

Speciation in nature: the threespine stickleback model systems

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The threespine stickleback *Gasterosteus aculeatus* species complex is an important natural model for speciation research because it includes several replicated sets of coexisting, divergent forms that are also experimentally tractable. Recent research has begun to emphasize lesser known divergences within the complex in addition to the well-studied limnetic–benthic pairs, as well as exploring a broader range of speciation mechanisms. With the goals of making general inferences about speciation in nature and bringing this body of research to a wider audience, we have surveyed studies from the entire species complex. We find that stickleback speciation is often rapid, that the geographical context of speciation is variable and often complex, and that many, diverse traits have often diverged early in the speciation process. We find no unambiguous evidence of founder-effect speciation, but much evidence that divergent natural and sexual selection have been central to the evolution of reproductive isolation in this species complex.

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In recent years, significant progress has been made in our understanding of how speciation occurs in nature [1]. An important component of this endeavor has been the study of the threespine stickleback *Gasterosteus aculeatus* complex, beginning with work by McPhail, Hagen and their colleagues [2,3]. Research in this species complex has focused on a diverse collection of distinct model ‘systems’, each involving a pair of phenotypically divergent forms that coexist in nature and exhibit various degrees of reproductive isolation (Fig. 1, Table 1). The limnetic–benthic lake pairs are the best studied but not the only example. Work has also been done on several other stickleback systems and, with an accelerating pace of research over the past decade, the literature is now extensive. Here, we update and expand upon McPhail’s 1994 review [4], integrating the results from a survey of the entire complex and presenting the general patterns and conclusions that emerge concerning speciation in nature.

The threespine stickleback species complex

The natural history of the threespine stickleback is characterized by repeated episodes of colonization by the marine stickleback (including freshwater-breeding anadromous populations) of various freshwater habitats in the northern hemisphere, including lakes and rivers of North America, Europe, Asia and numerous islands (Fig. 1) [4,5]. Colonization, often following glacial retreat, is followed by divergence in numerous traits, including morphology, behavior, physiology and life history. These divergent freshwater populations, however,

appear to suffer high extinction rates (often as a result of glacial advances) and rarely persist long enough to form widespread, distinct species [5]. Although most stickleback diversity exists in freshwater habitats and the marine form shows relatively little morphological variation, it has recently been established that divergent forms have also originated in the marine environment (Table 1).

Most research has focused on a set of six unusual systems within the complex. Each comprises two morphologically and ecologically divergent forms that, whilst often occurring in allopatry, include populations that are sympatric or parapatric during part or all of their life cycles (Fig. 1). In most cases, numerous phenotypic traits have diverged between the two forms, although the traits that are most conspicuously different vary among systems. The systems also vary in the age of the divergence of the two forms, as well as in the strength of reproductive isolation between them. In addition, in at least three of the systems, one or both forms have evolved repeatedly, independently of one another, with their phenotypic similarity being the product of parallel evolution (Tables 1, 2).

Stickleback speciation in nature

Speciation is frequently rapid

Results from laboratory experiments suggest that partial reproductive isolation can evolve extremely rapidly, on the scale of tens to hundreds of generations [6]. Little is known, however, about how quickly speciation occurs in nature [7]. A lower limit on the rate of evolution of reproductive isolation between two species can be determined from estimates of the age of their genetic divergence. Caution must be exercised when interpreting such rates, however, because gene flow between populations can bias genetic distances and estimates of divergence time, as can selection on genetic markers. Here and in Table 1, we focus mainly on data from allozymes, because microsatellite data are limited and uncalibrated [8,9] and results from mitochondrial DNA (mtDNA) data for the threespine stickleback complex appear to be particularly susceptible to biases. Specifically, mtDNA findings for the Japan marine, lake–stream and limnetic–benthic systems are inconsistent with other markers and some geological data. Unfortunately, no single explanation readily accounts for these disparities, although mtDNA gene flow has been implicated in the limnetic–benthic and Japan marine systems [8–15].

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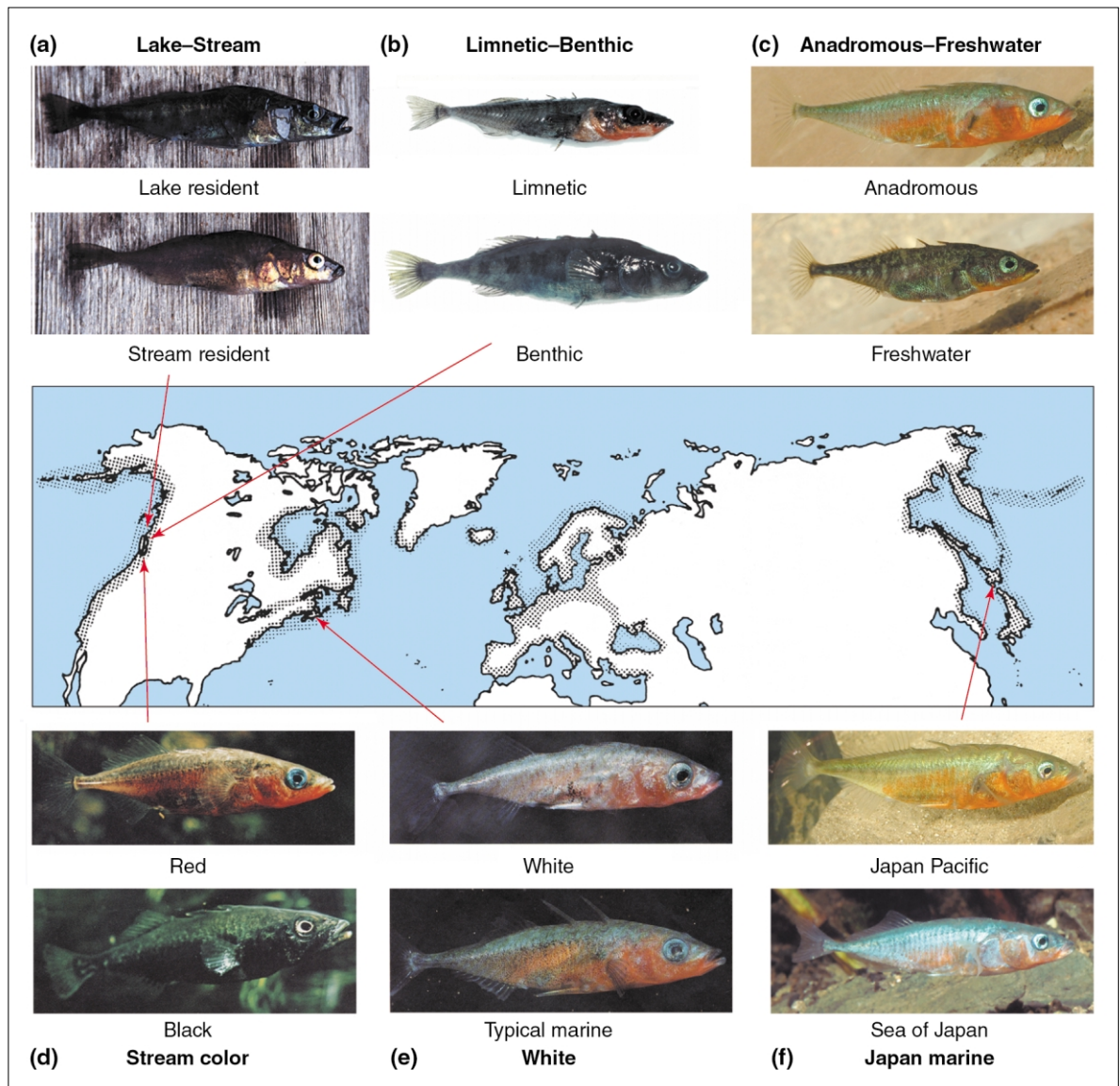


Fig. 1. Map of the distribution of the threespine stickleback *Gasterosteus aculeatus* species complex (shaded areas; modified from [57]) with photographs of males of each system. Males vary among sites within ecotypes (e.g. from weak red to black in benthics). The illustrated systems are: (a) lake-stream (found on Vancouver Island and the Queen Charlotte Islands, British Columbia; photos of Misty Lake fish by A. Hendry); (b) limnetic-benthic (found originally in lakes on Vancouver Island and two smaller nearby islands, British Columbia; photos of Enos Lake fish, reproduced with permission from [58]); (c) anadromous-freshwater (found throughout most of the range of the stickleback; photos of Alaskan fish by J.S. McKinnon); (d) stream color (found in Olympic Peninsula, WA, USA; photos reproduced with permission from [59]); (e) white (whites found along the coast of Nova Scotia and typical marines found throughout the range of the stickleback; both photos from Nova Scotia, reproduced with permission from [25]); (f) Japan marine (overlap along the coast of Hokkaido Island, Japan; photos by J.S. McKinnon and S. Mori). Arrows indicating distribution are omitted for the anadromous-freshwater system because it is found through much of the range of the threespine stickleback.

Genetic and geographical data suggest that at least three of the stickleback systems are young, requiring reproductive isolation between the pairs to have evolved rapidly (Table 1). Such is the case in the limnetic-benthic system, for which genetic divergences between the two forms are very low [8,16].

Also consistent with a recent origin, all pairs inhabit lakes that formed following the retreat of the Pleistocene glaciers ~10 000–12 000 years ago [4]. Similarly, allozyme estimates of divergence time (as well as microsatellite divergences) for some anadromous-freshwater pairs are also low [8,17,18], consistent with their presence in drainages from which glaciers have, in some cases, only recently retreated and again indicative of a recent, post-Pleistocene origin. Finally, allozyme studies reveal little or no significant divergence between the white stickleback and the 'typical' marine form, suggesting a recent origin also for this marine system [19]. Molecular estimates of divergence time are crudely calibrated, so it is unsurprising that they do not correspond perfectly with dates based on glacial history for some of these systems; however, the overall patterns are consistent.

The Japan marine system constitutes the only clear case of a more ancient origin within the species complex. This pair shows extensive divergence at allozyme loci, with an estimated time of divergence of ~two million years. This is supported by

Table 1. Geography and natural history of reproductive isolation in the threespine stickleback systems

System	Geographical context (breeding)	Hybrid frequency (data)	Nei's D (allozymes)	Estimated time since divergence (allozymes) ^a	No. of pairs (min. no. of independent origins)	Refs
Limnetic–benthic	Sympatric	<2% (morphology)	0.018	90 000 years	6(4)	[4,8,16]
Anadromous–freshwater	Sympatric or parapatric	0–21% (morphology)	0–0.008	0–40 000 years	Unknown, probably >1000 (5 ^c)	[4,17,18,60]
Lake–stream	Parapatric	<1% (morphology)	v. low ^b	Recent (quant. est. not avail.)	3 (2)	[4,9,10,13]
White	Sympatric	0% (observed spawnings)	0.000–0.003	0–15 000 years	1 (1)	[19,25,26,61]
Japan marine	Sympatric	0–3.1% (allozymes)	0.428	2 000 000 years	1 (1)	[11]
Stream color	Parapatric	60–70% in hybrid zone (male color)	Unknown	Unknown	>4 (1) ^d	[2,24]

^aGlacial history indicates that habitats of the limnetic–benthic, most anadromous–freshwater, and lake–stream systems became ice-free ~12 000 years ago [4,5]; if not provided, times of divergence calculated following [62].

^bOnly two of 25 loci polymorphic, with similar and not significantly different (unreported) allele frequencies [10].

^cAssumes independent origins in Japan, Kamchatka, British Columbia, the British Isles and Europe; actual number possibly much higher.

^dSeveral streams have a red downstream–black upstream pattern, but only one has been studied for reproductive isolation [2].

biogeographical evidence that suggests an extended period of low sea levels isolated the Sea of Japan from the Pacific Ocean around that time [11].

The geographical context of speciation is diverse

A long-standing controversy in evolutionary biology concerns whether new species can arise in sympatry. Whereas mainstream opinion has long favored the allopatric model, recent theory has demonstrated the feasibility of sympatric speciation [20,21]. Conclusive examples from nature, however, are few [21] and it is apparent that we have reached the point at which we are information limited; a better understanding of the geographical context of speciation will require data from real speciation events in nature.

The geographical context of speciation can be difficult to reconstruct, and it has not been addressed in two of the stickleback systems (the white and stream color). Nevertheless, evidence from the remaining systems suggests that the traditional sympatry–allopatry debate is overly simplistic and fails to capture the diversity of scenarios that occur in nature. An episode of allopatry is implicated in the speciation of the Japan marine and limnetic–benthic

systems. Such a result implies that present-day species ranges might be unreliable indicators of the geographical context of speciation (*sensu* [22]).

In the Japan marine system, biogeographical and allozyme data (Table 1) both indicate that the Sea of Japan form evolved after the Sea of Japan was isolated from the Pacific Ocean during an extended period of low sea level ~two million years ago [11]. Whether reinforcement (the strengthening of prezygotic isolation because of selection arising from reduced hybrid fitness) has occurred since their secondary contact has not yet been explored, although the weak and asymmetric assortative mating detected in the laboratory is not suggestive of a significant role (Table 3). An episode of allopatry is also implicated in the evolution of the limnetic–benthic system, in spite of the two forms being fully sympatric today. A combination of genetic, physiological, geological and geographical data suggests that the present-day limnetics and benthics are the result of two separate invasions of the marine stickleback into freshwater [23], as proposed by McPhail [4]. Reproductive isolation appears to have evolved during both allopatric and sympatric stages [23].

Table 2. Phenotypic differences between forms within each of the threespine stickleback systems

System	Principal morphological differences ^a				Male reproductive differences		Refs
	Body size	Shape ^b	Foraging traits ^c	Armor (antipredator) ^d	Nuptial color	Courtship behavior	
Limnetic–benthic	Yes	Yes	Yes	Yes	Yes	Yes	[4,16,27,36,40,63]
Anadromous–freshwater	Yes	Yes	Yes	Yes	Yes	Yes	[4,38,64,65 ^e]
Lake–stream	Yes	Yes	Yes	Yes	Yes	Unknown	[4,9,10]
White	Yes	Yes	Yes	Yes	Yes	Yes	[25,26,61]
Japan marine	Yes	Minor or none ^f	Minor or none ^f	Yes	Minor or none ^f	Yes	[11,37]
Stream color	Minor or none ^f	Minor or none ^f	Yes	Minor or none ^f	Yes	No (anecdotal)	[2,24,47]

^a'Yes' indicates that differences have been documented, although magnitude varies and there is variation among sites within a system.

^bBody depth, corrected for length.

^cJaw/gape width and/or gillraker number/length.

^dSpine length and/or lateral plate number.

^eMcKinnon and Aurit, unpublished.

^fMcPhail [2] (stream color) and Higuchi and Goto [11] (Japan marine) examined some aspects of morphology, but do not report differences for these traits; any differences that do exist will probably be small (Fig. 1).

Table 3. Components of reproductive isolation between forms within each of the threespine stickleback systems

System	Prezygotic isolation		Postzygotic isolation			Refs
	Breeding habitat differences	Assortative mating/courtship (% homospecific) ^a	Intrinsic genetic incompatibilities	Ecologically dependent isolation	Sexual selection against hybrid males	
Limnetic–benthic	Amount of cover, water color	Field: unknown Laboratory: 76–100%	No (possibly one backcross)	Yes: growth, survival	Laboratory: yes Field: yes	[4,31–34,40,42,45,54,63,66]
Anadromous–freshwater	Up/downstream, substrate, cover	Field: unknown Laboratory: 63–100%	No	Likely (indirect evidence ^b)	Laboratory: yes (preliminary) Field: unknown	[3,4,35,67,68]
Lake–stream	Lake or stream	Field: weak male preference Laboratory: lake females 83%	No	Likely (indirect evidence ^c)	Unknown	[4,9,10,69]
White	Amount of algae	Field: 100% Laboratory: 100%	Unlikely (hybrids easily raised)	Unknown	Unknown	[25,26,61]
Japan marine	Unknown	Laboratory: Pacific females 63%, Sea of Japan: NS ^d ; Field: unknown	Yes (sterility)	Unknown	Unknown	[11,29,30,37]
Stream color	Up/downstream, water color	Field: unknown Laboratory: red females 83%, black NS ^e	Yes (viability, parental care)	Likely (indirect evidence ^d)	Unknown	[2,70]

^a Most results are based on female nest entry or spawning, but data from the lake–stream and stream color systems are based on orientation tests, which might be less reliable.

^b Implied by intermediate hybrid morphology and ecology–morphology correlation, and by differences in salinity tolerance.

^c Implied by intermediate hybrid morphology and transplant experiments using parental forms.

^d Implied by differences in salinity tolerance of parental forms.

^e No statistically significant assortative mating and/or courtship.

The geographical context of speciation in the lake–stream system is unclear and not all data agree. Highly divergent mtDNA lineages dominate the separate forms in Misty Lake [13], suggesting a possible allopatric phase in their origin. However, the lake itself has a post-Pleistocene origin and evidence from microsatellites and allozymes suggests a more recent divergence [9,10].

In contrast to these cases, divergence in the face of gene flow appears much more probable for the anadromous–freshwater system if, as evidence suggests, freshwater sticklebacks have evolved many, perhaps hundreds, of times independently. It is difficult to envisage separate geographical barriers arising in all of these streams, with all barriers having since broken down to allow secondary contact [4].

Speciation involves divergence in many traits

By asking which traits diverge early in speciation, we can make inferences about the roles of various processes in the evolution of reproductive isolation. An examination of Table 2 reveals a striking pattern in which many different traits have diverged in each of the systems. Key differences shared by most systems include body size, body shape, trophic characters, antipredator traits and male reproductive characters. The heritability of most of these traits is known or inferred from data from other populations [2,4,9,10,24–27]. The overall pattern is consistent with Rice and Hostert's [6] claim that speciation is most likely, and proceeds most rapidly, when multiple traits are under divergent selection. Moreover, divergence has occurred in traits most likely to be under the influence of both natural (e.g. gillrakers) and sexual (e.g. male color) selection, suggesting a

possible role for both forms of selection in speciation. Nevertheless, the divergence of many traits, even early in the speciation process, does not eliminate the possibility that just one, or a few of them are responsible for the evolution of reproductive isolation.

The exceptions to the pattern could provide important additional insight. Morphological divergence appears to be least extensive in the Japan marine and stream color systems (Table 1), although more traits need to be measured in each. Similar to morphological divergence, prezygotic isolation appears to be weak in both cases and these are the only systems in which hybrids suffer from any apparent intrinsic genetic incompatibilities (Table 2). This suggests that reproductive isolation might have evolved by different processes in these cases.

Divergent selection is the dominant mechanism of speciation

Research to date strongly implicates ecologically based divergent selection, both natural and sexual, in the evolution of reproductive isolation in sticklebacks. By contrast, the contribution of other mechanisms, such as bottlenecks and/or founder events, appears to be minor, although unique predictions of these models are lacking and they have received little direct attention (Box 1). We begin by examining the evidence for the role of selection in the evolution of postzygotic isolation.

(i) Postzygotic isolation

Two forms of postzygotic isolation are generally recognized: intrinsic genetic and ecologically dependent [6,28]. In the first, hybrid fitness is reduced as a result of incompatibilities between the genomes of the parent species that are expressed

Box 1. Population bottlenecks appear to be unimportant in threespine stickleback speciation

There are several bottleneck-induced speciation models (e.g. founder flush and genetic transilience), but they all share the same basic tenet that a temporary bottleneck in population size can initiate a cascade of events resulting ultimately in reproductive isolation [a]. Experimental and theoretical support for these models is weak [b,c], yet they remain popular. Two common predictions of the bottleneck-induced models are met in the threespine stickleback complex; however, these predictions are not unique and are readily explained by other processes [b,d]. They are:

Founder events extreme enough to cause speciation should also cause a substantial and prolonged loss of genetic variability at neutral loci [b]

Freshwater sticklebacks exhibit reduced variation within, and greater variation among, populations than do marine populations [e]. Nevertheless, this is exactly what would be expected from colonization of novel habitats and does not require the additional postulation of extreme population bottlenecks. Strong selection can reduce variation at target loci as well as at other closely linked loci. In addition, selection itself reduces the effective population size [f]. The isolated nature of freshwater habitats also makes gene flow among populations difficult.

Speciation should occur in small, peripheral populations [g]

Stickleback populations on their way to full reproductive isolation are often at the periphery of the range of marine sticklebacks (e.g. in coastal, freshwater habitats). However, speciation in such localities is readily explained by invasion of novel habitats made available by the retreat of the glaciers and does not require unique population dynamics, such as extreme bottlenecks.

Three additional lines of evidence are inconsistent with population bottlenecks being important in stickleback speciation:

Intrinsic genetic incompatibilities between populations are rare

The extensive genetic reorganization proposed by the bottleneck models has commonly been hypothesized to generate assortative mating between the resulting species [a]. It is also reasonable to expect that it might cause genetic incompatibilities, although the theory has not been developed quantitatively. In the stickleback complex strong intrinsic genetic incompatibilities appear to be rare and are known to exist only for two pairs of populations. Rather, complete viability and fertility of hybrids is the norm (Table 3 main text).

Prezygotic isolation has evolved in parallel, in correlation with the environment

Prezygotic isolation has evolved in parallel among independent populations in the limnetic–benthic system, and possibly also in the anadromous–freshwater system. This is inconsistent with the

bottleneck-induced speciation models because only divergent natural selection can produce such concerted change, in multiple, independent lineages, in correlation with the environment [h].

Morphological divergence among lake populations has occurred in a predictable fashion

Analyses suggest that divergence among lake populations has occurred primarily along a single axis of phenotypic variation [i,j], which is not predicted if founder effects have led to haphazard change and unpredictable evolution. This axis of among-population variation also appears to be correlated with the axis of greatest additive genetic variation within a single lake population, suggesting that population divergence has been biased along the multivariate direction of greatest additive genetic variation within populations [i]. *Drosophila* experiments have demonstrated that the shape of the additive genetic variance/covariance matrix is highly variable among inbred lines [k], so such a bias in stickleback population divergence would not be expected if the genetic structure of separate populations had been reorganized through bottlenecks.

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when they are combined in hybrids. Such genetic incompatibilities act independently of environmental context and can be produced by all models of speciation. In the second, hybrid fitness is reduced as a result of an interaction between the phenotype of the hybrid and its ecological context. Hybrids might not perform well in either parental habitat and, in effect, fall between niches in the environment. Ecologically dependent isolation is a unique prediction of selection-based speciation models [28].

These two forms of postzygotic isolation are not equally represented among stickleback populations. Few examples of intrinsic genetic incompatibilities have been found, in spite of several tests specifically designed to detect them. To date, only two pairs of populations, one in the Japan marine system and the other in the stream color system, show any evidence of substantial genetic incompatibilities (Table 3). Even in these systems, the findings are of limited scope and further work is necessary to determine their generality.

The Japanese results come from crosses between a single anadromous population of one lineage and a single freshwater population of the other [29,30]; data from crosses between anadromous fish of each lineage are sorely needed. Results from the stream color system come from a single pair of populations in a region for which virtually all other interpopulation crosses yield viable offspring [4,31]. In the remaining systems, genetic incompatibilities have either been decisively rejected as important causes of isolation or appear unlikely, given the ease with which various hybrid crosses can be raised in the laboratory (Table 3).

In contrast to the rarity of intrinsic genetic incompatibilities, a variety of data suggests that ecologically dependent isolation is nearly ubiquitous, thus strongly implicating divergent natural selection in stickleback speciation. In the limnetic–benthic system, in spite of no detectable growth reduction in the laboratory, field transplant experiments documented reduced F1 hybrid growth rates that

appear to result from reduced foraging efficiencies in both parental habitats [1,31]. The ecological basis of postmating isolation is confirmed by a transplant experiment involving both limnetic and benthic backcrosses: limnetic backcrosses grew at approximately twice the rate of benthic backcrosses in the habitat of limnetics, whereas the reverse was true in the benthic habitat [32].

Evidence for ecologically dependent isolation in the other systems is circumstantial. The strongest case can be made for the lake–stream system, in which transplant experiments with one pair revealed a modest growth advantage of each parental form in its native environment [9]. Because F1 hybrids are known to be intermediate in morphology between the two forms for several characters [9], we would also expect them to be ecologically intermediate and disadvantaged in either environment.

Consistent with ecologically dependent isolation in the anadromous–freshwater system is the relationship between morphology and ecology that exists for independently derived or distantly related populations. This suggests an important role for natural selection in their parallel morphological evolution [4]. Because hybrids are morphologically intermediate [3,4], the implication is that hybrids in nature will suffer reduced fitness as a result of their poor fit to available niches, except perhaps in rare transitional habitats [3].

Sexual selection can also cause postzygotic isolation by reducing hybrid mating success. This has been clearly shown in the limnetic–benthic system where hybrids, which nest near limnetic males, are less successful at achieving matings than are limnetics [33,34]. Laboratory results suggest a similar pattern in the anadromous–freshwater system [35]. Given the widespread divergence in courtship-related characters in all sticklebacks (Table 2), sexual selection against hybrids might be common and is an important topic for further work.

(ii) *Prezygotic isolation*

Stickleback research has focussed on the role of divergent selection in the evolution of assortative mating and several unique, testable predictions have been evaluated in the various systems.

Assortative mating based on ecologically selected traits. Traits affecting assortative mating can also be strongly influenced by natural selection. If natural selection causes these traits to diverge in populations inhabiting different habitats or niches, prezygotic isolation will result. Detailed investigations in two stickleback systems, the anadromous–freshwater and limnetic–benthic, suggest such a situation. In both cases, laboratory experiments have revealed that assortative mating between the forms is based on body size [4,36,37]. In the limnetic–benthic system, evidence strongly indicates that body-size differences are adaptive for foraging in different niches [1]. The adaptive significance of body-size differences in

the anadromous–freshwater system is implied by the parallel evolution of body size in numerous populations [38], although phenotypic plasticity could also contribute to this pattern.

Similar to some insect systems [21], differences in preferred mating habitat might also contribute to reproductive isolation in several stickleback systems (Table 3); the possible adaptive origin of divergent habitat choice in sticklebacks deserves more explicit attention. Differences in the timing of spawning are also important in some anadromous–freshwater pairs and warrant similar consideration [3].

Assortative mating based on ecologically correlated courtship traits. This occurs when natural selection acts on traits that influence mating preferences and courtship; it is closely related to the more strictly ecological process described above, but also involves a central role for sexual selection. For example, as a by-product of adaptation of the female sensory system to different environments, female mate preferences can change. This, in turn, will generate sexual selection on male traits in a process known as ‘sensory drive’ [39]. Environmental differences by themselves can also cause divergent sexual selection on male traits, for example if alternative male nuptial colors differ in their conspicuousness depending on ambient light conditions. Such processes appear to have occurred in the limnetic–benthic system, suggesting a key role for both natural and sexual selection in limnetic–benthic speciation [40]. In addition, natural and sexual selection appear to have interacted in a different manner in the speciation of the white stickleback (Box 2).

Parallel evolution of reproductive isolation. Natural selection is strongly implicated when the same or similar traits evolve in independent lineages that inhabit similar environments. The parallel evolution of reproductive isolation (‘parallel speciation’) thus provides some of the strongest evidence for the role of selection in the speciation process [41]. The expected outcome is assortative mating according to environment (and hence selection regime), with reproductive compatibility between populations that inhabit similar environments and reproductive isolation between populations inhabiting different environments.

The first comprehensive test is a recent study in the limnetic–benthic system that used independently evolved pairs from three lakes [42]. Reproductive isolation was found to be strong between populations inhabiting different environments (i.e. between limnetics and benthics), independent of whether the two populations were from the same lake. By contrast, reproductive isolation was absent between populations inhabiting similar environments, even when they were from different lakes. Reproductive isolation has thus evolved in association with the environment, strongly implicating divergent selection in limnetic–benthic speciation. Preliminary results in the anadromous–freshwater system suggest a similar pattern [35,43].

Box 2. Selection and speciation in the white stickleback

The evolution of the white stickleback *Gasterosteus* spp. of Nova Scotia, Canada (Fig. 1 main text) suggests an interaction between natural and sexual selection in speciation. The white stickleback has abandoned the male parental care characteristic of typical *G. aculeatus* and constructs only rudimentary nests elevated within patches of filamentous algae [a]. After spawning, the male spreads the eggs into the algae, which probably provides better aeration for the eggs and also provides shelter from predators for both the eggs and the male when he is courting. The availability of this refuge, combined with release from the constraints of providing parental care, is suggested to have caused sexual selection to favor the exceptionally conspicuous coloration of the white males [a–c].

Assortative mating between typical marine sticklebacks and white sticklebacks is unusually strong and has been documented both in the laboratory and in nature [a,b]. In the early stages of courtship, however, both white and typical females respond strongly to white males whereas white females reject typical males. Thus, male coloration and the female preference, probably for color, both appear to be exaggerated in the white stickleback relative to typical sticklebacks [a,b]. Interestingly, a genetic correlation between male color and female preference has been documented within a European lake population [d], suggesting that sexual selection and an ensuing genetic correlation could have contributed to speciation in the white stickleback.

Additional initial findings suggest that the white stickleback deserves further attention. A second population might be present in the Baltic [a,e], raising the possibility of an evolutionary replication of the system that would enable more statistically powerful comparative studies to be made, such as tests for the parallel evolution of reproductive isolation. There is also considerable behavioral variation even within the white stickleback of Nova Scotia, with some populations spawning on rocks in the intertidal zone [f] rather than in algae, and assortative mating by spawning habitat is a possibility [g].

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Reproductive character displacement. This is the pattern of stronger assortative mating between incipient species in areas of sympatry than in areas of allopatry [44]. Although its occurrence has commonly been used to infer the process of reinforcement, it can also be produced by other processes involving selection [44]. For example, it can result as a by-product of ecological character displacement. More importantly, reproductive character displacement can also result from a biased extinction process in which two populations come together multiple times but persist in sympatry only in those cases in which prezygotic isolation between them was sufficiently strong. Consequently, using this pattern to infer a role for selection in speciation necessitates eliminating the alternative explanation of biased extinction [44].

Reproductive character displacement has been demonstrated in three of the stickleback systems: the anadromous–freshwater, limnetic–benthic,

and stream color. The most thoroughly studied case involves the limnetic–benthic pairs [23,45]. Laboratory mating trials reveal that benthic females from Priest Lake (BC, Canada) show a strong preference for benthic males from Paxton Lake (BC, Canada) over limnetic males from Paxton Lake; this preference is weaker and nonsignificant in ‘benthic-like’ females from both an allopatric lake and a stream population, from which limnetics are absent [45]. Similar results have been obtained in the anadromous–freshwater system. Stream-resident males from the upper portion of the Salmon river (BC, Canada), from which anadromous fish are absent, prefer larger females, as is generally observed in males of other populations of sticklebacks [46]. However, males from a downstream location, where the larger anadromous sticklebacks also breed, prefer smaller females [4]. Finally, in the stream red–black system, females from a black population, which is geographically isolated from red males, responded preferentially to red over black males. By contrast, females from a black population adjacent to a red population showed no preference for red, again indicating the pattern of reproductive character displacement [2, see also 47]. Biased extinction appears to be an unlikely explanation in the case of the limnetic–benthic system [45], but has not been considered in the other two.

Conclusions

Our review of the stickleback species complex suggests that speciation in nature can be rapid, can occur under a variety of geographical contexts, and generally involves the divergence of suites of traits. The primary message emerging from our review, however, is that selection is the principal cause of stickleback speciation. Evidence suggests a central role for divergent natural selection, and probably also sexual selection, in the evolution of both pre- and postzygotic isolation in nature. By contrast, the contribution of genetic drift and founder events to stickleback diversity appears to be minor (Box 1), although the lack of unique, testable predictions makes evaluation of these models difficult.

Many of these findings are not unique to sticklebacks and are supported by results emerging from other natural systems. For example, the rapid evolution of reproductive isolation has also been observed in some insect species upon colonization of novel hosts [21] and might also be occurring in introduced salmon [7]. Work in other organisms also suggests that the geographical context of speciation can be diverse. For example, at least partial reproductive isolation appears to have evolved in allopatry for present-day sympatric species of Darwin’s finches [48]. As in sticklebacks, hybrid inviability and sterility are also absent between finch species, whereas ecological mechanisms play a central role in their reproductive isolation [48]. Indeed, several studies have demonstrated that speciation can occur in the absence of genetic incompatibilities [1].

Nevertheless, the divergence in sticklebacks of many traits, both ecological and reproductive, early in the speciation process is not characteristic of all taxa. In cichlids, for example, divergence in male coloration appears to be more important in speciation than is divergence in ecological traits [49].

Evidence is also accumulating from several organisms that reproductive isolation is ultimately the result of divergent natural selection [1]. Evidence for the role of sexual selection remains primarily indirect [50], although it has been shown to cause postzygotic isolation in *Heliconius* butterflies [51]. With the possible exception of polyploid speciation [52], direct evidence of a role for genetic drift and founder events remains elusive [20].

Prospects

In future speciation research, both in sticklebacks and in other model systems, it will be important to widen our perspective on the role of selection. Ongoing stickleback research is exploring novel ecological causes of divergent selection, such as predation [53,54], and it is essential that this also be done in other systems. The contribution of sexual selection, for example, has received little direct attention yet recent stickleback work suggests that it plays a central role and can interact with natural selection to cause the rapid evolution of reproductive isolation. Not only ecological traits, but also courtship traits and female preferences can evolve by divergent natural and sexual selection stemming ultimately from environmental differences [1]. One possible example of such a pathway in sticklebacks has been suggested by Foster and colleagues [55]. As a result of differences in feeding

mode and body size, benthic females are able to cannibalize eggs from the nests of males, whereas limnetic females cannot. This difference in cannibalism is hypothesized to have contributed to the divergence in courtship behaviors between limnetics and benthics, and hence to their reproductive isolation.

It is important for future stickleback research to continue expanding into additional systems, to exploit the particular strengths of those systems and to test the generality of current patterns. A thorough understanding of the origin of new species will also require addressing topics that are, as yet, little studied, such as the genetic basis of reproductive isolation. With a recently published linkage map [27], the threespine stickleback is also poised to make important contributions to this area. In addition, the historical emphasis on natural history and field studies provides the opportunity to study the genetics of traits involved in the initial stages of reproductive isolation in an ecological context. With further elucidation of stickleback phylogeography, comparative approaches to trait genetics, as well as other important topics, will be strengthened.

It is only recently that divergent, reproductively isolated marine forms have been discovered [11,25] and additional surprises are likely to be in store from recent geographical surveys in several regions. Already, research in Iceland has revealed a previously unknown system comprising forms found on either lava or mud substrates [56]. These new systems are likely to generate unanticipated insights, much as the white stickleback has (Box 2); at the very least, they will provide opportunities for further evaluating current findings.

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