

## GEOGRAPHIC AND GENETIC VARIATION IN FEEDING PREFERENCE FOR CHEMICALLY DEFENDED SEAWEEDS

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**Abstract.**—Genetic variation in tolerance for seaweed chemical defenses among populations of marine herbivores is poorly described, either because few marine studies have addressed the issue or because the feeding preferences of populations of marine herbivores rarely differentiate. We address this using the polyphagous amphipod *Ampithoe longimana* (Crustacea), which commonly consumes, and lives on, terpene-rich brown seaweeds in the tropical genus *Dictyota*. Previous work demonstrated that in areas where the amphipod and seaweeds overlap geographically, these chemically deterrent seaweeds provide the amphipod with spatial refuges from predation by omnivorous fishes that avoid consuming *Dictyota*. However, the amphipod's distribution extends northward of the seaweed's distribution, making this benefit of association unavailable to more northerly populations of amphipods. On average, populations sympatric with *Dictyota* have stronger feeding preference for *Dictyota* species and greater fitness when raised on *Dictyota* than do populations that are outside *Dictyota*'s geographic endpoint. These results are consistent with the hypothesis that in areas where the amphipods co-exist with *Dictyota*, selection favors amphipods that tolerate *Dictyota*'s chemical defenses and thereby access its enemy-free space. Amphipods allopatric with *Dictyota* are unable to tolerate *Dictyota*'s chemical defenses, either because of selection, drift, or some mix of both forces. A trade-off between preference for *Dictyota* and for the chemically distinct seaweed *Hypnea musciformis* is suggested by the finding that populations with high preference for *Dictyota* tended to have lower preference for *Hypnea*. However, this population-level pattern appears to be the result of independent evolution and not a genetic trade-off: an analysis of full-sib families within a single population detected heritable variation in preferences for *Dictyota* and suggested this for *Hypnea* (although the latter was not statistically significant), yet no family-level covariation was detected. Phylogeographic analysis of mitochondrial DNA and nuclear sequences indicates a strong historical break between populations sympatric with *Dictyota* and populations more than 500 km beyond *Dictyota*'s geographic endpoint. The historical separation of these most northerly populations could have allowed a random accumulation of alleles to effectively degrade tolerance for *Dictyota*, although selection may also be responsible for the degradation.

**Key words.**—*Ampithoe longimana*, cytochrome oxidase I, *Dictyota*, heritability, phylogeography, plant-herbivore interaction, ribosomal internal transcribed spacer.

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Terrestrial plants play a fundamental role in the evolution of herbivorous insects, largely because they produce formidable arrays of traits that deter feeding by numerous insect species (Hartley and Jones 1997) and provide enemy-free space to others (Price et al. 1980). In response, herbivorous insects are thought to evolve host choices that minimize the costs of tolerating plant defenses (Dethier 1954) while enhancing the probability of escaping or deterring their own natural enemies (Bernays and Graham 1988). Over micro-evolutionary timescales, these selective forces can result in the genetic differentiation of groups of individuals, especially when host choices are coupled with mate choice (Via 2001). Over longer evolutionary timescales, these pressures appear to have promoted the evolution of more specialized feeding preferences and higher speciation rates among herbivorous insects than among lineages of insects that feed as predators or detritivores (Strong et al. 1984; Mitter et al. 1988).

By extension, traits of seaweeds could be shaping the evolution of small (insectlike; Hay et al. 1987) marine herbivores because, as with terrestrial plants, seaweeds contain chemical and morphological traits that deter their herbivores (Hay 1996; Paul et al. 2001) and seaweeds vary in their ability to

provide small herbivores with a refuge from their natural enemies (e.g., Hay et al. 1987; Duffy and Hay 1994; Sotka et al. 1999; Stachowicz and Hay 1999). Further, seaweed communities and their arsenal of deterrent chemistry vary spatially (e.g., Steinberg et al. 1995; Bolser and Hay 1996; Van Alstyne et al. 2001), providing an opportunity for the local adaptation of populations of marine herbivores.

However, there is little evidence on whether populations of marine herbivores commonly, or rarely, adapt to specific seaweed foods; as a consequence, our understanding of the roles that seaweeds play in the evolution of marine herbivores is incomplete. In particular, there are no published examples of population-level differentiation in feeding preferences among any marine herbivore, whereas there are dozens of examples of terrestrial insect populations displaying spatial variation in feeding preferences that suggest local adaptation to plant resources (for reviews, see Fox and Morrow 1981; Bernays and Chapman 1994; but see Thompson 1993). It is possible that marine examples of among-population variation in feeding preference are not described because such questions are rarely asked (see reviews of among-species variation in Hay 1992, 1996; Targett and Arnold 2001). To our knowledge, the only assessment of local genetic differentiation in host use by a marine herbivore is Poore and Steinberg (2001); they found that although the amphipod *Peramphithoe parmerong* displayed heritable variation in juvenile survival

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when feeding on different brown seaweeds, amphipod populations from sites dominated by each of these seaweeds did not differ in their fitnesses when reciprocally grown on each seaweed.

Alternatively, the apparent difference among marine and terrestrial herbivores in the frequency of population-level adaptation could be the consequence of fundamental differences in their biology. For example, the general reproductive and dispersal mechanisms of marine versus terrestrial herbivores (i.e., highly dispersive marine populations vs. relatively closed terrestrial populations; see Kinlan and Gaines 2003) may make local adaptation less likely for marine herbivores, since the potentially higher rates of gene flow among marine populations would reduce the likelihood of local genetic differentiation (Endler 1977). In a similar manner, it has been hypothesized that the tendency of marine herbivores to broadly disperse may impede the evolution of feeding specialization (Hay and Fenical 1988); most marine herbivores are feeding generalists whereas most terrestrial insects are restricted to a subset of available hosts (Bernays and Chapman 1994).

We tested for the presence of population-level variation in feeding preferences in the marine amphipod *Ampithoe longimana*, a small (<2-cm length) polyphagous herbivore that is found in temperate, high-salinity estuaries along the eastern coast of North America. The amphipod lives in tubes it builds on selected host plants, it broods its offspring, and it reaches sexual maturity within about three weeks when feeding on high quality foods. As such, these amphipods are similar to terrestrial insects (e.g., butterflies) whose females choose relatively large plants on which the herbivorous juveniles develop. This amphipod is a direct developer and lacks a dispersive planktonic stage, suggesting that its populations may exchange genes on fairly local scales; as such, amphipods could be more likely than other groups of marine herbivores to adapt to local seaweed communities. However, even direct developers have the ability to disperse over broad distances due to transport by drift seaweeds (for long-distance dispersal of *Ampithoe longimana* on floating *Sargassum*, see Fine 1970); this could, in principle, generate enough genetic mixing to prevent local adaptation.

As with most herbivores having broad distributions (Strong et al. 1984), the geographic range of *A. longimana* extends beyond that of its preferred host plants (Fig. 1). In North Carolina, the amphipod is found on seaweeds from several taxonomic classes, but it prefers brown seaweeds in the genus *Dictyota*. These seaweeds produce diterpene alcohols that deter most herbivores but have minimal effects on *A. longimana* (Hay et al. 1987; Cruz-Rivera and Hay 2001, 2003), thus allowing *A. longimana* to escape heavy predation by omnivorous fishes by living on this chemically rich seaweed (Duffy and Hay 1994, 2000). *Dictyota* does not regularly exist northward of the North Carolina/Virginia border (Fig. 1; Richardson 1979). However, populations of the amphipod are known from the Florida Bay to at least the Gulf of Maine (Bousfield 1973; E. E. Sotka, pers. obs.). Thus, amphipods from Virginia to Maine are not exposed to *Dictyota*, and would not experience selection to use it as either a food or a spatial refuge from consumers.

Previous work indicated that a single population sympatric

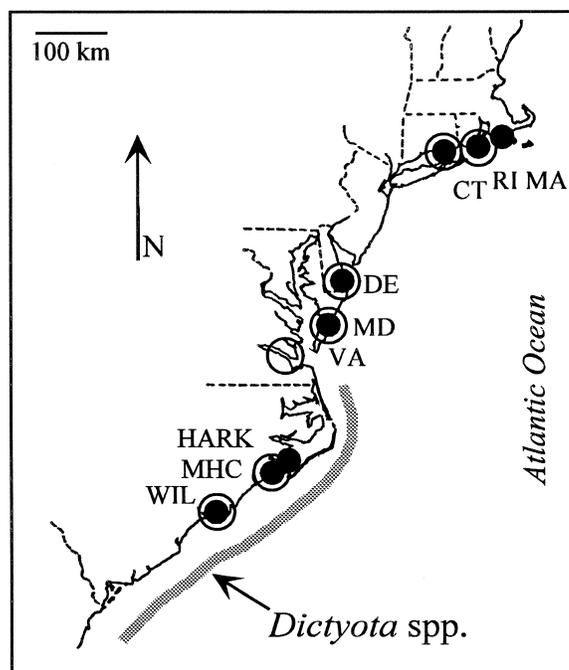


FIG. 1. Geographic distribution of the tropical seaweed *Dictyota* and the locations of collected populations of the amphipod *Ampithoe longimana* along the eastern coastline of North America. Open circles indicate populations collected for genetic analysis. Closed circles indicate populations collected for feeding and fitness assays. Three populations are sympatric with *Dictyota* [Harker's Island (HARK), Morehead City (MHC) and Wilmington (WIL), all in North Carolina], three populations are within about 250 km of *Dictyota*'s northern endpoint [Bottom Hills, Delaware (DE), Ocean City, Maryland (MD), York River, Virginia (VA)], and three populations are > 500 km from *Dictyota*'s endpoint [Westport, Massachusetts (MA), Jamestown, Rhode Island (RI), and Millstone, Connecticut (CT)].

with *Dictyota* had higher affinity for, and fitness on, *Dictyota* than did a single population outside the plants' geographic range, and that these feeding differences were mediated by the lipophilic diterpene alcohols produced by the seaweed (Sotka and Hay 2002). In this study we assess the robustness of this geographic pattern and attempt to identify the evolutionary forces responsible for the acquisition or loss of tolerance for *Dictyota* by different amphipod populations. First, we validate and expand our documentation of geographic variation by assaying the feeding preferences of eight populations of amphipods for two species of *Dictyota* and two species of red seaweeds (*Hypnea musciformis* and *Gracilaria tikvahiae*) that are phylogenetically and chemically distinct from *Dictyota*. We are particularly interested in whether populations sympatric with *Dictyota* have higher feeding preference for *Dictyota* than populations outside the geographic range of *Dictyota*. Second, we demonstrate the genetic potential for evolution by measuring the degree of variance and covariance in feeding preferences for two of these seaweeds (*D. menstrualis* and *Hypnea*) among full-sib families of a single population. Third, we test whether these different feeding preferences have fitness consequences (measured as survivorship, growth, and reproduction) for amphipods from different regions. Finally, we use mitochondrial

DNA (mtDNA) and nuclear sequences to estimate historical relationships among populations of amphipods, and compare the phylogeography of these neutral traits to the geographic variation in feeding preference for, and juvenile performance on, chemically rich seaweeds in the genus *Dictyota*.

## MATERIALS AND METHODS

### *Collection of Animals and Plants*

*Amphithoe longimana* were collected from nine populations located in estuaries along the eastern shore of North America (Fig. 1). The shallow subtidal sites (<3 m depth) were dominated by seagrass beds and epiphytic and drift macroalgae, except for the Wilmington site, which was a macrophyte-dominated floating dock (<1 m depth). Salinity ranged from 29 to 35‰ at the times of collection. We have information on seasonal variation in seawater temperature only near the geographic endpoints of our collection sites; the average monthly temperatures at the Connecticut site range from three to 20.5°C, and at North Carolina range from six to 28°C (Sutherland and Karlson 1977; R. Whitlatch, pers. comm. 2000).

Amphipods were collected from a variety of seaweeds that are found throughout the eastern coast of North America, primarily the brown seaweeds *Ectocarpus* spp. and green ephemeral seaweeds (e.g., *Enteromorpha* spp.) that are high quality foods for the amphipod (Cruz-Rivera and Hay 2001). Generally, seaweeds were immediately placed in buckets filled with seawater, returned to the laboratory, and sorted by hand for amphipods. North Carolina populations were returned immediately to the University of North Carolina at Chapel Hill's Institute of Marine Sciences (Morehead City, NC). Amphipods collected outside of North Carolina were sorted, placed into plastic containers or scintillation vials, and transported back to North Carolina in coolers.

All amphipods used for assays were collected in May 2000, except for the Harker's Island population, which was collected in July 2000. Field-collected adults were assayed to determine their feeding preferences for common seaweeds, and their offspring were used for feeding-based fitness assays. There are several reasons to believe that patterns documented in our feeding assays have a genetic basis. First, we ensured that patterns in adult feeding behavior and juvenile fitness were not affected by recent experience with *Dictyota*. With one exception, amphipods in North Carolina were never collected from *Dictyota* species or during times of the year when species of *Dictyota* were available (i.e., warm summer months). The single exception was the Harker's Island population of *A. longimana*, which was collected in July 2000, yet even these adults were collected from *Sargassum* (Fucales) and in areas depauperate in *Dictyota* (Dictyotales). In addition, adults were given non-*Dictyota* seaweeds (e.g., *Enteromorpha* spp., *Sargassum filipendula*) during acclimation and before any behavioral assays. Second, this paper documents a strong heritable component to the feeding preference for *Dictyota menstrualis* (see Results). Moreover, Sotka and Hay (2002) determined that geographic differences in feeding preference for, and juvenile performance on, *Dictyota* were maintained whether the assayed individuals were field-col-

lected or came from a second generation raised in a common laboratory environment.

Finally, we minimized environmental effects that may have caused stress. We raised water temperature of collected amphipods very slowly (about 0.5°C per day) to about 20°C (i.e., room temperature) before rearing and behavioral assays began. We then maintained the amphipods at this temperature and at a salinity of about 30–32‰, physical conditions experienced by both northern and southern populations during the course of a year. During this careful acclimation, we observed no signs of stress among adult or juvenile amphipods. If populations from different locations had been differentially stressed by transport to the laboratory, this likely would have affected feeding rates by adults or the fitness of their offspring. Instead, adult feeding rates were uniformly high among individuals collected from within or outside North Carolina (mean  $\pm$  SE tissue consumed = 10.7  $\pm$  0.3 mg per individual per day for individuals from outside North Carolina, and 9.9  $\pm$  0.5 mg per individual per day for individuals from within North Carolina; one-way ANOVA,  $df = 1$ ,  $F = 2.220$ ,  $P = 0.137$ ), and juveniles of field-collected adults from each region died at statistically indistinguishable rates when deprived of food (mean  $\pm$  SE number of days = 3.1  $\pm$  0.1 for individuals from outside North Carolina, and 2.8  $\pm$  0.2 for individuals from within North Carolina; one-way ANOVA,  $df = 1$ ,  $F = 1.831$ ,  $P = 0.178$ ).

The seaweed genera used in our feeding assays are found at every site, with the exception of *Dictyota* species, which are not regularly found north of the North Carolina/Virginia border (Richardson 1979). Seaweeds for assays were collected within a 12-km radius near Bogue Sound, North Carolina. The brown seaweeds *D. menstrualis* and *D. ciliolata* and the red seaweeds *Gracilaria tikvahiae* and *Hypnea musciformis* were collected from a subtidal boulder jetty (Radio Island Jetty; 34°42'N, 76°41'W), and the green seaweeds *Enteromorpha* sp. and *Ulva* sp. were collected from floating docks. With the exception of *D. ciliolata*, each of these seaweeds can be found at the seagrass site from which Morehead City, North Carolina populations of *A. longimana* were collected, but the density of seaweeds at the seagrass site was neither large nor persistent enough to harvest in sufficient quantities for these experiments.

### *Among-Population Variation in Feeding Preference*

To quantify adult feeding preference, amphipods were given a choice of two seaweeds and allowed to feed for three days or less. For most replicates, a single adult amphipod was simultaneously offered a choice between preweighed tissue from *Ulva* sp. and a "test" seaweed species. *Ulva* was chosen as the contrast for all other species because it was easy to collect, and because initial work had detected no differences in feeding preference or fitness on *Ulva* among Connecticut and North Carolina populations (Sotka and Hay 2002). The four test species offered were the southern seaweeds *Dictyota menstrualis* and *D. ciliolata* and the more broadly distributed red seaweeds *Hypnea musciformis* and *Gracilaria tikvahiae*; initial work indicated that amphipod populations might differ in their relative preference for these species (Sotka and Hay 2002). We added two small adults

rather than one large one when adequate numbers of large adults were not available (about 5% of replicates).

Tissues were removed from each plant, blotted dry, weighed to the nearest milligram, and placed in plastic bowls filled with about 200 ml of seawater. To minimize bias in feeding preference due to encounter rates, tissues from each seaweed were added to the choice assay bowls at a blotted wet mass equivalent to 3 cm<sup>2</sup> ( $\pm 10\%$ ), according to a surface-area-to-weight ratio calculated for each seaweed. Projected surface areas were determined using a Li-Cor (Lincoln, NE) 3000 surface area meter. Seven amphipod populations were assayed simultaneously, and an eighth (Harker's Island) was similarly assayed approximately two months later and grouped with the rest for analysis. Each population was given tissue from each replicate plant. For each replicate, a bowl with pieces from the same plant was run without amphipods; this acted as a control for autogenic changes in plant mass unrelated to amphipod feeding. Replicates were stopped after the amphipods had consumed at least half of one plant piece or when 72 hours had elapsed, whichever came first. Plant pieces were blotted and re-weighed, and the change in wet mass of each piece was scaled to account for autogenic changes in plant mass using the formula  $T_i \times (C_f/C_i) - T_f$ , where  $T_i$  and  $T_f$  represent the tissue subject to grazing and  $C_i$  and  $C_f$  represent the control tissue, before ( $i$ ) and after ( $f$ ) the experimental run. Of the 629 replicates that we set up, we dropped replicates from the subsequent analysis if the amphipod died during the assay (28 replicates), if the amphipod fed so little that our measurements of mass change would have had a significant weighing error relative to the amount consumed (defined as  $<10\%$  of the available plant mass being consumed; 53 replicates), or if the *Ulva* spawned (39 replicates).

Paired  $t$ -tests determined whether a single population consumed the two seaweeds at equal rates. This test is robust to deviations from normality and heteroscedasticity (Zar 1996). To directly test whether populations differed in relative preference for the test seaweed, we calculated a ratio of test seaweed consumed to the total amount of both seaweeds consumed within a given replicate. These ratios are sometimes less than zero and greater than one; this occurs when autogenic control plants and plants isolated with amphipods differ in growth rates, as when one plant degrades during the course of the assay. The proportion of cases with ratios greater than 1.10 or less than  $-0.10$  is relatively small (40 out of 509 replicates). Ratios were rank transformed to reduce, though not eliminate, heteroscedasticity (Bartlett's test  $P < 0.05$ ), and subjected to two sets of analyses. A two-way ANOVA detected an interaction between population source and seaweed offered and Tukey-Kramer post hoc tests detected pairwise differences among populations. Such ANOVAs are robust to deviations from normality and heteroscedasticity when sample sizes are relatively large (Underwood 1997).

We also tested the hypothesis that populations sympatric with *Dictyota* have feeding preferences that differ from those that are allopatric with *Dictyota* using a nested two-way ANOVA, with site nested within region. A series of nested one-way ANOVAs were used to detect post hoc differences among regions.

#### *Within-Population Variation in Feeding Preference*

To estimate the genetic variance and covariance of feeding preferences for *Dictyota* and *Hypnea* in a single population, the feeding preferences of offspring from full-sib families were assayed. Gravid females were collected from laboratory cultures of a Connecticut population. This population was chosen primarily because of the culture's high density ( $>500$  females), which reduces sampling error in estimating genetic variance and increases probability of random mating. For unknown reasons, cultures of other populations were not as robust.

The laboratory cultures were maintained at the University of North Carolina Institute of Marine Sciences, and kept at conditions that Connecticut populations would experience in the field during the course of a year (i.e., about 20°C and a salinity of about 30%). Seawater in plastic tubs (about 5 l) was aerated constantly and changed every two to four days by pouring through a 500- $\mu$ m Nitex screen (Tetko, Inc., Depew, NY) to prevent passage of cultured amphipods. All seaweeds added to the cultures (*Enteromorpha*, *Ectocarpus*, *Sargassum*, *Gracilaria*, and *Hypnea*) were first bathed in freshwater for at least two 30-sec intervals; this eliminates the introduction of local *A. longimana*, which immediately leave plants placed in freshwater. Juveniles used in these assays represent the approximately fifth generation raised in the laboratory. Thus, nongenetic effects on preference for *Dictyota* were minimized, but our estimate of heritability of *Hypnea* preference could be affected by recent exposure to this plant.

To set up this experiment, individual gravid females were left on *Sargassum* in small petri dishes until juveniles had left the brood pouch. Juveniles from 11 mothers (7–18 per mother) were placed individually within 40-ml petri dishes containing *Enteromorpha* sp., a palatable green seaweed devoid of known secondary metabolites that provides for high fitness of *A. longimana* juveniles (Sotka and Hay 2002). The amphipods were checked every two days for deaths, water was changed every four to five days, and *Enteromorpha* was added to dishes when depleted. During this time, several juveniles died of unknown causes (mean family survivorship = 79.7% range = 50–100%).

After approximately four weeks, the amphipods were offered a feeding choice between preweighed pieces of tissue from *Ulva* sp. paired with either *Dictyota* or *Hypnea*. These two seaweeds were chosen because of a negative correlation between feeding preferences for these seaweeds among populations of amphipods (see Results; Sotka and Hay 2002). Ten families were assayed for *Dictyota* preference and for *Hypnea* preference; an eleventh family was assayed for its *Hypnea* preference but not for its *Dictyota* preference, as there was some mortality that occurred between assays. Assays were performed as described in the previous section, with the exception that all 10 to 11 families could not be assayed on each replicate plant due to a lack of material. As a consequence, pieces from replicate plants were offered to only a subset of families. We made sure that each family was never confronted with more than one piece from any one of these plants. Less than 5% of replicates were dropped from the subsequent analysis, because the amphipod died during

the assay or the total mass of plant eaten was minimal (<10%).

The feeding choice results were translated into preference ratios as described earlier. Because these relative preferences for *Dictyota* and for *Hypnea* were normally distributed (Shapiro-Wilks  $W$  test,  $P > 0.05$ ) and homoscedastic (Bartlett's test,  $P > 0.05$ ), parametric one-way ANOVAs were performed on the relative preferences using full-sib family origin as an independent variable. Broad-sense heritability ( $h^2$ ) of the preference traits was estimated by  $2\sigma_s^2/(\sigma_s^2 + \sigma_w^2)$ , where  $\sigma_s^2$  is the covariance among full-sibs (i.e.,  $\frac{1}{2}$  of the additive variance,  $\frac{1}{4}$  of the dominance variance, and various amounts of epistatic variance) and  $\sigma_w^2$  is the remainder of the genetic variance plus environmental variance (Becker 1992). A 95% confidence interval was also calculated for the estimate. To test whether covariance was present, the mean family preferences for *Hypnea* were regressed onto preferences for *Dictyota* (cf. Via 1984).

#### Among-Population Variation in Fitness on *Dictyota*

To assess effects of foods on amphipod fitness, juvenile amphipods were isolated on single seaweed species two days after emergence from the mother's brood pouch. They were fed on these foods for the next 30 days. To set up this experiment, brooding females collected from the field were left on *Sargassum filipendula* in 40-ml petri dishes until juveniles left the brood pouch. Juveniles from each mother were placed in one of four treatments within individual 40-ml petri dishes; *D. menstrualis*, *D. ciliolata*, *Enteromorpha*, and a no-food control. A single mother provided one offspring to each treatment. Juveniles from 21–22 females were collected at each site, except for Maryland ( $n = 13$ ). The amphipods were checked daily for deaths and for reproductive maturity of females. Water was changed every two to three days, and food was added to dishes when needed to prevent depletion. Survivorship, size of surviving amphipods at the termination of the experiment (as measured by the straight-line length of the dorsal edge of the first three segments), and days to female maturity were recorded. The assay was conducted simultaneously with seven populations; the Harker's Island population was collected two months later and so was not assayed. During the course of changing seawater, a small number of juveniles (17 of 569 total) were lost and subsequently removed from all analyses.

As with the analysis of feeding preferences, we were interested in whether these fitness traits revealed an interaction between seaweed and site, as well as an interaction between seaweed and region. For each analysis, all individuals within a treatment were independent because mothers provided one juvenile per treatment and all individuals were held in separate petri dishes. Survivorship data were examined via sets of  $\chi^2$ -tests. Adult sizes at day 30 were square-root transformed to yield normal (Shapiro-Wilks  $W$  test,  $P > 0.5$ ) and homoscedastic (Bartlett's test,  $P > 0.15$ ) distributions. To detect an interaction between seaweed and site, a two-way parametric ANOVA was performed, followed by Tukey-Kramer post hoc tests. To detect an interaction between seaweed and regional source, a nested two-way ANOVA was performed, with site nested within region.

To analyze the female reproductive response, we combined data on the proportion of total females that went reproductive within 30 days with the number of days to maturity to yield a single failure-time curve (cf. Muenchow 1986) for each seaweed-region combination. These data fit a lognormal distribution better than exponential or Weibull distributions. We used parametric survival models to detect a significant interaction between seaweed and site, and analyzed post hoc pairwise comparisons of these curves in the same manner, applying a sequential Bonferroni correction factor (Rice 1989). To calculate a female response curve for each region, we calculated the average proportion of females that were reproductive each day by standardizing proportions within a population and averaging across populations. As with the site-by-seaweed curves, we used a parametric survival model based on lognormal distributions to detect interactions between seaweed and regional source, and to detect post hoc differences.

#### Mitochondrial DNA and Nuclear ITS Sequence Analysis

Specimens used in phylogenetic analysis were collected April–July 1999 and May 2000 and placed immediately into 95% ethanol. All individuals were collected on non-*Dictyota* species, except for five of the nine Morehead City, North Carolina individuals that were collected on *Dictyota* for genetic comparison. Methods used for DNA isolation, PCR amplification, and automated sequencing of the mitochondrial cytochrome oxidase I (COI) and nuclear ribosomal internal transcribed spacer (ITS-1) genes are described in Wares (2001a,b). In each dataset, characters that were missing or ambiguous were removed to generate datasets of constant-length sequences.

The set of maximum parsimony trees for this dataset, as well as bootstrap support for clades within the genealogy, were generated using PAUP\* 4.0b5 (<http://paup.csit.fsu.edu>). The best-fit maximum likelihood (ML) models were determined with standard likelihood-ratio methods using the programs ModelTest (Posada and Crandall 1998) and PAUP. For the COI data, the best-fit model is HKY (different rates for transitions and transversions, Ti:Tv = 9.94) with gamma-distributed rate variation ( $\alpha = 0.232$ ); similar parameter values were obtained with and without the inclusion of outgroup species *Ampithoe valida* and *Cymadusa compta*. The best-fit substitution model for the ITS-1 data was F81 (equal rates among sites).

To test for the presence of genetic structure, we performed an analysis of molecular variance (AMOVA) using Arlequin 2.0 (Schneider et al. 2000). This procedure evaluates the degree of genetic differentiation between predefined groups of populations using frequencies of haplotypes and their genetic relatedness. Significant relationships were tested against a distribution of 10,000 randomization replicates.

## RESULTS

#### Among-Population Variation in Feeding Preferences

In general, amphipod populations sympatric with *Dictyota* have higher affinity for *Dictyota* and lower affinity for *Hypnea* than do populations outside the range of *Dictyota* (Figs. 2,

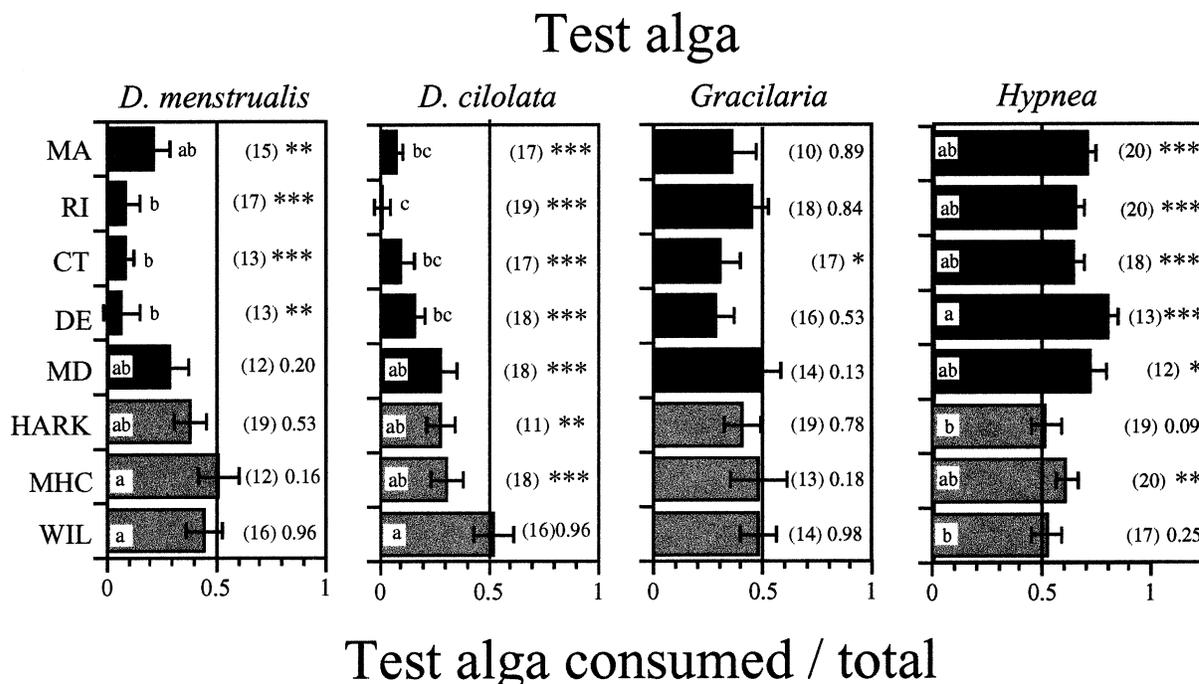


FIG. 2. Relative preference (mean  $\pm$  1 SE) of amphipod populations for each of four seaweeds. Population codes as in Figure 1. Gray bars represent the response of populations sympatric with *Dictyota* (HARK, MHC, WIL); black bars represent populations outside the geographic range of *Dictyota* (MA, RI, CT, DE, MD). For each replicate, a single adult amphipod was offered a choice of one test seaweed (*D. menstrualis*, *D. ciliolata*, *Gracilaria tikvahae*, or *Hypnea musciformis*) and *Ulva* sp. Relative preference is equal to the amount of the test seaweed eaten divided by the amount of both seaweeds eaten within that replicate. Sample sizes are given in parentheses, and the *P*-values of paired *t*-tests are indicated (\**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001). A significant interaction between site and seaweed species is present (see Table 1A). Lowercase letters indicate treatments that are statistically indistinguishable by post hoc analysis.

3). Populations differed significantly in their relative feeding preferences for seaweeds, as indicated by a two-way ANOVA (Fig. 2; Table 1a). The three sites sympatric with *Dictyota* (i.e., WIL, MHC, HARK) and a single but nearby population allopatric with *Dictyota* (MD) populations consumed *D. menstrualis* and *Ulva* at statistically indistinguishable rates; in contrast, the more geographically distant allopatric popula-

tions from MA, RI, CT, and DE significantly preferred *Ulva* over *D. menstrualis*. All populations but the most southerly one tested (WIL) avoided *D. ciliolata* relative to *Ulva*. All populations but CT did not distinguish between the red seaweed *Gracilaria* and the green control seaweed, *Ulva*. Two southern sites (HARK and WIL) did not distinguish between *Hypnea* and *Ulva*, whereas the other six populations significantly preferred *Hypnea*. Post hoc tests revealed complex patterns in the relative responses of amphipod populations to the seaweeds offered (Fig. 2); in general, however, populations sympatric with *Dictyota* tended to consume relatively more *Dictyota* and less *Hypnea* than did populations allopatric with *Dictyota* (Fig. 2).

These apparent regional patterns in feeding preference were confirmed by a direct test (Fig. 3; Table 1b); as a group, populations sympatric with *Dictyota* (WIL, MHC, WIL) had higher affinity for *D. menstrualis* and *D. ciliolata* and lower affinity for *Hypnea* than did populations that were allopatric with *Dictyota* (CT, RI, MA, DE, and MD). There was no significant difference among these regions in feeding preference for *Gracilaria*.

*Within-Population Variation in Feeding Preferences*

Phenotypic traits that evolve must vary among individuals and be heritable; the feeding preferences of *Ampithoe longimana* appear to satisfy both conditions for *Dictyota* (Fig. 4). When offered a choice between *D. menstrualis* and *Ulva*,

TABLE 1. ANOVA for preference assays. Degrees of freedom (df), mean sums of squares (MS), *F*-ratios, and *P*-values are indicated. Response variable is the percentage of target seaweed (*Dictyota menstrualis*, *D. ciliolata*, *Gracilaria*, *Hypnea*) versus *Ulva* in a choice assay. Data were rank transformed before analysis. Post hocs are indicated on the figure. (A) Represents data analyzed by site and seaweed. (B) The ANOVA nests site effect within two regions, sites that are either allopatric or sympatric with *Dictyota*.

Source	df	MS	F	P
A.				
Seaweed	3	921,996.1	66.14	<0.001
Site	7	82,180.5	5.90	<0.001
Seaweed $\times$ site	21	49,524.8	3.55	<0.001
Error	478	13,940.0		
B.				
Region	1	306,710.0	7.13	<0.050
Seaweed	3	695,690.5	16.18	<0.005
Region $\times$ seaweed	3	242,861.6	5.65	<0.050
Site {region}	6	43,002.5	3.06	<0.010
Error	496	14,062.0		

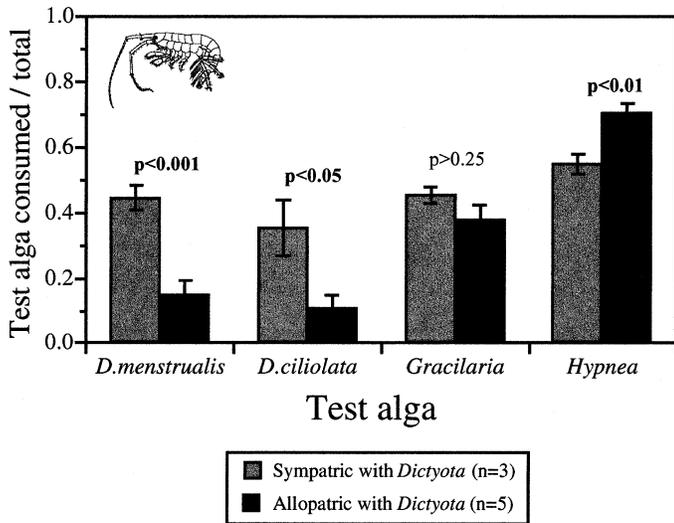


FIG. 3. Relative preference (mean  $\pm$  1 SE.) for each of four seaweeds averaged by whether amphipods were sympatric versus allopatric with *Dictyota*. A significant interaction between region (sympatric vs. allopatric) and seaweed species is present (see Table 1B), and results of post hoc tests are indicated.

individuals from a single population in Connecticut fed on only *D. menstrualis*, only *Ulva*, or tissue from both plants (i.e., the ratio of *D. menstrualis* to total amount consumed ranged from 0.96 to  $-0.19$ ). There was similar variation among these individuals in consumption of *Hypnea* versus *Ulva* (i.e., ratio of *Hypnea* to total amount consumed ranged from 0.95 to  $-0.35$ ).

This variation appears to have a significant heritable component for *Dictyota*; full-sib families differed strongly in relative feeding preference for this alga (Fig. 4A; range of family means = 0.01–0.58;  $df = 9$ ,  $F = 2.490$ ,  $P = 0.021$ ), and broad-sense heritability for preference was 0.42 (95% C.I. = 0.02, 1.16). Family-level variation in preference for the red seaweed *Hypnea musciformis* was not significant but was nearly so (Fig. 4B; range of family means = 0.19–0.60;  $df = 10$ ,  $F = 1.736$ ,  $P = 0.096$ ). Broad-sense heritability for *Hypnea* preference was 0.24 (95% C.I. =  $-0.09$ , 0.91). The variance in response among families and the relatively low  $P$ -value suggest that our power to detect significant variance may have been limited for this species. Given this potential limitation, we proceeded with a test for covariance; this produced no evidence for a relationship between family-level preference for *Dictyota* and *Hypnea* (Fig. 4C;  $r^2 = 0.035$ ,  $df = 9$ ,  $P = 0.605$ ).

#### Among-Population Variation in Fitness on *Dictyota*

To determine whether differences in feeding preference for *Dictyota* have consequences for fitness, we raised juveniles from seven populations for 30 days on *D. menstrualis*, *D. ciliolata*, and *Enteromorpha* sp., a palatable green seaweed devoid of known secondary metabolites. The latter served as a control for the basic physiological condition of the different populations.

Survivorships of sympatric and allopatric populations after 30 days were relatively high and did not differ among sea-

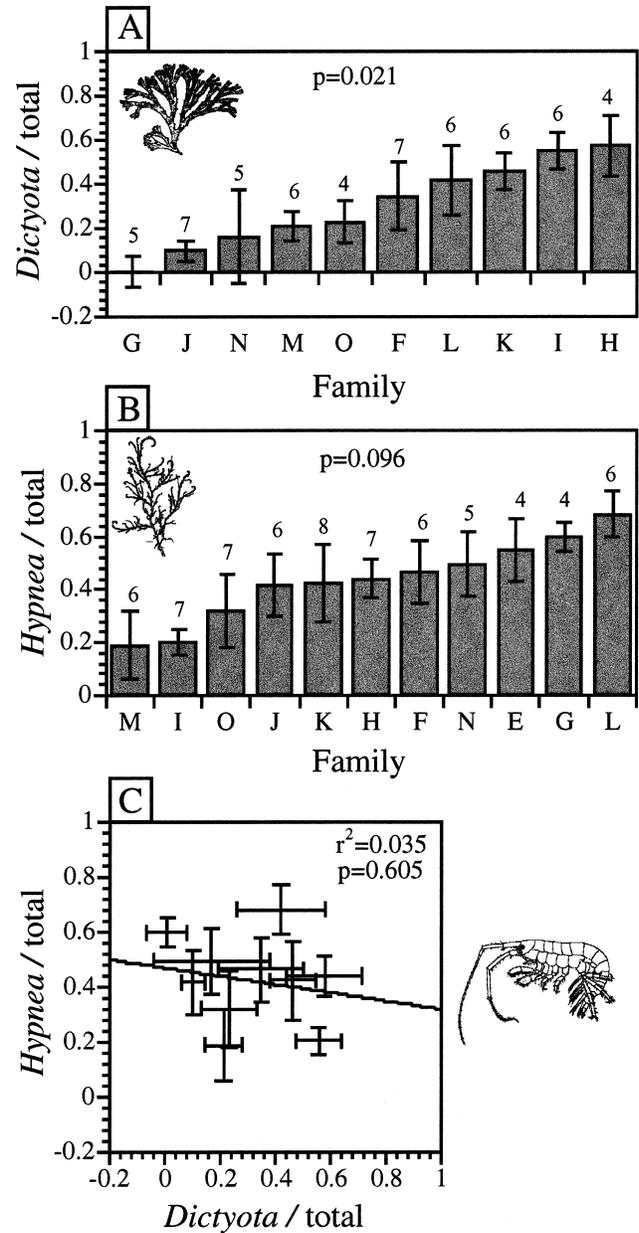
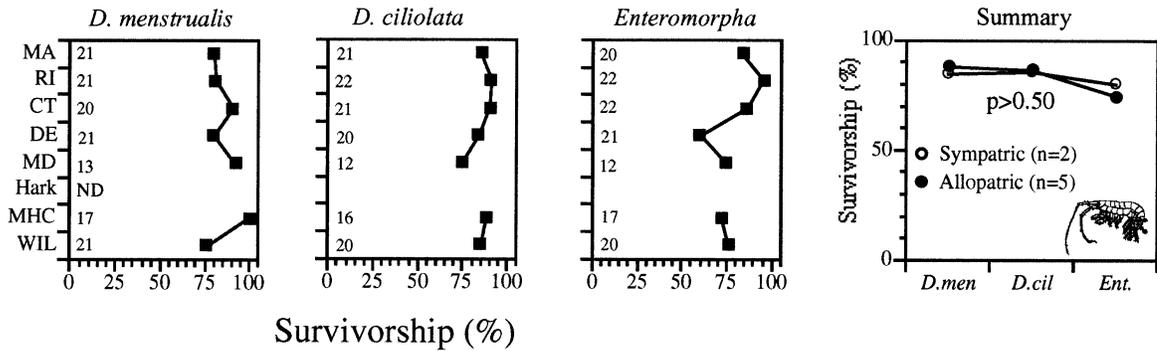


FIG. 4. Family-level variation in feeding preference (A) for *Dictyota* and (B) for *Hypnea*. Individuals of each full-sib family were offered a choice between *Ulva* sp. and either *Dictyota* or *Hypnea*. Sample sizes for each family are presented above the bars.  $P$ -values are from a one-way ANOVA with family as an independent variable. (C) Family-level covariation in feeding preference for the two seaweeds.

weeds (Fig. 5A; survivorship on *D. menstrualis*: 76–100%; on *D. ciliolata*: 75–91%; on *Enteromorpha*: 60–95%). As such, no interaction was detected between seaweed, site, and state (alive/dead;  $\chi^2 = 22.64$ ,  $df = 32$ ,  $P > 0.50$ ), nor between seaweed, region, and state ( $\chi^2 = 5.49$ ,  $df = 7$ ,  $P > 0.50$ ).

Amphipod populations did differ significantly in the growth rate of juveniles on alternative seaweeds and, for both species of *Dictyota*, in the growth of individuals from different populations on these specific seaweeds (Fig. 5B). There was a significant interaction between site and seaweed (Table

A. Survivorship



B. Adult size at day 30

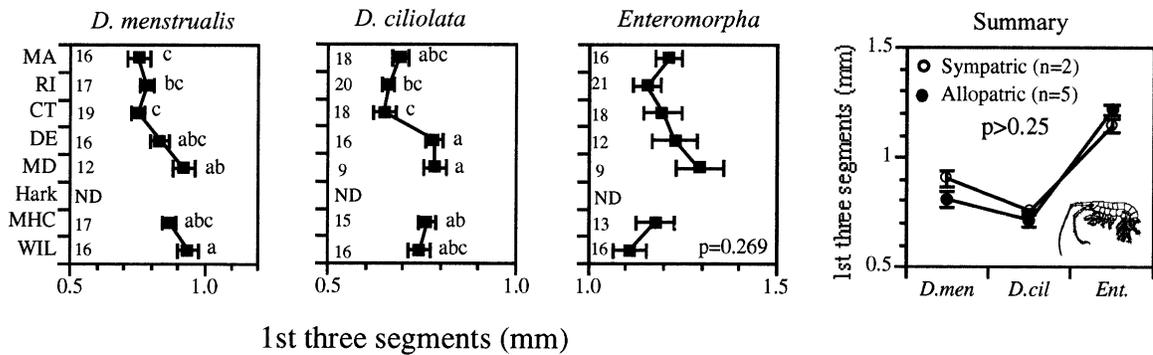


FIG. 5. Survivorship (A) and adult size (B) after 30 days. Sample sizes for each population are indicated on the graph. There were significant interactions in adult size between site and seaweed (see Table 2). The first three charts indicate population-level response to each seaweed: sample sizes per population are indicated inside the y-axis, and lowercase letters indicate populations that are statistically indistinguishable by post hoc analysis. The fourth chart summarizes these results as a function of populations that were sympatric versus allopatric with *Dictyota*. The performances of individuals from Harker’s Island (HARK) were not assayed.

2A). For both species of *Dictyota*, sympatric populations (WIL, MHC) tended to grow rapidly, as did the most southern allopatric populations (MD, DE), whereas the most northerly allopatric populations (MA, RI, CT) tended to show slower growth. However, given the large number of contrasts, the

significant groupings were complex and not invariant as a function of sympatry versus allopatry (Fig. 5; Table 2B). In contrast with patterns for *Dictyota*, performance on *Enteromorpha* did not differ significantly among amphipod populations (Fig. 5). Neither survivorship nor growth differed significantly as a function of populations sympatric vs. allopatric with *Dictyota* (Fig. 5A,B, right side figures).

TABLE 2. Analyses of variance for adult size at 30 days. Degrees of freedom (df), mean sums of squares (MS), *F*-ratios, and *P*-values are indicated. Data were square-root transformed before analysis. Post hocs are indicated on Fig. 5. (A) Represents data analyzed by site and seaweed. (B) The ANOVA nests site effect within two regions, sites that are either allopatric or sympatric with *Dictyota*.

Source	df	MS	F	P
A.				
Seaweed	2	1.041	304.21	<0.001
Site	6	0.019	5.55	<0.001
Site × seaweed	12	0.008	2.34	0.010
Error	309	0.003		
B.				
Seaweed	2	0.781	37.11	<0.001
Region	1	0.006	0.28	>0.5
Seaweed × region	2	0.030	1.44	>0.25
Site {region}	5	0.021	6.17	<0.001
Error	319	0.003		

In contrast to the patterns for survivorship and growth, patterns of reproduction did differ significantly between amphipod populations that were sympatric versus allopatric with *Dictyota* (Fig. 6). Reproduction of females from CT, MA, and RI was suppressed on *D. menstrualis* or *D. ciliolata* relative to *Enteromorpha*. Females from WIL, MHC, MD, and DE reproduced at similar rates on all three seaweeds. These patterns are confirmed by a significant interaction between site and seaweed (lognormal survival curve fit;  $\chi^2 = 135.29$ ,  $P < 0.001$ ). When the female reproductive response was averaged across populations that were sympatric versus allopatric with *Dictyota*, females outside the geographic range of *Dictyota* reproduced significantly more slowly when raised on *D. menstrualis* and *D. ciliolata* than on *Enteromorpha*; females sympatric with *Dictyota* reproduced at statistically indistinguishable rates when raised on *D. menstrualis* and *Enteromorpha*, and at slightly slower rates when raised on

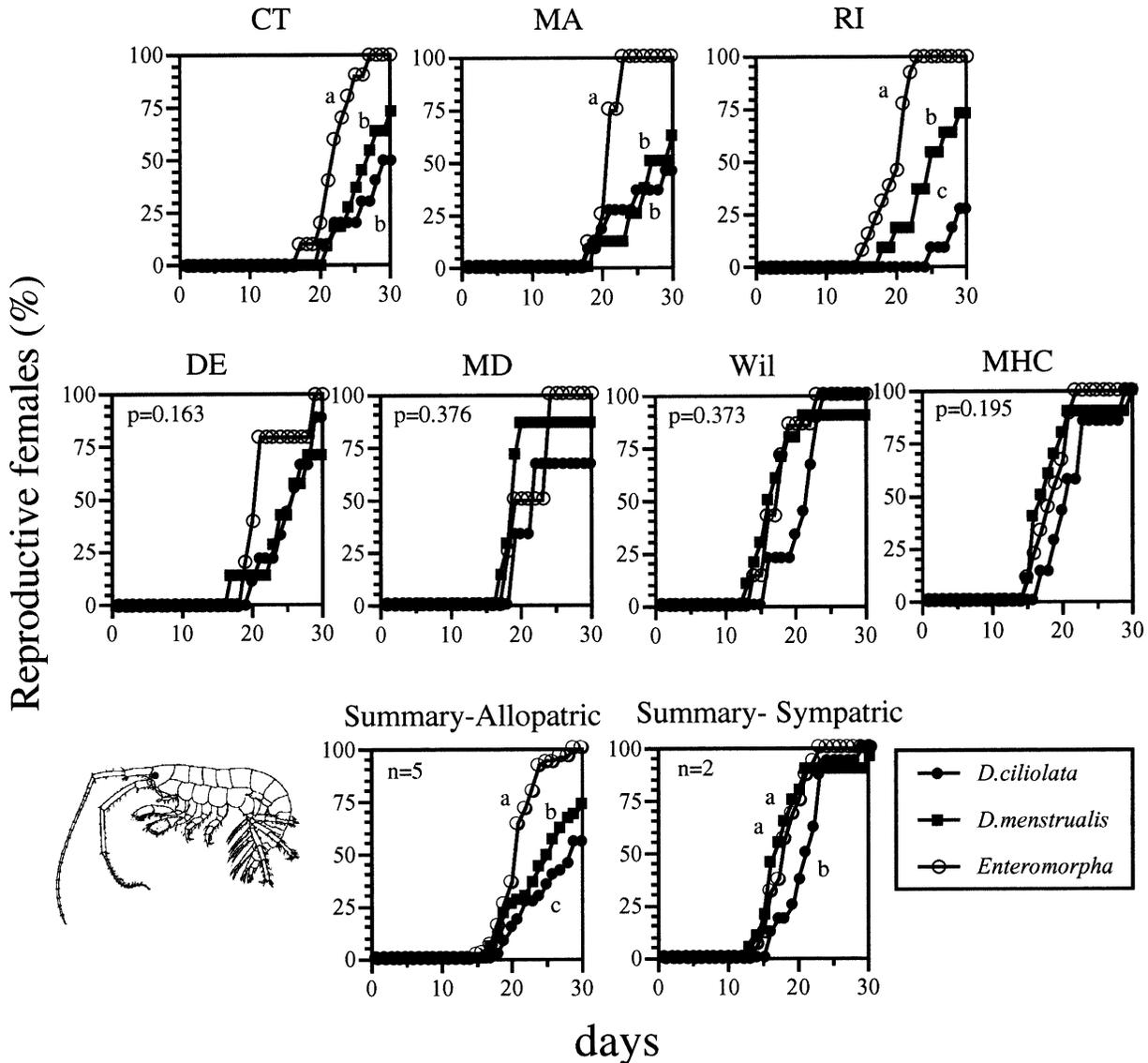


FIG. 6. Female reproductive response across 30 days incorporating both proportion of total females that became mature and the amount of time to maturity (see Materials and Methods). There was a significant interaction between site and seaweed. Letters (a,b,c) indicate statistically distinguishable groupings by post hoc analysis. There was also a significant interaction between region (sympatric vs. allopatric) and seaweed. The performances of juveniles from Harker's Island (HARK) were not assayed.

*D. ciliolata* (Fig. 6). These patterns are confirmed by a significant interaction between region and seaweed (lognormal survival fit;  $\chi^2 = 449.68$ ,  $P < 0.001$ ).

#### Molecular Phylogeography of *Ampithoe longimana* in the Northeastern Atlantic

We placed these geographic patterns of feeding preference and offspring performance into a historical context by delineating the molecular phylogeography of *A. longimana* from DNA sequences (Fig. 7). The cytochrome oxidase I dataset includes 282 characters (34 variable, 17 parsimony-informative) from 57 *A. longimana*, two *A. valida* individuals and one *Cymadusa compta* individual. The last two species serve as outgroups, because they are amphitoid amphipods that are

morphologically similar to *A. longimana* (Conlan 1982). A set of these sequences have been submitted to GenBank (AF265408–AF265438). The ribosomal internal transcribed spacer region ITS-1 was sequenced from six individuals of *A. longimana* (three from Morehead City, NC; three from Connecticut), obtaining two to three copies of ITS-1 via standard cloning techniques from each individual. A total of 14 clones yielded 12 distinct sequence types that varied in size from 591 to 688 bases. The sequences of individual clones were submitted to GenBank (AY004840–AY004853).

Several lines of analyses indicate the presence of a strong historical separation between the northern populations (i.e., CT and RI) and the others. First, a clade of COI haplotypes from CT and RI is separated from all other haplotypes by at



TABLE 3. Analysis of molecular variance (AMOVA) of cytochrome oxidase I sequences from amphipod populations. (A) Genetic differentiation between amphipod populations in CT and RI versus all others. Substantial genetic variation is partitioned between these two groups. (B) The genetic differentiation between amphipod populations within the geographic range of *Dictyota* (i.e., MHC and WIL) versus allopatric populations south of CT and RI (i.e., MD, DE, VA). No significant genetic differentiation is partitioned between these two groups.

Source	df	SS	Percentage of variation	P
A. Populations in CT and RI vs. all others				
Among groups	1	58.04	56.380	0.047
Among populations, within groups	5	20.1	6.460	<0.001
Within populations	50	84.23	37.150	<0.001
Total	56			
B. Populations within vs. outside range of <i>Dictyota</i> (excludes CT and RI)				
Among groups	1	1.897	-9.680	>0.50
Among populations, within groups	3	17.69	21.19	0.078
Within populations	38	75.42	88.490	0.005
Total	42			

Morehead City population, individuals collected from *D. menstrualis* were genetically indistinguishable from those collected on other species of seaweeds.

#### DISCUSSION

As with terrestrial plants and their insect herbivores, seaweeds produce morphological and chemical traits that could impose selection on their associated marine herbivores (Hay 1996; Paul et al. 2001), but current evidence is incomplete on whether marine herbivores respond evolutionarily to these seaweed traits. In particular, there are virtually no examples of genetic differentiation in host use within a single species of marine herbivore. This lack of studies contrasts sharply with the numerous demonstrations that terrestrial insects commonly adapt to locally abundant plants (Fox and Morrow 1981; Strong et al. 1984; Bernays and Chapman 1994). In part, the apparent lack of local differentiation among marine herbivores reflects a historical trend in the marine literature; most studies on the feeding ecology of marine herbivores have used large, relatively mobile animals (e.g., fishes, urchins, and large crabs) that are less likely to have intimate relationships with particular seaweeds and are thus less likely to adapt to traits of individual host species (Steneck 1982; Hay and Fenical 1988). These herbivores also usually produce young that disperse widely in the plankton, making adaptation to local resources less likely (Vermeij 1982). However, the larger and better-studied marine herbivores are more similar to large terrestrial herbivores such as buffalo and deer than to small terrestrial insects. A more appropriate test of local adaptation by marine herbivores to their seaweed hosts would focus on herbivorous amphipods; these small, brooding animals are ecologically similar to terrestrial insects (Hay et al. 1987), and thus more likely to respond to spatial variation in their host plant communities.

In this study of the herbivorous amphipod *Ampithoe longimana*, populations sympatric with chemically rich seaweeds in the genus *Dictyota* fed more readily on *Dictyota* (Figs. 2, 3) and reproduced faster (Fig. 6) when feeding on *Dictyota* than did populations beyond the northern endpoint of *Dictyota*'s range. These patterns have a significant genetic basis; feeding preference for *Dictyota* is heritable (Fig. 4)

and among-population variation in feeding preference and juvenile performance persists after several generations in laboratory cultures (Sotka and Hay 2002). To our knowledge, this study and Sotka and Hay (2002) represent the first reports of local genetic differentiation in feeding preferences for seaweeds within a single species of marine herbivore. Our findings suggest that seaweed traits can shape the evolution of their associated marine herbivores.

#### Geographic Variation in Evolution of Tolerance

The strength of selection acting on *A. longimana* changes with geographic location. In areas sympatric with *Dictyota*, selection appears to maintain the high feeding preference for, and juvenile performance on, *Dictyota* species (Figs. 2, 3, 6). Because several other local seaweeds are nutritionally equivalent, or superior, to *Dictyota* as foods for *Ampithoe longimana* (Cruz-Rivera and Hay 2001), it appears that selection for host use may be driven more by the value of the host as a predator-free habitat than by *Dictyota*'s value as a food. The primary predators of small herbivores in North Carolina are omnivorous fishes, which avoid consuming *Dictyota* because of diterpene alcohols produced by the plants (Hay et al. 1987). As a consequence, those herbivore species that associate with *Dictyota* gain protection from consumption by fishes (Duffy and Hay 1994; Stachowicz and Hay 1999). These species-level patterns of escape from predation likely mirror variation in susceptibility among genotypes within a single population; that is, genotypes with high affinity for *Dictyota* may gain protection from omnivorous fishes, whereas genotypes with low affinity suffer heavier predation. Analogous examples of plant-mediated susceptibility to predation are common to other marine (Hay et al. 1987; Sotka et al. 1999; Stachowicz 2001) and terrestrial (Bernays and Graham 1988) systems and can operate over relatively short, micro-evolutionary timescales (e.g., Feder 1995; Brown et al. 1996; Hufbauer and Via 1999).

In areas outside the geographic range of *Dictyota*, *A. longimana* does not accrue the benefit of association with *Dictyota*, and as a consequence, the genetic mechanisms responsible for feeding tolerance for *Dictyota* and its secondary metabolites are not maintained. It remains an open question

whether such degradation is due to selection, drift, or both. Feeding preference for *Dictyota* could be selected against if *Dictyota* preference displayed a negative genetic covariance with preference for an alternative plant whose use benefited the amphipod (cf. Via 1984). In the present case, populations that had high preference for *Dictyota* tended to have low preference for *Hypnea* (Figs. 2,3). However, there was no evidence of a negative correlation in feeding preferences for *Hypnea* and *Dictyota* among families of a single population (Fig. 4C). This result suggests that the evolution of feeding preferences for these seaweeds is occurring independently, and that there is no strong genetic trade-off. This result mirrors previous findings focused on juvenile performance; Sotka and Hay (2002) found that juveniles from a Connecticut population had lower feeding-based fitness on *Dictyota* than did juveniles from North Carolina, but the fitnesses of juveniles on all other seaweeds offered were statistically indistinguishable (the red seaweeds *Hypnea musciformis* and *Gracilaria tikvahiae*, the brown seaweeds *Fucus vesiculosus* and *Sargassum filipendula*, and the green seaweeds *Codium fragile*, *Enteromorpha* sp., and *Ulva* sp.). Thus, if selection lowers tolerance for *Dictyota* in areas outside the range of *Dictyota*, such selection may not be mediated by the use of alternate seaweeds. Instead, maintaining tolerance for *Dictyota*'s chemical defenses may prove to be metabolically expensive, as suggested by Cruz-Rivera and Hay (2003). Populations allopatric with *Dictyota* would be most susceptible to such metabolic costs because they do not gain the benefit of association that *Dictyota* provides.

Alternatively, traits that allow the amphipod to tolerate *Dictyota*'s chemical defenses may not be costly to populations outside the geographic range of *Dictyota*; if true, then the degradation of tolerance for *Dictyota* would be more strongly affected by genetic drift than selection. For instance, the most northerly populations sampled (i.e., CT and RI) are historically separated from populations sympatric with *Dictyota* (Fig. 7; Table 3A); a similar phylogeographic break at or north of Delaware has been documented for a number of estuarine animals (for review see Wares 2002) and its exact causes are unknown. As a consequence of this long separation, degradation of tolerance for *Dictyota* due to the random accumulation of deleterious mutations (i.e., genetic drift) could have progressed unimpeded in CT, MA, and RI without the introgression of "*Dictyota*-tolerant" genotypes from populations sympatric with *Dictyota* (cf. Slatkin 1985). Note that this evolutionary scenario does not preclude a role for selection acting to degrade tolerance.

Patterns of gene flow may also help explain geographic variation in tolerance for *Dictyota*, especially in areas close to the geographic endpoint of *Dictyota*. Populations in MD and DE, which are within about 250 km of the northern endpoint of *Dictyota*, show a tendency to feed more (Fig. 2) and grow better (Fig. 5) on *Dictyota* than do more northerly populations. Interestingly, these MD and DE populations are statistically indistinguishable by mtDNA sequences from populations sympatric with *Dictyota* (Table 3B); this suggests that populations from North Carolina to Delaware either are exchanging genes currently, or gene flow recently ceased and differentiation in COI sequences could not be detected with our sampling regime. If gene flow is ongoing, as suggested

by direct observation of long-distance dispersal on drifting *Sargassum* seaweeds (Fine 1970), then alleles that serve to increase tolerance for *Dictyota* are likely introgressing into the MD and DE populations. Overall then, the present data do not allow for a complete assessment of the relative roles that selection, drift, and gene flow play in shaping the evolution of tolerance of *Dictyota* by allopatric populations. Resolution of these alternatives will require a more exhaustive survey of the clines in tolerance for *Dictyota* and in neutral alleles (Barton and Hewitt 1985), as well as a closer examination of the potential mechanisms of selection.

#### *Heritable Variation of Feeding Preferences*

The marine literature has ascribed most phenotypic variation among consumers to interindividual differences in ontogeny, physiological state, environmental influences, and recent experience (e.g., Palmer 1984; Moran and Arrontes 1990; Lindquist and Hay 1995; Cronin and Hay 1996; Micheli 1997; Rovero et al. 1999; Thacker et al. 1997). Our results demonstrate that the feeding preferences of marine herbivores may also have substantial genetic variation, and may thus be able to evolve in response to their seaweed hosts. There was strong among-individual variation in preferences for *D. menstrualis*, and a full-sib analysis indicated that the variation was at least partly heritable (broad sense heritability = 0.42; Fig. 4A). A separate analysis of hybrids of populations with high and low preference for *Dictyota* indicates that feeding preference is inherited in a largely additive manner and is not sex-linked (E. E. Sotka, unpubl. data). To our knowledge, the only other published marine example of genetic variation in feeding preference comes from the amphipod *Gammarus palustris* (Guarna and Borowsky 1993).

Our estimate of heritability represents the upper bound of a genetic effect (Lynch and Walsh 1998), because the analyses of full-sibs cannot delineate genetic effects from non-additive genetic effects, maternally inherited effects, and unknown environmental effects. However, strong genetic component is suggested because environmental and maternally inherited effects were minimized; the mothers of amphipods used in these heritability assays came from the approximately fifth generation raised on non-*Dictyota* seaweeds in common temperature, light, and salinity conditions.

#### *Local Differentiation in Marine Organisms*

Although most examples of local adaptation in marine systems are for physical parameters, such as salinity, temperature, or pollution gradients (Behrens Yamada 1989; Hilbish 1996), there are a small but growing number of well-documented examples of differentiation in host use: pea crabs within molluscan hosts (Stevens 1990), an amphipod on estuarine substrata (Stanhope et al. 1993), shrimps on sponges (Duffy 1996), barnacles on fire corals (Mokady and Brickner 2001), whelks on mussels and barnacles (Sanford et al. 2003), and several examples of local differences in host use that may or may not be genetically mediated (e.g., a filter-feeding polychaete on seaweeds [Mackay and Doyle 1978], a decorator crab using *Dictyota* for protection rather than food [Stachowicz and Hay 2000], and a herbivorous sea slug on seaweeds [Trowbridge and Todd 2001]).

Though the number is small, we can glean some general trends among these studies. First, nearly all of the published marine examples of local differentiation in host use are among small organisms that have intimate relationships with larger hosts. These hosts act as isolated resource patches and habitats (and thus as selective regimes) for small herbivores in ways that are not possible for larger and more mobile consumers that forage among many different foods (Steneck 1982; Duffy and Hay 1994; Thompson 1994).

Second, life history is not unambiguously correlated with the tendency for local differences in host use. Most marine organisms spawn enormous numbers of larvae that develop over days to weeks in the water column (Thorson 1950). During this time, ocean currents can potentially transport larvae hundreds to thousands of kilometers away from natal populations. Such broad dispersal is thought to enhance gene flow among widely separated populations and impede the evolution of local genetic differences (Endler 1977). However, despite the logic of these assertions, they may not be completely true; growing evidence suggests that gene flow can be dramatically more restricted than the life history of the organism would predict (Swearer et al. 2002). Furthermore, local differentiation in host use can evolve in species of marine spawners (i.e., the barnacle and pea crabs listed earlier).

Moreover, it is equally clear that populations of brooding animals do not always differentiate. In the only published test of local differentiation in a marine herbivore, populations of the herbivorous amphipod *Peramphithoe parmerong* separated by tens of kilometers did not differ in juvenile performance on locally abundant seaweeds (Poore and Steinberg 2001). The amphipods *P. parmerong* and *A. longimana* both brood their offspring, yet only *A. longimana* displayed genetic differentiation in host use, albeit at a much larger spatial scale. In total, the limited number of examples of genetic differentiation in host use suggest that the strength of selection involved may be more influential in generating local differentiation than is the dispersal mode of the organism.

Third, these examples include species from more than one trophic level (i.e., herbivores, parasites, and commensals). Among terrestrial insects, lineages with herbivorous lifestyles tend to have significantly higher speciation rates than do lineages of carnivores (Mitter et al. 1988). Currently, an analogous relationship would be difficult to test among marine consumers, because of taxonomic uncertainties, the prevalence, often unrecognized, of sibling species (Knowlton 1993), and a general lack of information on the feeding ecology of small marine consumers (but see Duffy 1990; Vermeij 1992; Viejo 1999; Williams and Walker 1999; Cruz-Rivera and Hay 2000, 2003).

In summary, the polyphagous marine herbivore *A. longimana* displays strong population-level differentiation in preference for, and fitness on, species in the chemically rich genus *Dictyota*. There is selection acting to increase tolerance of *Dictyota* and its secondary metabolites in areas where the amphipod and seaweed overlap. Amphipods outside the geographic range of *Dictyota* have generally lost their ability to tolerate *Dictyota*, although it is unclear whether selection, drift, gene flow, or some mix of these forces are responsible. More broadly, it is clear that seaweeds can play an important

role in the evolution of mesograzers like *A. longimana*. It remains to be seen whether seaweeds frequently or rarely impose strong selection on other species of marine herbivores and whether such pressures commonly cause the evolution of local differentiation.

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