

# Correlated Evolution and Dietary Change in Fossil Stickleback

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Models, experiments, and field studies provide evidence of the ecological controls on evolution, but extrapolating results over longer time scales is a perennial problem in evolutionary biology. Trophic ecology and competition for food, for example, are thought to drive speciation through niche differentiation, character displacement, and phenotypic divergence (1). Yet direct evidence that feeding controls evolution over extended time scales,

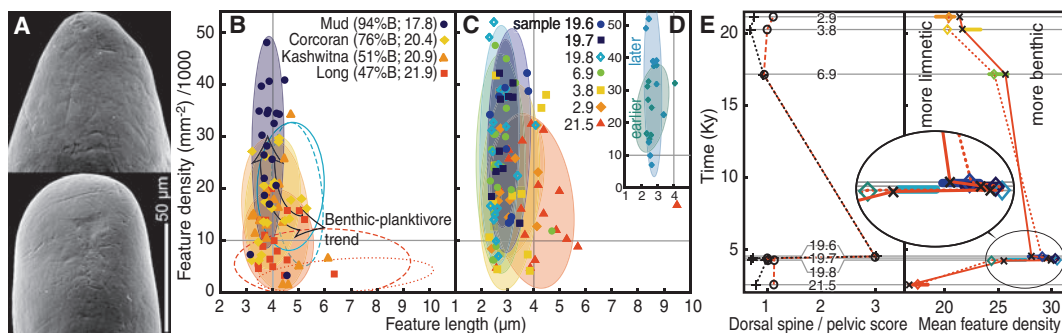
high-resolution record of evolutionary change within a lineage spanning tens of thousands of years (3).

We investigated the relationship between trophic resource use and evolutionary change through quantitative analysis of dental microwear (4). Laboratory feeding experiments and analyses of wild stickleback populations show that microwear exhibits a progressive shift from planktivores to benthic feeders (Fig. 1, A and B) (5). Discriminant

These changes in inferred trophic ecology are significantly correlated with evolutionary changes in armor phenotype through time (3) (Fig. 1E). DF scores are correlated with dorsal [nonparametric Spearman rank correlation ( $r_s$ ) = 0.23,  $P$  = 0.03,  $n$  = 89 fish] and pelvic armor ( $r_s$  = 0.21,  $P$  = 0.05), feature density with dorsal armor ( $r_s$  = 0.24,  $P$  = 0.02). Interestingly, the shift to a more benthic ecology within sample 19.8 (Fig. 1, D and E) precedes the increase in mean armor scores in 19.6 (a time lag of circa 100 years). This evidence of an ecological shift preceding phenotypic change suggests that this part of the sequence may record rapid evolution driven by shifts in trophic ecology and adaptation to benthic niches. If this hypothesis is correct, however, the low number of specimens displaying intermediate phenotypes is puzzling, and the scenario of replacement of one lineage by another (3) cannot be ruled out. The gradual shift

to less benthic ecology over the next 17,000 years supports the interpretation that a return to low-armor phenotypes reflects directional natural selection (3).

Our analysis shows that dental microwear analysis can provide direct evidence for changes in trophic niche and resource exploitation in fossil fishes. That changes in feeding can be detected independently of morphological change highlights the potential of this approach to provide important insights into trophic ecology during adaptive radiations of fishes and other evolutionary events.



**Fig. 1.** Microwear in stickleback teeth and correlated evolutionary change. (A) Scanning electron micrographs showing tooth microwear in fossil (top) and extant benthic feeding (bottom) stickleback. For details, see fig. S1. (B) Microwear in wild-caught and lab-raised stickleback. Open ellipses indicate lab distributions (blue, benthic treatments; red, planktivore; solid, dashed, and dotted lines are coarse, medium, and no sand substrate, respectively). In wild fish, microwear tracks trophic ecology as indicated by % benthic stomach contents and mean gill raker count (Mud Lake, most benthic; Long Lake, least benthic). (C) Fossil stickleback microwear; inset (D) shows sample 19.8 divided into earlier (1746 to 1753 years) and later (1757 to 1771 years) subsamples with shift toward more benthic trophic ecology in later interval. Ky, thousand years. (E) Trophic niche and morphology in fossil stickleback through time [○ dorsal armor; + pelvic armor; ◇ mean feature density; × DF scores (minimum of 0.38 and maximum of 2.51)]. Colored horizontal bars show niche scores reflecting the position of the samples in the benthic-planktivore microwear spectrum (C). Time scale follows (3).

available only from the fossil record, is difficult to obtain because it is rarely possible to directly analyze dietary change in long-dead animals. Functional changes must be inferred from changes in morphology, and attempts to determine whether morphological changes were caused by shifts in feeding can become circular.

Here, we report an investigation of trophic resource use in a fossil sequence preserving an evolving lineage of threespine stickleback (*Gasterosteus*). We focus on stickleback for two reasons. First, perhaps the best-known work on speciation in fishes concerns stickleback in postglacial coastal lakes in Canada, where planktivores and benthic feeders coexist as two reproductively isolated and phenotypically distinct trophic forms. The differences between these forms result from competition for food (1, 2). Second, fossil stickleback from the Miocene Truckee Formation (Nevada) provide a detailed,

analysis using feature length and density indicates that scores for the first discriminant function (DF) are a good predictor of trophic ecology. For wild fish populations ( $n$  = 4), mean scores were significantly correlated with diet ( $r$  = 0.95,  $P$  = 0.05) and gill raker number ( $r$  = -0.996,  $P$  = 0.004).

Analysis of fossil stickleback teeth revealed an overall range and pattern of feature densities and lengths similar to that of extant fish (Fig. 1C), suggesting that the fossil microwear records a similar benthic-planktonic feeding spectrum. This was supported by application of the DF derived from wild fish to the fossils: DF scores vary significantly between samples ( $F$  = 10.8, df of 7 and 87,  $P$  = 0.0001), and a Tukey-Kramer procedure revealed significant pairwise differences. This procedure also grouped some fossil samples with benthic-feeding wild populations (samples 19.6, 19.7, 19.6, and 6.9), others with planktivore populations (21.5), with some placed between (2.9 and 3.8).

## References and Notes

1. D. Schluter, *The Ecology of Adaptive Radiations* (Oxford Univ. Press, Oxford, 2000).
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4. Materials and methods are available as supporting material on Science Online.

5. M. A. Purnell, P. J. B. Hart, D. C. Baines, M. A. Bell, *J. Anim. Ecol.* **75**, 967 (2006).
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## Supporting Online Material

www.sciencemag.org/cgi/content/full/317/5846/1887/DC1  
Materials and Methods  
Fig. S1

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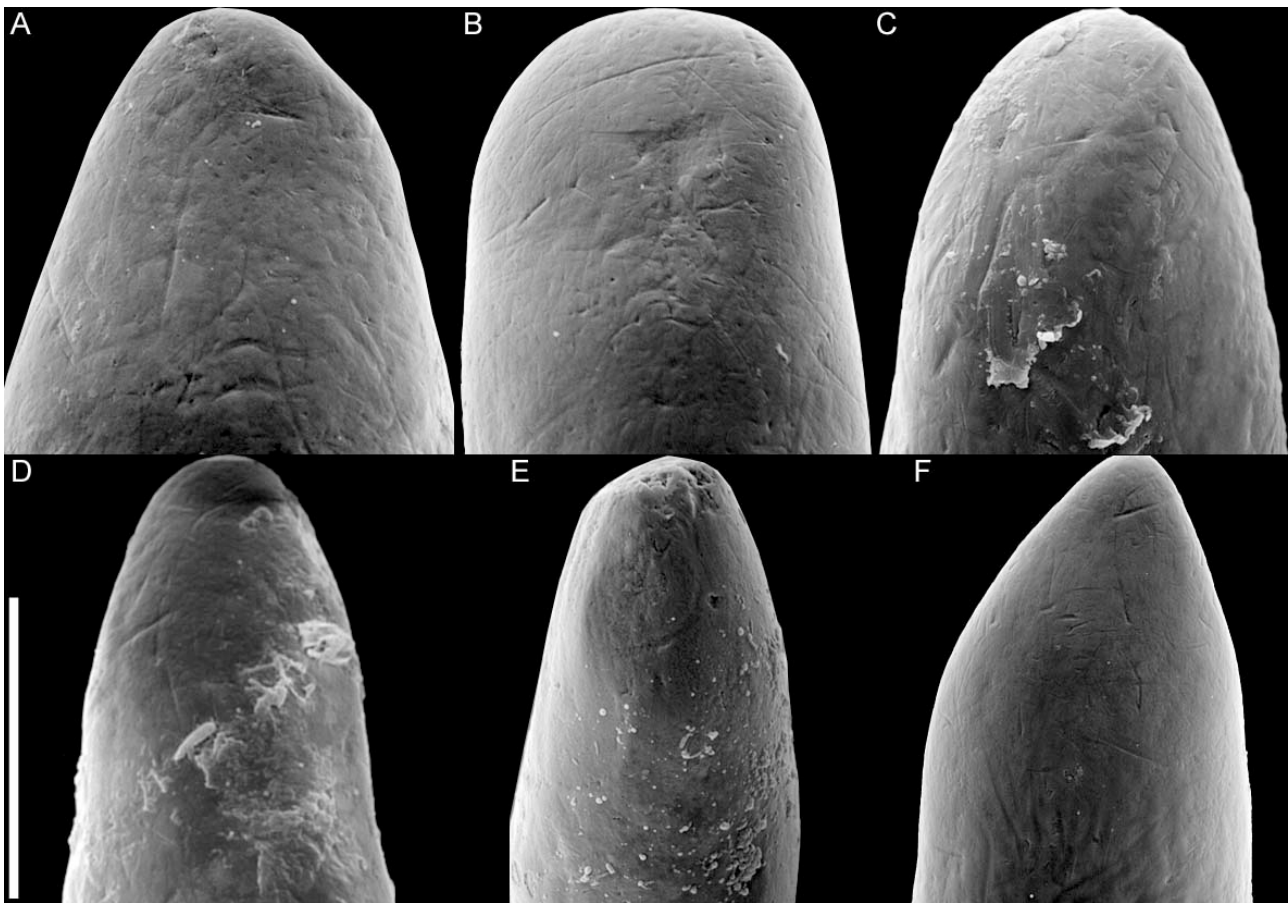
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data generated by the Microware software. For further details of our methods, see ref (1). Analysis of tooth microwear and trophic interpretation was carried out blind: the investigators who scored and interpreted microwear did not know the relative stratigraphic positions of the fossil fish horizons

sampled until the analysis and trophic interpretation were complete. Because pelvic armor phenotypes are scored using an ordinal scale, correlations between microwear and armor were tested using non-parametric Spearman Rank Correlation ( $r_s$ ). Density ellipses in Fig. 1B-C, intended only as a guide to the pattern of distribution of the data, are drawn to include 80% of the data for each sample, assuming a bivariate normal distribution (sample 21.5 extreme outlier excluded).

It is unlikely that changes in sediment are influencing fossil microwear to any significant degree because although experimental data indicate that microwear in treatments with coarse and medium sand substrates differs from those with fine sand or no substrates (1) there is no evidence for grain size changes of this magnitude in the Truckee Formation sequence (5). Difference between planktivore and benthic microwear in the fossils is unlikely to be the result of increased variance resulting from time averaging. Compared to samples exhibiting benthic microwear patterns, samples with planktivore microwear have lower minimum and lower maximum values for feature density (i.e. less variance), combined with greater range and higher values for feature length; this is not what would be expected from time averaging (6). Furthermore, recent work has shown that the effects of time averaging on phenotypic variance are less than has been thought, and that data are generally comparable to their population-level equivalents (7). Some of our samples have very different durations but the variance of the microwear data does not differ significantly. For example, samples 6.9 and 19.8 have similar microwear patterns, both falling between the mid-range and the benthic end of the spectrum. Results of ANOVA indicate that microwear data is not significantly different (for feature density and length, respectively,  $F = 0.78$ ,  $P = 0.38$ ;  $F = 0.02$ ,  $P = 0.88$ , d.f. 1, 37 in both cases), even though sample 6.9, spans 179 years, and sample 19.8 spans only 25 years.

The data for stomach contents of wild fish populations shown in Fig. 1 indicate trophic niche, but the mean of percentage food types found in each fish cannot capture some important aspects of the pattern of variation. For example, although by any measure it is the most planktivorous of the populations analysed, fish from Long Lake have a bimodal distribution of stomach contents (i.e. 30% of fish had 100% planktonic food items, 25% of fish had 100% benthic items). This is probably why the distribution of microwear data differs from the laboratory planktivore treatments. Kashwitna Lake fish were genuinely mixed feeders (20% of fish had 100% benthic items, the remainder spread across the 0-95% range). Fifty percent of Corcoran Lake fish had 100% benthic food items, and the remainder were spread. 80% of Mud Lake fish had 100% benthic prey, and of the remainder, none had less than 35%. Fish from Lynda Lake (1) exhibit no preferences regarding plankton/benthic food (no more than 11% of fish in any % prey bin) and were not included in the present analysis.



**Figure S1. Scanning electron micrographs of Miocene and recent stickleback teeth.** A. Tooth from fossil benthic sample 19.7 (image no. 04808). B. Tooth from wild benthic population from Mud Lake (image no. 92109). C. Tooth from laboratory benthic treatment, coarse sand substrate (image no. 84403). D. Tooth from fossil planktivore sample 21.5 (image no. 00832). E. Tooth from wild planktivore population from Long Lake (image no. 94309). F. Tooth from laboratory planktivore treatment, medium sand substrate (image no. 81106). Wild fish were obtained from lakes in the Matanuska-Susitna Valley, Alaska . Fossil fish were obtained from the Miocene Age Truckee Formation, Nevada. Scale bar 50  $\mu\text{m}$ .

#### Supplementary references

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