

# Sticklebacks and Humans Walk Hand in Hand in Fin to Lighter Skin

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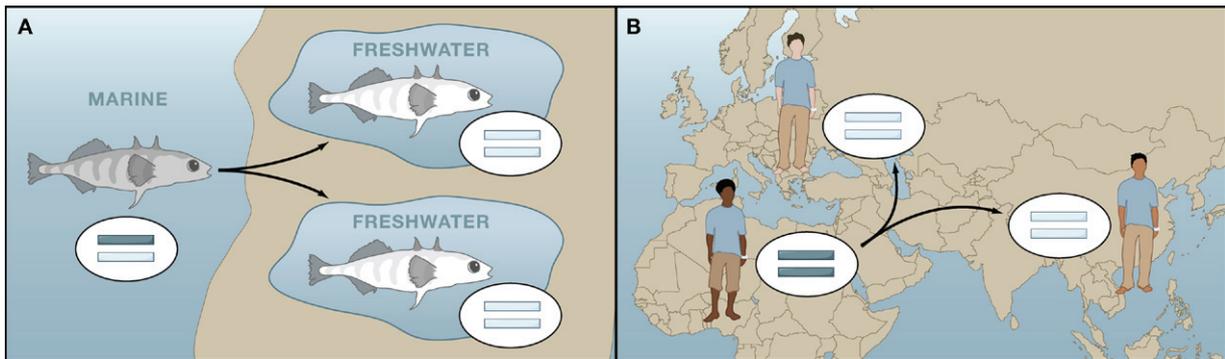
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In this issue, Miller et al. (2007) show that evolution makes repeated use of the same genes to produce light pigmentation in animals as divergent as stickleback fish and humans. This study indicates that analyzing parallel evolution at the genetic level could help to answer a number of outstanding questions in evolutionary genetics.

To what extent is evolution repeatable? One classic way to address this issue is to ask whether evolutionary change occurs in parallel, that is do the same traits evolve repeatedly in disparate taxa? Evidence of parallel evolution at the phenotypic level has come from many studies of experimental evolution in microbes (e.g., Travisano et al., 1995). These studies show overriding patterns of repeat-

able, predictable change; however, the details vary, muddying those patterns. Thus, it remains unclear how deep the parallel changes go. With the advent of the genomics era this question can now be asked at the genetic level for natural populations. Does evolution tinker with the same genes to produce parallel phenotypic change or are there many routes to the same phenotype?

In this issue, Miller and colleagues (2007) tackle this question by exploring genetic mechanisms underlying pigmentation differences between marine and freshwater species of threespine stickleback fish (*Gasterosteus* species complex). Sticklebacks are well known for their variation in color: from red and blue, to jet black, to translucent white. In the midst of large-scale



**Figure 1. Parallel Evolution of a Pigmentation Gene in Sticklebacks and Humans**

As sticklebacks and humans colonized new environments, they evolved an alternate *Kitlg* allele that produces light pigmentation due to changes in *cis*-regulatory regions. This occurred repeatedly and independently in multiple stickleback species and human populations. Alleles are represented as filled bars (fill color indicates dark or light allele). Arrows show direction of evolutionary change. (A) Marine sticklebacks are polymorphic for *Kitlg* alleles; dark alleles predominate, but a light allele is also present at low frequency. Multiple freshwater species that have evolved from marine sticklebacks possess the light allele and have light gills and ventral regions. (B) In humans, the ancestral dark *KITLG* allele predominates in African populations along with dark skin. Independently derived light alleles predominate in both European and East Asian populations along with light skin.

genomic work, Miller et al. noticed that marine sticklebacks have dark gills whereas freshwater fish have pale gills. This represents a loss of pigment because the marine sticklebacks are ancestral to the freshwater species. To track down the gene associated with this color loss, Miller et al. used genome-wide linkage mapping followed by fine mapping within the quantitative trait locus (QTL). The gene they found, *Kit ligand* (*Kitlg*)—also known as steel factor or mast cell growth factor—plays a key role in melanocyte development in mice where it controls the distribution and number of melanocytes. *Kitlg* affects several aspects of pigmentation patterns. For example, heterozygote mutant mice have reduced pigment in ventral (belly) regions. Miller et al. found that the fish with the freshwater haplotype (the light *Kitlg* allele) had both light gills and bellies, suggesting that *Kitlg* controls both gill and ventral pigmentation in sticklebacks.

Next, the authors turned to the question of parallel evolution by testing if the same patterns are found in other stickleback species that are independently derived from marine sticklebacks. They found the light *Kitlg* allele in two other freshwater species that have light gills and bellies (Figure 1). A third freshwater species has the dark marine

allele and dark gills and bellies. Thus, parallel genetic mechanisms underlie parallel phenotypic change in multiple stickleback species.

The authors find numerous mutations in noncoding regions of the light *Kitlg* allele, and expression studies showed reduced *Kitlg* expression associated with these changes in both gill tissue and ventral skin but not dorsal skin, which is heavily pigmented. Moreover, they show that reduced expression is due to *cis*-acting rather than *trans*-acting regulatory mutations. They also found mutations in the coding region but observed no differences in transcript size or putative protein structure, suggesting that these mutations are not likely to reflect functional differences in the protein. The difficult job of verifying the causal mutations awaits, but the authors provide strong evidence that *cis*-regulatory mutations in *Kitlg* alter pigmentation in multiple stickleback species.

The *KITLG* gene has also cropped up recently as a strong candidate for human skin color variation. The intergenic regions surrounding *KITLG* show a strong signature of selection in humans, primarily in light-skinned East Asian and European populations (Williamson et al., 2007), which suggests that humans harbor functional variation at this

gene. The most strongly selected regions are upstream of the gene itself, suggesting that selection altered the regulation of *KITLG*. Miller and colleagues took the next step and used admixture mapping in African-Americans to specifically test for associations between skin color and a single nucleotide polymorphism (SNP) that they suspected could modify *KITLG* expression. This SNP is at a site upstream of *KITLG* that is highly conserved in mammals, and both East Asians and Europeans differ dramatically from West Africans in the frequency of this SNP (Figure 1). The mapping results show that skin color in humans is strongly associated with this SNP.

Finding that the same gene is involved in the independent loss of pigment in multiple stickleback species is surprising, but more surprising is the discovery that this gene is also involved in pigment loss in humans. More surprising still is when we recognize that the parallel phenotypic changes are not likely to be the result of similar selection pressures. Pale skin in humans is unlikely to provide similar benefits as pale gills and bellies in sticklebacks.

A number of recent studies reveal repeated involvement of the same gene in phenotypic change even when the phenotype is subject to

different kinds of selection. For example, although over 100 genes have been shown to control pigmentation in mice, mutations in a single gene, *mc1r*, cause melanism in taxa as divergent as rock pocket mice, bananaquits (a tropical bird), and lesser snow geese (Hoekstra and Coyne, 2007). This suggests a very high degree of parallel evolution at the genetic level. Yet melanistic coloration has different functions in these species: it appears to be selected for camouflage in mice, for thermal tolerance in bananaquits, and for mate choice in lesser snow geese. If the functions are different, shared selection cannot produce the parallel change. In all cases, melanism appears to be advantageous (although this has not been directly shown), so it seems that it's the endpoint that matters more than the specific advantage conferred.

The Miller study does not identify the function of pigment in stickleback gills and bellies. Evolutionary geneticists focus more on parallel genetic change than the sources of selection on parallel phenotypes. However, identifying the selective causes of parallel genetic change will yield two benefits. First, we know that phenotypes will diverge in the absence of selection due purely to mutation and chance. Therefore, to demonstrate adaptive evolutionary change requires verifying that a phenotype is actually adaptive. Second, if similar phenotypes depend on the same genes even when selection is not shared, this suggests that evolution is highly constrained to follow the same path, and that genetic constraint determines that path. Incorporating direct tests of adaptive function into tests of parallel evolution at the genetic level offers a powerful way to dis-

entangle the contributions of shared selection and common ancestry to parallel evolution.

The light allele has a relatively high frequency of 12% in present day marine fish, indicating that parallel evolutionary change is from standing variation rather than new mutation. This suggests that selection has repeatedly favored this light allele in multiple freshwater species and highlights the role of shared selection to this story. This is the second such case from sticklebacks and, with a smattering of other cases, provides data to address yet another outstanding question in evolutionary genetics regarding the relative contribution of new mutation and standing variation to adaptation. Adaptation from standing variation may proceed more quickly and leave a different imprint on the genome (Barrett and Schuller, 2007), yet we have no idea how common it is.

Why do certain genes pop up again and again? For this study, having a polymorphic gene of large effect probably tipped the balance to *Kitlg*. If certain genes are more likely to be polymorphic, they might be used by selection repeatedly. Another popular idea is that genes with few pleiotropic effects are subject to fewer tradeoffs, which might make them more available for adaptive evolution. Two mechanisms to circumvent negative pleiotropy seem likely. Coding changes in duplicated genes can allow the original copy to retain ancestral function releasing the duplicate to take on new function (Hoekstra and Coyne, 2007). Alternatively, genes with highly modular regulatory elements can isolate mutational effects to specific tissues or points in development by regulating the location or timing of

gene expression (Prud'homme et al., 2007). However, for either of these ideas to explain the repeated use of the same gene, that gene needs to have higher rates of duplication or more modular regulation than other genes.

We have accumulated a number of examples of adaptive evolution due to coding changes (Hoekstra and Coyne, 2007) and a number due to regulatory changes (Wray, 2007). A few studies even show that both are involved in the same adaptive change (Hittinger and Carroll, 2007; Steiner et al., 2007). So the jury is still out on which is the primary driver of adaptive evolution or whether they share the driver's seat. In either case, studies like the present one show us that evolution is driving down the same path over and over again.

## REFERENCES

- Barrett, R.D.H., and Schuller, D. (2007). Trends Ecol. Evol. Published online November 12, 2007. 10.1016/j.tree.2007.09.008.
- Hittinger, C.T., and Carroll, S.B. (2007). Nature 449, 677–681.
- Hoekstra, H.E., and Coyne, J.A. (2007). Evolution Int. J. Org. Evolution 61, 995–1016.
- Miller, C.T., Beleza, S., Pollen, A.A., Schluter, D., Kittles, R.A., Shriver, M.D., and Kingsley, D.M. (2007). Cell, this issue.
- Prud'homme, B., Gompel, N., and Carroll, S.B. (2007). Proc. Natl. Acad. Sci. USA 104, 8605–8612.
- Steiner, C.C., Weber, J.N., and Hoekstra, H.E. (2007). PLoS Biol. 5, 1880–1889. 10.1371/journal.pbio.0050219.
- Travisano, M., Mongold, J.A., Bennett, A.F., and Lenski, R.E. (1995). Science 267, 87–90.
- Williamson, S.H., Hubisz, M.J., Clark, A.G., Payseur, B.A., Bustamante, C.D., and Nielsen, R. (2007). PLoS Genetics 3, 901–915.
- Wray, G.A. (2007). Nat. Rev. Genet. 8, 206–216.