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...
The presence of a relationship between two or more traits that may be causal. For example, Protas et al.\textsuperscript{6,8} reported that \textit{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.\textsuperscript{3} 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.\textsuperscript{3} Although it would seem obvious that correlated traits should exhibit QTL that cluster within the genome, comprehensive studies of multi-trait evolution in \textit{Astyanax} and other species reveals that this is not always the case.\textsuperscript{6,7} For example, in a separate study, Protas et al. (2007) also found that \textit{Astyanax} eye size and melanophore number were virtually uncorrelated ($r = -0.05$), yet the QTL for these traits formed six clusters within the \textit{Astyanax} genome.\textsuperscript{6,8}

Second, multi-trait QTL clustering may link suites of traits that are necessary for adaptation via so-called “supergenes.”\textsuperscript{9} For example, in \textit{Heliconius} butterflies, the supergene locus $P$ contains numerous tightly-linked polymorphisms that control aspects of wing coloration necessary for successful Müllerian mimicry.\textsuperscript{10} Similarly, although the biochemical pathways leading to increased EO SN/VAB and reduced eye size may be distinct,\textsuperscript{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).\textsuperscript{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. This conclusion was supported by theoretical models that predicted that the rate of adaptation would decrease with increasing organismal complexity. 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. Under these new findings, theoretical models predict that adaptation will actually proceed faster at an intermediate level of trait integration or pleiotropy. 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, as well as those from other rapidly evolving species—including domesticated lines such as honeybee, fowl and rice, as well as spectacular examples of adaption radiation such as stickleback and African cichlid fishes—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, E0 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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References
1. Yoshizawa M, Goricki S, Soares D, Jeffery WR. Evolution of a behavioral shift mediated by superficial neuromasts helps caye fish find food in darkness. Curr Biol 2010; 20:1631-6; PMID:20705469; http://dx.doi.org/10.1016/j.cub.2010.07.017
2. Abdel-Latif H, Hassan ES, von Campenhausen C. Sensory performance of blind Mexican cave fish after destruction of the canal neuromasts. Naturwissenschaften 1990; 77:237-9; PMID:2577253; http://dx.doi.org/10.1007/BF01384932
3. Yoshizawa M, Yamamoto Y, O‘Quinn KE, Jeffery WR. Evolution of an adaptive behavior and its sensory receptors promotes eye regression in blind cavefish. BMC Biol 2012; 10:189; PMID:23270452; http://dx.doi.org/10.1186/1741-7007-10-108
4. Yoshizawa M, Jeffery WR. Evolutionary tuning of an adaptive behavior requires enhancement of the neuromast sensory system. Commun Integr Biol 2011; 4:89-91; PMID:21509190
5. Yamamoto Y, Jeffery WR. Central role for the lens in cave fish eye degeneration. Science 2000; 289:631-3; PMID:10915628; http://dx.doi.org/10.1126/science.289.5479.631
6. Protas M, Tabansky I, Conrad M, Gross JB, Vidal O, Tabin CJ, et al. Multi-trait evolution in a cave fish, Astyanax mexicanus. Evol Dev 2008; 10:196-209; PMID:18335813; http://dx.doi.org/10.1111/j.1558-1424.2008.00227.x
7. Mougel F, Solignac M, Vaurin D, Baudry E, Ogden J, Tchapa A, et al. Quantitative traits loci (QTL) involved in body colour, wing morphometry, curvilinear hydrocarbons and venom components in honeybees. Apidologie (Celle) 2012; 43:162-81; http://dx.doi.org/10.1017/S135901090000-0
8. Protas M, Conrad M, Gross JB, Tabin C, Bonowsky R. Regressive evolution in the Mexican cave tetra, Astyanax mexicanus. Curr Biol 2007; 17:452-4; PMID:17306543; http://dx.doi.org/10.1016/j.cub.2007.01.051
9. Ernst A. Weitere Untersuchungen zur Phänanalyse, zum Fertilitätsproblem und zur Genetik heterostyler Primeln. II. Primula hortensis Wettst. Arch Julius Rassenhyg 1936; 11:1-280
10. Brown KS, Benson WW. Adaptive polymorphism associated with multiple Mullerian mimicry in Heliconius numata. Biotropica 1974; 6:205-28; http://dx.doi.org/10.2307/2898666
11. Mayr E. Animal Species and Evolution. Cambridge: Belknap Press, 1963
12. Fisher RA. The genetic theory of natural selection. Oxford: Claredon, 1930
13. Orr HA. Adaptation and the cost of complexity. Evolution 2000; 54:13-20; PMID:10937178; http://dx.doi.org/10.1111/j.1002-0859.2000.00002.x
14. Wagner GP, Kenney-Hunt JP, Pavlicev M, Peck JR, Waxman D, Cheverud JM. Pleotropic scaling of gene effects and the 'cost of complexity'. Nature 2008; 452:470-2; PMID:18368117; http://dx.doi.org/10.1038/nature06756
15. Wagner GP, Zhang J. The pleiotropic structure of the genome-phenotype map: the evolvability of complex organisms. Nat Rev Genet 2011; 12:204-13; PMID:21331091; http://dx.doi.org/10.1038/nrg2949
16. Wang Z, Liao BY, Zhang J. Genomic patterns of pleiotropy and the evolution of complexity. Proc Natl Acad Sci USA 2010; 107:18034-9; PMID:20876014; http://dx.doi.org/10.1073/pnas.100466107
17. Wright D, Kerje S, Brästandröm H, Schütz K, Kindmark A, Andersson L, et al. The genetic architecture of a female sexual ornament. Evolution 2008; 62:86-98; PMID:18053076; http://dx.doi.org/10.1111/j.1558-5646.2007.00281.x
18. Wright D, Rubin CJ, Martinez Barrio A, Schütz K, Kerje S, Brästandröm H, et al. The genetic architecture of domestication in the chicken: effects of pleiotropy and linkage. Mol Ecol 2010; 19:5140-56; PMID:20140053; http://dx.doi.org/10.1111/j.1365-294X.2010.04882.x
19. Albert AYK, Sawaya S, Vines TH, Knecht AK, Miller CT, Summers BR, et al. The genetics of adaptive shape shift in stickleback: pleiotropy and effect size. Evolution 2008; 62:76-85; PMID:18005154
20. Albertson RC, Streelman JT, Kocher TD. Directional selection has shaped the oral jaws of Lake Malawi cichlid fishes. Proc Natl Acad Sci USA 2003; 100:5252-7; PMID:12704237; http://dx.doi.org/10.1073/pnas.0309235100
21. Hansen TF. Is modularity necessary for evolvability? Remarks on the relationship between pleiotropy and evolvability. Biosystems 2003; 69:83-94; PMID:12689723; http://dx.doi.org/10.1016/S0006-2781(03)00132-6
22. Oshini K, Horinuchi Y, Ishigoh-Oka N, Takagi K, Ichikawa N, Matsuka M, et al. A QTL cluster for plant architecture and its ecological significance in asian wild rice. Breed Sci 2007; 57:7-16; http://dx.doi.org/10.1270/jsbsb.57.7
23. O‘Quinn KE, Schulte JE, Patel Z, Kahn N, Naseer Z, Wang H, et al. Evolution of cichlid vision via trans-regulatory divergence. BMC Evol Biol 2012; 12:251; PMID:23526765; http://dx.doi.org/10.1186/1471-2148-12-251
24. Kirkpatrick M, Barton N. Chromosome inversions, local adaptation and speciation. Genetics 2006; 173:419-34; PMID:16204214; http://dx.doi.org/10.1534/genetics.105.047985