EVOLUTIONARY BIOLOGY

A pharyngeal jaw evolutionary innovation facilitated extinction in Lake Victoria cichlids

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Evolutionary innovations, traits that give species access to previously unoccupied niches, may promote speciation and adaptive radiation. Here, we show that such innovations can also result in competitive inferiority and extinction. We present evidence that the modified pharyngeal jaws of cichlid fishes and several marine fish lineages, a classic example of evolutionary innovation, are not universally beneficial. A large-scale analysis of dietary evolution across marine fish lineages reveals that the innovation compromises access to energy-rich predator niches. We show that this competitive inferiority shaped the adaptive radiation of cichlids in Lake Tanganyika and played a pivotal and previously unrecognized role in the mass extinction of cichlid fishes in Lake Victoria after Nile perch invasion.

Evolutionary innovations are adaptive traits that allow a lineage to cross a functional barrier and gain access to new niches (1). They are often framed as "key innovations" that can promote rapid diversification in the groups that evolve them (2, 3), and the search for key innovations has become a major component of modern macroevolutionary studies (4, 5). However, despite the obvious importance of evolutionary innovations in the history of life on Earth, innovative traits rarely show a direct link with increased diversification (6–15).

Evolutionary innovations are also traditionally thought to reduce extinction rates (2), but this may not be the case if innovation facilitates the evolution of specialist phenotypes sensitive to ecological disturbance (16, 17). Innovation may also exhibit niche-specific effects on extinction rates if the innovative trait involves a performance trade-off (13). Specifically, performance may increase in new niches at the cost of competitive exclusion and eventual extirpation from previously accessible niches.

We examined the potential cost of evolutionary innovation by using a classic example: pharyngognathy (18). Pharyngognathy involves multiple modifications of the jaw apparatus in the back of the throat that allow a fish to generate high bite force, which likely enables pharyngognathous fishes to exploit hard-shelled and processing-intensive prey items (19). However, these modifications reduce pharyngeal gape, which may alter the maximum size of prey that can be easily swallowed (20).

Several lineages within the spiny-finned fishes have independently evolved pharyngognathy, including wrasses, surfperches, damselfish, marine halfbeaks, flyingfishes, and cichlids (20). Most of these lineages live in shallow marine habitats, except for cichlids, which occur mostly in tropical freshwaters. Cichlids are especially well known for their tendency to undergo rapid speciation and accumulate exceptionally large species richness in spatially confined assemblages, particularly in Lakes Victoria, Malawi, and Tanganyika in eastern Africa (21, 22).

Each of these lineages has interacted with nonpharyngognathous spiny-finned lineages in different ways. In marine habitats, pharyngognathous lineages such as wrasses, parrotfishes and damselfishes have existed alongside closely related nonpharyngognathous spiny-finned fishes for tens of millions of years (20). In Lakes Victoria and Malawi, cichlids initially radiated in the complete absence of any nonpharyngognathous spiny-finned fish lineages. Unfortunately, in the 1950s, a nonpharyngognathous predatory fish, the Nile perch, *Lates niloticus*, was introduced into Lake Victoria, facilitating a cichlid mass extinction (23). In Lake Tanganyika, which hosts an older cichlid radiation than Victoria and Malawi, nonpharyngognathous *Lates* species and the pharyngognathous cichlids coexist, albeit with many fewer cichlid species and a lower speciation rate than the other two radiations (22, 24).

Comparative dietary data reveal that pharyngognathy has ecological consequences for the marine lineages that possess the trait. Unlike cichlids, which can sometimes evolve into predatory niches free from competition with predators like Nile perch, marine pharyngognaths always occur alongside typical nonpharyngognathous fish-eating lineages (20). We surveyed diet data across a phylogeny of marine spiny-finned fishes, including four marine transitions to pharyngognathy as well as other spiny-finned species occurring in the same environments as those four lineages, and measured rates of dietary evolution for fish and processing-intensive prey like plants and hard-shelled animals.
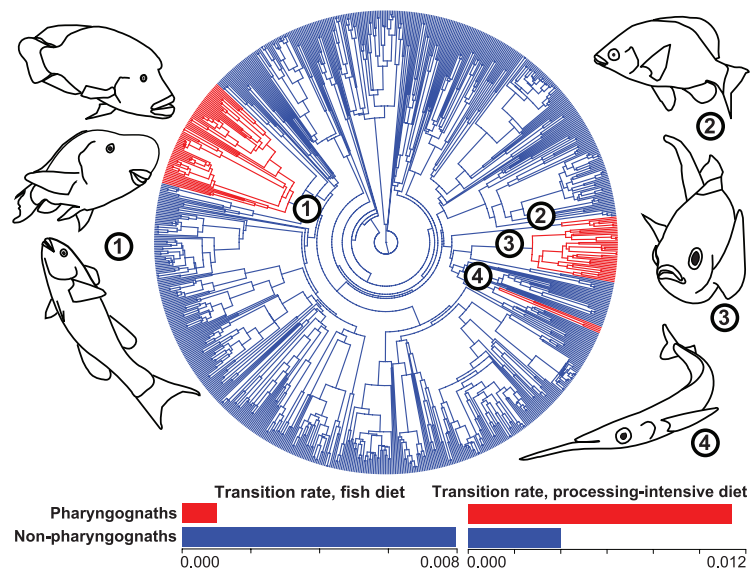
Fig. 1. Pharyngognathy affects dietary transitions in marine fishes. (**A**) Four transitions to pharyngognathy on a time-calibrated phylogeny of 851 marine spiny-finned fishes: (1) labroid fishes, including wrasses (Labridae), parrotfish (Scaridae), and weed whitings (Odacidae); (2) surfperches (Embiotocidae); (3) damselfishes (Pomacentridae); (4) marine halfbeaks (Hemirhamphidae). (**B**) Comparison of the transition rate for nonpharyngognathous and pharyngognathous fishes for fish prey and processing-intensive prey.

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We analyzed diet both as a continuous character using Brownian motion and as a categorical variable using stochastic character mapping (*25*). In both cases, we examined whether fishes with pharyngognathy had different rates of dietary evolution or rates of transition to a specialist diet for fish prey and processing-intensive prey. Pharyngognathous marine fishes evolved into niches favoring processing-intensive prey items at a much higher rate than other spiny-finned fishes (Fig. 1). However, pharyngognaths evolved into fish-eating niches more slowly, suggesting that the evolution of the innovation may compromise access to this niche.

To assess the impact of pharyngognathous predators on competition with nonpharyngognathous predators, we investigated feeding performance and functional morphology of Nile perch and cichlids. We measured pharyngeal gape in Nile perch, which possess unfused pharyngeal jaws typical of nonpharyngognathous spiny-finned fishes, as well as in every major lineage of fish-eating cichlid (*25*). We found that pharyngognathy reduced cichlid pharyngeal gape to half that of Nile perch (Fig. 2A). The only exception to this pattern was in the South American genus *Cichla*, an old fish-eating cichlid lineage that has independently

lost pharyngognathy via loss of fusion of the lower pharyngeal jaw.

Feeding experiments indicate that pharyngognathy drastically increases handling time in cichlid predators relative to Nile perch. We measured handling time (*25*) in four predatory Lake Victoria cichlids and similarly-sized Nile perch by using fish prey of sizes and shapes comparable to those consumed in the wild by both groups (*26*). Cichlids were considerably slower, often taking many hours to process a prey item that a Nile perch could swallow in a few minutes (Fig. 2B). If processing time is examined with respect to pharyngeal gape (*25*), the difference between Nile perch and cichlids disappears, suggesting that the narrower pharyngeal gape of the cichlids is the primary cause of their long prey-processing times (*25*). Our results here are limited to predatory cichlids and *Lates*, but we suggest that similar analyses across marine lineages are likely to be of great interest for understanding the role of pharyngognathy in marine ecosystems.

If pharyngognathy hinders cichlid feeding performance when processing fish prey, fish-eating cichlids may have been particularly disadvantaged after Nile perch were introduced into Lake Victoria. We used conditional inference forests with

corrections for correlated variables to explore how ecological variables predict extinction in Victorian cichlids (*25*). A fish diet is the most important predictor of extinction (Fig. 3A), suggesting that competition played an important role in addition to known factors like predation (*23*) and eutrophication (*27*, *28*).

Of the major functional morphological traits associated with the radiation, a large lower jaw length shows the strongest association with a fish diet in Victorian cichlids (*21*, *25*). We reveal a large morphological shift in this character when comparing all fish-eating Victorian cichlids to a representative sample of fish-eaters in the relict fauna of Lake Victoria after Nile perch invasion (Fig. 3B). The preextinction fish-eater community was highly diverse and species rich, with many species possessing jaws of equal or greater size to Nile perch and often consuming large prey (*26*). However, the few relict fish-eating individuals collected postextinction all have a less predatory morphology than was typical for predatory cichlids of this radiation before the extinction events. The relict Victorian cichlid species now more closely resemble the less-extreme fish-eaters from Lake Tanganyika, where Nile perch and cichlids have coexisted for millions of years.

Fig. 2. Cichlids exhibit reduced pharyngeal gape and increased handling times relative to Nile perch. (**A**) Pharyngeal gape comparison of Nile perch, which possesses typical pharyngeal jaws, and fish-eating cichlids, including the one known loss of pharyngognathy in cichlids (genus *Cichla*). From top, *Lates niloticus*, *Harpagochromis* sp. "orange rock hunter," *Pyxichromis orthostoma*, *Harpagochromis cf serranus*, *Harpagochromis* sp. "two stripe white lip," *Lipochromis* sp. "matumbi hunter," *Lipochromis parvidens*, *Champsochromis caeruleus*, *Nimbochromis* sp., *Rhamphochromis longiceps*, *Boulengerochromis microlepis*, *Bathybates minor*, *Lepidiolamprologus profundicola*, *Cyphotilapia frontosa*, *Cichla ocellaris*, *Parachromis* sp., *Petenia splendida*. (**B**) Handling time comparison between Nile perch and four species of fish-eating Victorian cichlids with respect to the ratio of prey length:predator length. Colors as in (A).
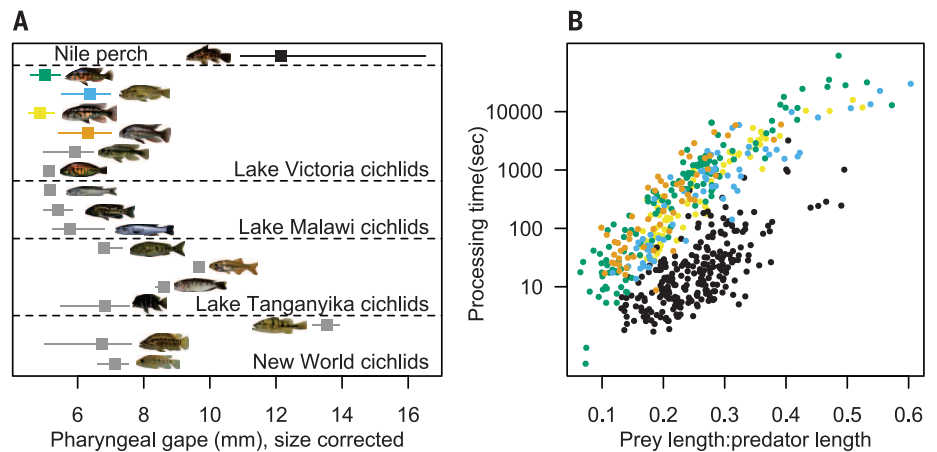
Fig. 3. Extinction of fish-eating Lake Victoria cichlids. (**A**) Relative importance of the four highest ecological variables predicting extinction in Victorian cichlids for the original 1992 data set (*23*) and an updated one (*25*). Pluses indicate increased risk; minuses indicate reduced risk. AUC, area under the curve. (**B**) Beeswarm plot of size-corrected lower-jaw length of Nile perch (NP), preinvasion Victorian cichlid fish-eaters (LV), postinvasion relict fish eaters (LVrelict), and eight transitions to fish-eating in Lake Tanganyika (LT) cichlids. Large bars indicate mean jaw length.
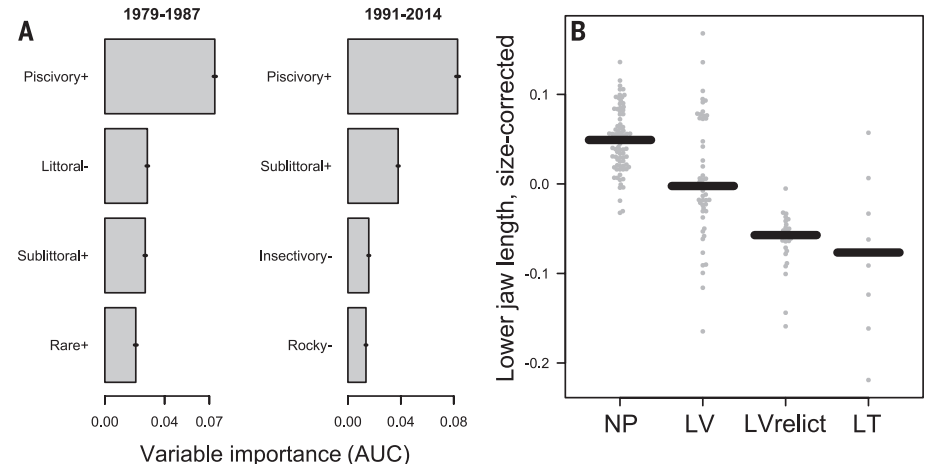
Interspecific competition is thought to be a pervasive force in evolution (29, 30), and we suggest that the pattern we observe across Lakes Victoria and Tanganyika is likely due to competition between Nile perch and cichlid predators.

For nearly half a century, the robust pharyngeal jaws of cichlids, wrasses, and other pharyngognathous fishes have been considered a classic example of evolutionary innovation that opened up new niches through increased trophic flexibility (18). Although this is almost certainly correct, our results suggest that the innovation involves a major trade-off that severely limits the size of prey that can be eaten, facilitating competitive inferiority in predatory niches and extinction in the presence of a predatory invader lacking the innovation. The evolutionary innovation of pharyngognathy is not a uniformly beneficial trait, but a specialization that can promote competitive exclusion and extinction depending on ecological context and community composition.

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CANCER IMMUNOTHERAPY

Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota

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Antibodies targeting CTLA-4 have been successfully used as cancer immunotherapy. We find that the antitumor effects of CTLA-4 blockade depend on distinct *Bacteroides* species. In mice and patients, T cell responses specific for *B. thetaiotaomicron* or *B. fragilis* were associated with the efficacy of CTLA-4 blockade. Tumors in antibiotic-treated or germ-free mice did not respond to CTLA blockade. This defect was overcome by gavage with *B. fragilis*, by immunization with *B. fragilis* polysaccharides, or by adoptive transfer of *B. fragilis*–specific T cells. Fecal microbial transplantation from humans to mice confirmed that treatment of melanoma patients with antibodies against CTLA-4 favored the outgrowth of *B. fragilis* with anticancer properties. This study reveals a key role for *Bacteroidales* in the immunostimulatory effects of CTLA-4 blockade.

Ipilimumab is a fully human monoclonal antibody (Ab) directed against CTLA-4, a major negative regulator of T cell activation (1), approved in 2011 for improving the overall survival of patients with metastatic melanoma (MM) (2). However, blockade of CTLA-4 by ipilimumab often results in immune-related adverse events at sites that are exposed to commensal microorganisms, mostly the gut (3). Patients treated with ipilimumab develop Abs to components of the enteric flora (4). Therefore, given our previous findings for other cancer therapies (5), addressing the role of gut microbiota in the immunomodulatory effects of CTLA-4 blockade is crucial for the future development of immune checkpoint blockers in oncology.

We compared the relative therapeutic efficacy of the CTLA-4–specific 9D9 Ab against established MCA205 sarcomas in mice housed in specific pathogen–free (SPF) versus germ-free (GF) conditions. Tumor progression was controlled by Ab against CTLA-4 in SPF but not in GF mice (Fig. 1, A and B). Moreover, a combination of broad-spectrum antibiotics [ampicillin + colistin + streptomycin (ACS)] (Fig. 1C), as well as imipenem alone (but not colistin) (Fig. 1C), compromised the antitumor effects of CTLA-4–specific Ab. These results, which suggest that the gut microbiota is required for the anticancer effects of CTLA-4 blockade, were confirmed in the Ret melanoma and the MC38 colon cancer models (fig. S1, A and B). In addition, in GF or ACS-treated mice, activation of splenic effector CD4$^+$ T cells and tumor-infiltrating lymphocytes (TILs) induced by Ab against CTLA-4 was significantly decreased (Fig. 1, D and E, and fig. S1, C to E).

We next addressed the impact of the gut microbiota on the incidence and severity of intestinal lesions induced by CTLA-4 Ab treatment. A "subclinical colitis" dependent on the gut microbiota was observed at late time points (figs. S2 to S5). However, shortly (by 24 hours) after the first administration of CTLA-4 Ab, we observed increased cell death and proliferation of intestinal epithelial cells (IECs) residing in the ileum and colon, as shown by immunohistochemistry using Ab-cleaved caspase-3 and Ki67 Ab, respectively (Fig. 2A and fig. S6A). The CTLA-4 Ab–induced IEC proliferation

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