

# Diversification on an ecologically constrained adaptive landscape

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## Abstract

We used phylogenetic analysis of body-size ecomorphs in a crustacean species complex to gain insight into how spatial complexity of ecological processes generates and maintains biological diversity. Studies of geographically widespread species of *Hyaletta* amphipods show that phenotypic evolution is tightly constrained in a manner consistent with adaptive responses to alternative predation regimes. A molecular phylogeny indicates that evolution of *Hyaletta* ecomorphs is characterized by parallel evolution and by phenotypic stasis despite substantial levels of underlying molecular change. The phylogeny suggests that species diversification sometimes occurs by niche shifts, and sometimes occurs without a change in niche. Moreover, diversification in the *Hyaletta* ecomorphs has involved the repeated evolution of similar phenotypic forms that exist in similar ecological settings, a hallmark of adaptive evolution. The evolutionary stasis observed in clades separated by substantial genetic divergence, but existing in similar habitats, is also suggestive of stabilizing natural selection acting to constrain phenotypic evolution within narrow bounds. We interpret the observed decoupling of genetic and phenotypic diversification in terms of adaptive radiation on an ecologically constrained adaptive landscape, and suggest that ecological constraints, perhaps acting together with genetic and functional constraints, may explain the parallel evolution and evolutionary stasis inferred by the phylogeny.

**Keywords:** cryptic species, evolution, evolutionary stasis, *Hyaletta*, phylogeny, predation

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Although Darwin (1859) suggested a central role for ecological processes in promoting biological diversity, our understanding of how the ecological landscape shapes diversity remains incomplete (Schluter 2000; Arnold *et al.* 2001). Much of our understanding of adaptive diversification comes through studies of adaptive radiation which indicate resource exploitation ecology drives ever increasing phenotypic diversity as a diversifying lineage fills new regions of niche space (Grant 1986; Baldwin & Sanderson 1998; Losos *et al.* 1998; Schluter 2000). Recent studies, however, challenge some conventional views of the association between phenotypic and systematic diversification. The growing application of molecular genetic analyses to natural populations has led to the frequent discovery of cryptic species (Taylor *et al.* 1998; De Vargas *et al.* 1999;

Martin & Bermingham 2000; Bond & Sierwald 2002; Hebert *et al.* 2004; Kozak *et al.* 2005). These studies often reveal not only hidden species diversity, but also surprising levels of molecular diversification among the species (Taylor *et al.* 1998; Jarman & Elliott 2000; Colborn *et al.* 2001; Mayer & von Helversen 2001), suggesting that phenotypic similarity of species is due to constraints, rather than limited time for phenotypes to diverge.

Of the several possible mechanisms for muted phenotypic change in a diversifying lineage (Schlichting & Pigliucci 1998), recent studies have favoured explanations based on ecological constraints (Taylor *et al.* 1998; Colborn *et al.* 2001; Hebert *et al.* 2004; Kassen *et al.* 2004; Kozak *et al.* 2005). This mechanism may be conceptualized using Simpson's (1953) adaptive landscape metaphor. Phenotypic and systematic diversification can be decoupled when the adaptive landscape is tightly constrained by ecological processes such that there exists only one or a few adaptive peaks (equivalent to niches in our conceptualization). Spatial structure of the

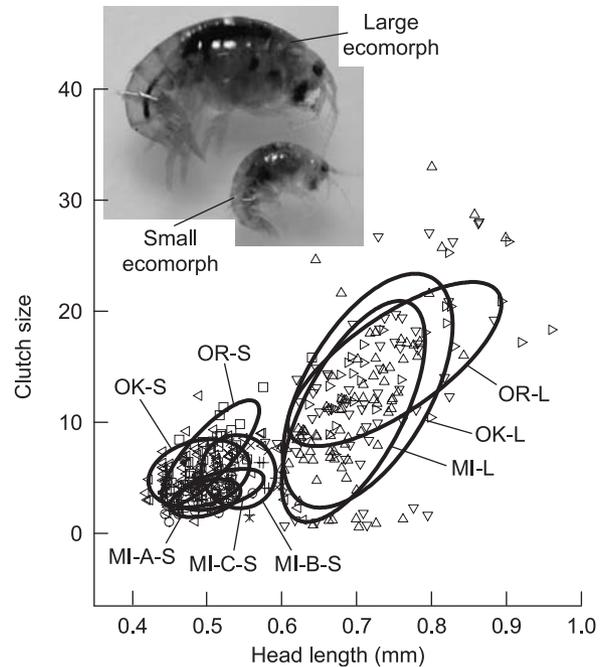
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environment might provide opportunities for speciation, allowing lineage radiation on a landscape with only one or few available niches, but diversification would involve the repeated evolution of the same adaptive phenotypic form(s) (Schluter & Nagel 1995; Losos *et al.* 1998; Gillespie 2004).

*Hyaella* amphipods are common littoral grazers and detritivores found throughout most of the New World (Bousfield 1996). Recent molecular evidence demonstrates that many North American *Hyaella* form a species complex characterized by extensive genetic evolution, but limited phenotypic diversity (Witt & Hebert 2000; Witt *et al.* 2003, 2006; Wellborn & Cothran 2004; Wellborn *et al.* 2005). Indeed, cryptic species diversity within North American *Hyaella* appears to be extensive, as evidenced in a recent study documenting more than 30 cryptic provisional species within the southern Great Basin of the United States (Witt *et al.* 2006). North American *Hyaella* form a monophyletic clade that comprises the subgenus *Hyaella* (*Hyaella*) (Bousfield 1996). Although eight species have been formally described, seven have greatly restricted geographical distributions, usually a single habitat (Gonzalez & Watling 2002). The remaining species, *Hyaella azteca*, was described in 1858 from specimens collected from southern Mexico, and its current distribution is not known (Gonzalez & Watling 2002). None of the species included in this study are described, but all are geographically widespread and common in the regions where they occur (Strong 1972; Wellborn *et al.* 2005).

In each of three geographical regions of the USA – Michigan, Oklahoma, and Oregon – *Hyaella* amphipods are represented by two ecomorphs. A small-bodied *Hyaella* ecomorph occurs exclusively in habitats with intensely size-selective predatory fish in the genus *Lepomis* (principally bluegill, *Lepomis macrochirus*), while habitats with no or less intense fish predation contain a large-bodied ecomorph (Fig. 1; Strong 1972; Wellborn *et al.* 2005). We refer to the two phenotypic groups (large and small body size) as 'ecomorphs' because each type appears to be adaptive in the ecological setting where it is found (Wellborn 1994; Wellborn *et al.* 2005). Body size and life-history differences between ecomorphs in the same region are genetic differences between reproductively isolated species rather than plastic responses to environmental variation (Wellborn 1994, 2002). The taxonomic status (species delimitation) of all lineages included in this study is not entirely understood, but we have a partial understanding based on genetic and interbreeding studies. In Michigan, there are four distinct species, with one species of the large ecomorph and three species of the small ecomorph (Wellborn & Cothran 2004; Wellborn *et al.* 2005). In Oklahoma, the large and small ecomorphs are each represented by one species (Wellborn *et al.* 2005), and the same is true for Oregon (current study). In most cases, we do not yet know whether individuals of the same or different ecomorph in one geographical region



**Fig. 1** Clutch size vs. body size (mm head length) for all regional ecomorphs. Note that both size at which females begin reproduction and adult body size differ substantially between ecomorphs, but are very similar within ecomorphs for the three geographical regions. Head length (an index of body size; Wellborn *et al.* 2005), and clutch size data for regional ecomorphs were compiled from published reports (Strong 1972; Wellborn 1995; Wellborn & Cothran 2004; Wellborn *et al.* 2005). Total head capsule length is reported. Because Strong (1972) measured head length from base of the first antenna to the back of the head capsule, and this method differed from the other studies, we converted his measurements to total head length using a regression equation derived from a sample of individuals measured for both dimensions. Symbols are individual females, and ellipses are bivariate standard deviations for each regional ecomorph. Labels indicate region (OK, Oklahoma; OR, Oregon; MI, Michigan), ecomorph type (S, small; L, large), and, for Michigan small ecomorphs, species identity (A, B, C). Symbol and ellipse colours correspond. Large and small ecomorphs from Oklahoma are shown in inset photograph.

are reproductively isolated from individuals in other geographical regions, but substantial nuclear genetic differences between large ecomorphs in Michigan and Oklahoma suggest that these are not the same species (Wellborn *et al.* 2005).

Across the three geographical regions, the ecomorphs differ distinctly in life history (Strong 1972; Wellborn 1994; Wellborn *et al.* 2005). Disparity in the size at which females initiate reproduction and in growth trajectory cause adults of the two ecomorphs to have nearly nonoverlapping adult size distributions, yet members of the same ecomorph are very similar in maturation size and body size (Fig. 1).

We conducted a molecular phylogenetic analysis to assess the alternative hypotheses that ecomorphs issue from a single evolutionary divergence vs. multiple, replicate divergence events (parallel evolution). Although a single origin is consistent with the dichotomous nature of the phenotypic diversification (Fig. 1), if evolutionary niche shifts have occurred in multiple lineages of *Hyalella*, similarity of ecomorphs across regions could instead reflect parallel evolution (*sensu* Fitch 2000).

## Materials and methods

### Study sites

Amphipods from habitats in Oklahoma, Oregon, and Michigan were collected with dip nets and placed in 95% ethanol or returned to the laboratory alive. Location and ecological conditions of collection sites are available in Strong (1972), Wellborn (1994), Wellborn (2000), Wellborn & Cothran (2004), and are reviewed in Wellborn *et al.* (2005). Briefly, the small ecomorph in Oregon is found in coastal lakes (Siltcoos Lake, Eel Lake) with a well-developed littoral zone, and small ecomorph species in Michigan occur in small kettle lakes (Deep Lake, Chief Noonday Lake, Duck Lake, Sullivan Lake, South Lake, Long Lake, Turner Lake) with extensive littoral vegetation. In Oklahoma, small ecomorph individuals were found in stream pools (Briar Creek, Blue River) containing algal mats and emergent plants. All small ecomorph habitats contain *Lepomis* sunfish (mostly *Lepomis macrochirus* in Oregon and Michigan, and mostly *Lepomis cyanellus* and *Lepomis megalotis* in Oklahoma) that prey on *Hyalella* (Strong 1972; Wellborn 1994; Wellborn *et al.* 2005). Habitats containing the large ecomorph are more ecologically diverse. In Oregon, the large ecomorph is found in Cascade Mountain lakes (Suttle Lake, Lost Lake) that do not contain *Lepomis*, but have trout that consume *Hyalella* only in the early spring, before the seasonal onset of *Hyalella* reproduction, and a time when *Hyalella* exhibit little size variation. In Michigan and Oklahoma, the large ecomorph is found in small fishless habitats (Michigan: Otis Marsh, George Pond; Oklahoma: UOBS creek), and sometimes occurs in sediments at the very shallow (1–2 cm) margin of large reservoirs (Lake Thunderbird), a micro-habitat that provides a refuge from fish predation (G. Wellborn, unpublished data).

### DNA analysis and phylogenetic methods

DNA was prepared by placing all or part of single individuals in microtubes containing a chelating resin (Chelex 100, Sigma Aldrich) and heating to 60 °C for 20 min, then 103 °C for 20 min. Fragments were amplified in 50- $\mu$ L polymerase chain reactions (PCR) using REDTaq (Sigma) and 1.1 mM MgCl<sub>2</sub> (primer information is provided in Tables 1 and 2).

**Table 1** Primers used for amplification of an 1187-bp region of the COI mtDNA gene. The region was amplified in three overlapping sequences. Although segments 1 and 3 overlap slightly, sequence near the primers was sometimes poor. We amplified segment 2 to confirm the sequence in this overlap region. Segment 1 was amplified with LCO1498/HCO2198. Primer sequences are given in Table 1

Regional ecomorph	Segment 2		Segment 3	
	Forward	Reverse	Forward	Reverse
OK-L	L2v1	H2v2	CO1f-L	CO1a-v2
OR-L	L2v2	H2ORMIL	CO1f-L	CO1a
MI-L	L2v2	H2ORMIL	CO1f-L	CO1a-v2
OK-S	L2v1	H2v1	CO1f-AC	CO1a-v2
OR-S	L2v2	H2v1	CO1f-AC	CO1a-v2
MI-A-S	L2MIA	H2MIA	CO1f-AC	CO1a
MI-B-S	L2MIB	H2MIB	CO1f-B	CO1a-v2
MI-C-S	L2v1	H2v1	CO1f-AC	CO1a-v2
<i>Platorchestia platensis</i>	L2Plat	H2Plat	CO1f-B	CO1a-v2
<i>Hyalella texana</i>	*		CO1f	CO1a-v2

\*Amplification of segment 2 was not necessary for *Hyalella texana*.

**Table 2** COI primers used in this study. Except where noted, primers were developed by the authors. Relative position of priming sites is provided

Primer name	Sequence (5'–3')	Position
LCO1490*	GGTCAACAAATCATAAAGATATTGG	1–25
HCO2198*	TAAACTTCAGGGTGACCAAAAATCA	684–709
L2v1	CATTTGGCAGGTGCCTCTTC	429–448
L2v2	CATTTGGCAGGTGCTTCTTC	429–448
L2MIA	AGGGGTAGATCAGTTGACATAGC	393–415
L2MIB	GGAGGTCGTGGATCTAGCTAT	397–418
L2Plat	GTGCTTCAGTAGACCTTGCT	397–416
H2v1	AATATGTGATGAGCCCATACAAT	834–856
H2v2	AATATATGGTGGGCTCACACAATG	833–856
H2ORMIL	TGTCAACGTCATATCCCACTGT	858–879
H2MIA	GTGTGTCTACGTCCATCCCTAC	861–882
H2MIB	TACGCTCGAGTGTCTACATCCAT	867–889
H2Plat	CCAATAAAAACCTTTGATACCGGT	924–947
CO1ft	CCAGCTGGAGGAGGAGAYCC	642–661
CO1f-L	GGCGATCCTATTTTATACCAGC	654–675
CO1f-AC	GGGGACCCTATTTTGTACCAGC	654–675
CO1f-B	GGGGACCCTATTCTTTACCAAC	654–675
CO1at	AGTATAAGCGTCTGGGTAGTC	1302–1322
CO1a-v2†	AGTRTARGCGTCTGGRTAATC	1302–1322

\*Folmer *et al.* 1994; †Palumbi 1996.

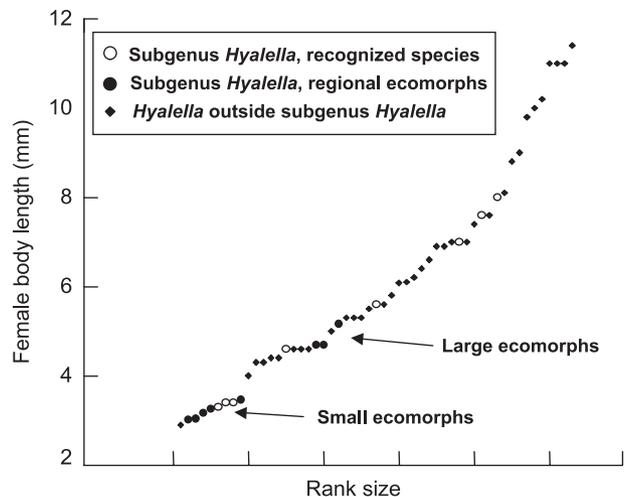
Sequencing employed BigDye version 3.0 and an Applied Biosystems PRISM 310 sequencer. Resulting sequences were manipulated and aligned with SEQUENCHER version 4.14 (Gene Codes, Inc.). Phylogenetic analysis was performed on an 1187-bp segment of the mitochondrial COI gene

(GenBank Accession nos EU621724–EU621762). The complete segment was amplified and sequenced in two to three parts. In most cases, complete aligned sequences were mosaics of two individuals of the same species, and usually from the same habitat; however, all subfragments and all complete fragments yielded the same monophyletic clades in preliminary analyses. The outgroup taxon was *Platorchestia platensis*, a species that, like *Hyalrella*, is included within the superfamily Talitroidea, and *Hyalrella texana*, a congener basal to the ingroup taxa (see Results), was included for purposes of rooting the ingroup.

Phylogenetic analyses were performed using maximum-likelihood and Bayesian methods with PAUP 4.10b (Swofford 2002) and MRBAYES 3.1.2 (Huelsenbeck & Ronquist 2001), respectively. Likelihood models were assessed with MODELTEST 3.7 (Posada & Crandall 1998) for the ingroup taxa. Model parameters for likelihood were either those indicated by MODELTEST or were estimated from the data on a minimum evolution tree with logdet distances (models are discussed in more detail under Results). Likelihood searches employed 10 random addition sequences with tree-bisection–reconnection (TBR) branch swapping. Bootstrapping was performed using ‘previous’ parameter values from the heuristic search for 1000 pseudoreplicates without branch swapping. Bayesian analyses were run for 5 000 000 generations on four concurrent chains starting with uniform priors and the defaults for all other settings.

#### Ancestral character state

The direction of body size and life-history evolution inferred by the phylogeny depends on the plesiomorphic character state of ingroup taxa. Because no relevant fossil evidence is known, we cannot be certain of the ancestral character state for the group. Moreover, the phylogeny of the genus *Hyalrella* is too poorly known to be certain of closest relatives of the ingroup, and thus, we cannot infer the ancestral state of the ingroup via outgroup comparison. To shed light on the probable ancestral character state, we examined body size of the undescribed ingroup species reported here in comparison to all described *Hyalrella* species (Fig. 2) and particularly species in the subgenus *Hyalrella* (*Hyalrella*), which comprises all described North American and Caribbean species and is considered on morphological grounds to be of more recent evolutionary origin than the other subgenera (Bousfield 1996). The small-bodied ecomorphs are smaller than all *Hyalrella* (*Hyalrella*) except *Hyalrella muerta*, *H. sandra*, and *H. meraspinosa*, each of which is known from single isolated habitats in southwestern North America (Baldinger *et al.* 2000; Baldinger 2004). Because of their limited distribution, we consider it unlikely that any of these species is the immediate ancestor of the ingroup taxa. Thus, given the generally large body size of described *Hyalrella* (*Hyalrella*) species, including *Hyalrella azteca*, we suggest

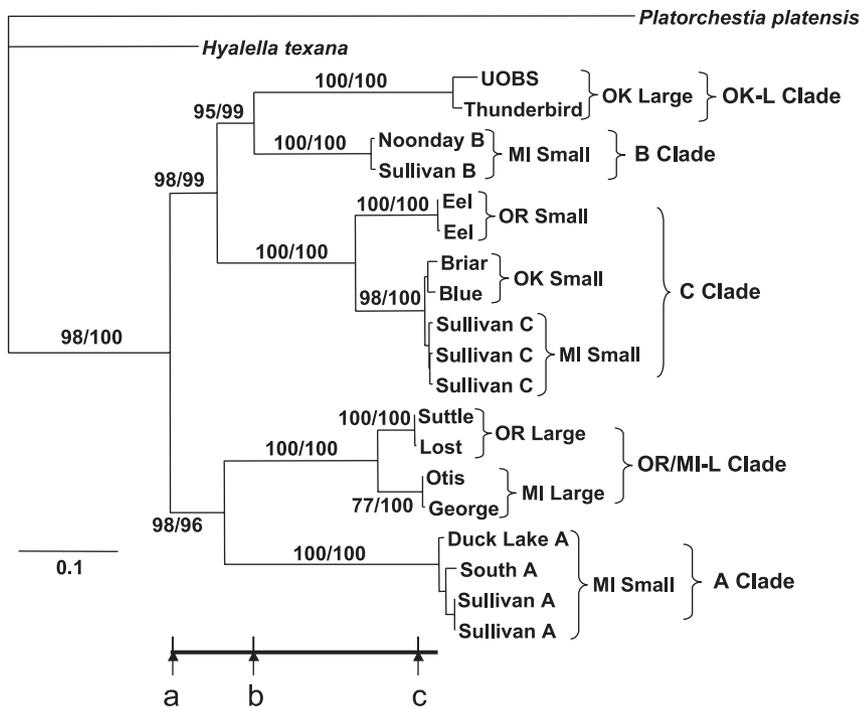


**Fig. 2** Female body lengths for described species of *Hyalrella* were taken from species descriptions (Gonzalez 2001; Baldinger 2004). Male body length is used in a few cases when female length is not available. Female body length for regional ecomorphs was determined from our samples, and average length is reported. Symbols indicate: (closed circle) regional ecomorphs, all of which fall within the subgenus *Hyalrella* (*Hyalrella*); (open circle) described species within *Hyalrella* (*Hyalrella*), all of which occur in North America; and (diamond) *Hyalrella* species outside *Hyalrella* (*Hyalrella*), all of which occur in Central or South America.

that large size is highly likely to be the plesiomorphic character state for our ingroup species. Because it is supported by available data, we assume here that large body size is the ancestral character state.

#### Results

MODELTEST results indicated that the data were best explained with the HKY + I + G model by likelihood-ratio tests and the TVM + I + G model by AIC. The GTR + I + G and K81uf + I + G models also scored high under both criteria. These four models, which differ only in the number and type of rate classes that are free to vary, account for 0.9763 of the cumulative AIC weights (out of 56 models examined). In likelihood searches, the same tree topology (Fig. 3) was optimal under all four models. Maximum-likelihood analyses of all taxa were consistent across all models in placement of the root, as indicated by the outgroup (Fig. 3). Although root position was supported, bootstrap support for the two branches diverging from this root node was not high (60–75%). We suggest that this result is due to the outgroup effectively splitting the characters on the central branch to either side of the root. As a consequence, there are fewer characters on the short deep branches, and the probability of sampling characters supporting either branch is reduced for any bootstrap pseudoreplicate. Because our primary



**Fig. 3** Maximum-likelihood phylogram (based on HKY + I + G model) for *Hyalella* regional ecomorphs and outgroup taxa. Numbers at nodes are maximum-likelihood bootstrap values/Bayesian posterior probabilities. Bootstrap values for ingroup nodes were determined in an analysis that included only ingroup taxa (see text). Tree rooting was determined in an analysis that included all taxa. Samples are labelled by collection locations (and A, B, or C species designations for Michigan small ecomorphs). Regional ecomorphs are indicated, and most inclusive brackets denote the five major clades. Bold line is tree length from inferred initial divergence from common ancestor to most distant regional ecomorph: (a) point of initial divergence, (b) point at which all ecomorph clades had formed, and (c) average point of terminal species (excluding intraspecific haplotype diversity).

goal, assessing monophyletic vs. parallel origin of ecomorphs, depends only on relationships among ingroup taxa, we conducted a second analysis that excluded outgroups. This analysis did not alter the arrangement of ingroup taxa, and yielded high bootstrap support for the tree topology (reported in Fig. 3). The Bayesian analysis, which included outgroup taxa, produced a tree topology identical to that recovered in the maximum-likelihood analysis. In addition, posterior probabilities generated in the Bayesian analysis were high for all nodes (Fig. 3).

Ingroup diversity is organized into five major clades (Fig. 3). Large ecomorphs occur in two clades, with Michigan and Oregon large ecomorphs together in the OR/MI-L clade, and Oklahoma large ecomorphs in the separate OK-L clade. Small ecomorphs occur in three clades. Oregon and Oklahoma small ecomorphs occur in the C clade along with one of the three small ecomorph species in Michigan. The other two small ecomorph species in Michigan occur in the separate A and B clades. Substantial levels of molecular evolution separate the five major clades, with nucleotide sequence divergence among clades of 16–20%, and amino acid sequence divergence of 7–10% (Table 3).

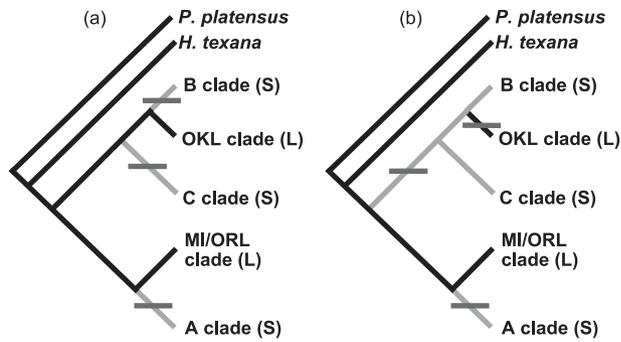
Because large and small ecomorphs do not fall within reciprocal monophyletic clades, the phylogeny supports a polyphyletic origin of ecomorphs. To assess confidence in the polyphyletic origin hypothesis, we used the Shimodaira–Hasegawa test (Shimodaira & Hasegawa 1999) to evaluate the a priori hypothesis that our unconstrained tree differed from the tree constrained to place large or small ecomorphs in monophyletic clades. These trees differed significantly

**Table 3** Mean proportion of DNA and amino acid differences among regional ecospecies (see Fig. 1 for clade identification). Nucleotide divergence (uncorrected 'p') is above diagonal, and amino acid divergence is below

	OKL	MIS-B	ORL	MIL	ORS	OKS	MIS-C	MIS-A
OKL	—	0.17	0.20	0.20	0.18	0.19	0.19	0.20
MIS-B	0.08	—	0.18	0.19	0.18	0.16	0.17	0.19
ORL	0.11	0.08	—	0.07	0.19	0.19	0.19	0.19
MIL	0.10	0.07	0.02	—	0.20	0.19	0.19	0.18
ORS	0.11	0.08	0.08	0.08	—	0.11	0.11	0.20
OKS	0.10	0.08	0.08	0.08	0.05	—	0.02	0.19
MIS-C	0.10	0.08	0.08	0.08	0.04	0.01	—	0.19
MIS-A	0.08	0.07	0.08	0.07	0.09	0.07	0.08	—

(1000 bootstrap pseudoreplicates;  $P = 0.007$ ), and thus, we reject the hypothesis of monophyly of large or small ecomorphs.

Character-state optimization indicated two equally parsimonious hypotheses for body size evolution of ecomorphs. In the first hypothesis (Fig. 4a), the ancestral condition of large body size is retained in the OK-L and MI/OR-L clades, while small body size in the A, B, and C clades evolved independently from large-bodied ancestors. Thus, under this hypothesis, phenotypic similarity among small ecomorphs is the result of parallel evolution, and the close phenotypic similarity of the two large ecomorph clades results from phenotypic stasis, despite substantial underlying molecular evolution. In the second hypothesis



**Fig. 4** Equally parsimonious hypotheses for character evolution in *Hyalella* ecomorphs. Ancestral character state is assumed to be large body size. Major clades and outgroups are shown. Black lines indicate the large body size; lightened lines indicate small body size. Horizontal bars denote evolutionary shift from one ecomorph to the alternative form. (a) Small-bodied ecomorph evolves independently in three clades. (b) Small-bodied ecomorph evolves independently in two clades, with reversal of OKL clade to large-bodied condition.

(Fig. 4b), small-bodied clades B and C are similar by evolutionary stasis, but these are similar to the small-bodied A species by parallel evolution. Additionally, in the second hypothesis the large-bodied OK-L species is similar to the large-bodied MI/OR-L clade by reversal to the plesiomorphic character state. Although we assume the ancestral character state is large body size, we note that if small size is the plesiomorphic character state, then the phylogeny suggests that all small-bodied species are similar by stasis, and species in the two large-bodied clades are similar by parallel evolution.

## Discussion

Phylogenetic analysis yielded strong support for a polyphyletic origin of ecomorphs, suggesting phenotypic similarity of *Hyalella* ecomorphs is due to the combined effects of parallel evolution among some lineages and stasis along other branches. Small ecomorph A and B clades were found only in Michigan lakes, and representatives from the C clade were found in each region. The three major clades containing small ecomorphs were separated by substantial divergence in nucleotide and amino acid sequence, and similarly large differences in nuclear allozyme markers (Wellborn *et al.* 2005). Nonetheless, species A and C in Michigan lakes are virtually identical in phenotype, with broad overlap in body size, life histories, and other traits (Wellborn & Cothran 2004). Species B is slightly larger in maturation size and mean body size (Wellborn & Cothran 2004), but size overlaps with the other small species (Fig. 1). The small species in Oklahoma, although similar to other small ecomorphs in body size, life history (Fig. 1) and

other traits (Wellborn *et al.* 2005), differs morphologically from other small species by the lack of a dorsal spine on the second pleon segment. High levels of evolutionary stasis or parallel evolution are also evident in the two major large ecomorph clades. The clade comprising Michigan and Oregon large ecomorphs is differentiated from the Oklahoma large species by approximately 20% mtDNA sequence divergence, and have also diverged substantially at allozyme loci, with multiple fixed, or nearly fixed, allelic differences (Wellborn *et al.* 2005). Despite the considerable genetic divergence, large ecomorphs in the two clades are virtually identical in life history (Fig. 1) and other traits (Wellborn *et al.* 2005), and are not phenotypically distinguishable in any readily apparent way.

Although our study relied solely on mitochondrial sequence data, studies of *Hyalella* that also assess nuclear genetic data suggest general congruence of mitochondrial and nuclear markers. A recent study by Witt *et al.* (2006) tested for incongruence of COI and 28S (nuclear) phylogenetic relationships among 33 provisional *Hyalella* species. Trees derived from each gene fragment were congruent at all well-resolved nodes, and there was no statistical evidence for incongruence (Witt *et al.* 2006). In addition, allozyme markers are congruent with COI sequence with respect to species delineation for several *Hyalella* species (Witt & Hebert 2000; Wellborn *et al.* 2005).

## Nature of constraints

The inferred independent origin of the small ecomorph phenotype (and possibly the large ecomorph phenotype), together with the extensive molecular divergence among major clades, makes the phenotypic similarity within ecomorphs especially noteworthy, and suggests tightly constrained evolution of these geographically widespread *Hyalella* species. In this section, we examine possible constraints acting in the group, and review evidence for each. Although we discuss them separately, it is probably naive to presume that any single source of constraint limits phenotypic diversity in a radiation (Wake 1991; Schlichting & Pigliucci 1998). Rather, observed phenotypic diversity may often involve the interaction of various forms of constraint. Genetic architecture may restrict the variety of potential phenotypic forms, for example, while ecological constraints may permit only a limited subset of these to succeed.

*Genetic variability and architecture.* The scope of phenotypic evolution may be constrained by the form of genetic and epigenetic variation expressed across ontogeny (Schlichting & Pigliucci 1998). In North American *Hyalella*, some evidence suggests that phenotypic diversification in the clade is not tightly limited by genetic architecture. Species endemic to ecologically unique habitats demonstrate evolutionary

potential within the group, and stand in contrast to the phenotypic conservatism of the geographically widespread species (Witt *et al.* 2003). For example, *Hyaella montezuma*, a species endemic to Montezuma Well, Arizona, is adapted morphologically and behaviourally to a pelagic filter-feeding lifestyle (Cole & Watkins 1977; Blinn & Johnson 1982), but is genetically similar to our OR/MI-L clade (based on our analysis of partial COI sequences of Witt *et al.* 2003). Similarly, *Hyaella muerta*, a hypogean species that is known only from one spring complex in Death Valley is eyeless, and has an unusually short second antenna (Baldinger *et al.* 2000). Also, an undescribed species known from a single spring in Oklahoma is phenotypically distinct from the large and small ecomorph species common throughout the region, having, for example, a small number of unusually large eggs relative to its body size (Wellborn *et al.* 2005). Such cases suggest that tight constraints on genetic variability and architecture are unlikely to fully explain the very close similarity of species within the two ecomorphs.

**Functional constraints.** Functional constraints (Schlichting & Pigliucci 1998) could limit extremes of body size among the species, such that under diversifying selection, the ecomorphs have simply accumulated at large and small extremes of feasible phenotypes (McShea 1994). We explored this hypothesis by examining the body size distribution of species in the genus (Fig. 2). Small ecomorph species do occur at the extreme of the *Hyaella* size distribution, suggesting that functional constraints may explain their similarity in size. For example, selection due to strong size-selective predation may push them down to an extreme small size, but other constraints, such as limited volume of the brood chamber, might prevent further reduction in body size. Large ecomorph species, in contrast, are not remarkably large, and thus provide no evidence that strict functional constraints influence their body size. Evaluating the importance of both functional and genetic constraints in the *Hyaella* radiation will require empirical studies, such as selection experiments, which can elucidate the form and causes of constraint (Fuller *et al.* 2005).

**Ecological constraints.** Ecological studies of *Hyaella* ecomorphs suggest that ecological constraints are likely to play a role in the limited phenotypic diversification of the geographically widespread species (reviewed in Wellborn *et al.* 2005). In all cases we are aware of, small ecomorph species co-exist with *Lepomis* sunfish, and these fish impose strong size-biased predation on *Hyaella* such that larger (adult) individuals are roughly fivefold more likely to be consumed than smaller (juvenile) individuals (Wellborn 1994). The early maturation and small adult body size of the small ecomorph species are therefore consistent with predictions of life-history theory for populations experiencing

this form of size-biased mortality (Taylor & Gabriel 1992). Large ecomorph species exist in more ecologically varied habitats (Wellborn *et al.* 2005), but ecological processes, including predation (Wellborn 1994) and competition (Wellborn 2002) foster the evolution of comparatively large body size. For example, larval dragonflies are the primary predators of large-ecomorph *Hyaella* in Michigan, and these predators preferentially prey on smaller individuals within the large-ecomorph population, a pattern that is also reflected in overall schedules of mortality in the habitat (Wellborn 1994). The larger maturation size and adult body size of the large ecomorph is also in accord with predictions of life-history theory (Taylor & Gabriel 1992), suggesting that predation imposes an ecological constraint.

Thus, for geographically widespread *Hyaella* species in North America, the adaptive landscape may offer only two major adaptive peaks, with peaks determined by natural selection arising from environmental factors (i.e. ecological constraints). If so, the dichotomous nature of phenotypic variation implies that the selective environment is itself dichotomous, at least when viewed at the scale of ecomorph divergence (see Wellborn & Cothran (2007) for analysis of fine-scale niche disparity within communities of co-existing small-ecomorph species). Although seemingly enigmatic, the bimodal adaptive landscape occupied by widespread *Hyaella* species appears to be commonplace in permanent freshwater habitats of North America (Wellborn *et al.* 1996; Taylor *et al.* 1998; Stoks *et al.* 2003), and is mediated by the presence vs. absence of some species of predatory fish, a condition associated with wholesale shifts in the community composition of these habitats (Wellborn *et al.* 1996). We suspect that this process forms a basis for the dichotomous phenotypic variation in *Hyaella* ecomorphs, but we currently have insufficient understanding of genetic and functional constraints to assess the extent to which these interact with ecological constraints to shape ecomorph evolution.

#### *Evolutionary patterns*

Our study suggests a biogeographical hypothesis for diversification of regional ecomorphs. *Hyaella* amphipods originated in South America, where they are represented by two relatively primitive subgenera that exhibit much species and morphological diversity (Bousfield 1996). All described North American *Hyaella* are thought to comprise a single subgenus with derived characters, implying a comparatively recent invasion of North America (Bousfield 1996). We suggest that parallel diversification of small ecomorph clades is consistent with evolution by niche shifts, as ancestral *Hyaella* species spread throughout North America and invaded lakes and streams containing *Lepomis* sunfish, which were evidently present well before the invasion of *Hyaella* (Cross *et al.* 1986; Witt & Hebert

2000; Witt 2004). Furthermore, evaluation of branch lengths and character evolution in the phylogenetic analysis suggests ecomorph diversification occurred in an early, temporally concentrated episode, followed by a comparatively long period of stasis within clades. Ecomorph diversification coincides with the formation of the five major clades, and these were established within a temporal window representing only 24% of total change along branches from the inferred common ancestor (Fig. 3). Although these results are consistent with a burst of phenotypic diversification early in the radiation, this interpretation must be made with caution because the limited number of taxa in the clade provide too little statistical power to assess this issue quantitatively.

The early diversification that generated the ecomorphs, together with evidence for invasion of North America by a monophyletic lineage, suggests a relatively rapid adaptive diversification of *Hyaella* during the initial colonization of North America. Adaptive radiation is often associated with colonization of new geographical regions, and is often defined by a period of rapid diversification as new ecological opportunities are exploited through niche shifts (Schluter 2000; Harmon *et al.* 2003). Although application of a commonly employed molecular-clock estimate for crustaceans (Knowlton & Weigt 1998) would place diversification of ecomorphs in the Miocene, this estimate is probably too early for North American *Hyaella* (Witt & Hebert 2000; Witt 2004). Evidence for elevated rates of molecular evolution in North American *Hyaella* (Witt 2004) suggests a Pliocene origin of the ecomorphs may be a more appropriate, albeit uncertain, estimate. Today, much of the distribution of *Hyaella* ecomorphs may be due to dispersal, explaining both the co-existence of multiple small ecomorph species in postglacial northern lakes (Witt & Hebert 2000; Wellborn & Cothran 2004), and the presence of small ecomorph *Hyaella* co-existing with *Lepomis* sunfish in Oregon lakes where these fish are not native but have been broadly introduced (Berra 2001).

Evolutionary patterns in the phylogeny are consistent with long periods of phenotypic stasis following an initially rapid diversification. Evolutionary stasis involves the decoupling of genotypic and phenotypic evolution. Some traditional explanations for stasis have focused on genetic or developmental constraints (Williamson 1987), but recent studies point to a growing view that stabilizing natural selection may be the primary cause of evolutionary stasis (Morris *et al.* 1995; Colborn *et al.* 2001; Mayer & von Helversen 2001; Kozak *et al.* 2005). For example, Morris *et al.* (1995) attributed community-wide stasis in fossil communities to 'ecological locking', in which long-term stability of species interactions maintains long-term phenotypic stability of component species even in the face of abiotic environmental change. Our study supports this emerging view. The ecological locking hypothesis suggests that species interactions,

and the stabilizing selection they impose, be constant over the period over phenotypic stasis observed (Morris *et al.* 1995; Kozak *et al.* 2005). For North American *Hyaella*, the specific habitats occupied by populations have changed over the period of observed stasis. Current Michigan populations and Cascade populations in Oregon, for example, must have colonized about 12 000 ago following retreat of the Wisconsin ice sheet (Pielou 1991). Although freshwater habitats may be transient over millennial scales (Wetzel 2001), strong interspecific interactions can cause species sorting during colonization of new habitats, thereby maintaining species associations over time (Wellborn *et al.* 1996). A contemporary parallel of this species sorting process may be the common occurrence of small ecomorph *Hyaella* in human-made farm ponds that have been stocked with *Lepomis* fish (Wellborn *et al.* 2005; G. Wellborn, personal observation).

### Cryptic diversification

The evolution of species that share close phenotypic similarity, commonly referred to as cryptic species, is much more common than previously anticipated (Sáez & Lozano 2005). Attempts to understand the origin and maintenance of cryptic biodiversity raise some fundamental questions about how genetic and ecological processes influence evolutionary dynamics. Here, we show that close phenotypic similarity of geographically widespread species of *Hyaella* amphipods may be shaped by ecological constraints that force adaptive evolution into either of two life-history phenotypes. Similar adaptation-based explanations have been proposed for other groups of cryptic species (Taylor *et al.* 1998; Colborn *et al.* 2001; Kozak *et al.* 2005), suggesting that adaptive radiations on ecologically constrained adaptive landscapes may be a common, but underappreciated, feature of biological diversity.

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