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LETTER TO THE EDITOR

Variability and loss of functionless traits in cave animals. Reply to Jeffery (2010)

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Studies of the evolution of cave animals mostly omit two important facts. Firstly, the focus is on eye regression and loss of melanin pigment, but the reduction of the many other traits, which have become functionless because of the absence of light as a transporter of information is often not considered. In *Astyanax* cave fish, these traits are various types of body colour, circadian rhythm, pineal organ, dorsal light reaction, schooling and visually released aggressive behaviour. Secondly, the conspicuous variability of regressive traits is rarely considered. Variability in eyes, pigmentation and aggressive behaviour develop at both the population and individual levels. It is enhanced by left–right asymmetry, because the eye rudiments of one specimen may diverge in size and show divergent structural differentiation. Asymmetry of the eye was described for brotulid, poeciliid and pimelodid cave fish (Wilkens, 2010), and also for cave isopods and mysids (Kosswig, 1940).

Attempts to explain this variability led to the proposal that such traits were no longer under selection (Kosswig, 1940). Their variability and ultimate regression was suggested to be caused by the accumulation of mutations, which were no longer eliminated by selection, which usually acts to preserve functional capability.

Because of the failure to detect specific selective forces responsible for the regression of functionless traits, selection was suggested to be mediated by pleiotropy. The most recent case was proposed by Yamamoto *et al*. (2009). They found, in *Astyanax*, by conditional over-expression of an injected *shh* transgene at specific times in development, that taste bud amplification and eye degeneration are sensitive to *shh* over-expression during the same early developmental period. From this observation, it was concluded that pleiotropy mediated developmental trade-offs between the regressive eye and oral/pharyngeal constructive traits, such as jaw size and taste bud number. They attempted to verify this inverse relationship using morphological techniques in an F3 generation, which is an unusual tool for genetic analysis. Whereas each individual in an F2 is an unbiased sample of alleles in the original F1, this is not true for an F3. It is a biased sample, which may actually create a spurious correlation. Furthermore, the findings of Yamamoto *et al*. (2009) are in contrast to QTL (Protas *et al*., 2007) and morphological studies, which did not reveal correlations between eye size and taste bud number. Jeffery (2010) cited a QTL study (Protas *et al*., 2008) as support for the pleiotropy hypothesis. However, this reflects a selective reading of the paper, in which it was shown that pleiotropy was nearly universal. Instead of finding genetic correlations specifically between regressive (R) and constructive (C) characters, wherein the evolution of R would be driven indirectly by selection for C, as in Jeffery's interpretation, what was seen was that the correlations were of all types, R with R, R with C, and C with C. (I am grateful to an anonymous referee for drawing my attention to this point.).

Furthermore, Varatharasan *et al*. (2009) showed in comparative studies of surface and cave fish that 'differences in taste bud numbers become notable at around 12 days post fertilisation, and are significantly different by 22 days post fertilisation, which is well after the stages investigated by Yamamoto *et al*. (2009)' at 6 days post fertilisation. They also 'believe the wider cavefish mouth cannot alone account for the substantially elevated numbers of taste buds in cave fish', as implied by Yamamoto *et al*. (2009), but confirmed that taste bud density in the cave fish is increased in comparison to the surface fish.

Yamamoto and Jeffery (2000) concluded from transplantation of surface lenses into cave fish eyes that there is a 'Central Role for the Lens in Cave Fish Eye Degeneration'. Later, they found a second factor located in the optic cup (possibly the pigment epithelium) that governs eye regression. One of the explanations could be that the chromophore *11-cis* retinal, which is produced in the pigment epithelium, together with the protein opsin, which is built in the visual cell, are a prerequisite for the folding of the outer segment disc membranes (Wilkens, 2007).

However, I doubt that the lens is 'necessary to prevent cell death in the retina': (1) After apoptosis, during early ontogeny, the cave fish rudimentary retinas subsequently returned to normal growth when left with the degenerate lenses (Wilkens, 2007). (2) The 'complete recovery' of the eye was concluded from the detection of many more opsin-expressing cells in the retina of the transplanted compared with the control eye by immunostaining. However, a really complete recovery would require the existence of visual cell outer segments, including the disc membranes. These are missing in the immunostained eye sections presented for the cave fish host retina in comparison with the surface fish, indicated by the cave fish host pigment epithelium being flat and not showing the cubic cell shape developing when combined with outer segments (Yamamoto and Jeffery, 2000, Figure 4g and e respectively). Such opsin signals also occurred transiently in the embryonic eye rudiments of the strongly reduced Piedras cave fish (Wilkens, 2010). Furthermore, the differences in the number of opsin-expressing cells probably resulted from left–right asymmetry, as described for the Pachón cave population (Peters and Peters, 1966; Figure 1).

Alternatively, I propose that after induction of the lens placode by the eye cup the dioptric (lens, pupil) and the retinal (including pigment epithelium) part develop independently from each other (see histological and

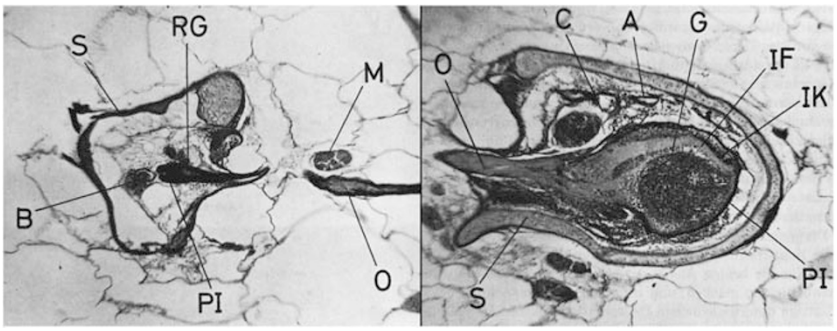

Figure 1 Histological sections of the left and the right eye of a Pachón cave fish specimen (from Peters and Peters, 1966). A, argentea; B, blood vessel; C, chorioid; G, ganglionic layer; IF, inner plexiform layer; IN, inner nuclear layer; M, muscle tissue; O, optic nerve; PI, pigment epithelium; RG, nerve tissue; S, sclera.

crossing analyses; Wilkens, 1988, 2007, 2010). This proposal fits into the general scheme of developmental independence of constructive and regressive traits at the phenotypic level in *Astyanax* cave fish that was suggested to be advantageous, because of the improved adaptive evolutionary flexibility it provides (Wilkens 2010).

As variability is a characteristic of other regressive traits, I suggest that eyes are subjected to similar patterns of regression. Therefore, I suggest that variability and loss of functionless traits are driven by the accumulation of neutral mutations. It might be argued that variability results from antagonistic and variable polarities of pleiotropic factors. However, this would not explain why all biologically functionless traits finally get completely reduced.

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References

Jeffery WR (2010). Pleiotropy and eye degeneration in cavefish. *Heredity* **105**: 495–496.

Kosswig C (1940). Die Variabilität bei *Asellus aquaticus*, unter besonderer Berücksichtigung der Variabilität in unter- und oberirdischen Populationen. *Fen Fakültesi Mecmuasi* **5**: 1–55.

Peters N, Peters G (1966). Das Auge zweier Höhlenformen von *Astyanax mexicanus* (Philippi) (Characidae, Pisces). *Roux Arch Dev Biol* **157**: 393–414.

Protas M, Conrad M, Gross JB, Tabin C, Borowsky R (2007). Regressive evolution in the Mexican cave tetra, *Astyanax mexicanus*. *Curr Biol* **18**: R27–R29.

Protas M, Tabansky I, Conrad M, Gross JB, Vidal O, Tabin CJ (2008). Multi-trait evolution in a cave fish, *Astyanax mexicanus*. *Evol Dev* **10**: 196–209.

Varatharasan N, Croll RP, Franz-Odendaal T (2009). Taste bud development and patterning in sighted and blind morphs of *Astyanax mexicanus*. *Dev Dynam* **238**: 3056–3064.

Wilkens H (1988). Evolution and genetics of epigean and cave *Astyanax fasciatus* (Characidae, Pisces). *Evol Biol* **23**: 271–367.

Wilkens H (2007). Regressive evolution: ontogeny and genetics of cave fish eye rudimentation. *Biol J Linnean Soc* **92**: 287–296.

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

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

Yamamoto Y, Jeffery WR (2000). Central role for the lens in cave fish eye degeneration. *Science* **289**: 631–633.