

# Widespread Parallel Evolution in Sticklebacks by Repeated Fixation of Ectodysplasin Alleles

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Major phenotypic changes evolve in parallel in nature by molecular mechanisms that are largely unknown. Here, we use positional cloning methods to identify the major chromosome locus controlling armor plate patterning in wild threespine sticklebacks. Mapping, sequencing, and transgenic studies show that the Ectodysplasin (EDA) signaling pathway plays a key role in evolutionary change in natural populations and that parallel evolution of stickleback low-plated phenotypes at most freshwater locations around the world has occurred by repeated selection of *Eda* alleles derived from an ancestral low-plated haplotype that first appeared more than two million years ago. Members of this clade of low-plated alleles are present at low frequencies in marine fish, which suggests that standing genetic variation can provide a molecular basis for rapid, parallel evolution of dramatic phenotypic change in nature.

Particular phenotypic traits often evolve repeatedly when independent populations are exposed to similar ecological conditions (1, 2). The threespine stickleback species complex (*Gasterosteus aculeatus*) provides an ideal system for further studying the molecular mechanisms that underlie widespread parallel evolution of phenotypic traits in nature. Parallel evolution within this species complex has occurred in countless freshwater lake and stream environments colonized by marine sticklebacks after widespread melting of glaciers 10,000 to 20,000 years ago. The young age of the freshwater populations, the ability to generate fertile hybrids between divergent populations, and the recent development of stickleback genomic resources make it possible to map the genes that control evolutionary change and to compare the genetic basis of similar traits that have evolved in different locations (3–7).

Several marine phenotypes have changed repeatedly in new freshwater environments, which includes reduction of the extensive bony armor found in ocean fish (8, 9). Marine sticklebacks typically have a continuous row of 32 to 36 armor plates extending from head

to tail (“complete” morph, Fig. 1A). In contrast, innumerable freshwater populations have a gap in the middle of the row of plates (“partial” morph), or retain only zero to nine plates at the anterior end (“low” morph, Fig. 1A) (10, 11). Several factors have been proposed that could contribute to the selective advantage of armor plate reduction after colonization of new lakes and streams by completely plated marine ancestors, including low calcium levels, increased body flexibility and maneuverability, changes in swimming performance, and changes in predation regimes in freshwater environments (12–17).

**Positional cloning of the plate morph region.** Further understanding of the molecular basis of parallel evolution requires the identification of the genes and mutations that underlie major phenotypic change. We previously used genome-wide linkage mapping in a marine by freshwater  $F_2$  cross to show that armor plate patterns are controlled primarily by a single major QTL that maps to linkage group IV and by four minor modifier QTL that cause smaller quantitative variation in the size and number of plates (4). To refine the position of the major locus, we screened for new amplified fragment-length polymorphism markers (18) that differed in allele size or frequency in pools of low and completely plated progeny from a marine by freshwater cross. Newly isolated markers *Stn345* and *Stn346* were much more closely linked than previous markers, defining a new interval of 0.68 cM for the plate morph locus (Fig. 1B).

*Stn345* and *Stn346* were used to screen a bacterial artificial chromosome (BAC) library derived from completely plated marine fish from Salmon River, Canada (SRMA) (7). Three rounds of chromosome walking led to the isolation of six overlapping BAC clones spanning the interval of interest (Fig. 1B). In a total of 1166 chromosomes, *Stn345* and BAC end marker *Stn347* mapped two recombination events proximal and one recombination event distal of the plate morph locus, respectively. These recombination events define a physical region of about 539 kb that must contain the plate morph locus. BAC clones A and B (Fig. 1B) were completely sequenced during the chromosome walk, generating a contiguous sequence assembly of 407,051 base pairs (bp) derived from the plate morph region.

**Linkage disequilibrium screening.** Meioses and interbreeding in natural populations tend to homogenize allele frequencies at most loci. However, particular alleles that are very closely linked to a mutation of interest may remain in linkage disequilibrium with that mutation for many generations (19). To test for possible linkage disequilibrium in the region controlling plate morph phenotypes, we designed microsatellite markers at intervals of ~12 kb throughout the BAC sequence assembly and examined the distribution of alleles at each marker in a sample of 46 completely and 45 low-plated fish from a single interbreeding wild population from Friant, CA (FRI) (4, 20). The difference in allele distribution at *Stn365* in completely and low-plated fish was  $10^7$  times more significant than the differences at flanking markers *Stn364* and *Stn366*, which define a region of peak linkage disequilibrium of ~16 kb (Fig. 1C).

Gene predictions show that the marker at the peak of linkage disequilibrium is located within intron 2 of the stickleback *Ectodysplasin* (*Eda*) gene. EDA is a member of the tumor necrosis family of secreted signaling molecules and, in mammals, is required for proper development of a number of ectodermal derivatives (e.g., teeth, hair, and sweat glands) and dermal bones (21, 22). Previous studies have shown that a mutation in the *Ectodysplasin receptor* (*Edar*) gene in medaka (*Oryzias latipes*) causes loss of most scales (23), which are elements of the dermal skeleton (24). Many elements of the dermal skeleton in fishes, including scales and the dermal lateral plates of sticklebacks, have likely evolved from a common ancestral element (24). The position of *Eda* at the peak of linkage disequilibrium in the stickleback candidate interval and the known role of EDA signaling in scale formation suggested that changes in the *Eda* locus may underlie the molecular basis of plate morph evolution in sticklebacks.

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***Eda* structure in low and complete**

**morphs.** To compare the structure of *Eda* and linked genes in marine and freshwater sticklebacks, we isolated two overlapping BAC clones from a library derived from the low-plated benthic Paxton Lake, Canada population (PAXB) (7), and completely sequenced the *Eda* region. Comparison to the sequence of the completely plated SRMA marine population identified numerous non-coding changes (table S2), and four mutations that lead to amino acid changes in EDA protein (Fig. 2). None of the amino acid changes occur at sites that are highly conserved between mammals and fish or in residues that have previously been associated with defects in humans (25, 26). Three of the four sites also vary among other fish species.

Previous studies in mammals have shown that *Eda* undergoes alternative splicing to produce two protein isoforms that differ in length by two amino acids and bind different receptors (27, 28). Both splicing isoforms were recovered from developing low- and completely plated fish (10- to 20-mm standard length) with the use of reverse transcription polymerase chain reaction (RT-PCR), and no other splicing changes were seen in the low-plated fish.

We also generated probes to examine the spatial pattern of expression of *Eda* in sticklebacks, but we were unable to detect significant *Eda* expression in any samples using whole-mount in situ hybridization, even

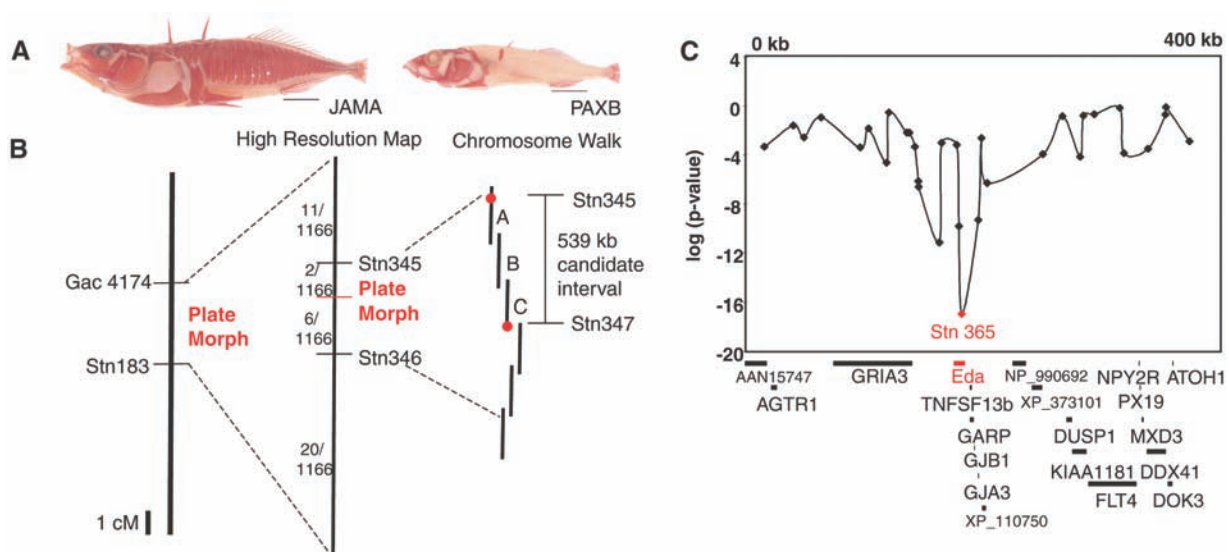
at stages where we could recover spliced *Eda* mRNA using RT-PCR. Difficulties in detecting *Eda* expression by in situ hybridization have also been reported in several other organisms, presumably because of low levels of expression during normal development (29, 30).

**Molecular basis of parallel evolution.**

Because previous complementation and genetic mapping results suggest that the same major locus controls lateral plate patterning in multiple freshwater populations around the world (2, 4, 6), we expanded the survey of *Eda* sequence to 10 completely plated marine populations and 15 low-plated populations collected from sites throughout the Northern Hemisphere (Fig. 3A, table S1). It was interesting that most of the low-plated populations, although from diverse regions, shared many of the base-pair changes previously seen in the PAXB population. One clear exception was a low-morph population from Nakagawa Creek, Japan (NAKA), which had no mutations that would alter the EDA amino acid sequence found in marine sticklebacks (SRMA) (Fig. 2). Previous genetic crosses show that low-plated fish from the NAKA population fail to complement the low-plated phenotype of fish from Salmon River, British Columbia (SRST) (2), a population that does share the characteristic *Eda* sequence changes seen in PAXB and most other low-plated populations. These data suggest that the low-plated phenotype in NAKA is due to an independently derived allele of *Eda*.

To further analyze the history of sequence changes in the plate morph region, we built phylogenetic trees using 1328 bp of exon and intron sequence from the *Eda* locus (Fig. 3B). Bayesian, maximum parsimony, and maximum likelihood trees all showed that *Eda* sequences from every low-plated population except NAKA belonged to a distinct clade (posterior probability of 1.00 in MrBayes trees and bootstrap support of 99.7 and 100% in parsimony and maximum likelihood trees, respectively). These results show that *Eda* alleles of most low-plated populations share a common ancestry. The exception is the low-plated allele within the NAKA population, which clearly has a separate origin.

Despite the shared ancestry of *Eda* alleles, it is highly unlikely that an ancestral population of low-plated fish migrated through the ocean to found most other low-plated populations in freshwater lakes and streams around the world. Ocean sticklebacks are virtually always completely plated. In addition, previous phylogenetic analysis of mitochondrial sequences reject the possibility that low-plated fish have a single origin (9, 31, 32). To further test whether low-plated populations have multiple origins, we amplified sequences from 25 random nuclear genes and scored single-nucleotide polymorphisms (SNPs) at 193 sites from 20 different completely and low-plated populations. Trees built from the nuclear sequences showed no evidence for a single origin of low-plated populations (Fig. 3C). The



**Fig. 1.** Genetic, physical, and linkage disequilibrium map of the plate morph interval. (A) Complete and low-morph phenotypes in alizarin red-stained specimens (4) of Japanese Marine (JAMA, left) and benthic Paxton Lake (PAXB, right) sticklebacks, the parent populations of a large  $F_2$  mapping cross. Other skeletal changes evident in PAXB fish, such as reduction of pelvis and spines, map to different chromosomes (5). Scale bars, 1 cm. (B) High-resolution genetic and physical mapping. Newly isolated markers *Stn345* and *Stn346* rarely recombine with the plate morph locus (numbers of recombinants in 1166 chromosomes shown). Six overlapping BAC clones span the genetic interval, and markers *Stn345* and *Stn347* (red dots) map to opposite sides of the plate morph locus.

(C) Linkage disequilibrium screening. Two sequenced BAC clones were used to develop new microsatellite markers (*Stn348–Stn379*) located 15, 39, 48, 63, 96, 103, 118, 120, 135, 137, 142, 145, 145, 163, 165, 177, 179, 182, 195, 198, 203, 250, 267, 282, 284, 293, 315, 319, 339, 353, 354, and 374 kilobases from *Stn345* (black dots). *Stn365*, located in the stickleback *Eda* locus, showed large differences in allele frequency in completely and low-plated fish from Friant, CA. Positions of other genes in the sequenced interval are shown, with human genome nomenclature committee (HGNC) designations, or accession numbers of the best matches in National Center for Biotechnology Information (NCBI) BLAST searches.



basis of genetic and geological estimates (33)], but smaller than the number between *G. aculeatus* (SRMA) and the sister species *G. wheatlandi* [thought to have diverged ~ten million years on the basis of the split between Pacific and Atlantic populations (8)] ( $K_s$  values of 0.01938, 0.01384, and 0.05875, respectively). The common clade of low-plated alleles thus probably arose between 2 and 10 million years ago. This date is at least 100 to 1000 times older than the recent divergence of modern stickleback populations in post-glacial lakes and streams that formed at the end of the last Ice Age.

**EDA-A1 transgenic sticklebacks develop extra lateral plates.** Although the previously established role of EDA signaling in dermal bone and scale development (23) makes *Eda* a compelling candidate for the locus controlling lateral plate phenotypes, several other genes are present in the shared haplotype found in most low-plated populations. To test directly whether changing levels of EDA signaling could alter plate development in sticklebacks, we injected one-cell embryos from low-plated parents with a full-length mouse EDA-A1 cDNA under the control of the broadly expressed human

cytomegalovirus (CMV) promoter. This construct has previously been shown to restore development of teeth, hair, and sweat glands when introduced into *Tabby* mutant mice carrying a null mutation at the *Eda* locus (34). Because of the mosaic inheritance of injected DNA constructs in transgenic sticklebacks (35), we scored injected and control siblings for mosaic patches of ectopic lateral plate formation after raising animals to 33- to 40-mm standard length. PCR genotyping confirmed that all fish from the cross were homozygous for an indel marker in intron 1 of *Eda* that is characteristic of the low-morph allele. Of 23 injected animals, 14 were also positive for the transgene (Fig. 4B). All 33 control siblings displayed the expected low-morph phenotype, having eight or fewer anterior plates per side (Fig. 4A). However, 3 out of 14 transgene-positive animals developed extra plates on their sides and caudal peduncles. The animal shown in Fig. 4, C and D, had six extra plates on its left side and one keel plate on its right peduncle. The other transgenic fish with additional plates also displayed them in a mosaic fashion, with one fish having one extra plate on the right side and two keel plates on the left peduncle and

the other animal having one extra plate on the right side and two extra plates on the left side. No extra plates developed after injection of low-plated embryos with constructs containing the same vector backbone and a GFP insert instead of the EDA cDNA (35). These results confirm that EDA signaling is sufficient to trigger lateral plate formation on both the flank and tail regions of the body and that introduction of *Eda* transgenes can partially rescue the low-plated phenotype of freshwater sticklebacks.

**Mutational spectrum in mammals and sticklebacks.** In both humans and mice, a diverse set of mutations in three different genes of the EDA signaling pathway cause nearly indistinguishable defects in hair, teeth, sweat glands, and bone (21, 22). In contrast, our studies show that widespread evolution of low-plated phenotypes in sticklebacks has occurred by repeated selection of a small number of related alleles at *Eda*. Several factors may account for this narrow spectrum of alleles that produce the low-plated phenotype. The diverse human *Eda* mutations (25, 26), originally identified in rare patients with a medical disorder, have pleiotropic effects that would decrease fitness in the wild. By contrast, the low-plated phenotype in sticklebacks represents the first known example whereby specific *Eda* alleles have been repeatedly fixed by natural selection in the wild. The limited amino acid changes seen in most low-plated fish and the absence of any amino acid changes in the NAKA population suggest that natural variation in lateral plates is likely caused by coding or regulatory alleles of *Eda* that are not null mutations. Such limited changes may avoid the pleiotropic effects of a complete loss-of-function of *Eda* and thereby produce a spectrum of mutations very different from those found in human patients.

In addition, the repeated fixation of a particular haplotype at the *Eda* locus could be due to coselection for additional phenotypes produced by genes closely linked to *Eda*. Low-plated populations show correlated changes in salt tolerance (36), parasite susceptibility (37), and behavior (38, 39). Although our transgenic results show that changes in lateral plate patterning are controlled by *Eda* itself, the multiple linked coding and noncoding changes in the common haplotype could also influence the structure or regulation of *Tnfrsf13b*, *Garp*, or *Gjb1*. *Tnfrsf13b* has previously been implicated in B cell development (40) and parasitic worm load in humans (41). The functions of *GARP* are unknown, but *Gjb1* belongs to a family of connexins that can produce changes in myelination and conduction velocity of neurons, hearing, skin thickness, and salt secretion (42). Related nonplate phenotypes caused by closely linked genes may contribute to the overall selective advantage of the ancient low-morph haplotype in freshwater (36).

**Table 1.** Distinctive haplotypes surrounding *Eda* in completely and low-plated sticklebacks. Genotypes are shown at 22 selected SNP positions throughout the plate morph region. Ten different completely plated (red) populations (AKMA, GJOG, JAMA, JASE, LITC, LLOY, NEU, NHR, SRMA, NAV) and four low-plated (blue) populations (BLAU, PAXB, WMSO, SFC) differ at all 22 positions, defining a shared haplotype characteristic of most complete and low populations. Recombinant haplotypes in SCX, FADA, PAXL, NOST, WALL, AKST, COND, SRST, and FRIL populations define a minimum shared haplotype, including SNPs 5-15. SNP genotypes in the completely plated LITC58 animal confirm that low-plated alleles are present in migratory marine populations. SNPs are either intergenic (I), synonymous (S), or nonsynonymous (N) changes and are located at the following nucleotide positions in the CHORI213-2D11 BAC sequence: 195422 (EDA Ser7Pro), 195496 (EDA Ser31Ser), 195649 (EDA Leu79Pro), 195692 (EDA Ile97Val), 203488 (EDA Ser140Leu), 203815 (EDA Pro172Pro), 207116 (TNFSF13B Leu69Phe), 208941 (TNFSF13B Gly197Gly), 208557 (TNFSF13B Ile219Ile), 210237 (GARP Met197Leu), 210240 (GARP Leu198Leu), 211414 (GARP Ser589Ile), 211415 (GARP Ser589Ile), 211573 (GARP Phe642Cys), 211615, 211677, 221853, 221944 (GJB1 His16His), 222209 (GJB1 Val105Ile), 222692 (GJB1 Thr266Ala), 222758, and 222759.

	EDA				TNFSF13B			GARP				Intergenic		GJB1			Intergenic					
Exon:	1	1	1	1	3	4	2	6	6	2	2	2	2	2	-	-	1	1	1	-	-	
Type of Change:	N	S	N	N	N	S	N	S	S	N	S	N	S	N			S	N	N			
SNP:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
10 COMPLETE POPULATIONS	T	T	T	A	C	G	G	C	C	A	T	G	C	T	T	C	T	C	G	A	G	T
4 LOW POPULATIONS	C	C	C	G	T	A	C	T	T	C	C	T	T	G	A	G	A	T	A	G	A	G
SCX (26)	T	T	T	G	T	A	C	T	T	C	C	T	T	G	A	G	A	T	A	G	A	G
FADA (23)	T	T	T	A	T	A	C	T	T	C	C	T	T	G	A	G	A	T	A	G	A	G
PAXL (8)	T	T	T	A	T	A	C	T	T	C	C	T	T	G	A	G	A	T	G	A	G	T
NOST (24)	C	C	C	G	T	A	C	T	T	C	C	T	T	G	A	C	T	C	G	A	G	T
WALL (4), AKST (5), & COND (12)	C	C	C	G	T	A	C	T	T	C	C	T	T	G	A	G	A	T	G	A	G	T
SRST (9)	C	C	C	G	T	A	C	T	T	C	C	T	T	G	A	G	A	T	A	A/G	G/A	T/G
FRIL (15)	C	C	C	G	T	A	C	T	T	C	C	T	T	G	A	G	A	T	A	G	G/A	T/G
OMPL(19)	C	C	T	G	T	A	C	T	T	C	C	T	T	G	A	G	A	T	A	G	A	G
LITC58 (10)	T/C	T/C	T/C	A/G	C/T	G/A	G/C	C/T	C/T	A/C	T/C	G/T	C/T	T/G	T/A	C/G	T/A	C/T	G/A	A/G	G/A	T/G
NAKA (1)	T	T	T	A	C	G	G	C	C	A	T	G	C	T	T	C	T	C	G	A	G	T

**Mechanism for rapid parallel evolution.**

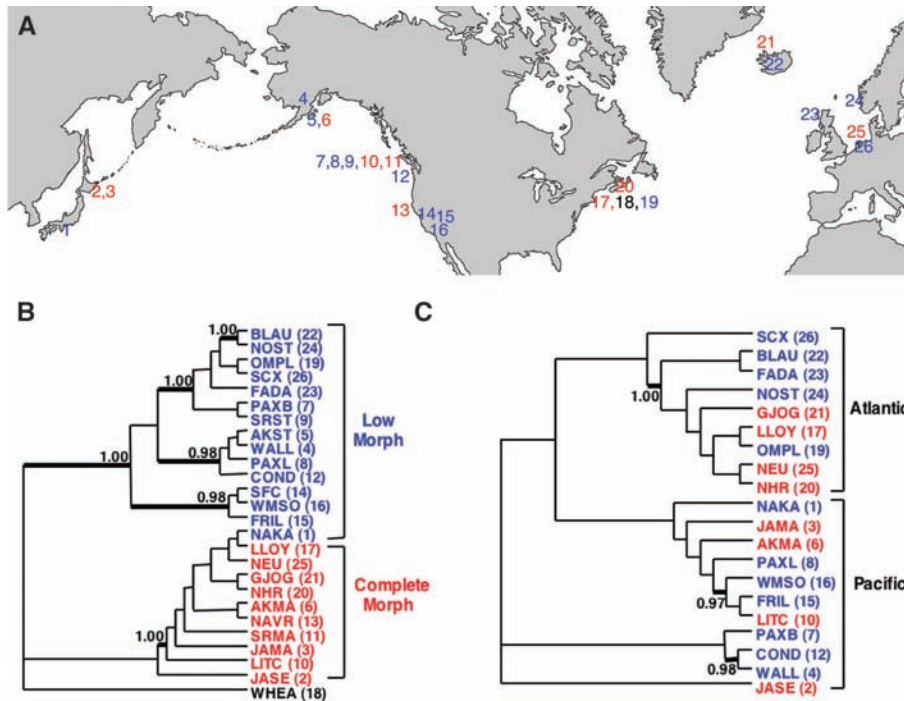
Forty years ago, Lindsey proposed that parallel evolution of stickleback lateral plate patterns could occur either by independent mutations in different populations or by repeated selec-

tion on the standing genetic variation already present in marine ancestors (43). Our studies show that both mechanisms have contributed. The EDA sequences in low-plated NAKA fish are distinct from those in other low popula-

tions (Fig. 3B). However, the presence of a shared haplotype in most low-plated populations suggests that selection on standing variation is the predominant mechanism underlying the recent rapid evolution of changes in lateral plate patterns in wild sticklebacks.

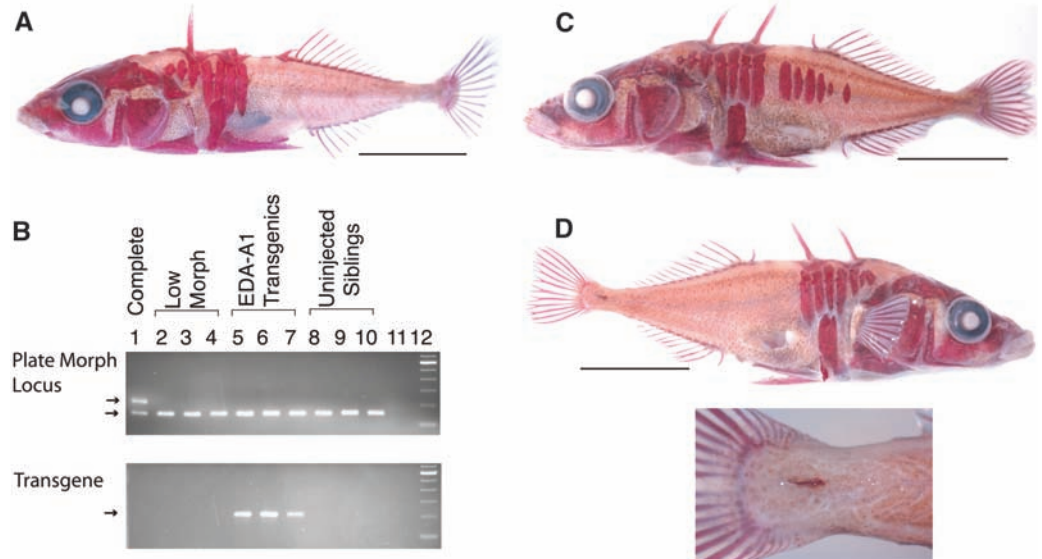
One surprising finding of this study is the enormous geographical distance over which related low-plated alleles are shared. Both nuclear and mitochondrial data (9, 31, 32) rule out the possibility that these alleles have spread by migration of a single low-plated population of fish throughout the world. In contrast, our surveys of large numbers of marine fish show that low-plated alleles are present at detectable frequencies in completely plated sticklebacks. This low frequency is likely maintained by the occasional hybridization that occurs when marine sticklebacks come into contact with low-plated freshwater populations in coastal streams during the breeding season (38) or perhaps by fitness effects in heterozygotes. The annual migration of marine sticklebacks from ocean to freshwater could provide a mechanism for repeated flow of the ancient low-morph alleles back into marine populations and, from there, into new freshwater environments. Fish heterozygous for completely and low-plated alleles at *Eda* typically have either complete or partial lateral plates (4, 6), facilitating the persistence of a cryptic genetic variant within marine populations, and providing a genetic mechanism that could lead to rapid emergence of the low-plated phenotype after colonization of new environments.

The differences in armor plate patterns of sticklebacks are so large that Cuvier originally classified plate morphs as distinct species (10).



**Fig. 3.** Most low-plated populations have a shared history at the *Eda* locus, but not at other nuclear genes. (A) Geographic origin of 25 low-plated (blue numbers) and completely plated (red numbers) stickleback populations used for sequencing. (B) MrBayes tree of *Eda* sequences. *G. wheatlandi*, a sister species of *G. aculeatus*, was used as an outgroup (black, number 18). *Eda* alleles in most low populations share a common origin. Posterior probabilities greater than 95% are shown (branches in bold). (C) MrBayes phylogeny based on genetic differences in 193 SNPs from 25 random nuclear loci. Tree topology is significantly different from the EDA tree, ruling out the possibility that all present-day low-plated populations are derived from a single population sharing alleles at most genes.

**Fig. 4.** EDA-A1 stimulates lateral plate formation in transgenic sticklebacks. (A) Typical low-plated pattern in control fish stained with alizarin red. (C and D) A sibling from the same clutch after introduction of a transgene containing a full-length mouse EDA-A1 cDNA driven by a CMV promoter (34). Note six extra lateral plates that have formed in the flank region on the left side (C), and the new keel plate on the right tail (D). Differences in spine number are due to clipping for DNA recovery. Scale bars, 1 cm. (B) (top) PCR genotyping confirms that transgenic animals are homozygous for a 150-bp allele characteristic of the ancestral low-plated allele at the endogenous *Eda* locus, just like wild-caught low-plated fish from FRI, San Francisquito Creek (SFC) and uninjected siblings. In contrast, completely plated fish from FRI are either heterozygous for the 218-bp allele and the 150-bp allele (lane 1) or homozygous for the 218-bp allele (not shown). (B) (bottom) Primers specific for mouse EDA-A1 amplify a 211-bp fragment from transgenic fish, but not from uninjected control animals. Genomic DNA samples:



Lane 1, FRI Complete; 2, FRI Low; lanes 3 to 4, SFC Low; lanes 5 to 7, EDA-A1 transgenic animals with additional lateral plates; lanes 8 to 10, uninjected siblings of EDA-A1 transgenic animals; lane 11, water control; lane 12, 100-bp DNA size ladder (Invitrogen).

In the past, it has been difficult to trace such large-scale changes in body form to the action of particular genes and mutations. Our results show that large changes in vertebrate skeletal morphology in the wild can be created by relatively simple genetic mechanisms. Natural populations have variants of the same major signaling genes already known to play a crucial role in normal development and human disease. The variant alleles preexist at low frequency in ancestral marine populations and can be repeatedly fixed in new environments to produce a rapid shift to an alternative body form. Many other dramatic morphological, physiological, and behavioral changes are known in sticklebacks. With the recent advent of forward genetic approaches in this system, it should now be possible to identify the genes that underlie many ecologically important traits, which will bring a new molecular dimension to the study of how phenotypic changes arise during vertebrate evolution.

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#### Supporting Online Material

[www.sciencemag.org/cgi/content/full/307/5717/1928/DC1](http://www.sciencemag.org/cgi/content/full/307/5717/1928/DC1)

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## Temporal Relationships of Carbon Cycling and Ocean Circulation at Glacial Boundaries

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Evidence from high-sedimentation-rate South Atlantic deep-sea cores indicates that global and Southern Ocean carbon budget shifts preceded thermohaline circulation changes during the last ice age initiation and termination and that these were preceded by ice-sheet growth and retreat, respectively. No consistent lead-lag relationships are observed during abrupt millennial warming events during the last ice age, allowing for the possibility that ocean circulation triggered some millennial climate changes. At the major glacial-interglacial transitions, the global carbon budget and thermohaline ocean circulation responded sequentially to the climate changes that forced the growth and decline of continental ice sheets.

Records of past global climate preserve evidence of large-scale changes in temperature and ice volume at glacial-interglacial boundaries. Although the timing of ice ages is broadly driven by Milankovich orbital cycles, the small insolation changes require amplifying mechanisms to produce the large glacial-interglacial climate changes. Fluctuation in North Atlantic Deep Water (NADW) production is a potential amplifier and has been suggested as a trigger for rapid global climate

shifts (1). Carbon dioxide (CO<sub>2</sub>) is another possible amplifier on glacial-interglacial time scales, because its atmospheric concentration is predominantly controlled by changes in deep-ocean storage (2) and the terrestrial carbon reservoir (e.g., 3–5). Carbon isotope ratios are distinct in different reservoirs, making it a powerful tool to constrain the timing of global carbon budget reorganizations relative to other changes in the climate system. In the oceans, carbon isotope ratios of benthic foraminifera

(benthic δ<sup>13</sup>C) are commonly used as a proxy for ocean circulation because they vary systematically in water masses [e.g., (6–8)]. However, temporal benthic δ<sup>13</sup>C changes at any location reflect a combination of the global carbon mass balance, ocean-circulation changes, air-sea equilibration, and productivity changes. If carbon budget and ocean-circulation signals can be deconvolved, the temporal sequence of major shifts in global ice volume, carbon mass balance, and ocean circulation can help to clarify the ocean's role as a trigger of, or a response to, major climate changes. In this study, we compare the temporal sequence of these changes since the last interglacial period. Chronological ambiguities are obviated through study of different proxy signals in the same core, and thus the sequence of events associated with climate change and ocean circulation can be extracted.

**Nd isotopic systematics.** We use Nd isotopes as a proxy of the balance between NADW and southern-sourced waters in the South Atlantic. <sup>143</sup>Nd/<sup>144</sup>Nd ratios vary in the

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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.

## EVOLUTION

# The Synthesis and Evolution of a Supermodel

Greg Gibson

**S**ynthesis 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

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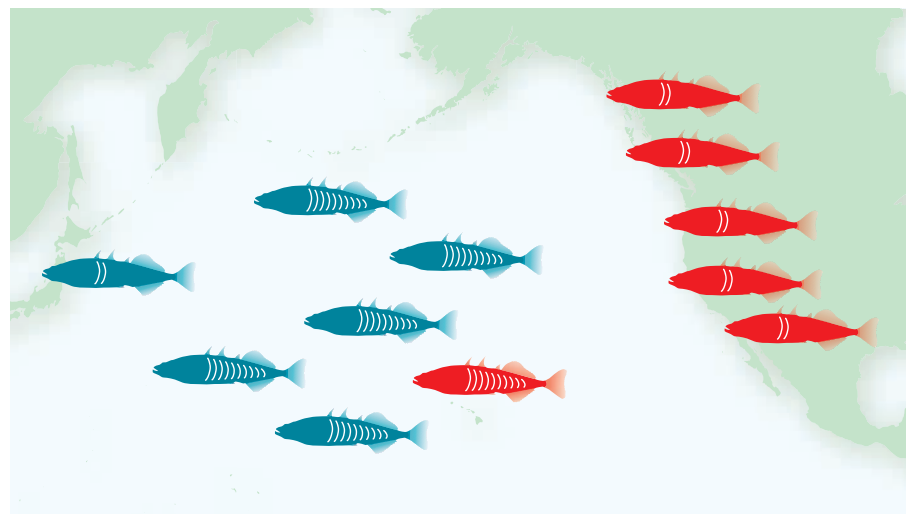
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rather than a single stickleback population with reduced body armor founding the populations in all of these lakes, parallel evolution must have occurred. He attributed parallel evolution to selection either on independent mutations or on a rare allele 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 dis-

tinct. 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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