974) Changes in oxygen 18 as a measure of long-term fluctuations in tropical lake levels and molluscan populations. Limnol Oceanogr 19:682–691

Crish SD, Dengler-Crish C, Catania KC (2006) Central visual system of the naked mole-rat (*Heterocephalus glaber*). Anat Rec A Discov Mol Cell Evol Biol 288(2):205–212

Culver DC (1987) Eye morphometrics of cave and spring populations of *Gammarus minus* (Amphipoda: Gammaridae). J Crustac Biol 7:136–147

Culver DC, Kane TC, Fong DW (1995) Adaptation and natural selection in caves. Harvard University Press, Cambridge

Davies WL (2011) Adaptive gene loss in vertebrates: photosensitivity as a model case. In: Encyclopedia of life sciences (ELS). Wiley, Chichester. doi:10.1002/9780470015902.a0022890

Deharveng L, Bedos A (2000) The cave fauna of Southeast Asia. Origin, evolution and ecology. In: Wilkens H, Culver DC, Humphreys WF (eds) Ecosystems of the world: subterranean ecosystems, vol 30. Elsevier, Amsterdam, pp 603–632

Dillman CB, Bergstrom DE, Noltie DB et al (2010) Regressive progression, progressive regression or neither? Phylogeny and evolution of the Percopsiformes (Teleostei, Paracanthopterygii). Zool Scr 40:45–60

Dollo L (1893) Les lois de l'évolution. Bull Soc Belge Geol Pal Hydr VII:164–166

Duboué ER, Keene AC, Borowsky RL (2011) Evolutionary convergence on sleep loss in cavefish populations. Curr Biol 21:671–676

Eigenmann CH (1909) Cave vertebrates of America. Carnegie Inst Wash Publ 104:1–241

Elipot Y, Hinaux H, Callebert J et al (2013) Evolutionary shift from fighting to foraging in blind cavefish through changes in the serotonin network. Curr Biol 23:1–10

Ellis R (1996) Deep Atlantic: life, death, and exploration in the abyss. The Lions Press, New York

Emerling CA, Springer MS (2014) Eyes underground: regression of visual protein networks in subterranean mammals. Mol Phylogenet Evol 78:260–270

Espinasa L, Borowsky RL (2000) Eyed cave fish in a karst window. J Cave Karst Stud 62:180–183

Fernandes CS, Batalha MA, Bichuette ME (2016) Does the cave environment reduce functional diversity? PLoS One 11(3):e0151958. doi:10.1371/journal.pone.0151958

Freese E, Yoshida A (1965) The role of mutations in evolution. In: Bryson V, Vogel HJ (eds) Evolving genes and proteins. Academic, New York, pp 341–355

Gnaspini P, Trajano E (2000) Guano communities in tropical caves. In: Wilkens H, Culver DC, Humphreys WF (eds) Ecosystems of the world: subterranean ecosystems, vol 30. Elsevier, Amsterdam, pp 251–268

Gross JM, Perkins BD (2008) Zebrafish mutants as models for congenital ocular disorders in humans. Mol Reprod Dev 75:547–555

Gross JB, Borowsky RL, Tabin CJ (2009) A novel role for *Mc1r* in the parallel evolution of depigmentation in independent populations of the cave fish, *Astyanax mexicanus*. PLoS Genet 5:1–14

Hausberg C (1995) Das Aggressionsverhalten von *Astyanax fasciatus* (Cuvier 1819; Characidae, Teleostei): Ontogenie, Genetik und Evolution bei der epigäischen und hypogäischen Form. Dissertation, University of Hamburg

Hinaux H, Poulain J, Da Silva C et al (2013) De novo sequencing of *Astyanax mexicanus* surface fish and Pachón cavefish transcriptomes reveals enrichment of mutations in cavefish putative eye genes. PLoS One 8(1):e53553. doi:10.1371/journal.pone.0053553

Hinaux H, Blin M, Fumey J et al (2015) Lens defects in Astyanax mexicanus cavefish: evolution of crystallins and a role for alphaA-crystallin. Dev Neurobiol 75(5):505–521

Hinaux H, Devos L, Blin M et al (2016) Sensory evolution in blind cavefish is driven by early embryonic events during gastrulation and neurulation. Development 143:4521–4532. doi:10.1242/dev.141291

Hoch H (2000) Acoustic communication in darkness. In: Wilkens H, Culver DC, Humphreys WF (eds) Ecosystems of the world: subterranean ecosystems, vol 30. Elsevier, Amsterdam, pp 211–220

Hodell DA, Curtis JH, Brenner M (1995) Possible role of climate in the collapse of classic maya civilization. Nature 375:391–394

Hodell DA, Brenner M, Curtis JH et al (2001) Solar forcing of drought frequency in the Maya lowlands. Science 292:1367–1370

Hoffman S, Hausberg C (1993) The aggressive behavior of the Micos cave population *(Astyanax fasciatus,* Characidae, Teleostei) after selection for functional eyes in comparison to an epigean one. Mémoires de Biospéléologie 20:101–103

Horstkotte J, Strecker U (2005) Trophic differentiation in the phylogenetically young *Cyprinodon* species flock (Cyprinodontidae, Teleostei) from Laguna Chichancanab (Mexico). Biol J Linn Soc 85:125–134

Huber R, Staaden MJ van, Kaufman LS et al (1997) Microhabitat use, trophic patterns, and the evolution of brain structure in African cichlids. Brain Behav Evol 50:167–182

Humphries JM (1984) *Cyprinodon verecundus* n. sp., a fifth species of pupfish from Laguna Chichancanab. Copeia 1984:58–68

Humphries JM, Miller RR (1981) A remarkable species flock of *Cyprinodon* from Lake Chichancanab, Yukatan, Mexico. Copeia 1981:52–64

Hüppop K (1989) Genetic analysis of oxygen consumption rate in cave and surface fish of *Astyanax fasciatus* (Characidae, Pisces). Further support for the neutral mutation theory. Mémoires de Biospéléologie 16:163–168

Hüppop K (2000) How do cave animals cope with the food scarcity in caves? In: Wilkens H, Culver DC, Humphreys WF (eds) Ecosystems of the world: subterranean ecosystems, vol 30. Elsevier, Amsterdam, pp 159–188

Hüppop K (2012) Adaptation to low food. In: White WB, Culver DC (eds) Encyclopedia of caves, 2nd edn. Elsevier, Amsterdam, pp 1–9

Hüppop K, Wilkens H (1991) Bigger eggs in subterranean *Astyanax fasciatus*. Z Zool Syst Evol 29:280–288

Jeffery WR (2005) Adaptive evolution of eye degeneration in the Mexican blind cavefish. J Hered 96:185–196

Jeffery WR, Ma L, Parkhurst A et al (2015) Pigment regression and albinism in *Astyanax* cavefish. In: Keene AC, Yoshizawa M, McGaugh SE (eds) Biology and evolution of the Mexican cavefish. Elsevier, Amsterdam, pp 155–171

Jónasson PM (1992) The ecosystem of Thingvallavatn: a synthesis. Oikos 64:405–434

Jónasson PM (1993) Continental rifting-energy pathways of two contrasting rift lakes. Verhandlungen des Internationalen Vereins für Limnologie 25:1–14

Jónasson PM (1998) Continental rifting and habitat formation: Arena for resource polymorphism in Arctic charr. Ambio 27:162–169

Juberthie-Jupeau L (1976) Sur le système neurosécréteur du pédoncule oculaire d'un décapode souterrain microphthalme *Typhlatya garciai* Chace. Ann Spéléol 31:107–114

Katz PS (2011) Neural mechanisms underlying the evolvability of behavior. Philos Trans R Soc Lond B Biol Sci 366:2086–2090

Katz PS, Lillvis JL (2014) Reconciling the deep homology of neuromodulation with the evolution of behavior. Curr Opin Neurobiol 29:39–47. doi:10.1016/j.conb.2014.05.002

Kim B-M, Kang S, Ahn DH et al (2017) First insights into the subterranean crustacean Bathynellacea transcriptome: transcriptionally reduced opsin repertoire and evidence of conserved homeostasis regulatory mechanisms. PLoS One 12(1):e0170424. doi:10.1371/journal

Kimura M (1968) Evolutionary rate at the molecular level. Nature 217:624–626

King JL, Jukes TH (1969) Non-Darwinian evolution. Science 164:788–797

Kodric-Brown A, Strecker U (2001) Responses of *Cyprinodon maya* and *C. labiosus* females to visual and olfactory cues of conspecific and heterospecific males. Biol J Linn Soc 74:541–548

Konec M, Delić T, Trontelj P (2016) DNA barcoding sheds light on hidden subterranean boundary between Adriatic and Danubian drainage basins. Ecohydrology 9:1304–1312. doi:10.1002/eco.1727

Kosswig C (1935) Die Evolution von "Anpassungs"-Merkmalen bei Höhlentieren in genetischer Betrachtung. Zool Anz 112:148–155

Kosswig C (1949) Phänomene der regressiven Evolution im Lichte der Genetik. Communications de la Faculté des Sciences de l'Université d'Ankara 8:110–150

Kosswig C (1960a) Darwin und die degenerative evolution. Abhandlungen und Verhandlungen des Naturwissenschaftlichen Vereins Hamburg NF 4:21–42

Kosswig C (1960b) Zur Phylogenese sogenannter Anpassungsmerkmale bei Höhlentieren. Int Rev Ges Hydrobiol 45:493–512

Kosswig C (1976) Génétique et l'évolution regressive - Mécanismes de la rudimentation des organes chez les embryons de vertébrés. Colloq Int CNRSN 266:19–29

Kosswig C, Kosswig L (1940) Die Variabilität bei *Asellus aquaticus*, unter besonderer Berücksichtigung der Variabilität in isolierten unter- und oberirdischen Populationen. Révue de la Faculté des Sciences Istanbul 5:1–55

Kowalko JE, Rohner N, Linden TA et al (2013a) Convergence in feeding posture occurs through different loci in independently evolved cave populations of *Astyanax mexicanus*. Proc Natl Acad Sci U S A 110:16933–16938

Kowalko JE, Rohner N, Rompani SB et al (2013b) Loss of schooling behavior in cavefish through sight-independent and sight-dependent mechanisms. Curr Biol 23:1874–1883

Krishnan J, Rohner N (2017) Cavefish and the basis for eye loss. Philos Trans R Soc B 372(1713):20150487. doi:10.1098/rstb.2015.0487

Langecker TG (1989) Studies on the light reaction of epigean and cave populations of *Astyanax fasciatus* (Characidae, Pisces). Mem Biospeleol 16:169–176

Langecker TG (1993) Genetic analysis of the dorsal light reaction in epigean and cave-dwelling *Astynnax fasciatus* (Teleostei, Characidae). Ethol Ecol Evol 3:357–364

Langecker TG (2000) The effects of continuous darkness on cave ecology and cavernicolous evolution. In: Wilkens H, Culver DC, Humphreys WF (eds) Ecosystems of the world: subterranean ecosystems, vol 30. Elsevier, Amsterdam, pp 135–157

Langecker TG, Neumann B, Hausberg C et al (1995) Evolution of the optical releasers for aggressive behavior in cave-dwelling *Astyanax fasciatus* (Teleostei, Characidae). Behav Process 34:161–168

Langecker TG, Wilkens H, Parzefall J (1996) Studies of the trophic structure of an energy-rich Mexican cave (Cueva de las Sardinas) containing sulfurous water. Mém Biospéléol 23: 121–125

Lao O, de Gruijter JM, Duijn K v et al (2007) Signatures of positive selection in genes associated with human skin pigmentation as revealed from analyses of single nucleotide polymorphisms. Ann Hum Genet 71:354–369

Menuet A, Alunni A, Joly JS et al (2007) Expanded expression of Sonic Hedgehog in *Astyanax* cavefish: multiple consequences on forebrain development and evolution. Development 134:845–855. doi:10.1242/dev.02780

Meyer-Rochow VB, Juberthie-Jupeau L (1987) An electron microscope study of the eye of the cave mysid Heteromysoides cotti from the island of Lanzarote (Canary Islands). Stygologia 3:24–33

Mitchell RW, Russell WH, Elliott WR (1977) Mexican eyeless characin fishes, genus *Astyanax*: environment, distribution, and evolution. Special publications of the Museum Texas Tech University 12:1–89

Moran D, Softley R, Warrant EJ (2015) The energetic cost of vision and the evolution of eyeless Mexican cavefish. Sci Adv 1(8):e1500363. doi:10.1126/sciadv.1500363

Muller HJ (1949) The Darwinian and modern conceptions of natural selection. Proc Am Philos Soc 93:459–470

Nei M (2013) Mutation-driven evolution. Oxford University Press, Oxford

Nei M, Suzuki Y, Nozawa M (2010) The neutral theory of molecular evolution in the genomic era. Annu Rev Genomics Hum Genet 11:265–289

Niemiller ML, Fitzpatrick BM, Shah P et al (2012) Evidence for repeated loss of selective constraint in rhodopsin of amblyopsid cavefishes (Teleostei: Amblyopsidae). Evolution 67:732–748

Niven JE, Anderson JC, Laughlin SB (2007) Fly photoreceptors demonstrate energy-information trade-offs in neural coding. PLoS Biol 5:828–840

Orr HA (1998) Testing natural selection vs. genetic drift in phenotypic evolution using quantitative trait loci. Genetics 149:2099–2104

Palacios M, Voelker G, Rodriguez LA et al (2016) Phylogenetic analyses of the subgenus *Mollienesia* (Poecilia, Poeciliidae, Teleostei) reveal taxonomic inconsistencies, cryptic biodiversity, and spatio-temporal aspects of diversification in Middle America. Mol Phylogenet Evol 103:230–244

Parzefall J (1993) Schooling behaviour in population-hybrids of *Astyanax fasciatus* and *Poecilia mexicana* (Pisces, Characidae and Poeciliidae). In: Schröder JH, Bauer J (eds) Trends in ichthyology: an international perspective. Blackwell Scientific, Oxford, pp 297–303

Parzefall J (2001) A review of morphological and behavioural changes in the cave molly, *Poecilia mexicana*, from Tabasco, Mexico. Environ Biol Fishes 62:263–275

Pennisi E (2016) Blind cave fish may provide insights into human health. Science 352(6293): 1502–1503. doi:10.1126/science.352.6293.1502

Peters N, Peters G (1968) Zur genetischen interpretation morphologischer Gesetzmäßigkeiten der degenerativen evolution. Zeitschrift Morphologie der Tiere 62:211–244

Peters N, Peters G (1973) Genetic problems in the regressive evolution of cavernicolous fish. In: Schröder JH (ed) Genetics and mutagenesis of fish. Springer, Berlin, pp 187–201

Peters N, Scholl A, Wilkens H (1975) Der Micos-Fisch, Höhlenfisch in statu nascendi oder Bastard ? Ein Beitrag zur Evolution der Höhlentiere. J Zool Syst Evol Res 13:110–124

Peters N, Schacht V, Schmidt W et al (1993) Gehirnproportionen und Ausprägungsgrad der Sinnesorgane von *Astyanax mexicanus* (Pisces, Characinidae). Z Zool Syst Evol 31:144–159

Poulson TL, White WB (1969) The cave environment: limestone caves provide unique natural laboratc for studying biological and geological processes. Science 165:971–981

Protas M, Conrad M, Gross JB et al (2007) Regressive evolution in the Mexican Cave Tetra, *Astyanax mexicanus*. Curr Biol 17:452–454. doi:10.1016/j.cub.2007.01.051

Protas ME, Trontelj P, Patela NH (2011) Genetic basis of eye and pigment loss in the cave crustacean, *Asellus aquaticus*. Proc Natl Acad Sci U S A 108:5702–5707. doi:10.1073/pnas.1013850108

Rétaux S, Casane D (2013) Evolution of eye development in the darkness of caves: adaptation, drift, or both? EvoDevo 4(1):1–12

Rétaux S, Pottin K, Alunni A (2008) *Shh* and forebrain evolution in the blind cavefish *Astyanax mexicanus*. Biol Cell 100:139–147

Rodrigues FR (2013) *Comparison of brain and cranial nerve morphology between eyed surface fish and blind cave fish of the species Astyanax mexicanus.* Dissertation, Universidade de Lisboa

Rohner N, Jarosz DF, Kowalko JE et al (2013) Cryptic variation in morphological evolution: HSP90 as a capacitor for loss of eyes in cavefish. Science 342:1372–1375

Schemmel C (1967) Vergleichende Untersuchungen an den Hautsinnesorganen ober- und unterirdisch lebender *Astyanax*-Formen. Zeitschrift Morphologie der Tiere 61:255–316

Schemmel C (1980) Studies on the genetics of feeding behavior in the cave fish *Astyanax mexicanus* f. Anopthichthys. An example of apparent monofactorial inheritance by polygenes. Z Tierpsychol 53:9–22

Schluter D (1996) Ecological speciation in postglacial fishes. Philos Trans Biol Sci 351:807–814

Schön I, Martens K (2004) Adaptive, pre-adaptive and non-adaptive components of radiations in ancient lakes: a review. Organ Divers Evol 4:137–156

Stevenson MM (1992) Food habits within the Laguna Chichancanab *Cyprinodon* (Pisces: Cyprinodontidae) species flock. Southwest Nat 37:337–343

Strecker U (1996) Molekulargenetische und ethologische Untersuchungen zur Speziation eines Artenschwarmes der Gattung *Cyprinodon* (Cyprinodontidae, Teleostei). Universität, Hamburg

Strecker U (2002) *Cyprinodon esconditus,* a new pupfish from Laguna Chichancanab, Yucatan, Mexico (Cyprinodontidae). Cybium 26:301–307

Strecker U (2006) Genetic differentiation and reproductive isolation in a *Cyprinodon* fish species flock from Laguna Chichancanab, Mexico. Mol Phylogenet Evol 39:865–872

Strecker U, Kodric-Brown A (2000) Mating preferences in a species flock of Mexican pupfishes (Cyprinodon, Teleostei). Biol J Linn Soc 71:677–687

Strecker U, Meyer CG, Sturmbauer C et al (1996) Genetic divergence and speciation in an extremely young species flock in Mexico formed by the genus *Cyprinodon* (Cyprinodontidae, Teleostei). Mol Phylogenet Evol 6:143–149

Strecker U, Hausdorf B, Wilkens H (2012) Parallel speciation in *Astyanax* cave fish (Teleostei) in Northern Mexico. Mol Phylogenet Evol 62:62–70

Strickler AG, Yamamoto Y, Jeffery WB (2007) The lens controls cell survival in the retina: evidence from the blind cavefish *Astyanax*. Dev Biol 311:512–523

Turgeon J, Estoup A, Bernatchez L (1999) Species flock in the North American Great Lakes: molecular ecology of Lake Nipigon ciscoes (Teleostei, Coregonidae: *Coregonus*). Evolution 53:1857–1871

Verovnik R, Prevorčnik S, Jugovic J (2009) Description of a neotype for *Asellus aquaticus* Linné, 1758 (Crustacea: Isopoda: Asellidae), with description of a new subterranean Asellus species from Europe. Zool Anz - J Comp Zool 248(2):101–118

Villwock W (1986) Speciation and adaptive radiation in Andean *Orestias* fishes. In: Vuilleumier F, Monasterio MM (eds) High altitude tropical biogeography. University Press, Oxford, pp 387–403

Wilkens H (1971) Genetic interpretation of regressive evolutionary processes: studies on hybrid eyes of two *Astyanax* cave populations (Characidae, Pisces). Evolution 25:530–544

Wilkens H (1976) Genotypic and phenotypic variability in cave animals. Studies on a phylogenetically young cave population of *Astyanax mexicanus* (Fillippi). Annales de Spéléologie 3:137–148

Wilkens H (1988) Evolution and genetics of epigean and cave *Astyanax fasciatus* (Characidae, Pisces). Support for the Neutral Mutation Theory. In: Hecht MK, Wallace B (eds) Evolutionary biology, vol 23. Plenum, New York, pp 271–367

Wilkens H (2001) Convergent adaptations to cave life in the *Rhamdia laticauda* catfish group (Pimelodidae, Teleostei). Environ Biol Fishes 62:251–261

Wilkens H (2010) Genes, modules and the evolution of cave fish. Heredity 105:413–422

Wilkens H (2016) Genetics and hybridization in surface and cave *Astyanax* (Teleostei): a comparison of regressive and constructive traits. Biol J Linn Soc 118:911–928

Wilkens H, Strecker U (2003) Convergent evolution of the cave fish *Astyanax* (Characidae, Teleostei): genetic evidence from reduced eye size and pigmentation. Biol J Linn Soc 80: 545–554

Wilkens H, Peters N, Schemmel C (1979) Gesetzmäßigkeiten der regressiven Evolution. Verh Dtsch Zool Ges 1979:123–140

Wilkens H, Strecker U, Yager J (1989) Eye reduction and phylogenetic age in ophidiiform cave fish. Z Zool Syst Evol 27:126–134

Yamamoto Y, Stock DW, Jeffery WR (2004) Hedgehog signalling controls eye degeneration in blind cavefish. Nature 431:844–847

Yamamoto Y, Byerly MS, Jackman WR et al (2009) Pleiotropic functions of embryonic sonic hedgehog expression link jaw and taste bud amplification with eye loss during cavefish evolution. Dev Biol 330:200–211

Yang X-L, Tornquist K, Dowling JE (1988a) Modulation of cone horizontal cell activity in the teleost fish retina. I. Effects of prolonged darkness and background illumination on light responsiveness. J Neurosci 8:2259–2268

Yang X-L, Tornquist K, Dowling JE (1988b) Modulation of cone horizontal cell activity in the teleost fish retina. II. Role of interplexifoem cells and dopamine in regulating light responsiveness. J Neurosci 8:2269–2278

Yoshizawa M, Yamamoto Y, O'Quin KE et al (2012) Evolution of an adaptive behavior and its sensory receptors promotes eye regression in blind cavefish. BMC Biol 10:108–123

Yoshizawa M, Robinson BG, Duboué ER (2015) Distinct genetic architecture underlies the emergence of sleep loss and prey-seeking behavior in the Mexican cavefish. BMC Biol 13: 15–27

Zeutzius I, Probst W, Rahmann H (1984) Influence of dark-rearing on the ontogenetic development of Sarotherodon mossambicus (Cichlidae, Teleostei): II. Effects on allometric growth relations and differentiation of the optic tectum. Exp Biol 43:87–96