designed to address. Examples are a new estimate of the flux of meteors entering the atmosphere (*9*), the idea that mixed organic/salt particles could have had a role in the origin of life (*14*), and the tracking of smoke from fireworks (*15*). Yet public policy questions continue to provide a focus to the researchers involved in developing instrumentation to measure the composition of particles in the atmosphere.

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EVOLUTION

The Synthesis and Evolution of a Supermodel

Greg Gibson

Synthesis is the merging of disparate sources of knowledge to create a stronger, more compelling whole. In the biological sciences, barriers to synthesis—including the specialization of subdisciplines and their fractionation into departments and curricula—have increased over the past few decades. Developmentalists dissect their favorite ecologically irrelevant models with exquisite detail, while evolutionists tap away at hundreds of fascinating species with genetically toothless tools. Organismal biologists can take great solace in a report on page 1928 of this issue by Colosimo and colleagues (*1*) that shows how genomics can be used to buck this trend and lead us to new insights into fundamental evolutionary problems.

The threespine stickleback (*Gasterosteus aculeatus*) is a finger-sized species of fish that exhibits multiple examples of parallel evolution. The threespine stickleback populations that inhabit the streams and lakes of the northern Pacific and Atlantic rims show intriguing variations in morphology and behavior compared to marine populations (*2*). A particularly striking instance is the reduction in body armor exhibited by freshwater populations. Whereas marine sticklebacks carry a row of up to 36 armor plates extending from head to tail (complete morph), freshwater sticklebacks either carry a gap in the row of plates (partial morph) or retain only a few plates at their anterior end (low morph). Sticklebacks reside in diverse freshwater habitats that include numerous glacial lakes in western Canada that were formed as the last ice age retreated 10,000 or so years ago (*3*). Lindsey hypothesized in 1962 (*4*) that

rather than a single stickleback population with reduced body armor founding the populations in all of these lakes, parallel evolution must have occurred. He attributed parallel evolution to selection either on independent mutations or on a rare allele whose phenotypic effect is cryptic, that is, remains hidden in the marine population. The new study (*1*) combines quantitative genetics, genomics, population genetics, molecular evolution, field studies, and molecular developmental biology to demonstrate that both new mutations and cryptic variation have contributed to body armor reduction. In so doing, this study provides one of the first dissections of a skeletal polymorphism to the gene level, and thereby elevates the

stickleback to the status of supermodel for the study of developmental evolution.

The story begins with high-resolution linkage mapping of a major effect locus for armor reduction in a cross between "complete" and "low" body plate morphs of the threespine stickleback. This locus was mapped to an approximately 0.7-cM interval of the genome. Although expression of the phenotype varies in different crosses because of the segregation of modifier loci, loss of body plates is largely dependent on a generally recessive allele that accounts for as much as 75% of the difference between morphs (*5*). To positionally clone the gene responsible for this effect, Colosimo *et al.* (*1*) performed a chromosome walk across the region of interest, tiling six bacterial artificial chromosome clones covering more than a megabase. Half of this walk was completely sequenced, and microsatellites at 12-kb intervals were typed in a set of 46 of the complete-armor morphs and 45 of the low-plate-number morphs from an interbreeding stream population in California. This so-called linkage disequi-
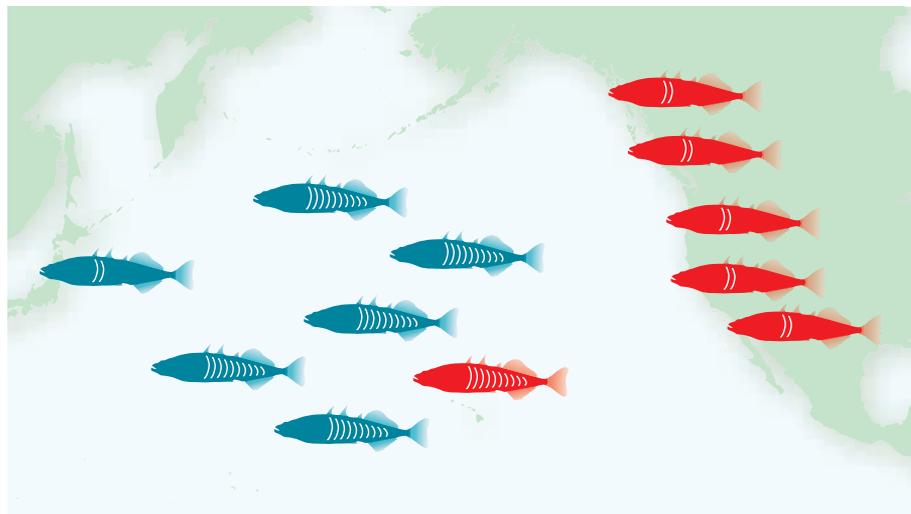
Lightening the load. Marine threespine sticklebacks (blue) have a robust set of body armor (indicated as multiple rays in each body), whereas multiple freshwater populations on either side of the north Pacific rim have independently lost their body armor during the course of evolution. Each low-morph stickleback population in lakes and streams of western North America carries an *Eda* allele (red coloration) that resembles the rare allele found in marine populations. This finding suggests that the *Eda* allele has increased in frequency under adaptive selection. By contrast, a Japanese marine population has a different *Eda* allele on the common background, implying that this case of armor reduction evolved through an independent mutation.

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librium approach—now the standard in mapping human disease genes—reduced the peak of the candidate interval to just 16 kb, centered on a marker in the second intron of a gene called *Ectodysplasin* (*Eda*).

Eda encodes a member of the tumor necrosis factor (TNF) family of secreted signaling proteins (*6*). The gene has a history of involvement in abnormal development of skin. Mutations in this gene cause a variety of human syndromes (*7*) and the Tabby phenotype in mice (*8*); meanwhile, absence of the EDA receptor results in loss of scales in medaka fish (*9*). Similarly severe mutations in these genes in natural populations would be likely to have such deleterious pleiotropic effects that selection would preclude their reaching high frequencies. Sequencing of a low-morph *Eda* allele detected multiple nucleotide differences with respect to the complete-morph allele, but only four of these led to an amino acid change in the EDA protein, and none of these changes obviously affected EDA's function (*1*). Thus, the precise polymorphism that leads to the quantitative skeletal phenotype is still unknown. Reverse transcription polymerase chain reaction methods detected the expected alternative splice products in both morphs of stickleback, but because the transcript abundance in developing epidermis was too low to detect by whole-mount in situ hybridization, it is not yet clear whether there is a quantitative or spatial difference in expression that might explain the phenotypic effect of the low-morph allele.

It remains possible that *Eda* is not the causative gene, particularly because the refined interval includes another TNF ligand and two other genes of interest (these may also, or alternatively, influence a couple of correlated physiological traits). Kingsley's group (*1*) has established more direct evidence for the involvement of EDA in plate development by generating transgenic sticklebacks that transiently overexpress a murine version of the gene. A handful of these fish exhibit partial rescue of plate development in a homozygous low-morph background. Thus, there is little doubt that Colosimo and co-workers have nailed the source of parallel morphological evolution in the threespine stickleback to a single gene.

The authors next performed a population survey of *Eda* genotypes to address the question of whether adaptive evolution has favored multiple independent mutations or repeated selection of an allele that is rare in marine populations (see the figure). All of the North American and European low-morph populations share a haplotype consisting of a set of single-nucleotide polymorphisms covering the interval centered on *Eda*, whereas the Japanese low-morph allele is clearly distinct. Because the Japanese allele fails to complement the North American one, this implies that at least two independent mutations have led to a reduction in body armor on either side of the Pacific. By contrast, detection of at least 14 instances of a similar haplotype in North America—one that is rare in the marine population from which the stream and lake populations of Canada and California were founded—strongly implies a single genetic basis for these instances of parallel evolution.

Counting up the number of nucleotide substitutions between the two sequenced high- and low-morph alleles suggests a date of 2 million years for their separation, which is two orders of magnitude longer than the inferred age of the postglacial populations. This method does not actually date the causative mutation, which could have arisen on the low-morph haplotype at any time before the founding of the freshwater populations in which it has risen to a high frequency. The haplotype has an estimated allele frequency of 0.6 to 3.8% in populations of marine fish sampled from coastal British Columbia and California. This is too low to produce an appreciable number of homozygotes, but large enough to create a pool of alleles that would be available for selection upon introgression into freshwater environments. Preliminary sequence comparisons suggest that the causal site itself is quite ancient, as there is considerable diversity even within the low-morph haplotype. Had the mutation appeared relatively recently in an isolated stream, then found its way back into the marine pool from whence the other populations were founded, sequence diversity in the haplotype would be much lower than that observed in the prevalent marine haplotype.

Ancient or recent, the more important point is that for the first time we have a clear demonstration that after alteration of environmental circumstances, adaptive evolution can act independently on an allele that is present in but has little effect on the morphology of the ancestral species. Colosimo *et al.* (*1*) refer to this phenomenon as selection on cryptic variation. A more technical definition of cryptic variation allows us to expand the scope of the potential impact of standing variation on rapid morphological evolution (*10*). In marine sticklebacks, the low-morph effect is hidden by the fact that only rare fish carry the relevant allele, and these fish are heterozygous rather than homozygous for the low-morph variant. Strictly speaking, though, cryptic variation refers to the situation where the phenotype of individuals is modified by the genetic background or environment such that a previously neutral variant becomes functional and adaptive.

Because plate reduction is modified by other loci (*5*) and may be influenced by environmental factors such as calcium concentration in the water, it is likely that more than just selection on *Eda* is contributing to the uncovering of this hidden variation.

These results should provide fuel for those who wish to emphasize the distinction between soft and hard selection. Hard selection—positive selection on new mutations—is known to lead to a substantial reduction in nucleotide diversity around the focal site, which can be used as the basis for detection of selective sweeps (*11*). By contrast, soft selection acts on standing variation that has been in the population for some time, as a result of a change in the environment or genetic background (both of which should occur when marine sticklebacks admix with those inhabiting streams). It is expected to leave a very different genetic footprint (*12*, *13*), and this system provides a superb opportunity to contrast these scenarios. There is also a lovely symmetry in the fact that the mouse Tabby mutation, now known to be due to a mutation in the *Eda* gene (*8*), provides a classic example of canalization (*14*). Canalization refers to the buffering of genetic variation and promotes the maintenance of cryptic variation.

Studies such as that by Colosimo and colleagues highlight how the disparate branches of biology can be synthesized to provide fresh perspectives on fundamental evolutionary phenomena. The National Evolutionary Synthesis Center (*15*) in Durham, North Carolina, has just received NSF funding to promote synthetic research. Few groups have the capacity to pull off such an integrative accomplishment, but there is little reason why interactive teams cannot contribute to the emergence of numerous other supermodel organisms.

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