Evolutionary inference: the value of viewing evolution through stickleback-tinted glasses

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The threespine stickleback species complex possesses a number of attributes that make it particularly suitable for evolutionary studies. This stickleback has undergone remarkable phenotypic diversification in freshwater habitats since the deglaciation of north-western North America, Scotland, and other less extensively studied regions (reviewed in Bell and Foster page 16 this volume). In some instances, speciation has occurred since the last deglaciation (McPhail Chapter 14 this volume). In addition, there exist many seemingly older, freshwater populations, and the marine, estuarine, and anadromous ancestors (hereafter referred to as marine ancestors) of the freshwater populations are extant in both the Atlantic and Pacific basins (Bell and Foster page 2). Extensive postglacial diversity, coupled with a limited, though long and informative, fossil record, provides a chronological dimension that is lacking in other species. The diversity of phenotypes, population ages, and interrelationships makes possible interpopulation comparisons that will shed light upon a variety of issues in evolutionary biology and that would be impossible in species with more obscure intraspecific phylogeny.

Research on the evolution of morphological phenotypes has in many respects progressed beyond that on other aspects of the phenotype in threespine stickleback. This advance is undoubtedly due to the relative ease with which morphological character states can be sampled. Because much of the special value of the threespine stickleback for evolutionary research lies in the extensive population diversification and well-documented racemic phylogeny of the species complex (Bell 1987, 1988; Williams 1992; Bell and Foster page 13 this volume), the ability to compare large numbers of individuals from many populations with relative ease facilitates evolutionary inference considerably. Early research on population biology (e.g. Hagen 1967; McPhail 1969; Hagen and McPhail 1970) was of critical importance because it highlighted evolutionary explanations and generated questions regarding the phylogenetic history of the species complex. Although most research has concentrated on the interpretation of morphological differentiation, efforts are now being made to interpret population differences in
behaviour and life history in an evolutionary context as well (Chapters by Baker, Huntingford et al., and Foster this volume). Evolutionary research on physiology is in a still earlier stage of development, but there is excellent potential for comparative studies and for research in physiological ecology (Chapters by Guderley, Wootton this volume).

The threespine stickleback species complex possesses a number of attributes other than its unusual phylogenetic history that enhance its value for evolutionary research. Not only is this stickleback readily reared in the laboratory, making genetic and physiological research possible, it is also abundant and easily collected and observed in the field. Finally, as amply demonstrated by the chapters in this volume, there already exists substantial information on many aspects of the biology of threespine stickleback.

A primary purpose of this closing chapter is to highlight the particular value of the threespine stickleback complex for evolutionary research. This topic is discussed to varying degrees in earlier chapters, and most of the authors have suggested important future research directions in their fields. We will attempt to bring together these ideas in an effort to identify major questions that must be addressed if this species complex is to be exploited fully as a source of evolutionary insights. We close with a brief comparison of this diversification with better-studied adaptive radiations, and with a plea for the conservation of the populations that constitute this remarkable species complex.

ADAPTATION AND THE EVOLUTION OF FRESHWATER POPULATIONS

The chapters in this volume provide convincing evidence of a prominent role for natural selection in the diversification of postglacial freshwater populations of threespine stickleback. In large part, this inference has been possible because of the unusual phylogenetic history (racemic phylogeny) of the group. One advantage of this history for phylogenetic inference is that many of the freshwater populations have been independently derived from the marine ancestor and have thus been exposed independently to similar selection regimes (e.g. Bell and Foster page 20, McPhail page 401 this volume). In effect, numerous ‘replicate experiments’ have been established, permitting examination of correlations between iterative apomorphies and environmental variables with more certainty of statistical independence among the populations than is usually possible. The value of the postglacial freshwater diversification for correlative studies of adaptation is also increased by the low level of gene flow between populations, a condition that should facilitate adaptation to local environments (e.g. Mayr 1942; Endler 1973, 1977; Slatkin 1985, 1987). Similarly, the recency of the diversification reduces the likelihood that historical events such as dispersal or stream capture have affected many populations.
There are, of course, pitfalls inherent in the use of correlative studies for adaptive inference (e.g. Gould 1984; Endler 1986). Especially when only a few populations are sampled, correlations with some environmental variables are likely to be detected by chance alone (e.g. Clarke 1975). This problem can be alleviated if a large number of populations can be sampled (Endler 1986). Geographical complexity in the pattern of environmental variation also reduces the probability of covariation among environmental variables, thereby reducing the likelihood that phenotypic variation will be ascribed to the wrong environmental factor (e.g. Endler 1977). Both requirements can be met readily in many regions in which freshwater stickleback have diversified since the last glacial recession. Finally, it is essential that the variation in the traits being studied have known heritability to avoid confusing environmental induction of phenotypically plastic traits with selection on heritable variation (e.g. Endler 1986). Because stickleback can readily be reared in the laboratory, phenotype heritabilities can be readily assessed. Despite this potential, our knowledge of trait heritabilities is extremely limited except for a handful of morphological (Lindsey 1962; Hagen 1973; Hagen and Gilbertson 1973a; McPhail 1977) and life history (Snyder and Dingle 1989; Snyder 1991b) traits.

Even when all the above conditions are met, correlative studies can, at best, suggest a causal relationship between an environmental variable and phenotypic variation. Other methods are required to demonstrate a causal relationship (Endler 1986). At present, evidence of adaptive variation in behaviour and life history is primarily correlational. Evidence for adaptive diversification of several morphological phenotypes is far stronger.

**Morphological phenotypes**

Variation in armour structure has been particularly well studied in an evolutionary context, beginning with the seminal work of Hagen and his colleagues. Hagen and Gilbertson (1972) originally detected an association between the presence of predatory fishes in lakes and a modal number of seven bony lateral plates on each side of the abdomen. Because they also demonstrated that plate number was heritable (Hagen and Gilbertson 1973b) and that predation by fish favours an increase in the frequency of seven-plated stickleback in natural populations (Hagen and Gilbertson 1973a) and in the laboratory (Moodie et al. 1973), they provided convincing evidence that selection by predatory fish maintains this plate number phenotype in natural populations. Reimchen (1983) subsequently showed that specific lateral plates interact functionally with the dorsal spines and pelvis, but the functional significance of the most anterior plates, which are present in seven-plated individuals, is currently being investigated (Reimchen pers. comm.). Such combined approaches are sufficient to meet Endler's (1986) criteria for demonstration of selection in natural populations.

The structure of the pelvic girdle also varies in relation to environmental
variables. Predation regime (Reimchen 1980) and calcium concentration (Giles 1983a) have been proposed as factors influencing evolution of pelvic structure. The pelvic girdle and spines are generally robust and play an important part in the deterrence of predation by vertebrates (e.g. Hagen and Gilbertson 1972; Gross 1978), but in rare cases the pelvic spines and girdle may be partially or totally lost (Bell 1987). Pelvic structure is highly heritable in the related ninespine stickleback, *Pungitius pungitius* (Blouw and Boyd 1992), and probably has a strong genetic basis in *Gasterosteus* as well.

Reimchen's work on the functional interactions of the pelvic girdle and lateral plates (Reimchen 1983), and on the relation between gape size of trout, *Oncorhynchus clarki*, and the distance between the tips of the dorsal and pelvic spines (Reimchen 1991, page 271 this volume), demonstrates the functional importance of the pelvis in deterring ingestion and mastication by predatory fishes. In contrast, Reist's (1980b) study of brook stickleback, *Culaea inconstans*, with variable pelvic structure indicated that individuals with pelvic reduction were less susceptible to predation by dytiscid beetles than were those with the primitive fully formed pelvis. Analysis of pelvic reduction has the added dimension of a fossil record, which suggests that it is an ancient phenomenon but can occur very rapidly (Bell et al. 1985b; Bell page 459 this volume).

Consideration of the functional morphology of this armour complex reveals why this combination of the pelvic girdle and seven lateral plates is maintained by fish predation. Threespine stickleback possess two large pelvic spines and three dorsal spines, the anterior two of which are large and serrated. Four of the seven lateral plates retained in populations exposed to vertebrate predation buttress the large, locking dorsal spines, while the pelvic spines, which also lock in an erect position, are supported on the pelvic girdle. Together the lateral plates and pelvic girdle enclose the abdomen of the stickleback in a flexible segmented armour complex that is an effective deterrent to many piscivorous vertebrates (Hoogland et al. 1957; Reimchen 1983 page 248 this volume).

These studies provide compelling evidence that this armour complex is maintained in populations as a consequence of selection imposed by predatory vertebrates (Reimchen Chapter 9 this volume). There is evidence that both lateral plate number and pelvic girdle expression are associated with vertebrate predation across a diversity of populations in geographically disparate locations. Both traits are known to be heritable, and selective predation by a piscivorous fish has been shown to favour the seven-plate phenotype. Finally, full expression of the pelvic girdle and lateral plates that support the dorsal spines is essential for the armour complex to act as an effective deterrent to predatory vertebrates.

In light of this evidence, variation in lateral plate number and pelvic girdle expression seems extremely unlikely to represent a 'non-adaptive product of history' (Gould and Lewontin 1979; Gould and Vrba 1982). Although
other factors, such as the presence of abundant predatory invertebrates (Reimchen 1980; Reist 1980b) and low calcium levels (Giles 1983a; Bell et al. 1985a, in press), may favour the loss of these structures, selection by vertebrate predators is clearly influential in maintaining the pelvic girdle and a high frequency of specimens with seven lateral plates per side.

Evidence of adaptive diversification in morphology is not limited to armour structures. Hagen and McPhail and their colleagues have provided evidence that population differences in morphological characters including mouth size, body shape, and the number and length of gill rakers are inherited (Hagen 1973; Lavin and McPhail 1987). They have also provided evidence that trait differences are strongly associated with specific ecological factors (Hagen and Gilbertson 1972; Lavin and McPhail 1985, 1986; McPhail Chapter 14 this volume). As in the case of the armour complex, similar associations are found over a wide geographic range, throughout which the different phenotypes are interspersed in a complex geographic pattern. Therefore divergence in these traits is also likely to be a consequence of evolution in response to local selection pressures, and similarities among geographically distant populations seem to be the products of parallel evolution. A similar combination of field, genetic, and laboratory research was used to explain the evolution of black nuptial coloration in populations of stickleback in the Chehalis River drainage in western Washington (McPhail 1969; Hagen and Moodie 1979; Hagen et al. 1980).

**Non-morphological phenotypes**

It is only recently that these methods have been used to infer the adaptive values of specific behavioural phenotypes. Because data on behavioural phenotypes are more difficult to collect in the field and laboratory than are morphological data, comparatively few populations have been studied. Nevertheless, these more limited comparisons provide compelling evidence of ecotypic differentiation in aggressive (Bakker Chapter 12 this volume), foraging (Hart and Gill Chapter 8, McPhail Chapter 14 this volume), antipredator (Huntingford et al. Chapter 10 this volume), and reproductive (Foster Chapter 13 this volume) behaviour. Efforts to assess the extent to which population differences in behaviour are mediated by genetic differences are few, but population differences in both the aggressive and the antipredator behaviours that have been examined have proven to be genetically based (Bakker Chapter 12, Huntingford et al. Chapter 10 this volume). Although the adaptive values of many of the behavioural patterns that have been studied seem evident (e.g. antipredator behaviours are best developed in populations exposed to relatively high levels of predation by vertebrates), in no instance has it been shown directly that particular behavioural traits contribute to fitness.

In summary, although the data are limited, evidence available to date suggests that behavioural phenotypes have undergone adaptive diversification akin to that observed for morphological phenotypes. Clearly, additional
research on behavioural phenotypes, including direct measures of natural selection on behaviour and assessments of the genetic underpinnings of population differences in behaviour, would do much to advance our understanding of the extent to which natural selection has influenced the evolution of behavioural phenotypes of a vertebrate in nature.

One approach to understanding the role of adaptation in behavioural diversification involves testing game theory models of the evolution of behaviour (e.g. Maynard Smith 1982) and optimal foraging models (e.g. Stephens and Krebs 1986) through interpopulation comparisons. Many of these models predict the evolution of different behavioural phenotypes under different ecological and social conditions. Judicious comparisons of populations exposed to appropriate selection regimes can permit tests of these evolutionary models (e.g. Riechert 1986). This approach has not been taken in research on stickleback, but should be.

The comparative approach to understanding adaptation is even more poorly developed in the study of life history evolution in stickleback (Baker Chapter 6 this volume), and has rarely been used to elucidate the adaptive values of variable physiological traits (Guderley Chapter 4 this volume) or of population differences in patterns of somatic and gonadal allocation (Wootton page 115 this volume). Baker’s chapter provides clear evidence of the potential of this approach for understanding the ways in which natural selection has moulded life history characteristics. He also stresses the importance of uniform methodology if data from different studies are to be of use in comparisons among populations. This concern applies not only to the study of life history data, but also to research on any phenotype.

Similarly, our understanding of adaptation is only as good as our understanding of the selection regimes to which populations are exposed. We have detailed information on the ecology of few of the habitats in which stickleback live. Equally, the measures of selection pressures that we use are not necessarily accurate (Reimchen page 273 this volume) or complete (e.g. Arnold and Wade 1984). Our understanding of the causes of diversification among populations of threespine stickleback clearly would benefit from a more complete ecological assessment of the habitats in which stickleback live and from a clearer perception of how the fitness of specific phenotypes is affected by specific ecological processes.

By arguing for a prominent role of natural selection in moulding many phenotypic components in the postglacial diversification of threespine stickleback, we are not uncritically invoking the adaptationist programme (Gould and Lewontin 1979). We feel that the research summarized above and in the chapters in this volume provides ample evidence for adaptive diversification in many aspects of phenotype. In making this argument we do not intend to exclude effects of genetic drift (including founder effects and population bottlenecks), ontogenetic constraints, or allometry as factors in population differentiation (Bell 1981, 1987). We wish only to reiterate
the point made by McPhail (page 435 this volume) and Reimchen (page 276 this volume) that a remarkable proportion of the diversification in post-glacial populations of threespine stickleback has been shown to be adaptive.

**PATTERNS OF EVOLUTIONARY CHANGE**

**The direction of evolutionary change**

The radiation of threespine stickleback that has taken place in freshwater habitats in recently deglaciated regions is of particular value for evolutionary research because plesiomorphic character states can be inferred with remarkable certainty. In the case of morphological traits this inference is relatively straightforward. Virtually all marine and anadromous stickleback, the ancestors of freshwater stickleback, have a complete set of lateral plates (complete morphs), a fully developed pelvic girdle, three dorsal spines, and paired pelvic spines (Bell and Foster page 5 this volume). Furthermore, marine stickleback appear to have possessed this morphology for at least the last 10 million years (Bell 1977, page 441 this volume). There can be little question that these are plesiomorphic character states relative to the postglacial freshwater diversification.

What is particularly unusual, however, is the ability to infer plesiomorphy for behavioural and life history traits. The fossil record rarely can provide evidence of stasis in either kind of trait (but see Boucot 1990), and it is plausible that behavioural or life history phenotypes have changed in marine populations since the last glacial maximum. However, the cladogram for populations of *G. aculeatus* (Haglund *et al.* 1992a; Buth and Haglund page 78 this volume) suggests that the behavioural and life history phenotypes of marine *G. aculeatus* are also ancient. A set of Japanese populations forms the sister group to all other *G. aculeatus*, which in turn are divided into separate monophyletic groups in the Atlantic and Pacific basins. The fossil record shows that *G. aculeatus* has been in the Pacific basin for at least 10 million years and possibly 16 million years, but the earliest record of threespine stickleback in the Atlantic basin is about 1.9 million years (Bell page 444 this volume). Consequently, character states shared between Pacific and Atlantic marine populations are presumably primitive character states relative to the postglacial diversifications in either basin.

The quality of such inference with respect to morphological, behavioural, or life history phenotypes is dependent on the accuracy of the assumption of relative uniformity of character states in marine and anadromous populations. The evidence of morphological uniformity is strong. Of the many reports on the morphology of marine threespine stickleback (reviewed in Bell 1984a), there are only a few reports of unusual morphological phenotypes in marine and anadromous populations (Münzing 1963; Black 1977; Borg 1985). In contrast, we are glaringly deficient in our knowledge of variation in other aspects of phenotype in marine populations.
The ability to infer the direction of evolutionary change enables much more accurate adaptive inference. For example, adaptive explanations for the loss of diversionary displays in freshwater populations are very different from those that might explain iterative evolution of novel diversionary display behaviour in freshwater populations (Foster 1988, page 394 this volume). Knowledge of character polarity can insure that the appropriate explanation is provided. Inference of character polarity can also facilitate tests of specific evolutionary hypotheses, such as Kaneshiro's (1976) speciation hypothesis (see Foster page 393 this volume).

Perhaps one of the most intriguing benefits of the ability to identify derived character states is that it should be possible to determine whether the independent evolution of similar, derived phenotypes is the consequence of the same or different genetic and developmental modifications. For example, Wilkens' (1971) interpopulation crosses of cave fish, *Anoptichthys* (= *Astyanax*) *mexicanus*, suggested that reduction of the eyes had evolved independently and depended on different genes in different populations. Such comparisons could provide a unique test of two alternative hypotheses using populations that have diversified under natural conditions. The first holds that uniform selection on closely related lineages tends to produce the same genetic changes because it favours the same shared alleles (e.g. Muller 1939). The second holds that uniform selection on closely related lineages (even populations drawn from the same source population) can act as a diversifying force in evolution (Cohan 1984; Cohan and Hoffman 1989). These alternatives can potentially be discriminated through judicious comparisons of populations in an adaptive diversification with the unusual characteristics of the threespine stickleback postglacial diversification.

Similarly, analysis of the role of changes in the timing of developmental processes in evolution (heterochrony) requires placement of phenotypic differences in a phylogenetic context (Fink 1982). Interpopulation comparisons have already demonstrated a tendency for simplification or reduction of phenotypic states of freshwater populations relative to their marine ancestors (Bell 1981, 1987). Undoubtedly, additional insights could be derived from more detailed comparative analyses of changes in developmental programmes.

**Molecular methods in the inference of evolutionary pattern**

Uncertainties about character polarity in endemic radiations of *G. aculeatus* would benefit from construction of regional population-level cladograms based on molecular data. Phenotypic characters are unsuitable for this purpose because similar phenotypes are often the products of iterative evolution and are therefore homoplasies. Furthermore, if phenotypic integration is as important as we think it is (Foster et al. 1992; Bell and Foster page 22 this volume), use of any phenotypic data to construct a phylogenetic framework for inference of character polarity would be circular (Brooks and
McLennan 1991). At present, however, the appropriate combination of methodology and molecules to resolve cladistic relations among postglacial populations is unavailable.

Postglacial populations are too young for a sufficient number of apomorphic allozymes to have been produced by mutational processes. Withler and McPhail (1985) conducted an allozyme survey of G. aculeatus populations in south-western British Columbia. Although their data provided evidence of lower polymorphism and greater population differentiation in freshwater populations than in anadromous populations, the electromorphs of freshwater populations included only one allele that was absent from anadromous populations (present in 11 of 40 freshwater populations). Thus, their data provided no evidence of sufficient, appropriate electromorph variation for use in reconstruction of phylogenetic relationships among these populations.

Similarly, restriction fragment length polymorphisms (RFLPs) appear to evolve too slowly to resolve cladistic relations among populations that have been isolated postglacially (Avise et al. 1987). Gach and Reimchen (1989) distinguished two groups of freshwater threespine stickleback populations in the Queen Charlotte Islands using RFLP analysis, but finer phylogenetic resolution was impossible. They seem to have detected the presence of an older group that had persisted in a glacial refugium, as well as populations resulting from postglacial colonization of freshwater.

Analysis of the control region (i.e. 'D-loop') of the mitochondrial genome using the polymerase chain reaction (PCR) and direct sequencing does not appear promising either. 'Universal primers' for the D-loop (Kocher et al. 1989; Meyer et al. 1990), which amplify this region in most species, do not appear to work in G. aculeatus (Ortí pers. comm.), but other effective primers for this region in threespine stickleback probably will be developed. The more fundamental problem, however, is that postglacial populations may be too young for a sufficient number of nucleotide substitutions to have occurred within the D-loop to produce a cladogram. For example, Meyer et al. (1990) analysed an 803 base-pair region that includes the most variable region of the D-loop in 32 cichlid species endemic to Lake Victoria, East Africa. They found a total of only 15 positions in the sequence that varied between at least two species, and estimated the age of the group at less than 200,000 yr. However, this age is an order of magnitude greater than that of postglacial threespine stickleback, in which only a few substitutions should be expected. If other fast-evolving sequences are identified, it might become possible to infer population-level cladograms for postglacial threespine stickleback populations, but the amount of sequence necessary to make such inferences for populations that have diverged so recently may make it impracticable using currently available methods.

Molecular methods for phylogenetic inference are developing at an astonishing pace, and a suitable method for cladistic analyses of postglacial
populations may be developed soon. For example, a method called RAPD (Williams et al. 1990) uses the entire genome and appeared to be promising for cladistic analyses. However, this method evidently is unreliable because binding of primer to the DNA sample is sensitive to a variety of experimental conditions (Eanes pers. comm.), and failure of a primer to bind will alter the result of this analysis.

Although it seems likely that the methodological impediments to inference of local interpopulation relations are temporary, there may be a more fundamental problem. If freshwater stickleback populations of a region were derived by means of sequential branching within a clade as lakes were colonized, the time between successive branch points may have been so brief that few base substitutions occurred between branches (i.e. nodes in a cladogram). Even if enough base substitutions occurred between successive branch points to permit a cladistic analysis, subsequent substitutions within the same DNA sequence might obscure synapomorphy substitutions needed for group formation in a cladogram. Alternatively, freshwater populations of a region might have been derived independently from a common marine ancestor. In either case, the resulting cladogram will be totally unresolved, a polytomy in which each population branches from a common point representing the hypothetical ancestor. Thus, even large quantities of molecular data may not produce a regional phylogenetic framework for inference of character polarities. This outcome would necessitate reliance on marine populations alone for inference of character polarities. Until appropriate methodologies are developed, these issues must remain unresolved.

The tempo of evolutionary change

There are serious problems inherent in the quantitative comparison of evolutionary rates for phenotypic traits (Simpson 1944). Differences in phenotypic complexity among taxa complicate estimation of evolutionary rates (Schopf et al. 1975), and imprecise estimates of the time since divergence between populations limit the reliability of rate estimates. Even if time since divergence could be estimated reliably, and a valid common phenotypic scale could be developed (e.g. variance units of properly scaled data), computed rates of evolution are inversely related to the time interval over which they are measured (Gingerich 1983).

When evolutionary rates are calculated using extant radiations, inferences are made based on 'molecular clock' models (Zuckerkandl and Pauling 1965) or on maximum habitat age (e.g. Carson 1970; Fryer and Iles 1972; Grant 1986). Molecular clock calibrations are proving remarkably variable, however, and are therefore of less value in the inference of evolutionary rates than was originally hoped (e.g. Avise and Aquadro 1982; Vawter and Brown 1986; Harrison 1991). Use of maximum habitat age is also problematic, because colonization could have occurred well after the habitat first became
available for colonization and evolutionary rates may have varied over the postcolonization interval. For example, the fossil record suggests that time since deglaciation under-estimates the time necessary for morphological divergence of postglacial threespine stickleback populations, and that evolutionary rates may have slowed significantly in postglacial populations as they approached an adaptive peak (Bell page 470 this volume).

Despite these serious methodological limitations, it appears that phenotypic evolution and speciation occur more rapidly in *G. aculeatus* than is usually assumed (e.g. Simpson 1944; Stanley 1979; McPhail page 433 this volume). Postglacial freshwater populations of threespine stickleback that have been studied range in age from about 22,000 yr in parts of Cook Inlet, Alaska, USA (Reger pers. comm.) down to about 11,000 yr in British Columbia, Canada (McPhail page 401 this volume) and 8000 yr in the Outer Hebrides, Scotland, UK (Campbell and Williamson 1979). Thus, the maximum age of initiation of the postglacial radiation of *G. aculeatus* is much less than those cited in other radiations (Grant 1986). Consequently, it is not clear whether *G. aculeatus* evolves unusually rapidly or whether rapid evolution is a common phenomenon that becomes detectable only with the unusually fine time scale available for postglacial stickleback populations (Gingerich 1983). Differentiation of other boreal fishes, including *Pungitius pungitius* (McPhail 1963), *Myoxocephalus* (Johnson 1964), and a variety of other northern freshwater fishes (Smith and Todd 1984; Mina 1991), suggests that the high rates of phenotypic evolution in *Gasterosteus* may not be as unusual as often assumed (e.g. McPhail page 433 this volume).

**THE EVOLUTION OF CORRELATED CHARACTERS**

Character correlations can result from pleiotropy, linkage disequilibrium, common mechanisms of developmental or physiological regulation, forced association by natural selection, or a common response to environmental conditions (e.g. Olson and Miller 1958; Thorpe 1976; Sokal 1978). Although genetic correlations among traits undoubtedly constrain the evolutionary responses of the traits to selection in the short term, there can be little doubt that genetic correlations can evolve, as do the traits themselves (Riska *et al.* 1989; reviewed in Barton and Turelli 1989).

The threespine stickleback radiation could provide unusually detailed insights into the ways in which selection in natural environments affects genetic correlations. Because many of the populations in recently deglaciated regions are thought to have been derived from a relatively uniform ancestor within a short time span, such populations can be treated as replicate lines drawn from a common ancestral gene pool. Consequently, comparison of laboratory estimates of genetic correlations in threespine stickleback populations could provide tests of a variety of hypotheses concerning the ways in which genetic correlations evolve in natural populations.
and about the causes of character correlations.

Although the value of this approach to the study of correlated characters in threespine stickleback has not been widely recognized, Bakker’s review (Chapter 12 this volume) emphasized this potential. For example, Bakker demonstrated that unexpected correlations among behavioural and life history traits are partly a consequence of common influences of the hormones of the pituitary–gonadal axis. Additionally he pointed out that population comparisons can be used to examine the ways in which selection on life history, for example, can affect reproductive behaviour through common hormonal controls.

**PHENOTYPIC INTEGRATION**

When subsets of a relatively uniform ancestral population are independently subjected to similar novel selection regimes, the outcome may be a set of populations that are phenotypically similar in a diverse array of characters (e.g. Muller 1939). Alternatively, it is possible that the populations will diverge in at least some character states (Cohan 1984; Cohan and Hoffman 1989), possibly in a compensatory manner. For example, it is possible that behavioural and morphological defences will always be positively correlated with one another and with the intensity of predation. Alternatively, populations that evolve effective antipredator behaviour may be more likely to exhibit armour reductions than are those that evolve no such behaviour. Phenotypic integration encompasses both possibilities, in so far as both reflect interactive responses of multiple characters to specific selection regimes.

The postglacial diversification of populations of threespine stickleback provides an excellent system in which to examine patterns of phenotypic integration, as exemplified by the limnetic–benthic contrast (Foster *et al.* 1992; Bell and Foster page 21, McPhail page 418 this volume). In this case, limnetic forms appear to possess a set of common phenotypic traits that differ from those that typify benthic forms (Fig. 1.8). There is no evidence of compensation. However, if more kinds of phenotypes are evaluated simultaneously in more populations, evidence of compensatory relationships among some aspects of the phenotype across populations might emerge.

Comparisons among postglacial freshwater populations can be used to test specific hypotheses about the patterns and causes of phenotypic integration. For example, Thorpe (1976) and Sokal (1978) suggested that characters with strong intrapopulation correlations should also have strong interpopulation correlations. If this proposal is correct, populations with strong intrapopulation correlations but weak interpopulation correlations (or strong correlations with opposite sign) would presumably reflect selection against the correlation. This hypothesis has rarely been tested (Riska 1985). Francis *et al.* (1986) found that in threespine stickleback populations,
four pairs of morphological characters with high intrapopulation correlations also had high interpopulation correlations, as expected. However, three, the correlation of body depth with eye length, head length, and standard length, are strong within \( r \geq 0.85 \) and weaker among populations \( r \leq 0.65 \), indicating that any correlated response of these characters has been broken in the course of postglacial differentiation of these populations.

Clearly, comparison of a diversity of characters across populations of freshwater and marine stickleback in recently deglaciated areas has the potential to provide substantial insights into the patterns and mechanisms of phenotypic integration among populations independently subjected to similar selection regimes. It should be possible to learn a great deal about the evolution of complexes of functionally integrated traits, especially when such research is combined with that on the genetic, developmental, and physiological bases of the differences in phenotype.

**CLOSING THOUGHTS**

Diversification of the threespine stickleback contrasts sharply with other radiations of freshwater fishes (Echelle and Kornfield 1984). Most other endemic radiations of freshwater fishes comprise numerous sympatric species. McPhail (Chapter 14 this volume) reports only three types of sympatry or parapatry in Gasterosteus: (1) benthic–limnetic lacustrine, (2) stream–lake, and (3) anadromous–stream-resident species pairs. However, there is never more than a pair of species, one of which concentrates on plankton and the other on benthic prey. The only known exception to this rule is sympathy of the ‘white stickleback’ and the common marine G. aculeatus in Nova Scotia, Canada (Blouw and Hagen 1990). Otherwise, trophic divergence appears to be the only means for coexistence of biological species of threespine stickleback.

The exceptional youth of threespine stickleback radiations does not appear to explain the absence of multiple sympatric threespine stickleback species. McCune et al. (1984; see also McCune 1987a,b) reported cyclical occurrence of intralacustrine radiations of semionotid fishes. These radiations occurred in Mesozoic rift lakes during pluvial cycles with periods of 22,000 yr. At least 21 semionotid species evolved during one cycle (P4 cycle of McCune et al. 1984) within one early Jurassic lake (McCune 1987b). Thus, the age of postglacial radiation does not appear to account for generation of only pairs of sympatric species of the G. aculeatus complex.

Dominey (1984b) suggested that mating systems that promote sexual selection should facilitate the evolution of reproductive isolation. Although this view is not held universally (reviewed in Greenwood 1991), if it is correct, evolution of isolating mechanisms should be facilitated in threespine stickleback. The elaborate courtship behaviour in which threespine stickleback often engage (Rowland page 313, Foster page 382 this volume) offers
ample opportunity for sexual selection. Other commonly cited factors, such as small body size and the potential to become isolated in small habitats, also apply to *G. aculeatus*. It appears that the most likely hypothesis for coexistence of no more than two biological species of *G. aculeatus* anywhere is their limited potential for divergence in use of trophic resources.

Most evolutionary radiations that have been studied in detail have been termed adaptive radiations because of the seemingly major role of natural selection in causing diversification (e.g. Simpson 1953; Carlequist 1980; Grant 1986; Simon 1987). Although exceptions may exist (Gittenberger 1991), radiations tend to be associated with adaptive shifts into novel environments or with novel patterns of resource use, and they are often attributed to underutilized ecological niche space. These qualities characteristic of most isolated archipelagos because many taxonomic groups are poor at dispersing across ocean expanses. These qualities also characterize freshwater habitats created by glacial recession that are now occupied by threespine stickleback (e.g. Bell 1984a; McPhail and Lindsey 1970, 1986; Bell and Foster page 17). Depauperate faunas combined with novel ecological conditions seem to have favoured rapid evolutionary responses of marine threespine stickleback to differences in selection regimes among habitats, resulting in population differentiation.

In closing, we would like to make a plea for the conservation of threespine stickleback populations. Threespine stickleback are most abundant and diverse in far northern boreal habitats that have been relatively undisturbed by human activities. Thus, the fascinating endemic species and phenotypically divergent populations, such as those reported by McPhail and Reimchen in this volume, remain largely untouched. However, serious losses have occurred in more heavily disturbed regions of western Europe and North America, and this history should serve as a warning of the vulnerability of this fascinating fish to human activity.

The ‘unarmoured threespine stickleback’, *G. a. williamsoni*, was once abundant and widespread in coastal streams around Los Angeles, California, USA, but is now represented only by relict populations in a few small headwater habitats that are threatened by urbanization and introduction of exotic aquatic species (Ono and Williams 1983). Less unusual threespine stickleback have disappeared throughout much of southern California (Swift pers. comm.). Similarly, threespine stickleback have declined and disappeared in European habitats where they once abounded (Lelek 1987), and have all but disappeared from some Dutch habitats that supplied specimens for classical ethological experiments (van Alphen pers. comm.). This decline appears to reflect a general decline in water quality and construction of barriers to entry by anadromous stickleback.

In boreal habitats, where the greatest concentration of phenotypic diversity occurs, regional habitat degradation owing to timber exploitation and point disturbances, especially from fisheries management, represent serious
threats to stickleback. For example, planning for management of natural resources in the Tongass National Forest, Alaska, USA, considers at length the impact of timber exploitation on salmonid fisheries and the value of enhancing salmon stocks, but ignores threespine stickleback entirely (US Department of Agriculture, Forest Service 1979).

Use of ichthyocides to eliminate stickleback and other 'trash fish' to improve game fish production was a common practice throughout western North America. Although this practice was generally unsuccessful, the stickleback that repopulated treated habitats are of uncertain ancestry. However, even without poisoning, sport fish introduction can by itself do serious damage. Kynard (1979b) reported simultaneous increase of an introduced sunfish, _Lepomis gibbosus_, and decline of threespine stickleback. Similarly, trout, _Oncorhynchus mykiss_, introductions to small lakes around Cook Inlet, Alaska, USA, have seemingly resulted in virtual extinction of some threespine stickleback populations with extreme pelvic reduction (Bell, unpubl. data). Because of the high degree of endemism in northern lakes, loss of even a single population may represent a significant decline in diversity. Therefore, some morphologically divergent endemic populations in British Columbia, Canada, have received special attention (Moodie 1984; Reimchen 1984; McAllister _et al._ 1985).

Threespine stickleback are often abundant and seem to represent an inexhaustible resource. Nevertheless, experience has proven that they are easily eliminated by human activities and therefore deserve serious conservation efforts. This volume amply demonstrates their value as a source of information on evolutionary processes. If populations continue to be extinguished at the current rate, we will have lost a remarkable source of information on pattern and mechanism in evolutionary change. We cannot afford this loss.

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