

Biogeographic and phylogenetic effects on feeding resistance of generalist herbivores toward plant chemical defenses

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Abstract. Many terrestrial and most marine herbivores have generalist diets, yet the role that evolutionary history plays in their foraging behaviors is poorly documented. On tropical hard-bottom reefs, generalist fishes and sea urchins readily consume seaweeds that produce lipophilic secondary metabolites. In contrast, herbivores on temperate reefs less commonly encounter seaweeds with analogous metabolites. This biogeographic pattern suggests that tropical herbivores should evolve greater feeding resistance to lipophilic defenses relative to temperate herbivores, but tests of this biogeographic pattern are rare. We offered lipophilic extracts from nine subtropical seaweeds at two concentrations to sea urchins (four subtropical and three cold-temperate populations) and quantified urchin feeding resistance. Patterns of feeding resistance toward lipophilic defenses were more similar within genera than across genera of urchins, indicating a substantial role for phylogenetic history in the feeding ecology of these generalist herbivores. The biogeographic origin of urchins also influenced feeding resistance, as subtropical species displayed greater feeding resistance than did temperate species. Similarly, a subtropical population of *Arbacia punctulata* had greater feeding resistance for *Dictyota* and *Styopodium* extracts relative to temperate *A. punctulata*. We conclude that evolutionary history plays a more central role in the foraging ecology of generalist herbivores than is currently appreciated.

Key words: arms race; generalist herbivore; herbivore feeding preference; latitudinal patterns of biotic interaction; lipophilic compounds; local adaptation; sea urchins; secondary metabolites; tropical vs. temperate herbivory.

INTRODUCTION

Interactions between plants and herbivores play central roles in regulating and structuring ecosystems, determining spatial patterns of biodiversity, and cycling of nutrients and materials through ecosystems (Thompson 2005, Stachowicz et al. 2007, Stephens et al. 2007). Predicting these effects depends in part on our ability to understand plant defenses and herbivore offenses. In the sea, marine plants defend themselves via an arsenal of physical and chemical defenses, while herbivores utilize multiple strategies (termed “herbivore offenses”; cf. Karban and Agrawal 2002) that allow them to tolerate or avoid poorer quality foods (Hay and Fenical 1988, Paul et al. 2001, Clements et al. 2009, Sotka et al. 2009). Despite decades of research on plant–herbivore interactions, the offensive traits of herbivores remain bewilderingly variable. In part, some of the variation reflects local ecological conditions that alter herbivore foraging

patterns, but a large portion of unexplained variation reflects our almost complete ignorance of genetically based traits that vary across individuals, populations, and species (e.g., digestive physiology, detoxification ability, taste receptors) and how these traits evolved (Sotka et al. 2009; but see Dearing et al. [2005] for terrestrial herbivores).

The influence of evolutionary history on herbivore foraging ecology is detected in at least two ways. Traits that are nonrandomly distributed across a phylogeny of related herbivores can indicate phylogenetic “baggage” (cf. Price 2003), as when herbivores are constrained to consume particular plants because their ancestors did (Vermeij 1992, Winkler and Mitter 2008, Futuyama and Agrawal 2009). Nearly all such examples come from specialist insects, despite the fact that many terrestrial insects (Novotny et al. 2002) and marine herbivores (Poore et al. 2008) are generalists. Evolutionary influence can also be inferred from biogeographic patterns. For example, a biogeographic comparison of the Australian vs. North American Pacific coastlines indicate that Australian brown seaweeds have stronger chemical defenses and face stronger herbivore offenses relative to North American seaweeds (Steinberg et al. 1995). These biogeographic patterns likely reflect a diffuse coevolutionary arms race in Australasia, in

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which reciprocal evolutionary change occurs among guilds of multiple species (Vermeij 1994, Strauss et al. 2005, Thompson 2005). To our knowledge, this remains the only well-documented example of marine plant–herbivore coevolution in the sea.

Tropical regions represent another biogeographic region in which marine plant–herbivore interactions may be coevolving. Bryopsidalean green algae and dictyotalean brown algae commonly occur on tropical reefs and produce high levels of lipophilic chemical defenses, while laminarian and fucallean brown algae are more common on extratropical reefs and mainly produce water-soluble (i.e., phlorotannin-based) metabolites (Hay and Fenical 1988, Steinberg et al. 1995, Bolser and Hay 1996, Paul et al. 2001, Van Alstyne et al. 2001, Amsler et al. 2009). Although there are exceptions to these generalities (e.g., instances of *Sargassum* on tropical reefs and *Dictyota* on temperate reefs), tropical seaweeds appear to be better defended by lipophilic compounds than are temperate seaweeds (Bolser and Hay 1996). As a consequence, diffuse coevolutionary theory predicts that tropical herbivores should respond by increasing their feeding resistance toward lipophilic secondary metabolites. However, this biogeographic prediction is rarely tested. Previous studies are based on relatively few herbivores, few compounds or both (Targett et al. 1995, Cronin et al. 1997). Similarly, there remain relatively few direct experimental tests of latitudinal gradients in chemical defense and herbivore offense within terrestrial and freshwater systems (Schemske et al. 2009, Morrison and Hay 2012).

Here, we tested for the presence of phylogenetic and biogeographic effects on the feeding behavior of sea urchins (Echinodermata; Echinoidea) with generalist diets (Cobb and Lawrence 2005). Inspired by the latitudinal decline in seaweed chemical defenses, we collected nine subtropical populations of seaweeds, and offered their lipophilic metabolites at two concentrations to four subtropical (*Arbacia punctulata* [see Plate 1], *Diadema antillarum*, *Echinometra lucunter*, and *E. viridis*) and three temperate urchin populations (*Arbacia punctulata*, *Strongylocentrotus droebachiensis*, and *S. purpuratus*) across 126 independent feeding assays. We used multiple analytical approaches to detect effects of urchin phylogenetic lineage and biogeographic region on urchin feeding resistance, or the willingness of urchins to consume lipophilic extracts.

MATERIALS AND METHODS

Collection of organisms

We collected seaweeds whose genera are common to Caribbean habitats, rare to absent in cold-temperate regions (Abbott and Hollenberg 1976, Villalard-Bohn-sack 2003), and known to produce lipophilic compounds (primarily terpenes) that can deter feeding by herbivores: Chlorophyta *Caulerpa sertularioides*, *Halimeda tuna*, *H. discoidea*, and *Penicillus dumetosus*; Ochrophyta *Dictyota ciliolata*, *D. pulchella*, *Spatoglossum schroederi*,

and *Styopodium zonale*; Rhodophyta *Palisada* (formerly *Laurencia*) *poiteaui*. All seaweeds are reported as “abundant” within the northeastern Caribbean (e.g., Lirman and Biber 2000, Littler and Littler 2000, Lapointe et al. 2005). *Dictyota* and *Halimeda* species have become particularly abundant in recent decades, covering 20–40% of Caribbean forereefs (e.g., Williams and Polunin 2001, Paddock et al. 2006, Burkepile and Hay 2011). In the summer of 2008, approximately 6 L (by volume) of each seaweed were collected via snorkeling or scuba diving and promptly returned to the Smithsonian Marine Station in Fort Pierce, Florida, USA (Appendix: Table A1). Subtropical sea urchins were collected by hand or by licensed collectors in Fort Pierce and the Florida Keys and brought immediately to the Smithsonian Marine Station. Temperate urchins were collected by licensed collectors in Woods Hole, Massachusetts, USA (*S. droebachiensis*, *A. punctulata*) and Santa Barbara, California, USA (*S. purpuratus*) and shipped to Grice Marine Laboratory (Charleston, South Carolina, USA). Urchins were held at temperatures and salinities typical for their collection sites and fed ad libitum palatable seaweeds that do not produce lipophilic feeding deterrents (*Enteromorpha*, *Gracilaria*, and *Hypnea* spp.). *Gracilaria tikvahiae* was lyophilized and ground to a fine powder using a Wiley mill and used as experimental food in all assays.

Seaweed extractions

Immediately upon collection, seaweeds were cleaned of epibionts, flash-frozen on dry ice within 12 h, and held in storage at -20°C . We extracted metabolites from 100 g of lyophilized seaweed tissue using a 1:1 ethyl acetate:methanol solvent mixture. This crude extraction targets lipophilic metabolites, but can yield moderately polar metabolites. Natural concentration ($1\times$) was calculated as dry extract mass per dry tissue mass.

Feeding assays

All urchin populations were offered each seaweed extract twice in two independent feeding assays. The first assay was a pairwise feeding choice between a control food and food coated with lipophilic extract at natural concentration ($1\times$; following Bolser and Hay 1996). If the $1\times$ concentration significantly deterred the urchin population (as determined by a two-tailed paired *t* test ($\alpha = 0.05$; $n = 15\text{--}36$), then a second assay was conducted at half concentration ($0.5\times$). If the $1\times$ concentration did not deter the urchin population, the extract was assayed at twice the natural concentration ($2\times$). We used these results to calculate an Herbivore Resistance Scale (HRS), which ranges from weakly resistant to seaweed chemical defenses (scored at 1; herbivore deterred at extract concentrations of $0.5\times$ and $1\times$) to strongly resistant (scored at 4; not deterred at $1\times$ or $2\times$; see scale key, Fig. 1). We pursued this novel strategy because it is clear from the terrestrial plant–herbivore literature that herbivore responses to plant secondary metabolites are

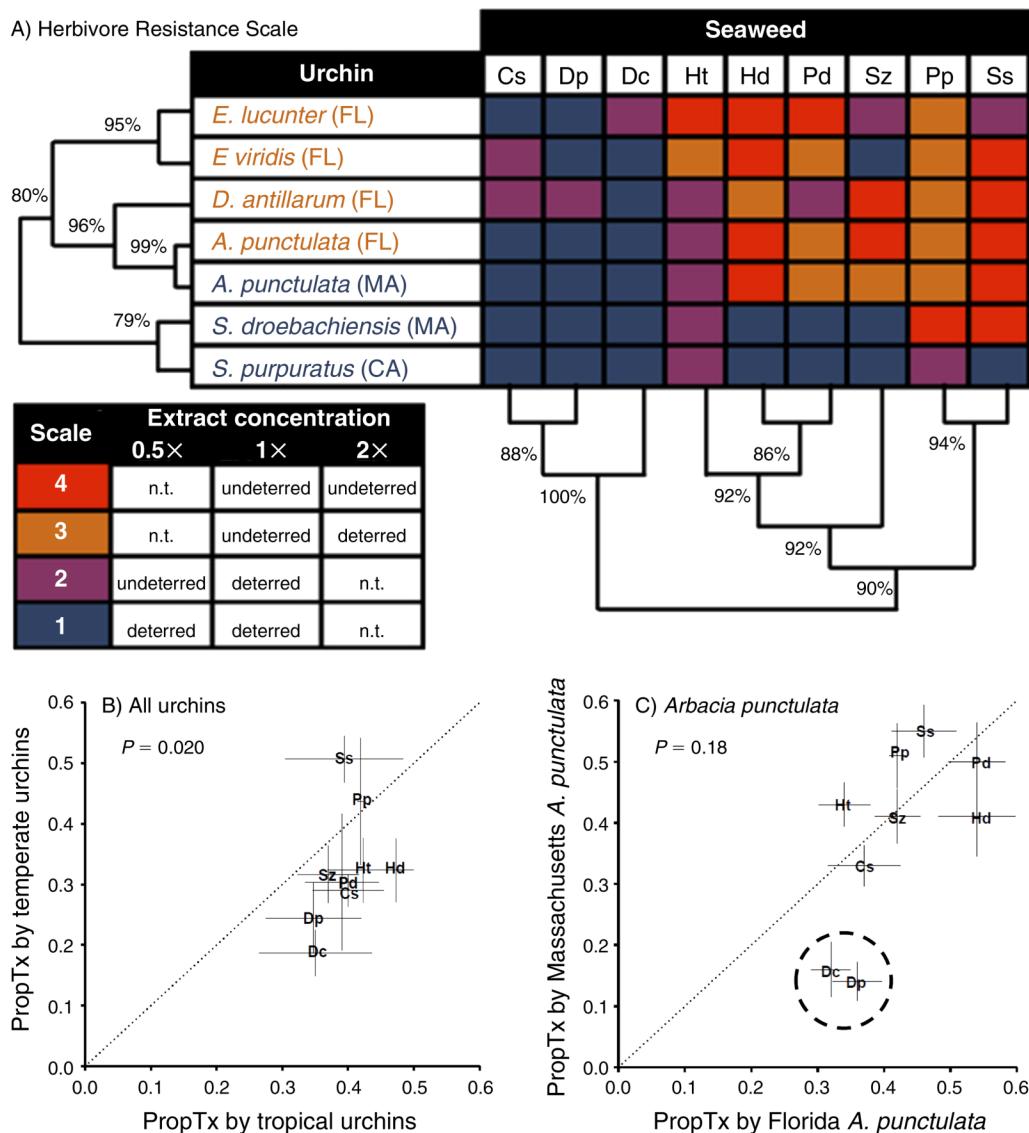


FIG. 1. (A) The Herbivore Resistance Scale generated across nine seaweed and seven urchin populations. The names of urchins from temperate regions (*Arbacia punctulata*, *Strongylocentrotus droebachiensis*, and *S. purpuratus*; collected in Massachusetts or California, USA [MA or CA, respectively]) are in blue, and tropical urchins (*A. punctulata*, *Diadema antillarum*, *Echinometra lucunter*, and *E. viridis*; collected in Florida, USA, [FL]) are in orange. Bootstrap probabilities (%) are indicated on dendrogram branches. Scale key abbreviations are as follows: "n.t." indicates a seaweed extract concentration that was not tested, and "deterred" and "undeterred" indicate seaweed extract concentrations that significantly deterred (or did not deter, respectively) feeding relative to control foods. Seaweed abbreviations are: Cs, *Caulerpa sertularioides*; Dp, *Dictyota pulchella*; Dc, *Dictyota ciliolata*; Ht, *Halimeda tuna*; Hd, *Halimeda discoidea*; Pd, *Penicillus dumetosus*; Sz, *Stypopodium zonale*; Pp, *Palisada poiteaui*; and Ss, *Spatoglossum schroederi*. The proportion of food consumed that was extract laden is compared between (B) all urchin populations and (C) only *Arbacia punctulata* populations. The relative consumption of treatment foods (or PropTx) is plotted for each seaweed species (mean \pm SE). The dotted line indicates concordance; P values from one-sided paired t tests are given. The dashed circle indicates the extracts from *Dictyota* spp. that tropical *A. punctulata* consumed significantly more than temperate *A. punctulata*.

dose dependent (Sotka et al. 2009), and using multiple concentrations is generally more effective in measuring herbivore responses than is a single concentration.

To generate the treatment (Tx) food at natural concentrations, the lipophilic extract from 2 g of dried tissue was solubilized in 2 mL ethyl acetate and mixed

with 2 g of dried, powdered *Gracilaria tikvahiae*. Ethyl acetate was removed via rotary evaporation. The seaweed powder was added to 7 mL diH₂O and a molten agar mixture (0.5 g agar and 18 mL deionized water at 68°C) and poured into a mold on window screen. The control (Ctrl) food was treated similarly, but

without adding extract. Foods were poured side-by-side (separated by 2 cm) into two lanes and cut into strips such that foods were $4 \text{ cm}^2 \times 1.5 \text{ mm}$.

Between 50 and 60 urchins were isolated within perforated containers in a water table with flowing seawater and allowed to feed for up to 24 h. We quantified consumption (Tx or Ctrl) as the number of window screen squares that were no longer coated by food. Assays were checked every half hour and replicates were terminated before 90% of the total amount of food offered was consumed. Assays in which individuals consumed <10% or >90% of food offered were excluded from final analyses (Bolser and Hay 1996).

Statistical analyses

We analyzed these data using three approaches. The first was a clustering algorithm of the Herbivore Resistance Scale. Dendrograms were constructed using Euclidean distance and Ward's clustering method (Suzuki and Shimodaira 2006), and approximately unbiased bootstrap probabilities (%) from 10 000 iterations were used to confirm clusters.

The second approach was a generalized linear mixed-effects model (GLMM) on the Herbivore Resistance Scale. We pursued GLMM because urchin and seaweed species are random effects (i.e., we did not sample all species of urchin or subtropical seaweeds) and the imbalanced design (four subtropical and three temperate urchins) means that a true F value cannot be calculated. The independent variable "urchin region" was a fixed effect, while the remaining independent variables were considered random (e.g., seaweed identity, urchin identity, the interaction of urchin identity with regional source, and so on). A stepwise model selection approach used likelihood ratio tests (LRTs), the Akaike information criterion (AIC), and the Bayesian information criterion (BIC). Parameter estimates for the best model were calculated with restricted maximum likelihood (REML) and evaluated using both the t value and the posterior distribution generated by Markov Chain Monte Carlo (MCMC) sampling. All analyses utilized the lmer function of the R package lme4 (R Development Core Team 2010).

The third approach focused on a summary statistic, PropTx, or the proportion of treatment (i.e., extract-coated) food divided by the total consumption within a replicate ($\text{PropTx} = \text{Tx} \times (\text{Tx} + \text{Ctrl})^{-1}$). We calculated a PropTx in all assays in which seaweed extracts were at $1\times$ concentration, with the exception of assays using three seaweeds (*Caulerpa sertularioides*, *Dictyota ciliolata*, and *D. pulchella*) that we analyzed at $0.5\times$. The extracts of these three seaweeds were deterrent to all urchins at $1\times$ (mean PropTx ranged from 0.07–0.36; Appendix: Table A2) and there was greater variance to detect patterns in feeding resistance at $0.5\times$ (mean PropTx ranged from 0.12 to 0.56). A one-sided paired t test on PropTx (averaged within each urchin–seaweed combination; $n = 9$) tested the a priori hypothesis that

subtropical urchins are more resistant to extracts than are temperate urchins.

RESULTS

The Herbivore Resistance Scale (or HRS) is a proxy for the feeding resistance of an herbivore toward a seaweed extract. From the perspective of the seaweeds, HRS clustered into two groups (Fig. 1A). One group of seaweed extracts strongly deterred all urchin species at natural concentrations (i.e., HRS = 1 or 2; *Caulerpa sertularioides*, *Dictyota ciliolata*, and *D. pulchella*), while the remaining extracts were deterrent to some but not all urchins at natural concentrations (Appendix: Table A2).

We also detected a strong effect of phylogenetic history on the feeding resistance of these generalist herbivores, as HRS clustered congeneric pairs (*Echinometra* and *Strongylocentrotus*) and populations of *Arbacia punctulata* (Fig. 1A). The consistency of feeding resistance within urchin genus is remarkable given that urchin populations were collected from disparate locations. As examples, *Arbacia* populations were collected 1800 km apart, while *Strongylocentrotus purpuratus* and *S. droebachiensis* were collected from the northeast Pacific and northwest Atlantic Oceans, respectively.

In general, subtropical urchins more readily consumed extract-laden foods relative to temperate urchins. A GLMM analysis of the HRS data revealed a significant effect of region (Model 4; t distribution $P = 0.002$, MCMC $P = 0.004$; Appendix: Table A3). The GLMM analysis also detected a significant effect of seaweed identity, but models that included urchin identity or a region–seaweed interaction, or nest urchin identity within region, did not statistically improve the fit. When PropTx data were averaged across all subtropical vs. temperate urchins, subtropical populations tended to consume more extract-coated foods than did temperate populations ($n = 9$; paired t test $P = 0.020$; Fig. 1B). The latitudinal decline was apparent for seven of nine seaweeds, and the two exceptions (*Spatoglossum schroederi* and *Palisada poiteaui*) were among the most readily consumed (and by inference, the least deterrent) of the nine seaweeds tested (Fig. 1A, B; Appendix: Table A2).

We also tested whether the latitudinal decline was reflected in subtropical vs. temperate populations of a single urchin species (Fig. 1C). While populations of *Arbacia punctulata* showed similar feeding responses across most seaweeds, subtropical populations consumed significantly more extract from three seaweeds. Subtropical *A. punctulata* consumed significantly more *Styopodium zonale* extract (at $2\times$ concentration) than did temperate *A. punctulata* (one-way ANOVA $F_{1,40} = 4.45$, $P = 0.041$). Similarly, subtropical *A. punctulata* consumed significantly more extract from *Dictyota ciliolata* and *D. pulchella* at natural concentrations than did temperate *A. punctulata* ($F_{1,53} = 6.58$ and $F_{1,44} = 18.3$; $P = 0.013$ and $P < 0.001$, respectively).

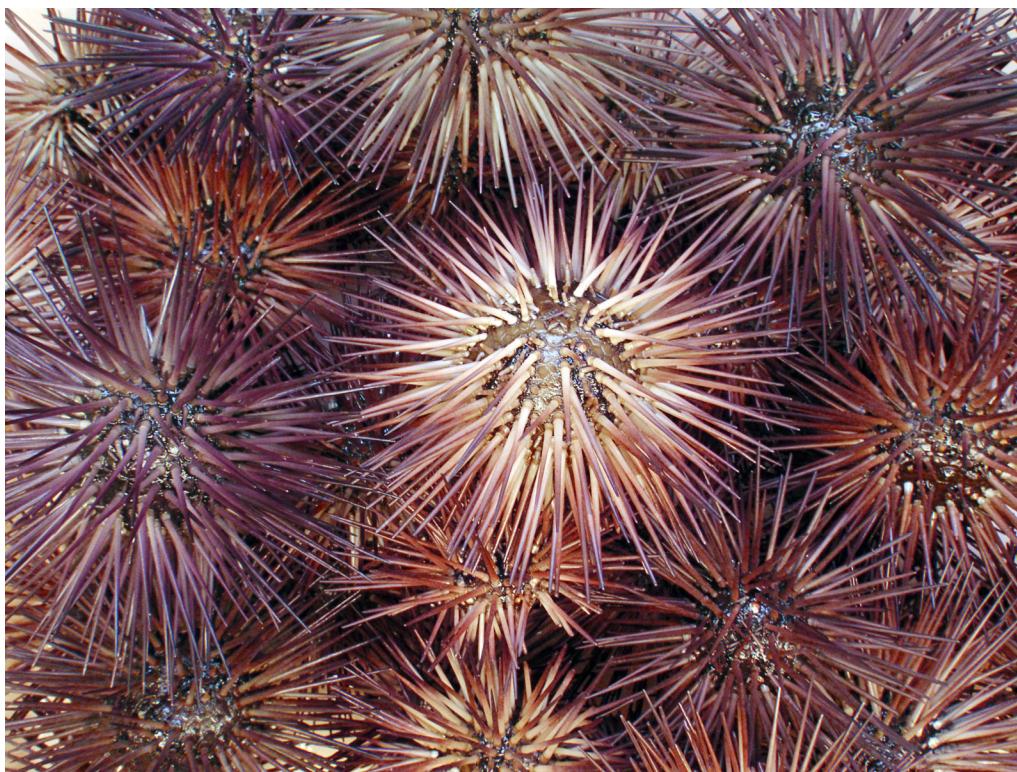


PLATE 1. The purple sea urchin *Arbacia punctulata*. Photo credit: J. D. Craft.

DISCUSSION

The feeding behaviors of herbivorous sea urchins with generalized diets are influenced by their phylogenetic lineage and biogeographic origin. The literature on phylogenetic effects largely neglect generalist herbivores (Winkler and Mitter 2008, Futuyma and Agrawal 2009) despite the fact that many terrestrial insects (Novotny et al. 2002) and most marine herbivores (Poore et al. 2008) are generalists. It is now clear that the feeding preferences of generalist sea urchins (as we found) and generalist amphipods (Poore et al. 2008) are strongly influenced by phylogenetic lineage, and we suggest such macroevolutionary effects may be common within other generalist herbivores, either terrestrial or marine.

Biogeographic origin also predicted urchin feeding resistance. In particular, subtropical urchin populations tend to consume more lipophilic extract than do temperate urchins. In no instance does a temperate urchin consume significantly more lipophilic extract than does a subtropical urchin (Appendix: Table A2). The biogeographic signal in feeding resistance is driven mostly, but not wholly, by differences in feeding resistance among urchin genera. While this between-genus pattern partially confounds biogeographic patterns, our experimental design is constrained by the fact that Atlantic genera of urchins are either temperate or tropical/subtropical in species distribution, and rarely span across all biogeographic regions. To a limited

degree, we can control for phylogenetic constraints by analyzing only urchins in the Order Echinoida, and find that *Echinometra* urchins have greater feeding resistance than do *Strongylocentrotus* urchins ($P < 0.05$; one-sided paired t test, analogous to Fig. 1B).

Biogeographic origin also influences the feeding resistance within a single urchin species (*Arbacia punctulata*) that spans tropical and temperate habitats. This among-population variation in herbivore resistance is one of a small number of examples among marine herbivores (Sotka and Hay 2002, Vesakoski et al. 2009), but suggests that microevolution of feeding behavior may more frequently evolve among generalist consumers than is currently appreciated (Sotka 2005). One might be tempted to view our simultaneous interpretation of phylogenetic conservatism and local adaptation in *Arbacia* as paradoxical. However, the literature is replete with examples of microevolution in herbivore feeding preference and host use in taxa that are under strong phylogenetic constraints (Price 2003).

There are several reasons to believe that the biogeographic differences in feeding resistance have a substantial genetic basis. First, it is hard to envision how recent experience with subtropical seaweeds or other plastic mechanisms can explain phylogenetic conservatism in feeding resistance. This is particularly true for *Arbacia* and *Strongylocentrotus* populations, which cluster despite being collected from distinct biogeographic zones or ocean basins. Second, urchins were fed ad libitum a

mixture of palatable seaweeds before and in-between feeding assays in order to minimize effects of feeding history. The extract-laden foods constituted a fraction of the animals' daily intake, minimizing the chance that feeding resistance would be induced over time in the laboratory. Finally, we repeated *D. pulchella* feeding assays at multiple temperatures and after repeated exposure to seaweed extracts and found no change in urchin feeding responses (Craft 2011). Thus, it is unlikely that differences in water temperatures or recent experience easily account for these results.

It is possible, although not certain, that the biogeographic differences in feeding resistance reflect a latitudinal decline in herbivore resistance. Our study was inspired by a documented latitudinal decline in the strength of lipophilic chemical defenses. Such defenses are produced by both tropical and temperate seaweeds, yet tropical genera have higher concentrations and diversity of lipophilic secondary metabolites relative to temperate genera (Bolser and Hay 1996). As a consequence, trait-specific coevolutionary theory (cf. Strauss et al. 2005) predicts that tropical herbivores should respond evolutionarily by increasing their feeding resistance toward these chemical defenses. While our biogeographic signal of feeding resistance is consistent with this coevolutionary prediction, a thorough test of latitudinal patterns requires assaying seaweeds and sea urchins from multiple locations along a latitudinal gradient in multiple ocean basins and in both hemispheres. This will require an enormous effort beyond the scope of this single study.

We did not test water-soluble portions of seaweed extracts or feeding resistance to those compounds. The tropical-temperate pattern in concentration of water-soluble compounds is more ambiguous than that for lipophilic metabolites, as high levels have been described from temperate Australasian and Arctic habitats, as well as some tropical habitats (Van Alstyne et al. 2001, Amsler et al. 2009) and a direct test revealed no latitudinal pattern in the strength of water-soluble deterrence (Bolser and Hay 1996). Because herbivores respond to phlorotannins on microevolutionary (Vesakoski et al. 2009) and macroevolutionary (Steinberg et al. 1995) timescales, these latitudinal gradients deserve increased attention.

Our study may help to resolve an emerging paradox on latitudinal patterns in herbivore impact. Herbivores (particularly fishes) are more numerous in tropical relative to temperate regions, yet recent meta-reviews indicate that the ecological impact of herbivores is statistically equivalent across latitude (Gruner et al. 2008, Hillebrand 2009, Poore et al. 2012). While there are several non-exclusive hypotheses that may explain the lack of a latitudinal pattern in herbivore impact, our data reinforce the role of seaweed defenses: If stronger chemical defenses minimize the impact of greater numbers of herbivores on tropical reefs, then the net herbivore impacts will be similar across latitude.

Finally, the strong influence of evolutionary history on marine plant-herbivore interactions that we detect here implies that evolution may play important roles in species range expansion. One way to view successful range expansions is as an evolutionary mismatch, where expanding populations of herbivores can devastate local seaweeds unaccustomed to these novel herbivores (e.g., Ling 2008). Predicting the effects of consumer or seaweed invader will be facilitated by understanding the evolutionary dynamics that operated in their native ranges (Parker et al. 2006, Gilman et al. 2010).

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SUPPLEMENTAL MATERIAL

Appendix

Tables that summarize collection and handling information, feeding preference assays, and model statistics ([Ecological Archives E094-003-A1](#)).