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Speciation through the lens of biomechanics: locomotion, prey capture and reproductive isolation

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Speciation is a multifaceted process that involves numerous aspects of the biological sciences and occurs for multiple reasons. Ecology plays a major role, including both abiotic and biotic factors. Whether populations experience similar or divergent ecological environments, they often adapt to local conditions through divergence in biomechanical traits. We investigate the role of biomechanics in speciation using fish predator–prey interactions, a primary driver of fitness for both predators and prey. We highlight specific groups of fishes, or specific species, that have been particularly valuable for understanding these dynamic interactions and offer the best opportunities for future studies that link genetic architecture to biomechanics and reproductive isolation (RI). In addition to emphasizing the key biomechanical techniques that will be instrumental, we also propose that the movement towards linking biomechanics and speciation will include (i) establishing the genetic basis of biomechanical traits, (ii) testing whether similar and divergent selection lead to biomechanical divergence, and (iii) testing whether/how biomechanical traits affect RI. Future investigations that examine speciation through the lens of biomechanics will propel our understanding of this key process.

1. Introduction

The quest to understand the origin of species requires integration across all facets of the biological sciences. Biochemical, molecular, behavioural, physiological and morphological levels of variation routinely contribute to the process of speciation. Although not all species evolve from natural selection, most do, even in the face of gene flow. The evolution of complex genetic architectures (numbers, location and effects of genes) contributing to reproductive barriers can be rapid, and involve similar or different solutions to the same problem [1]. Ecology, therefore, plays a critical role in speciation [2–4].

Selection is often multifaceted, from abiotic habitat characteristics to biotic interactions. Yet, when looking to the fossil record and across extant taxa, substantial evidence suggests that predator–prey interactions have repeatedly and consistently produced long-term behavioural and morphological (e.g. locomotion and feeding) trends in various clades (e.g. [5,6]). Evolutionary branching has been commonly induced by ecological interactions between predators and their prey [7,8]. Alternatively, predator culls of prey can reduce interspecific competition and stifle speciation under some circumstances

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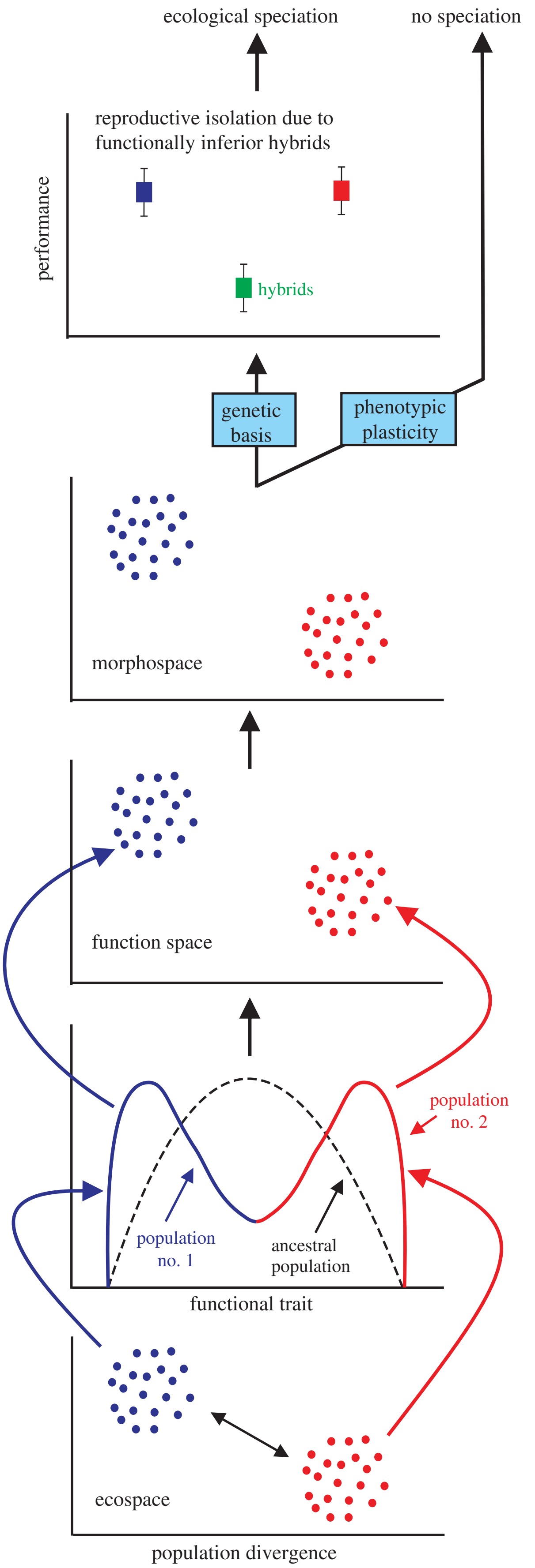


Figure 1. (*Caption opposite.*)

(e.g. [9]). Understanding the mechanistic nature of these interactions and their evolutionary consequences requires a multidisciplinary approach that integrates structure, function and performance—that is, a biomechanical approach.

Figure 1. (*Opposite*.) The theoretical framework for ecological speciation. A species will be divided by an abiotic or biotic isolating mechanism (bottom panel). This will result in the occupation of different regions of ecospace (e.g. two lakes with completely different structural and biotic attributes), followed by divergence of the two populations away from the ancestral population, resulting in the occupation of two distinct regions of function space. The differential functional demands will ultimately drive the alteration of underlying physiological (not shown) and morphological traits. If this is a result of phenotypic plasticity, no speciation will likely occur. With a genetic basis, and assuming reduced fitness of hybrids, speciation will likely occur. However, variation in morphology and biomechanics will likely exhibit a combination of plasticity and genetic-basis. In addition, we are not implying that some plasticity will hinder speciation.

Biomechanics represents the study of biological structure and function using physical principles. Organismal performance represents the primary substrate upon which selection acts [10–12], and variation in performance often arises via variation in biomechanics. For instance, changes in organismal performance are often reflected in morphological shifts, such as muscle and bone size, shape, and arrangement, ultimately leading to an alteration of the forces acting within an animal, or between an animal and its environment. Such changes can occur during adaptation to new ecological conditions [13]. For example, consider a single fish population that is split into two new habitats (figure 1). One habitat is a low-flow environment, much like the ancestral condition. The other is a high-flow environment, imparting new selective pressures. The biomechanical demands in a high-flow environment favour a more streamlined and slender body to minimize drag, and higher aspect-ratio caudal fins to maximize thrust [14–16]. Drag is a force that resists the forward motion of an animal, and thrust is a force that propels an animal forward. Thus, the response to changes in selective pressures is directly related to the resistance and/or production of force. As these two populations diverge over time, they may become reproductively isolated for several reasons, including decreased fitness of immigrants and hybrids if these individuals show maladaptive functional traits compared with residents. This simple illustration of the biomechanical basis of reproductive isolation (RI) highlights the potential for biomechanical approaches to enlighten our understanding of the *mechanisms* of speciation.

The tight fits between form and function suggest the influence of adaptive evolution; however, the prevalence of adaptive traits, the mechanisms by which they arise and the corresponding phenotypic and molecular responses to selection are subjects of extensive debate. Here, we present a unique multidimensional approach to studying how natural selection influences speciation, with the ultimate goal of building an understanding of the origin of species through the study of the adaptive evolution of biomechanical traits and their effects on RI. The lens of biomechanics can open up new predictions about the evolution of whole-organism performance in particular ecological environments. Moreover, biomechanical consequences of phenotypic variation are not always straightforward, sometimes leading to mismatches between morphological changes and functional changes [17]. Thus, assumptions of functional inferiority based on morphology alone are not adequate for predictions about speciation.

We highlight a quantitative framework for understanding population divergence and speciation built on a biomechanical foundation—i.e. study the evolution of organismal function to uncover insights into the evolution of RI. As function diverges, as a result of altered or similar selective pressures, lower-level morphological and physiological traits also diverge (figure 1). Population divergence can lead to reproductive incompatibility, either in the presence or absence of gene flow, and can occur during the expansion of populations into new habitats [18,19] or as habitats are fragmented or modified [20,21]. Although genetic drift and intrinsic incompatibilities may contribute to RI in these circumstances, here we focus on extrinsic forms of isolation resulting from functional mismatches (e.g. functional inferiority of migrants and hybrids in foraging, feeding, avoiding predation, attracting mates and mating). That is, functional divergence begets lineage splitting via functional incompatibility of the diverging populations, although additional (non-biomechanical) mechanisms also could hasten or restrain the evolution of RI. Of course, not all hybrids or migrants will be functionally inviable or even inferior, as in hybrid vigour [22], highlighting the need for empirical investigation of organismal function in the context of speciation.

Our thesis that the 'lens of biomechanics' provides insight into the speciation process relies on the following well-supported assumptions: (i) changes in ecological factors will result in differential selective pressures on one or more functional systems [23]. (ii) Multiple solutions to a functional problem are probably common [24], and can lead to functional divergence between populations experiencing similar selective pressures. (iii) Functional capabilities of animals emerge from the combination of underlying physiological and morphological traits [25]. (iv) Functional and morpho-physiological traits are commonly genetically based (e.g. [26]). (v) As function diverges between populations, immigrant and intermediate forms may be functionally inferior to resident forms [27], and thus speciation can occur by reducing migration and excluding any hybrids that might form between populations, resulting in RI.

Unlike other studies that have discussed biomechanics and speciation [13], we leverage the strong foundation of knowledge in fishes to describe approaches that directly link biomechanics and speciation, detailing multiple modes of selection, multiple isolating barriers and modern biomechanical techniques that are critical for quantifying function. While applicable to a wide range of animal systems, we focus on fishes because of their extensive ecological, phylogenetic and phenotypic diversity, as well as their prevalence as model systems for studying speciation, many-to-one mapping, and biomechanics. Predator–prey interactions in fishes have been a major focus of research over the past several decades [28,29], where survival depends on both the ability to escape from predators and to catch prey [30]. Locomotor and feeding traits underlie predator–prey interactions, and both respond to selection and contribute to RI [31,32], making predator–prey interactions central to the study of speciation. Despite the incredible diversity among fishes, common biomechanical links between form and function persist in the evolution of feeding and locomotion across broad phylogenetic groupings [33,34]. The groups that we propose as model systems are outlined in the electronic supplementary material and highlighted in figure 2. We illustrate a framework that identifies the key ecological variables shaping predator–prey interactions, links genetic architecture to phenotype, biomechanics and performance, determines the fitness consequences of functional variation and quantifies its effects on RI (figure 3).

2. Predator–prey interactions

(a) Prey capture

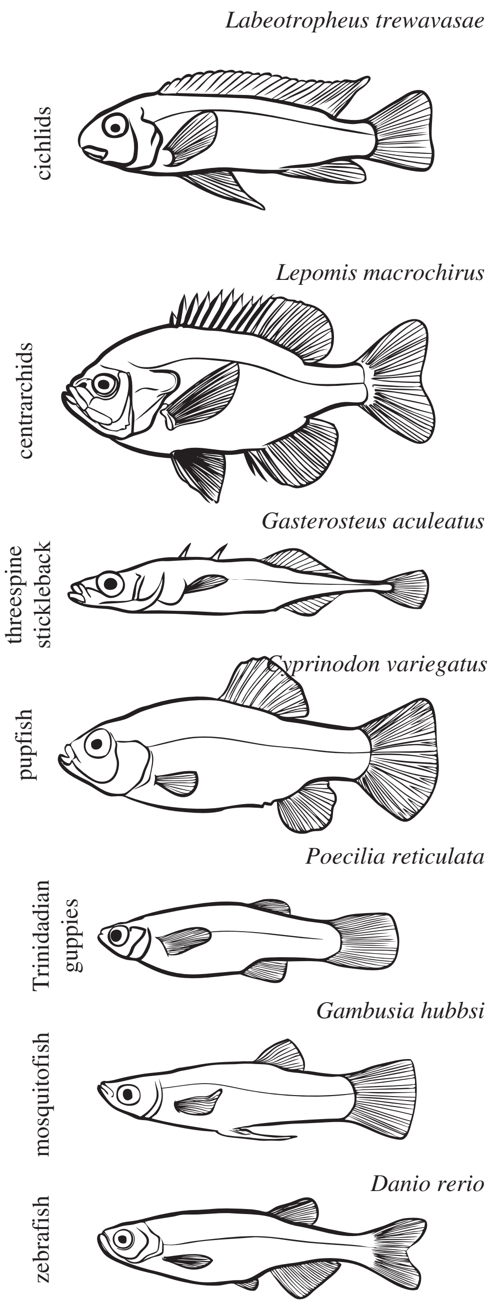
Suction feeding, the primary mode of prey capture among fishes, involves the rapid expansion of the mouth cavity that causes a sharp drop in pressure [55], driving nearby water and prey towards the mouth. Suction affects only a small area near the jaws [56], meaning that the fish must use locomotion to accurately position the mouth close to the prey for successful capture [57]. Thus, prey capture involves the tight functional integration of locomotion and feeding [58–61]. Key locomotor factors include approach speed, acceleration/deceleration, trajectory, stability and timing [59]. The functional divergence in response to selection for enhanced feeding performance on different prey can lead to a wide array of multivariate phenotypic changes. For example, *Gobiomorus dormitor* populations that have colonized inland blue holes in the Bahamas experience shifts in the available prey, driving changes in body shape, mouth morphology, suction generation capacity, strike kinematics and feeding performance on different prey types [62].

(b) Predator evasion

Fishes evade predation attempts using rapid escape behaviours. An example is the C-start, whereby powerful muscle contractions bend the fish into a C-shape and rapidly accelerate the animal [63]. Much research has focused on describing escape behaviours induced by controlled stimuli, yet in reality, changes in ecological and predatory parameters can significantly alter these patterns. The sensory signals that mediate the prey's response and the motor behaviours leading to escape have been investigated for decades. Research on zebrafish found that prey are startled by the visual cues produced by an approaching predator. Specifically, fish initiate a C-start when the appearance of the predator, from the perspective of the prey, increases in size above a critical rate (apparent looming threshold) [28], meaning that fish will most probably respond to a close and fast-moving predator. The flow-sensitive lateral line system is also crucial for detecting a predator's attack [64–66]. Zebrafish larvae use the lateral line to detect the subtle disturbance of water ahead of a swimming predator [65], and larvae without the lateral line are over three times more likely to be captured [64]. Ecologically divergent populations of three spine stickleback exhibit considerable differences in lateral line morphology [67] that are related to their ecological conditions (e.g. vegetation, amount of visual cues, habitat complexity), potentially impacting the fitness of migrants or hybrids.

3. Key ecological variables

Many environmental factors can affect whole-organism performance capabilities, and can influence selection on functional traits (figure 3). Here, we focus on the set of factors that represent the most widespread importance for speciation in fishes. Substantial evidence points to predator–prey interactions as major drivers of diversification in fishes, strongly influencing the evolution of locomotion and feeding [4,29,68]. Important factors that can affect predator–prey interactions in fishes include abiotic variables such as temperature, flow conditions, dissolved oxygen, salinity and pH, as well as biotic variables such as predator density and type, interspecific competitors, population density and prey resource quality and type (figure 3).



		interspecific ecological divergence	intraspecific ecological divergence	morphological divergence	biomechanics associated with ecological divergence	genetic basis of biomechanical trait divergence	reproductive isolation linked to biomechanics
cichlids	*Labeotropheus trewavasae*	A, B [36]		C, PC [35–37]	L, F [35,37,38]	Q [38]	R [36]
centrarchids	*Lepomis macrochirus*	A, B [96]	A, B [39]	C, PC [39,41]	L, F [40,57]	CG [39]	—
threespine stickleback	*Gasterosteus aculeatus*		A, B [4]	C, PC [43–45]	L, F [43,45]	Q, CG [42–44]	R [42]
pupfish	*Cyprinodon variegatus*	A, B [46]	A, B [49]	C, PC [47,50]	F [47,48]	RAD [48,49]	R [49]
Trinidadian guppies	*Poecilia reticulata*		A, B [51]	PC [51]	L [51,97]	CG [97]	—
mosquitofish	*Gambusia hubbsi*	A, B [14,31]	A, B [31]	C, PC [31]	L, F [68,69]	CG [31,69]	R [31,69]
zebrafish	*Danio rerio*		A, B [52]	—	—	AS [53]	—

Figure 2. Representative line drawings of the seven species/groups of fishes highlighted as model systems for locomotion and feeding. Species names are listed by each drawing. Tabular information indicates whether the group has been examined in each of the categories. The citations are merely examples [35–53]. A, abiotic; B, biotic; C, cranial; PC, post-cranial; L, locomotion; F, feeding, Q, quantitative trait loci; CG, common garden; RAD, RADseq; AS, artificial selection; R, reproductive isolation confirmed.

4. How to obtain and quantify phenotypic variation?

Understanding phenotypic variation is critical for assessing which forms provide an advantage in a given set of conditions. To obtain this information, we can (i) directly assess existing phenotypic variation in natural populations and test how this translates into differences in performance and fitness [69], (ii) manipulate animals by altering their morphology (including sensory systems) [64], (iii) use robotics/physical models [70], theoretical models and computational fluid dynamics to explore phenotypic space [71], and (iv) segregate phenotypic differences using experimental crosses between genetically and phenotypically distinct populations (e.g. [72]) (figure 3).

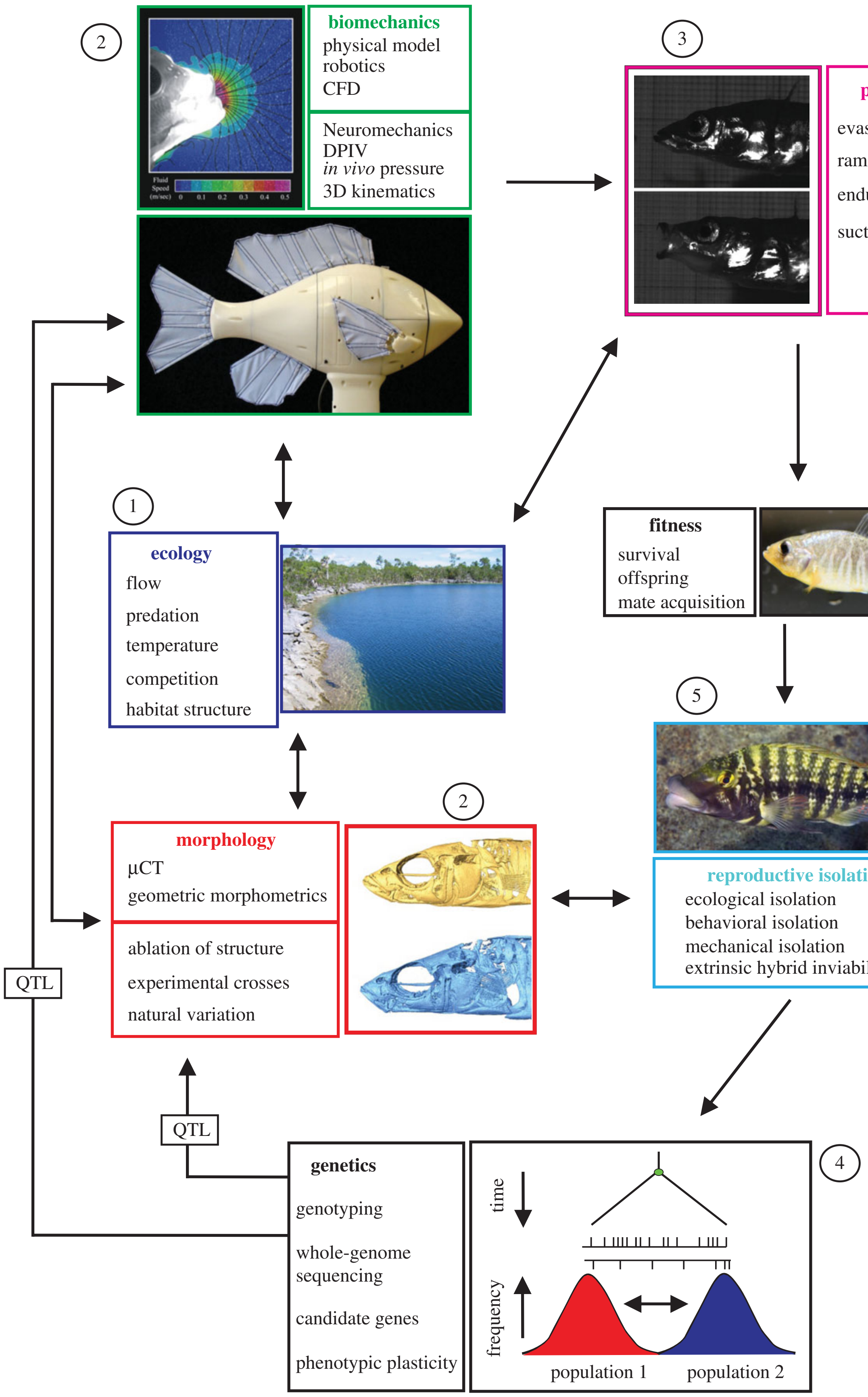


Figure 3. Our proposed methodological framework. The general flow is genetics—morphology—ecology—biomechanics—performance—fitness—reproductive isolation. However, multiple categories interact along the path. For each category, we highlight some of the factors that should or could be quantified. For morphology, the top box represents ways to quantify the phenotype, and the lower box represents ways to alter morphology. For biomechanics, the top box represents ways to mimic the biomechanics of the species or population of interest, and the lower box represents ways to quantify biomechanics in fishes. The robotic fish in this section is from [54]. The circled numbers represent the order in which particular components may be quantified when seeking to understand the biomechanics of speciation, and this is described in more detail in the text. (Online version in colour.)

Determining the phenotypic changes that produce biomechanical differences affecting performance, as well as the genetic underpinnings of these changes, requires quantification of morphology in different regions of morphospace, which is a multivariate representation of shape and structure of a species or multiple species. Photography, microscopy and radiography are commonly employed for quantifying morphology. More recently, techniques such as microcomputed tomography (μCT) allow three-dimensional modelling and visualization of hard and soft tissue components.

Measuring phenotypic covariation patterns is key to capturing the nature and extent of variation present in a system, and in understanding evolutionary responses of multiple traits to selection. The action of selection on the developmental-genetic architecture underlying functionally correlated traits relatively stronger covariation between such traits as a unit, in comparison to the rest of the phenotype [73,74]. Covariation is also influenced by drift and gene flow [75,76], and can constrain the range of possible phenotypes available for selection [77,78] and bias the direction of evolution [79]. Alternatively, patterns of phenotypic covariation can facilitate adaptive change without compromising function [74,80–82].

5. Quantifying functional consequences of phenotypic variation among fishes

Two important steps in understanding how different phenotypes differ in function or performance are first, to quantify organismal function and any differences among populations or species, and second, to generate testable hypotheses about both the consequences and causes of these functional differences. This process is often quite challenging, but in recent years a number of techniques (below) have become available that permit a much better understanding of organismal function and enable testing of the causes of differences among species.

(a) Three-dimensional kinematics

High-speed videography can be used to capture extremely small or rapid motions to quantify kinematics and ultimately performance. When coupled with approaches explained below, this can provide a powerful tool for understanding the biomechanics of fish locomotion and prey capture [57].

(b) Hydrodynamics

Fishes exert forces on the surrounding fluid using multiple control surfaces (locomotion) or by the rapid expansion of the mouth (feeding). Force production in fluids involves the transfer of momentum from the animal to the fluid, leading to the shedding of vorticity [83]. Quantifying the motions of fluid around moving structures can be achieved with engineering techniques such as digital particle image velocimetry (DPIV). With DPIV, water surrounding the fish is seeded with neutrally buoyant particles, a laser sheet illuminates those particles, and the movement of the particles can then be imaged with high-speed video. The two-dimensional and three-dimensional global flow fields can be calculated from spatial cross-correlation techniques to help reveal the fluid basis of fish function and behaviour [84]. For example, three-dimensional suction accuracy in centrarchid fishes was recently modelled and related to capture success [85].

(c) Robotics

One of the most challenging aspects of organismal biomechanics is separating cause from effect, and identifying the specific functional consequences of phenotypic traits in live animals. It is difficult to fully and accurately understand functional observations given the inability to control all relevant variables: individuals and species always differ in numerous traits other than the one of interest. One avenue of research that minimizes such confounding factors is the use of a robotic system to alter only the parameters of interest. Robotic systems offer the advantage of facilitating force measurement, the ability to explore a large parameter space of possible parameters, and greater control over flow visualization measurements. We believe that there will be increasing use of robotic systems in comparative biology to allow more precise understanding of the relationship between the phenotype and performance [86,87], especially where interspecific comparisons involve such distantly related species that one cannot have confidence in comparisons of biological systems or can serve as ‘surrogate organisms’ in cases where animal function cannot be directly observed. The design of robotic models that capture key phenotypic features of these hard-to-get species may be of use in testing the performance consequences of interspecific phenotypic differences that arise during the process of speciation.

(d) Computational fluid dynamics

Computational approaches share some of the same advantages that robotic systems have in serving as an abstracted version of biological reality that can be manipulated with relative ease to explore a large parameter space. Computational fluid dynamics mathematically simulates how fluids interact with surfaces using the Navier–Stokes equations. The main challenge associated with computational models of swimming and feeding in fishes is the rapidly developing and unsteady nature of the flow patterns that are produced (e.g. [88]). And the phenotypic features of fishes involved in feeding and swimming are flexible and complex biomechanically, making development of an accurate three-dimensional structural model challenging and the analysis of structure–fluid interactions difficult. Centrarchid fishes have served as the basis for computational models of both feeding [89] and locomotion [90], and these have provided considerable insight into the link between structure and function. For example, sunfish (*Lepomis*) pectoral fins deform in a complex way during slow speed labriform swimming and computational fluid dynamic analysis showed, unexpectedly, that this deformation pattern results in thrust generation on both the outstroke and instroke of the fin beat cycle.

(e) Neuromechanics

Our understanding of how fish trigger escape responses has been advanced by a wide variety of techniques, including electrophysiological recordings of the Mauthner cells [91], laser ablations of the Mauthner cells [92], and the addition of extra neurons during development [93]. Work on fish as predators has helped us understand how visual information is processed for hunting [94]. Work on the lateral line system is revealing how information encoded by a single mechanoreceptor elicits behaviour [95]. Further investigation of the neuromechanics of predator–prey encounters promises to yield insight into the unique demands of different habitats. For example, using calcium imaging or electrophysiology to measure the differential activity of nerves in different environmental conditions (e.g. still versus turbulent water) will help us understand the basis of how habitat affects performance.

6. Biomechanics of locomotion and feeding in fishes

Fish typically respond to two ecological shifts related to predator–prey interactions: (i) changes in trophic niche and (ii) changes in predation pressure. For example, rapid jaw evolution is observed in pupfish as they specialize on different types of prey (e.g. hard prey or scales). Comparable patterns are observed in African cichlids. In stickleback, divergence within a lake due to competition or among lakes due to predation pressure has led to shifts and divergence in the type of prey consumed. Across most groups, fish that become more pelagic will tend to eat zooplankton whereas benthic ecotypes tend to focus on benthic macroinvertebrates. Although shifts in trophic niche can occur as a result of competition [96], sometimes leading to sympatric divergence in feeding structures (e.g. stickleback, centrarchids), predation pressure can induce a trophic shift in prey by driving a change in habitat use. The latter is common in a number of the groups outlined in the electronic supplementary material. And in guppies and mosquitofish, variation in predation risk also leads to evolution of functional divergence independent of trophic niche (e.g. predator evasion, [69,97]).

A major question in evolutionary biology concerns the predictability and repeatability of evolutionary change and its role in the origin of species. With divergent fish lineages repeatedly experiencing similar environmental/ecological gradients, this provides an opportunity to gain insight into the predictability of functional divergence at multiple scales (e.g. genetics, morphology, kinematics, performance, RI). The bright future in this area is exemplified by the fact that we were able to highlight seven model systems in this paper (figure 2). Thus, there is great promise for shedding light on the extent of parallelism in functional evolutionary patterns at different scales among disparate groups.

7. Framework for the biomechanics of speciation: the functional link from genetics to reproductive isolation

Selection is a common driver of speciation [2,3], but the functional mechanisms linking adaptive changes in genotype and phenotype to the evolution of RI are still largely unknown [1]. Relatively recently, a framework for linking morphology, performance and fitness was solidified [10,11]. But little work has extended this framework to speciation. We propose that biomechanics provides a necessary piece as it bridges morphology and performance (figure 3), and generates testable predictions for evolutionary divergence and RI [13,14,68]. Biomechanics is critical for defining the limits (constraints) to performance, and morphology is defined, at least in part, by genetics. Using an integrative framework that recognizes connections from genetics to RI, we can identify functional mechanisms of speciation: e.g. using model fish groups to predict the evolution of divergent morphologies and post-zygotic isolation based on biomechanical and ecological knowledge, and test the genetic basis of the reproductive isolating barriers [98]. The primary impediment to such an integrative analysis is the lack of study system for which all of the variables can be studied, but locomotion and feeding in fishes represents a promising avenue as they represent a suite of integrated characters that routinely exhibit convergent evolution in association with adaptations to similar environments or ecological niches. Thus, our suggestion to focus on fish predator–prey interactions is based on the vast amount of existing information and the utility of the system. Insights gleaned from fish into the biomechanical basis of speciation will be applicable for almost all animals that capture prey or get eaten by a predator.

The primary reasoning for including genetics in this framework is not necessarily to pinpoint the genes for particular traits *per se*, but rather to uncover the nature of multi-trait divergence (e.g. genetic correlations versus independent evolution) and establish the extent to which population divergence reflects genetic differentiation, phenotypic plasticity or both (e.g. common-garden experiments). If performance exhibits adaptive plasticity, this could minimize genetic divergence and slow speciation. By contrast, identifying a genetic basis for a critical biomechanical trait will potentially reveal the functional basis of speciation. Once the extent of the genetic basis has been characterized, testing the outcomes of hybridization or migration will be more productive with biomechanical approaches, because these traits have definitive links to function in association with the environment and, as a consequence, more probably represent targets of selection.

Adaptive divergence in biomechanical traits can facilitate speciation under two primary scenarios: (i) divergent selection favours different aspects of performance in different ecological environments, and divergence in traits increases RI among populations (i.e. ecological speciation), and (ii) populations respond to similar selection on performance by evolving different adaptive solutions that enhance RI among populations (i.e. mutation-order speciation). Under both scenarios, populations must persist following adaptive peak shift [26] (figure 1) and the biomechanical traits involved in adaptive divergence must directly or indirectly cause RI (e.g. immigrant inviability, extrinsic hybrid inviability, behavioural isolation via mate choice (reject individuals with ‘wrong’ form or performance), mechanical isolation). Prior work has so far centred on the first scenario, revealing that divergent selection appears to drive functional divergence, with some studies linking biomechanical traits to RI—e.g. Bahamas mosquitofish that have evolved different body forms to accommodate different swimming abilities in different predatory environments have consequently evolved enhanced RI due to immigrant inviability and assortative mating for body shape [31,69,99]. Little research to date has addressed the second scenario, although given the ubiquity of non-parallel phenotypic responses to similar environmental gradients [1], combined with the potentially widespread phenomenon of many-to-one mapping of form to function [100], this could prove quite important. That is, the selection surface for biomechanical traits might often be quite complex, with multiple adaptive peaks of similarly high fitness levels—and different populations could traverse different peaks. This is because performance reflects how good an animal is at executing an ecologically relevant task [101], and this execution emerges from the integration of multiple underlying traits that could be combined in various ways to create similar levels of performance.

To determine the biomechanical basis of RI, we must do the following things: (i) identify ecological divergence (e.g. lake versus stream), (ii) identify divergent morphological and biomechanical traits across populations/species inhabiting similar/different environments (population divergence), (iii) quantify the performance outcomes, (iv) determine the genetic basis or plasticity of these traits and (v) uncover the role of these traits in speciation by linking them to fitness, RI or lineage diversification rates. Several of these steps will necessarily exhibit complex interactions, such as performance driving ecological differences and ecology driving performance differences. Figure 3 illustrates and expands on this framework.

Studies of recent divergence are best suited to test hypotheses of the effects of adaptive biomechanical variation on RI. This is because the observed phenotypes and genotypes involved are more likely to reflect RI that evolved in association with divergence rather than variation that evolved following the evolution of RI and speciation. Recent studies of adaptive traits provide frameworks for testing RI in fishes, such as immigrant inviability [102,103], extrinsic hybrid inviability [98], behavioural isolation via mate choice [99] and mating incompatibility (mechanical isolation) [104]. Examining the role of physiological and biomechanical divergence among nascent populations will be important for examining mechanistic underpinnings of RI [105]. Studies of older divergence can use phylogenetic comparative methods to test for associations between evolution of biomechanical traits and lineage diversification.

8. Conclusion

From bee pollination to the function of the heart, biomechanics is crucial for understanding evolution. We provide a specific framework for incorporating biomechanics into the study of ecological and mutation-order speciation. Considering speciation through the lens of biomechanics, specifically through measuring biomechanical traits associated with locomotion and prey capture, offers a holistic way of measuring traits that are often the targets of selection in fishes, and indeed across taxa. Although the groups of fishes presented here represent the best targets for understanding speciation through the lens of biomechanics, it should by no means exclude other fishes that clearly contribute to these questions (e.g. salmonids [106]). We propose that the low-hanging fruit in the movement towards linking biomechanics and speciation will include (i) establishing the genetic basis of biomechanical traits, (ii) testing whether similar and divergent selection lead to biomechanical divergence, and (iii) testing whether/how biomechanical traits affect RI. The next steps could be experimental tests that directly demonstrate links with RI. For example, using controlled crosses under a common-garden design, the genetic basis of biomechanical traits could be established while performance trials could test the prediction that hybrids are functionally mismatched for these traits. Because of the strong link of biomechanical traits with function in association with the environment, the opportunities to test the alternative consequences of hybridization and migration with these approaches will contribute to the quest for the origin of species.

Authors' contributions. T.E.H., S.M.R. and H.A.J. developed the idea for this manuscript, with subsequent input from all authors. T.E.H. wrote the first draft, and all authors provided subsequent text, comments and edits.

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Supplementary information

Key groups of fishes

There are several reasons why certain groups of fishes have received relatively more attention from researchers, including accessibility, viability in a laboratory setting, short generation time, size, degree of phenotypic variation, or simply the interesting questions that can be addressed with the specific group. To investigate the role of biomechanics in speciation, we focus on several groups of fishes in this review that have received considerable attention with respect to evolutionary ecology [1, 2], genetics [3, 4], biomechanics [5, 6], hydrodynamics [7, 8], functional morphology [9, 10], neuromechanics [11], and reproductive isolation [12, 13]. We fully acknowledge that not every potential group is covered by our study, but our aim is to highlight a range of species and groups that have been studied thoroughly and that will provide a foundation for future research. For each group, we will focus on WHY it is important and what it can offer toward understanding speciation through the lens of biomechanics. By integrating the information across the groups, we can also identify the gaps and shortfalls that exist. Topics will include adaptive trait variation, ecology, morphology, biomechanics, and neuromechanics.

Zebrafish

Zebrafish, *Danio rerio*, are now a valuable model system in several fields of biology, including biomedicine, developmental biology, genetics, neurophysiology, and behavior, because they are small, tractable, optically transparent as larvae, and relatively easy to keep and breed in the lab. Zebrafish have emerged as a model for understanding predator-prey interactions because their behavior as both predator and prey are quite typical of many other fishes. Zebrafish naturally inhabit slow-moving and still bodies of shallow freshwater in Southeast Asia [14]. They are small in size (body length < 4 cm), exhibit a fusiform body that is slightly compressed laterally, and face predation from larger fish and birds. Zebrafish are omnivorous and mainly feed within the water column [15] on a variety of plankton, such as small aquatic insects, insect larvae, zooplankton, algae, plant material [16], and even zebrafish larvae [14]. To catch prey, zebrafish swim at a moderate speed [11] before protruding their jaws [17] and executing a rapid suction-feeding strike in less than 20 ms [11], which drives nearby prey into the mouth.

Work on zebrafish has helped us understand how fish detect and evade predators. Zebrafish adults are startled by the visual stimulus produced by an approaching predator, and the timing of the fish's response depends on the perceived speed of the predator [18]. In addition to the visual system, the flow-sensitive lateral line system is crucial for prey. Disabling the lateral line system in zebrafish larvae prevents them from detecting suction, like that produced by a predator [19], and reduces their chances of evading a predator's attack by 80% [11]. A study employing a robotic predator and larval zebrafish revealed that prey detect the subtle disturbance of flow ahead of an approaching predator [20]. Once startled, prey are most successful when they initiate the c-start at an intermediate distance from the predator, [11], and orient their swimming in an optimal direction that depends on the predator's speed [21].

Studying zebrafish as predators has advanced our understanding of how fish feed effectively. Zebrafish larvae employ both vision and flow sensing when foraging, but in order to successfully capture prey in the dark, larval zebrafish must learn how to sense water flow over the first few weeks of life [22]. Adult zebrafish have also revealed cryptic hunting strategies, whereby they disguise the bow wave by slowly pulling water into the mouth when approaching prey [23], which decreases the chances of startling invertebrates. Work on zebrafish has helped us understand the evolution of complex traits, as zebrafish have evolved a unique method for protruding their jaws using a novel bone called the kinethmoid [24].

Leveraging the genetic and neurophysiological advantages of the zebrafish model system will be instrumental for future investigations on prey fish. Studies that investigate how zebrafish larvae encode sensory stimuli at the cellular level [25] are now increasing our understanding of how prey detect flow stimuli. Transgenic lines of zebrafish that express calcium-indicators in the hair cells and afferent neurons of the lateral line system now permit the visualization of nervous signals *in vivo* [26], which will allow future investigations of how fish integrate and translate sensory information from different parts of the body. Developing future lines of zebrafish in which some sensory pathways are genetically disabled (e.g., the efferent system of the lateral line) will be invaluable for determining how different components of the nervous system affect behavior.

Trinidadian guppies

Guppies that live in streams that drain the Northern Range Mountains of Trinidad co-occur with a diversity of predators in the higher order streams. Waterfalls exclude predators, but not guppies and a few less predatory species of fish from the upper portions of these streams [27, 28]. The presence of guppies in high versus low predation communities is repeated in multiple streams and provides the equivalent of a naturally replicated experiment.

Guppies that live with predators experience significantly higher mortality rates [29] and display a diversity of traits that represent adaptations to life with predators that have evolved independently in different rivers. These adaptations include behavior [30], life histories [31, 32], neuromuscular performance [33], survivorship in the presence of predators [34], and diet [35, 36].

Ghalambor et al. (2004) quantified c-start (reflexive alarm response) performance in second generation, lab reared guppies [37]. The use of lab reared, as opposed to wild-caught, guppies means that differences between populations in performance are likely to have a genetic basis. Ghalambor et al. evaluated high (HP) versus low (LP) predation guppies from two different rivers (Yarra and Oropuche) and hence performed two paired comparisons that represent independent instances in which guppies adapted to life with and without predators. HP guppies had faster acceleration and higher maximum swimming velocities. However, there was also evidence of a tradeoff with reproduction. The volume of developing embryos increases as they progress through development, causing a decline in all aspects of C-start performance. HP guppies produce more offspring and have a correspondingly higher rate of decline in performance as their offspring develop. The net result is that they are significantly faster when not pregnant and when their offspring are early in development, but lose this advantage as development proceeds. Walker et al. [38] show that the magnitude of the differences in performance of HP and LP guppies is sufficient to make the difference between life and death in an encounter with a real predator.

Zandona et al. [36] compared the diets of wild-caught HP and LP guppies from two rivers (Aripo and Guanapo). HP guppies consumed a more invertebrates and less diatoms and detritus than LP guppies. HP guppies prey selectively on higher quality prey (ones with lower carbon/nitrogen body ratios) while LP guppies consumed invertebrates in proportion to their abundance. Bassar et al. [35] compared the diets of wild-caught from the same four localities in an experiment performed in replicate, artificial streams and obtained the same results. The important distinction between the two studies is that Zandona et al. evaluated fish collected from their natural environment, where there were confounding differences in food availability. Bassar et al. compared fish in artificial streams that had comparable resource bases, yet they obtained the same result.

O'Steen et al. [34] quantified the survivorship of HP and LP guppies housed in a 1.8 m diameter wading pool with an adult pike cichlid (*Crenicichla alta*). Each experiment was run until the predator had consumed half of the prey; the duration ranged from 15 to 240 minutes. They compared HP and LP guppies from three rivers (Yarra, El Cedro, Aripo). In addition, they included guppies three introduction experiments in their comparisons – two were populations of guppies that had been transplanted from HP communities to previously guppy-free low predation sites above barrier waterfall 16 to 22 years earlier (Aripo and El Cedro Rivers). These fish were compared to representatives from the HP locality from which the introduced ancestors were derived. The third experiment was the introduction of a predator into a low predation site on the Aripo River. These fish were compared to a population found further upstream, above a barrier waterfall that excluded the introduced predators. O'Steen et al. repeated this experiment on the second generation of laboratory reared guppies from the HP and LP sites and two introduction experiments on the Aripo River.

HP guppies had higher survival than LP guppies in all three paired comparisons among wild-caught fish. Likewise, guppies from the two guppy introduction experiments had lower survival than those from the ancestral HP site. Guppies from the predator introduction site had higher survival than those from the control site upstream. The results for the second generation lab reared guppies were the same, although the magnitude of the differences was smaller. Together, these results show that there are genetic differences among populations in their ability to escape predators. There is some environmental component to escape ability because the lab reared fish from HP environments had a smaller advantage than the wild-caught fish from the same localities. Finally, the ability to escape predation evolved since guppies were transplanted from a HP to LP environment (22 years in the Aripo introduction and 16 years in the El Cedro Introduction) or when predators were introduced to a low predation environment (16 years).

These experiments do not tell us is why HP guppies are better at escaping predators than LP guppies. In addition to neuromuscular performance, HP and LP guppies also differ in behavior. For example, Seghers and Magurran [39] show that HP guppies are genetically predisposed to aggregate more than LP guppies. Aggregation behavior is known to increase a fish's ability to escape predators, so performance and behavior contribute to susceptibility, but we do not know their relative importance. The combination of these attributes, and all of the other aspects of guppy biology that differ among HP and LP environments, is a testament to the profound and pervasive ways in which predators shape the evolution of their prey.

Gambusia (mosquitofishes)

Gambusia fishes are small, livebearing fish in the family Poeciliidae (typically < 60 mm standard length), commonly referred to as mosquitofishes. *Gambusia* is the most speciose poeciliid genus, and species are distributed in the New World from northern Colombia to the central and south-eastern U.S.A., and across numerous Caribbean Islands [40]. These fish inhabit many diverse types of aquatic habitats, including nearshore marine environments, estuaries, lakes, hypersaline lakes, marshes, ponds, temporary ponds, rivers, springs, and even waters with high levels of toxic hydrogen sulfide. Indeed, *Gambusia* are well known for their successful colonization of a wide range of aquatic environments, and two species are notorious as some of the most highly invasive species on earth (IUCN 100 worst list).

Importantly, variation in these environmental variables exists at multiple timescales: across species separated by millions of years of evolution, across populations / incipient species with thousands of years of divergence, and across populations experiencing environmental change over decades to centuries. This situation has led to research addressing the ecological causes and predictability of phenotypic evolution and speciation in this group. Combined with their amenability for laboratory and field work, and the ecological and biomechanical knowledge about the group, they represent a remarkable model system for studying evolution, and using functional approaches to understand the causes of speciation.

Although numerous environmental factors have been implicated in some aspect of *Gambusia* evolution, existing evidence clearly points to predation as being the most important driver of diversification in the group. Similar to guppies, *Gambusia* populations/species show a wide range of adaptations to divergent predation regimes (living either with or without major piscivorous fish; e.g. [2]). This includes diversification in male coloration [41], a variety of behaviors [42], life histories [43], body and fin morphology [12, 44], trophic morphology [45], male genital morphology [42], female genital morphology [46], steady and unsteady locomotor performance [12, 47], survivorship in the presence of predators [47], and diet [48]. The majority of these traits have been shown to have a genetic basis, although plasticity in some traits occurs as well.

One particular study system that offers a striking opportunity for studying the role of predator-prey interactions in trait diversification and speciation is the adaptive radiation of Bahamas mosquitofish (*G. hubbsi*) in blue holes. Bahamas mosquitofish colonized inland blue holes of Andros Island during the past ~15,000 years, and repeatedly evolved different adaptive traits in either the presence (high-predation) or absence (low-predation) of the predatory fish, bigmouth sleeper (*Gobiomorus dormitor*). Molecular genetic evidence indicates that similar phenotypes independently evolved in similar environments multiple times [12, 42, 43]. These populations are further undergoing speciation, with significant reproductive isolation among many populations, especially populations that differ in the presence of predatory fish [12, 49]. Variation in the presence of piscivorous fish represents the primary source of environmental variation in these populations, with no known environmental factor co-varying with the presence of predatory fish (e.g., productivity, salinity, turbidity, water transparency, depth, dissolved oxygen, temperature, pH; [12, 42]. This system thus permits the more-or-less exclusive investigation of the effects of predation regime in driving trait divergence and speciation in the wild. Combined with biomechanical knowledge, this could prove especially insightful regarding the biomechanics of speciation.

Considerable biomechanical work has been conducted in this group, and continues to grow. Functional research in *Gambusia* has investigated steady swimming capacities, c-start escape performance, mating, feeding, and terrestrial jumping (e.g. [47, 50, 51]. Because variation in predation risk has repeatedly driven parallel changes in body morphology and steady and unsteady swimming abilities, a major line of inquiry is understanding how this divergence influences the evolution of reproductive isolation. Work has already demonstrated fitness consequences of locomotor performance and their morphological underpinnings, showing the effects of functional morphology and whole-organism performance on reproductive isolation due to immigrant inviability, behavioral isolation (mating preferences partially based on body morphology), and has led to clear predictions of reduced hybrid fitness [12, 47, 49]. Moreover, evidence for an important role of body morphology in speciation comes from multiple timescales of analysis [12]. Work in this genus is poised to shed new light on speciation through the lens of biomechanics, with the one clear limitation in this system being the relative paucity of work on the genomics of adaptation and speciation in *Gambusia*.

Threespine stickleback

Threespine stickleback (*Gasterosteus aculeatus*) are euryhaline fish circumpolar in their distribution and occupying many coastal marine environments and recently formed freshwater lakes and streams following the last glacial recession (10 – 15 000 years ago). Populations are derived from two distinct marine lineages (Euro North American Clade and the Trans North Pacific Clade) that diverged during a period of allopatry ~1 mya [52]. In general, they are a small streamlined fish (up to ~75 mm in length) with a body form similar to most teleosts, albeit with unique body armour. They have modified dorsal fins in the form of three dorsal stickles (spines), modified pelvic fins in the form of pelvic spines and modified scales in the form of bony lateral plates running dorsoventrally along the abdomen [53]. All of these traits are heritable but may vary with the environment, and indeed stickleback have undergone a multitude of phenotypic transitions in association with freshwater environments, in many cases evolving parallel changes with respect to behavior [54], physiology and morphology [3, 53, 55]. They eat a variety of insect larvae, amphipods, ostracods, and copepods [56], with the latter being capable of escape maneuvers [57].

Threespine stickleback have emerged as an excellent vertebrate organism for the study of evolution for a number of reasons. Females are highly fecund and husbandry techniques for breeding and maintaining crosses in the lab, including hybrid marine-freshwater forms, have been well developed. They have a relatively short generation time (nine months from hatching to maturity). In addition, the genome has been sequenced and partly annotated [58].

Adaptive peak shifts upon colonization of fresh water environments, at least with respect to morphology, are predictable and leave a genetic signature in the genomes of stickleback evolving in these environments [3]. In addition, many studies have emerged that have characterized striking population divergence among and between freshwater and marine environments (e.g. [58-60]). Phenotypic divergence in tight association with distinct freshwater environments has resulted in speciation within a lake (e.g., benthic and limnetic species pairs), often closely linked with specific ecological conditions [56]. For example, open water stickleback tend to consume water column plankton, whereas benthic stickleback tend to consume benthic macroinvertebrates [61].

What are the functional consequences of feeding on zooplankton *versus* benthic macroinvertebrates? Among the open water plankton are calanoid copepods, which have the ability to evade predatory attacks from stickleback [62]. Suction performance is likely elevated when feeding on benthic prey given that the prey are often embedded in, or attached to, the substrate [63]. A recent morphological investigation confirmed this by quantifying suction index, a reliable proxy for suction performance [9], for benthic and anadromous stickleback populations [64]. Conversely, those fishes that feed on zooplankton in the water column should exhibit a streamlined body (dorso-ventrally compressed), elongated jaws, and a terminal mouth [65]. These characteristics enable faster swimming during prey capture and a mouth adapted for capturing prey in front of the fish. Stickleback have been found to be more pelagic in the presence of a predator and competitor, the prickly sculpin (*Cottus asper*), which also inhabit a number of the same lakes that were isolated following the recession of the Cordilleran Ice Sheet [66-69]. Prickly sculpin not only feed on stickleback, they also compete with them for invertebrate prey [69-71]. Interestingly, not all lakes with stickleback contain sculpin predators [67], which has resulted in both phenotypic and genetic shifts in accordance with predation by sculpin [3]. Recent work has also noted the behavioral shifts that occur when stickleback are in the presence of different predators [72]. Open-water stickleback morphotypes exhibit greater capture success than benthic morphotypes in open-water feeding trials [61]. Additionally, morphological analyses have confirmed that pelagic populations that are sympatric with sculpin exhibit decreased body depth, a narrower caudal peduncle, larger eye diameter, and a longer jaw [69]. Collectively, these observations reinforce the extent to which investigation of biomechanic phenotypes in stickleback can elucidate the genetics of adaptive divergence and potentially speciation.

Centrarchids

Centrarchidae is a clade of North American perch-like freshwater fishes with 34 extant species [73]. They are often top predators in the freshwater habitats in which they live, and they have been the subjects of numerous fields of research including biomechanics [9, 74-78], ecomorphology (reviewed in [79]), evolution [80, 81], and ecology [82-85].

Given that centrarchid fishes are relatively large and top predators, they are not as amenable to laboratory studies aiming to develop hybrids and rear multiple generations. That said, there are countless cases of hybridization between species in natural populations of centrarchids [86]. Although they often make up a small percentage, there are locations where hybrids can make up over 75% of the population. Interestingly, hybrid viability declines with the age of the node separating parental species, supporting the "speciation clock" idea [87]

Trophic polymorphisms have been identified and studied in various populations of pumpkinseed sunfish, *Lepomis gibbosus* [88-90]. In Ontario lakes, for example, littoral pumpkinseed populations are more generalist feeders, consistently feeding on benthic prey. Pelagic pumpkinseeds were highly specialized on zooplankton [88]. These differences in ecology could be exploited in future laboratory studies of biomechanics and performance, especially in light of the large amount of data that already exists for centrarchids. Several studies have assessed whether the differences between populations are a result of phenotypic plasticity or evolution, and the results are mixed [90-93]. That said, aspects of both feeding and locomotion vary substantially between populations, between regions of a single habitat, and between species. These differences are often associated with biotic and abiotic environmental factors, including water flow, competition, prey type, and other factors.

In terms of biomechanics, centrarchids are the predominant model system among fishes, and this includes both feeding and locomotion. This role likely stems from their predominance in North American freshwater systems and their relatively large size. Suction feeding performance in bluegill sunfish and largemouth bass has been explored for decades, and techniques have included kinematics [94], DPIV [95-98], sonomicrometry [99] and *in vivo* pressure recordings [77, 100, 101]. Centrarchids are a very profitable system in terms of interspecific variation in feeding performance given that they include a range of functional types. Largemouth bass, *Micropterus salmoides*, occupy one end of the spectrum in which ram speed dominates and suction-induced fluid speeds are relatively low [98, 102]. They exhibit a large mouth that aids in the capture of evasive fish prey by ingesting a lot of water during suction [98]. This alleviates the constraints involved with accurately positioning the prey item relative to the mouth. Recent work with largemouth bass has explored muscle function during feeding using XROMM [103], sonomicrometry, and electromyography [104-107]. In contrast with largemouth bass, bluegill, *Lepomis macrochirus*, fall on the other end of the spectrum with low ram speeds and higher suction-induced fluid speeds [98]. This leads to the need for high accuracy during feeding in order to entrain the prey within a very small ingested volume of water.

Locomotor hydrodynamics have been studied extensively among centrarchids [5, 7, 74, 108]. Most centrarchids, especially sunfish, have relatively large median and pectoral fins that are used for swimming at low speeds, although the body and caudal fin are employed during faster bursts of locomotion. Thus, much of the work on centrachids has focused on the function of the fins. A gait transition in smallmouth bass (*Micropterus* *dolomieu*) is observed at intermediate speeds, and this gait is thought to involve the recruitment of fast-glycolytic white muscle [109].

The integration of locomotion and feeding during prey capture is critical for centrarchids [76, 98, 110-113]. Selective pressures acting on one system will, therefore, undoubtedly impact the other. Divergence between populations of centrarchids is commonly associated with trophic shifts, and biomechanics is very likely to play a major role in this divergence and ultimate speciation. Only recently are studies teasing apart the functional changes that accompany divergence in microhabitat use among populations [114]. Future studies will be very fruitful in this area.

African cichlids

Cichlidae are the most functionally and ecologically diverse group of freshwater fishes, including more than 3,000 species throughout the Neotropics, Africa, Madagascar, the Middle East, India, and Sri Lanka with a crown age of 57-65 Ma [115-117]. They are best known for their spectacular adaptive radiations in the East African Great Lakes of Malawi, Tanganyika, and Victoria, rapidly filling nearly all ecological niches in these lakes from one or a few common ancestors [115, 118, 119], but have also diversified in hundreds of smaller lakes [4, 120], paleolakes [121], alkali flats [122], rivers [123], rapids [124], and estuaries [125], and have crossed oceans [117]. Their physiological tolerances span the extremes of salinity (0 – 180% seawater) and pH (4.5 – 12) known in any fish group [126], but they are limited to tropical and subtropical environments, reaching as far north as Texas and Israel [115].

Cichlids are unrivaled in their morphological, ecological, and behavioral diversity, occupying nearly every ecological niche and body shape known in freshwater fishes, from elongated rapids specialists, algae-scrapers, piscivores, and open-water planktivores to more specialized niches such as scale-eaters, paedophages, and sponge-eaters [124]. More fine-scale partitioning may also exist. For example, three different sympatric species of paedophage specialize on ramming mouth-brooding females from different ventral or dorsal angles [127]. These specialized niches are all the more striking due to the large amount of convergent evolution. For example, scale-eating has evolved at least four different times in cichlids [128] and hypertrophic lips at least eight times on three continents [128]. Cichlids also exhibit a diverse array of parental care behaviors, from biparental mucus-feeding to mouthbrooding [129, 130], complex social signaling [131], and male courtship displays, including over 200 species that build display platforms out of sand and rock, analogous to bowerbirds [132, 133].

A sister clade to the monotypic engineer ‘goby’ (Pholidichthyidae), cichlids are defined by pharyngognathy: pharyngeal jaws composed of fused left and right fifth ceratobranchial bones encased in a muscular sling articulating with the neurocranium [134]. This key innovation freed up the cichlid oral jaws to specialize on prey capture [135]. Accordingly, most studies of cichlid functional morphology focus on the pharyngeal jaw, including its biomechanics [136], plasticity [137] and strength [138]. However, this innovation is not sufficient to explain the highly variable rates of *in situ* diversification across cichlid lineages [139]. Recent work also suggests pharyngognathy may slow prey processing by piscivores, facilitating the invasion of the Nile Perch and the largest human-induced vertebrate extinction of over 300 cichlid species in Lake Victoria ([140] ; also see [141]. Additional functional studies have focused on crevice-feeding [142], jaw protrusion [143], scale-eating [144], and locomotion [145], but surprisingly little is known about cichlid performance given their diversity.

Most cichlid research centers on explanations for their extraordinary diversification. Many cichlid lineages have failed to diversify, while others do so repeatedly and ‘explosively’, even within the same environments [139]. Numerous hypotheses have been proposed for this pattern, including egg spots on the anal fins

[146], hybridization [147], and repeated sweeps of sex determination loci [148], but a recent comparative analysis found that lake depth and sexual dichromatism best predicted the occurrence of sympatric cichlid radiations [149]. However, there are notable exceptions to this pattern, such as the sympatric diversification of two different monochromatic cichlid lineages within a tiny, shallow lake [150], and future work will need to address these alternative routes to diversification.

The evolution of reproductive isolation in sympatry may also contribute to the prevalence of cichlid radiations. Cameroon and Nicaraguan crater lake cichlids are the most widely celebrated examples of sympatric speciation due to replicated speciose radiations within uniform crater basins [151]. However, recent work indicates repeated colonization of these craters by riverine cichlids [152] and suggests that assortative mating by habitat may be necessary to complete speciation in sympatry [152]. Indeed, the only solid remaining examples of sympatric speciation in cichlids involve steep depth gradients [4] while sympatric shallow-water species pairs exhibit incomplete genetic and phenotypic divergence, suggestive of stalled speciation [150].

Considerable recent progress has been made in characterizing the genomic basis of species differences and the genetic architecture of ecologically relevant traits in African cichlids [4, 153], yet it is still unclear if hybridization has played a driving role in their diversification or is simply pervasive in all young taxa [154]. Similarly, the substantial behavioral complexity of cichlids affects speciation through mechanisms such as male-male competition [155], phenotype-matching [156], multimodal displays [157], and deceptive signaling [132], yet more work is needed to connect these findings to patterns of diversification across the group.

There is still surprisingly little knowledge of the ecology of cichlids, which mostly comes from a few large-scale studies [128]. For example, in addition to their celebrated ecological diversity, many sympatric species appear to be ecologically equivalent [158], inspiring Liem's paradox: highly specialized morphologies may only be useful during times of scarcity [159]. However, a year-long study found that sympatric cichlid specialists did not partition resources during periods of scarcity and still managed to invade new habitats [158]. Much remains unknown about the stages of ecological and sexual divergence across cichlid radiations which exhibit complex dynamics (contra [160]). Indeed, existing knowledge of selection on cichlid morphology comes almost entirely from its genetic architecture [161]. Much more work is needed to connect emerging cichlid genomic and behavioral insights with functional morphology, performance, fitness landscapes, and ecology across diverse radiations and non-radiations in nature.

Pupfish

In contrast to cichlids, *Cyprinodon* pupfishes (family Cyprinodontidae) comprise a very recent radiation (crown age: 25 kya) which rarely diversified in sympatry despite their wide distribution across the western Atlantic, Caribbean, and Mojave and Sonoran deserts [10, 162]. Only two sympatric *Cyprinodon* radiations are known from hypersaline lakes on San Salvador Island, Bahamas and a brackish lake in the Yucatan, Laguna Chichancanab [10]. These two independent radiations of generalist and specialist pupfishes occur in remarkably similar habitats with abundant ecological opportunity due to lack of predatory fishes and only 1-2 co-occurring fish species. However, the paradox in this system is that generalist pupfish species have also colonized thousands of similar lakes throughout the Caribbean with identical depauperate fish communities and comparable levels of resource diversity, genetic diversity, population size, lake area, and island size, and yet have failed to diversify in all these environments (Martin in revision). Thus, in contrast to classic adaptive radiations found in unique environments (e.g. African rift lakes) and incipient speciation replicated across many similar environments, pupfish adaptive radiations pose a different problem: why are sympatric radiations of ecological specialists so localized, despite so many ecologically and genetically similar generalist populations on neighboring islands? Answering this question is key to understanding the origins of adaptive radiation.

Despite their young age, pupfish radiations exhibit striking divergence in trophic morphology and niche specialization. One species on San Salvador Island is a specialized scale-eater and has evolved much larger jaws, larger adductor mandibulae muscle mass, and an elongated body, driving rates of jaw diversification 50 – 1,000 times faster than other pupfish clades (Martin in revision; [10, 163]). To quantify the novelty of this trophic niche, we note that the most closely related scale-eating specialists are found within the African cichlids, separated by thousands of species and 168 million years of evolution from the scale-eating pupfish [164]. A second hard-shelled prey specialist on San Salvador exhibits an enlarged nasal skeletal protrusion, convergent with the anatomy of a unique reef fish, the black musselcracker *Cymatoceps nasutus*, which may stabilize its oral jaws for shell-crushing [165]. Laguna Chichancanab species have specialized on a different set of resources, including zooplankton and other fish [166], but their extinction in the wild has constrained laboratory studies of these endangered species [10]. Although there is some work on the functional morphology of scale-eating [167, 168], which may be facilitated by the decoupled jaw protrusion mechanics of Cyprinodontiform fishes [169, 170], future work is needed to connect divergent pupfish morphology to performance.

Field measurements of the fitness landscape for the San Salvador pupfish radiation provide a central focus for research on this system and offer an unexpected explanation for their ecological, behavioral, and genetic divergence. Pupfishes are nearly ideal for fitness experiments due to their hardiness, fecundity, high densities, and short generation time (four months). Furthermore, most species can be hybridized in the lab and produce viable and fertile hybrids [171], enabling manipulation of the phenotype using laboratory crosses among divergent species. Field experiments measuring the growth and survival of F2 hybrids among all three species in the San Salvador radiation demonstrated a complex landscape with two fitness peaks corresponding to the phenotypes of the generalist and molluscivore species. A small fitness valley separated the intermediate generalist phenotypes from a higher fitness peak corresponding to mollusk-eating hybrids with nasal protrusion, whereas a large fitness valley isolated the scale-eating large-jawed phenotypes from the other two species [172]. This fitness valley asymmetry makes biomechanical sense given the divergent performance demands of non-evasive (molluscs) versus evasive prey (biting scales) and high efficiency necessary for scale-eating [173]. The topography of the fitness landscape thus suggests an explanation for the rarity of scale-eating. Different-sized fitness valleys separating the two specialist species from the ancestral generalist phenotype also predict reduced gene flow between the scale-eater and other species in sympatry (supported by [165]), reinforcement of scale-eater pre-mating isolation (supported by [174]), rapid trait diversification of specialist phenotypes ([10]; Martin in revision) and a greater number of large-effect alleles underlying the scale-eater phenotype (Martin et al. in prep). Interestingly, hybrid fitness appears to reflect the foraging performance of different phenotypes, rather than the frequency of similar competitors, and varies in complex ways across different trait subsets, even within the oral jaws (Martin in press). In summary, measurement of the complex fitness landscape spanning an incipient adaptive radiation can inform patterns of speciation, gene flow, and divergence in ecology and performance.

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