ARTICLE ADDENDUM

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QTL clustering as a mechanism for rapid multi-trait evolution

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Cave-dwelling animals exhibit remarkable convergence in multiple cave-related traits, yet the genetic mechanisms responsible for the evolution and integration of many such traits remain unclear. *Astyanax mexicanus* is a model cave-dwelling fish with sighted surface-dwelling (surface fish) and blind cave-dwelling (cavefish) forms. Using a genetic cross between these morphs, we discovered significant correlations among several cave-related traits, including reduced eyes, increased superficial neuromast receptors located within the eye orbit (EO SN) and a vibration-attraction behavior (VAB) that facilitates foraging in darkness. Furthermore, we discovered that the quantitative trait loci (QTL) underlying these traits are clustered within the *Astyanax* genome. Following an ablation experiment that demonstrated that the EO SN contribute to VAB, we concluded that the adaptive evolution of VAB and EO SN has likely contributed to eye loss in cavefish. In this addendum, we further discuss the possible role of multi-trait QTL clustering in facilitating rapid adaptation.

Multi-Trait Evolution in Cave Animals

Astyanax cavefish have evolved multiple cave-related traits, including Vibration Attraction Behavior (VAB), a novel foraging behavior that is defined as the swimming of cavefish toward an oscillating object. This behavior is rarely seen in surface fish but is pronounced in cavefish and has evolved repeatedly among different cavefish populations.[1,2] VAB is correlated with several cave-related traits, including an increase in the number of cranial superficial neuromasts located within the cavefish eye orbit (EO SN) (Pearson's correlation coefficient, $r = 0.29$, $p < 0.001$, **Fig. 1A**). The experimental ablation of EO SN confirms that these sensory receptors contribute to VAB.[3] VAB is likely adaptive since it increases foraging efficiency in the cave environment, which is devoid of light, contains sparse food resources and lacks other macroscopic predators.[1,4]

Through the series of quantitative genetic studies, we investigated the genetic basis VAB and EO SN to evaluate their possible relationship to the most conspicuous cave-related trait, eye reduction. We found that VAB and EO SN were both strongly correlated with eye size ($r = -0.26$ and $r = -0.44$, respectively, both $p < 0.001$, **Fig. 1A**). We then performed a genome scan for quantitative trait loci (QTL) underlying these traits and found multiple QTL for VAB, EO SN and eye size.

Importantly, we found that the QTL for all three traits overlapped each other in two regions of the *Astyanax* genome (linkage groups 2 and 17, **Fig. 1A**), and that this clustering was significantly greater than expected by chance. Since the genetic clustering of multiple traits can provide a mechanism for their correlated evolution, we concluded that the adaptive evolution of VAB and EO SN has likely contributed to the correlated loss of eyes in cavefish, either as a result of pleiotropy or tight physical linkage of the mutations underlying these traits.[3]

The Significance of Genetic Correlations and Multi-Trait QTL

The significance of genetic correlations among traits and multi-trait QTL clusters is 2-fold. First, genetic correlations indicate

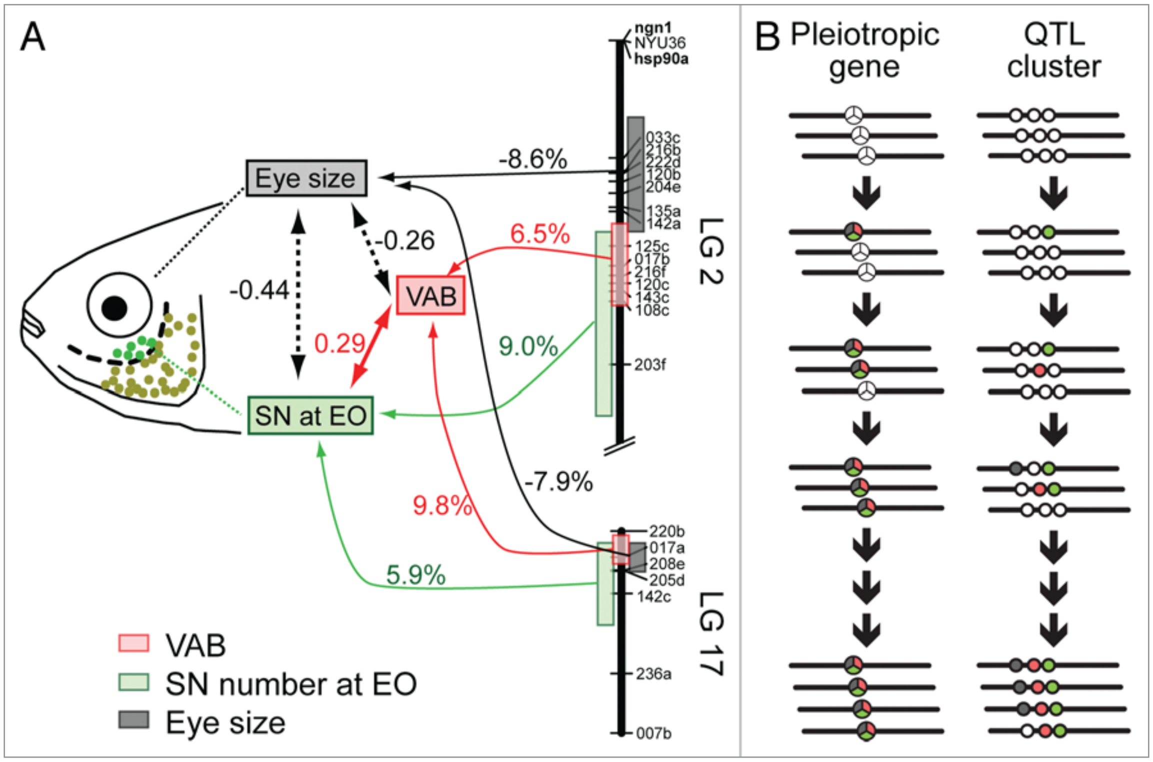

Figure 1. The evolution of multiple cave-related traits in *Astyanax*. (**A**) Schematic diagram of correlations and QTL among three cave-related traits in *Astyanax*: the Vibration Attraction Behavior (VAB, red), superficial neuromasts located within the eye orbit (EO SN green) and eye size (black). Thick arrows represent genetic correlations among traits (red = positive; black = negative).[3] Thin arrows indicate QTL effects (percent of phenotypic variance explained) of VAB, EO SN and eye size QTL on linkage groups (LG) 2 and 17, respectively. Negative effect sizes indicate that cavefish alleles reduce the trait, while positive values indicate that cavefish alleles increase it. Colored bars on each LG denote 95% Bayesian credible intervals for the location of each QTL. (**B**) Possible scenarios for the evolution of VAB, EO SN and eye size due to the action of a single pleiotropic mutation (left) or multiple linked mutations (right). Bars indicate chromosomes representing the frequency of surface fish (white circles) and cavefish (colored circles) alleles in a population of *Astyanax*. Colors denote traits as in (**A**). Thick arrows indicate passing generations, while bars represent possible haplotype pools following allelic fixation in subsequent generations. A single pleiotropic mutation can lead to the rapid evolution of correlated cave-related traits; however the rate of evolution will depend on the both the strength of selection and any negative effects on other traits. Multiple linked loci acting as "supergenes," arising either de novo or through chromosomal rearrangement, can also lead to the rapid evolution of multiple traits through the combined fitness of each allele;[24] however, rare recombination events may maintain genetic diversity within this region, as in the final haplotype pool shown at the bottom of the QTL cluster in 1B. Either pleiotropic genes or multiple linked QTL could contribute to the correlated evolution of VAB, EO SN and eye size in *Astyanax*.

the presence of a relationship between two or more traits that may be causal. For example, Protas et al.[6,8] reported that *Astyanax* lens size is positively correlated with eye size ($r = 0.71$), and that two sets of QTL for these traits overlap in the genome. Far from a coincidence, these phenotypic and genetic correlations confirmed the direct role that the lens plays in controlling cavefish eye growth.[5] Similarly, the importance of the positive genetic correlation between VAB and EO SN was confirmed by our experimental manipulation of EO SN, which demonstrated that these sensory receptors are necessary for VAB.[3] Although it would seem obvious that correlated traits should exhibit QTL that cluster within the genome, comprehensive studies of multi-trait evolution in *Astyanax* and other species reveals that this is not always the case.[6,7] For example, in a separate study, Protas et al. (2007) also found that *Astyanax* eye size and melanophore number were virtually uncorrelated ($r = -0.05$), yet the QTL for these traits formed six clusters within the *Astyanax* genome.[6,8]

Second, multi-trait QTL clustering may link suites of traits that are necessary for adaptation via so-called "supergenes."[9] For example, in *Heliconius* butterflies, the supergene locus *P* contains numerous tightly-linked polymorphisms that control aspects of wing coloration necessary for successful Müllerian mimicry.[10] Similarly, although the biochemical pathways leading to increased EO SN/VAB and reduced eye size may be distinct,[3] the correlations between these traits suggests that eye size must be reduced in order to increase the number of EO SN and therefore VAB. Thus, the clustering of QTL for eye size, VAB and EO SN provides a genetic mechanism for the co-inheritance and correlated evolution of these beneficial cave-related traits, e.g., the preservation of coadapted gene complexes (**Fig. 1A**).[11]

The Significance of Multi-Trait QTL Clustering for Evolutionary Change

Since multi-trait QTL clusters facilitate the co-inheritance of traits, either as a result of pleiotropy or tight physical linkage of their underlying mutations, these clusters provide a mechanism for integrating traits necessary for adaptation. Historically, such linkages have been interpreted as an impediment to evolution, in what was known as the "cost of complexity." Fisher first outlined this cost in his geometric model of adaptation, in which he calculated the probability of fixation for beneficial mutations in both simple and complex organisms. Fisher concluded that mutations affecting complex traits or organisms would be less likely to reach fixation because a mutation that was advantageous to one trait would likely be harmful to others.[12] This conclusion was supported by theoretical models that predicted that the rate of adaptation would decrease with increasing organismal complexity.[13] However, recent studies of pleiotropy have revealed that the distribution of such pleiotropic loci is actually quite limited and that their effect sizes can vary dramatically, even increasing with the number of traits affected.[14-20] Under these new findings, theoretical models predict that adaptation will actually proceed faster at an intermediate level of trait integration or pleiotropy.[16,21] Furthermore, the extent of harmfulness may be minimized by relaxed selection for the major regressive traits exhibited by cave-adapted animals.

Indeed, far from being an impediment, the results of our latest study,[3] as well as those from other rapidly evolving species—including domesticated lines such as honeybee, fowl and rice,[7,18,22] as well as spectacular examples of adaption radiation such as stickleback and African cichlid fishes[6,19,20,23]—reveal that multi-trait QTL clustering may be a common feature of rapid adaptation. Although some of these multi-trait QTL clusters represent the action of pleiotropic loci, others likely represent tightly-linked polymorphisms or supergenes. While both genetic mechanisms can promote the evolution of complex, integrated adaptations, one advantage of non-pleiotropic linkage (i.e., supergenes) is that the underlying alleles can still be broken up by recombination in order to maintain genetic and phenotypic diversity (**Fig. 1B**). This rare genetic and phenotypic variation may play a major role in continued adaptation to environmental changes. In the future, we plan to identify the genes and mutations responsible for the evolution of VAB, EO SN and eye size in *Astyanax* in order to elucidate the genetic mechanisms that are responsible for the evolution of these complex cave-related traits.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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