

Regressive Evolution in the Mexican Cave Tetra, *Astyanax mexicanus*

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Summary

The evolutionary forces driving the reduction of eyes and pigmentation in cave-adapted animals are unknown; Darwin famously questioned the role of natural selection in eye loss in cave fishes: “As it is difficult to imagine that eyes, although useless, could be in any way injurious to animals living in darkness, I attribute their loss wholly to disuse” [1]. We studied the genetics of eye and pigmentation regression in the Mexican cave tetra, *Astyanax mexicanus*, by mapping and quantitative trait loci (QTL) analysis. We also mapped QTL for the putatively constructive traits of jaw size, tooth number, and numbers of taste buds. The data suggest that eyes and pigmentation regressed through different mechanisms. Cave alleles at every eye or lens QTL we detected caused size reductions, consistent with evolution by natural selection but not with drift. QTL polarities for melanophore number were mixed, however, consistent with genetic drift. Arguments against a role for selection in the regression of cave-fish eyes cited the insignificant cost of their development [2, 3], but we argue that the energetic cost of their maintenance is sufficiently high for eyes to be detrimental in the cave environment. Regression can be caused either by selection or drift.

Results and Discussion

Absence of light drives the evolution of cave animals toward a suite of characteristic, cave-related (troglomorphic) phenotypes. In the dark, eyes and pigmentation lose their functions and tend to regress or disappear over the generations. Without light there is no photosynthesis, and the trophic base of many cave communities is narrow. Cave animals typically cope with the scarcity of food by evolving more sensitive tactile and chemical senses and slower or more efficient metabolisms. Compensatory changes like these probably evolve because of strong selection, but what causes the regression of eyes and pigmentation? The three modern competing

hypotheses for eye regression are natural selection, recurrent mutation with genetic drift, and pleiotropy [2].

Astyanax mexicanus is an ideal species for studying the genetics of troglomorphy because it has both eyed surface and cave-adapted populations, all of which are interfertile. Cave fish were collected from Pachón cave in NE Mexico (locality map in [4]), and surface fish were collected from nearby streams (Figure S1 in the Supplemental Data available with this article online). We hybridized Pachón-cave and surface fish and thus created a mapping progeny of 539 F₂ siblings. We mapped 178 loci in the cross (2191 cM) for an average distance between adjacent markers of 14.7 cM. We phenotyped the F₂ fish by measuring eye size and lens size, counting the density of melanophores in four places on the bodies, measuring the lengths of the dentary and maxillary bones in the jaw apparatus, and counting maxillary teeth and taste buds (Table S1 lists sample sizes for the different traits). This gave us a set of standardized phenotypes that could be correlated with genotypes. Phenotypic and genotypic data (see Supplemental Data) were used for identifying chromosome regions where genes affecting the traits were located. Quantitative trait loci (QTL) were detected in two phases: first by simple interval mapping (SIM) of putative QTL and then by a refinement phase with multiple interval mapping algorithms (MIM). We used MultiQTL software (www.multiqtl.com), with $p < 0.05$ and a false detection rate < 0.10 (see Supplemental Experimental Procedures).

With few exceptions, phenotypic correlations among traits in the F₂ are weak or nonexistent (Table S1). Not all correlations could be determined because some traits (notably lens sizes) were assessed in different siblings, but of the 26 correlations we calculated, only six were significant at the $p = 0.01$ level, and three others were significant at the $p = 0.05$ level. Eye size was significantly negatively correlated with three melanophore traits and the number of maxillary teeth and positively correlated with lens size. MeIE and MeID were strongly correlated, and the length of the maxillary bone was significantly correlated with the length of the dentary and the number of taste buds. It is notable that eye size was not significantly correlated with the lengths of the dentary or maxillary or the number of taste buds.

We detected 48 QTL for these traits: eight affecting eye size, six affecting lens size, 18 affecting pigmentation, seven affecting lengths of the jaw bones, six affecting the number of maxillary teeth, and three affecting the number of taste buds (Table S2). The total proportions of variance explained by QTL for each trait ranged 0.11–0.77 (mean = 0.44) and the total proportions of additive variance explained ranged 0.03–0.52 (mean = 0.28).

Some of the QTL comapped and might represent the effects of single genes or tightly clustered genes (Figure S2). On LgP7, LgP9, and LgP15, there were two, four, and two comapping QTL for different melanophore traits. On LgP8 and LgP20, QTL for eye and lens

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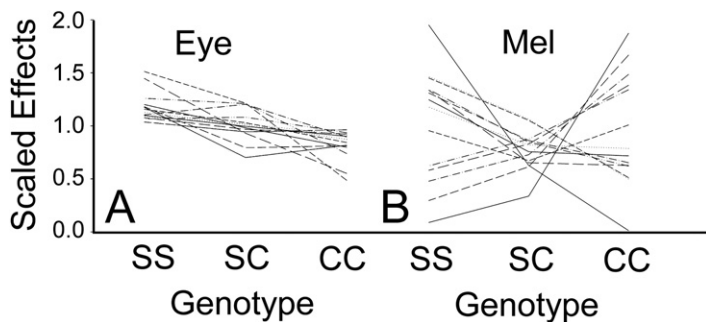


Figure 1. Standardized Trait Values for Surface Homozygotes, Heterozygotes, and Cave Homozygotes for Eye or Lens and Melanophore QTL

Scaled phenotypic values for genotypes at QTL affecting eye or lens sizes (A) or melanophore numbers (B). SS stands for surface allele homozygote, SC stands for heterozygote, and CC stands for cave allele homozygote.

size comap. Because of the possibility that these sets of comapping QTL each represent single loci, for statistical comparisons of eye and melanophore QTL, we counted each region only once. On LgP13, QTL for maxillary size and the number of maxillary teeth comap. Although these may represent one gene, the polarities of substitution effects are in disagreement, with smaller maxillae associated with more teeth. On LgP27, QTL for eye size and the number of maxillary teeth comap. On LgP5 and LgP25, QTL for eye or lens size comap with QTL for taste buds. Other examples of comapping traits can be seen in Figure S2.

Trait means (μ) are given in Table S3, and estimates of allelic substitution (d) and heterozygous effects (h) are given in Table S2. Expected trait values for cave and surface homozygotes and heterozygotes are $\mu + d$, $\mu - d$, and $\mu + h$, respectively. We calculated trait values for all 48 QTL and identified loci at which the heterozygote fell between the two homozygotes as intermediate in dominance. Based on this criterion, 36 of the cave alleles are of intermediate dominance. The remaining 12 loci cannot be classified unambiguously because the standard errors of estimate sometimes exceeded the differences in trait values among genotypes, but at four of the loci the cave allele seems recessive, at two others it seems dominant, and at another two others it seems clearly overdominant. We calculated a measure of dominance as the absolute value of the ratio of h/d and found the median value to be 0.44, or semidominant.

In order to compare patterns of substitution between eye or lens and melanophore QTL, the two regressive trait classes, we calculated trait values for all three genotypes at each QTL by using estimates of d, h, and μ . To standardize the scales, we divided expected trait values by their trait means. In the three cases in which two or more melanophore QTL comapped and it was possible that single genes were affecting multiple traits, the scaled trait values were averaged for each genotypic class. This reduced the number of melanophore QTL to 13 for statistical testing. In the two cases where eye and lens QTL comapped, we chose the one with the higher LOD score to represent the QTL.

The patterns of substitution effects differ radically between QTL for eye or lens size and melanophore numbers. Cave alleles at all 12 eye or lens QTL effect relatively modest but steady decreases of eye or lens size (Figure 1A). In contrast, cave alleles at QTL affecting melanophore number have positive ($n = 5$) as well as negative slopes ($n = 8$), and their substitution affects are much larger (Figure 1B). The distributions of polarities

differ significantly between the two classes of traits (12:0 versus 8:5, two-tail $p = 0.039$, Fisher's exact test). A comparison of the slopes for the two trait classes (Figure 1) also reveals an obvious difference in dispersion (Wilcoxon two-sample statistic for testing homogeneity of variances, $R_{11,13} = 186$, $p = 0.005$).

Our interpretation of these differences in QTL effects between the two classes is that regression of eyes came about primarily through selection, whereas decreases in numbers of melanophores resulted mainly from recurrent mutation with genetic drift or indirectly through pleiotropy. If there were strong direct selection against melanophores, it is unlikely that five QTL, all with major effects, would have cave alleles increasing the numbers of melanophores. If eye or lens reduction were accomplished through genetic drift, it is unlikely that the pattern of effects would contrast so radically with that for melanophores.

If eyes regressed through selection, was the selection directed against the eye itself or was it indirect, through negative pleiotropy of alleles selected for affects on other traits? Hedgehog signaling pathways direct the development of midline structures, including jaws, teeth, and taste buds (reviewed in [5]). Hedgehog activities also have important affects on eye development, in part, because *Hh* expression is antagonistic to that of *PAX6* and alters patterns of expression of *PAX2*. Yamamoto et al. [5] have shown that hedgehog activity is a strong determinant of eye size through experimental alteration of gene activity in *A. mexicanus* embryos. Increased unilateral expression of *sonic hedgehog* (*shh*) and *tiggy-winkle hedgehog* (*twhh*) in surface fish suppresses the development of the treated eye. Thus, one hypothesis is that eye regression is an indirect consequence of adaptive improvements in feeding efficiency brought about through upregulation of hedgehog signaling [6].

The *Hh* hypothesis has two parts. The first is that upregulation of hedgehog activity suppresses development of the eyes; the second is that hedgehog activity was upregulated during cave-fish evolution by selection to improve feeding efficiency and that this was the primary cause of eye regression. The evidence linking hedgehog activity to eye development seems compelling, but our data do not yet provide a definitive test of the second part of the hypothesis, although they suggest that it cannot be the sole explanation of eye regression. Six QTL for eye or lens size comap with QTL affecting feeding traits (jaw-bone sizes, numbers of teeth, and taste buds), but six others do not, and the QTL in the

latter group control a much greater proportion of explained additive variance than those in the former (non-comap versus comap groups: Eye: 0.233 versus 0.087; LensE: 0.364 versus 0.070; LensL: 0.014 versus 0.015). Furthermore, it is not just feeding trait QTL and eye or lens QTL that comap. Feeding trait QTL comap with QTL for melanophore numbers three times, and QTL for eye or lens size and melanophore number comap four times. We attribute this comapping to a general tendency toward pleiotropy with these traits [7] rather than to any specific relationship between feeding efficiency and eye loss. In addition, if the QTL affecting feeding traits were major contributors to eye regression, we might expect to see strong negative phenotypic correlations between these traits and eye size in the F₂. Such correlations are weak or nonexistent (Table S1). In sum, definitive tests of the generality of the second part of the Hh hypothesis await the molecular identification of the genes underlying eye loss and feeding morphology and characterization of the fitness effects of their alleles.

We also mapped candidate genes *shh* (LgP28), *twhh* (LgP15), and *PAX6* (LgP10). The fact that no eye QTL are located near these loci makes it unlikely that mutations in any of them are directly responsible for eye regression. One eye QTL maps to a point near the gene for ocular and cutaneous albinism (*OCA2*, LgP5).

Is it possible that Darwin's premise was simply incorrect? Are eyes in a cave disadvantageous, and if so, why? In essence, the argument against selection is that the cost of making an eye is trivial compared to the cost of its replacement tissue in the socket [2, 3] or that the developmental cost is paid by cave fish anyway because the eyes start developing and only degenerate after many cell cycles of tissue growth and replacement [6]. However, modern physiology and molecular biology suggest that these arguments might address the wrong costs. The vertebrate retina is one of the most energetically expensive tissues, with a metabolism surpassing even that of the brain [8]. Underscoring this high metabolic demand is the observation that one manifestation of genetic defects decreasing the efficiency of mitochondria is blindness (e.g., Leber's hereditary optical neuropathy [9]). Thus, maintenance of eyes might pose a significant burden in the cave environment. Increasing this burden, the vertebrate retina uses more energy in the dark than in the light because the membranes of the photoreceptor disks must be maintained in the hyperpolarized state until they are depolarized in response to light [10, 11]. Oxygen consumption by the vertebrate retina is approximately 50% greater in the dark than in the light [8]. Adding further to the retina's cost is its structural maintenance. Ten percent of the photoreceptor outer disks in vertebrates are shed and renewed each day, and the structure may be completely replaced over 35 times yearly [12].

Thus, although the energetic cost of making an eye may be trivial, the expense of maintaining one is much greater. In the dark, it may be costly enough to create effective selection for eye regression. In contrast, the argument of metabolic cost cannot be made for regression of pigmentation, and the QTL trait-value data (Figure 1) show that the two traits have regressed through different mechanisms.

This study shows that regression may be effected by active selection as well as by the passive accumulation and fixation of damaging mutations and that the various possibilities can be distinguished by the patterns of allelic substitutions involved. Thus, regression, an integral part of the progress of evolutionary change, can be accomplished in a variety of ways.

Supplemental Data

Supplemental Data include Experimental Procedures, two figures, three tables, and a zip file and are available with this article online at <http://www.current-biology.com/cgi/content/full/17/5/452/DC1/>.

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