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Phenotypic evidence for local adaptation to heat stress in the marine snail *Chlorostoma* (formerly *Tegula*) *funebralis*

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ABSTRACT

Southern California (USA) populations of the intertidal marine snail *Chlorostoma* (formerly *Tegula*) *funebralis* generally occupy warmer climates and are exposed to high air temperatures during low tides more often than northern California populations. Available genetic data suggest there is extensive gene flow across a broad range of *C. funebralis* populations, so it is unclear if populations can adapt to differences in local environments. To test for population-specific responses to heat stress, three phenotypic assays were performed on three northern and on three southern populations of *C. funebralis*, after acclimation to common-garden conditions in the laboratory. Thermal drop-down, heat stress mortality, and heat stress reattachment assays were designed to evaluate ecologically relevant phenotypic responses to heat stress; these assays assessed tolerance during, mortality following, and speed of recovery following heat stress. The latter two tests indicate that southern populations consistently suffer significantly lower mortality and recover significantly more quickly following heat stress compared to northern populations. Hierarchical cluster analysis of stress response data clearly identified northern California and southern California regional groupings of populations. Thus, these results indicate that southern populations have higher tolerance to heat stress than northern populations and suggest that adaptation to local environmental differences can evolve despite moderate potential for larval dispersal in this species. Accounting for intraspecific population variation in thermal tolerance may provide important insights for predicting how species distributions will respond to global warming.

1. Introduction

The geographic ranges of many marine organisms span from hundreds to thousands of kilometers. Across these ranges, populations frequently experience significant variation in both biotic and abiotic environments. Persistent variation can promote genetic divergence among conspecific populations as natural selection acts to favor locally adapted phenotypes. However, evolution of local adaptation may be impeded if high rates of migration homogenize the gene pool among populations (Lenormand, 2002; Lewontin, 1974; Mayr, 1963; Slatkin, 1985). In many marine invertebrates with planktonic larvae, the potential for local adaptation is unclear because the balance between selection for local adaptation and the rate of interpopulation gene flow is largely unknown. Although numerous studies suggest that marine populations are not as connected as might be presumed (Burton, 1983; Kyle and Boulding, 2000; Levin, 2006; Marshall et al., 2010) and that adaptive differentiation often occurs in species with planktonic dispersal (Sanford and Kelly, 2010), local adaptation in the sea remains understudied. As rates of environmental change are accelerating due to stressors such as global warming and ocean acidification, predicting future distributions of marine organisms requires increased understanding of the balance of local adaptation and gene flow among populations.

One such marine invertebrate with planktonic larvae, the intertidal snail *Chlorostoma funebralis*, has the widest distribution of the five species in its genus (Bouchet, 2013). *C. funebralis* can be found along the Pacific coast of North America from Vancouver Island, British Columbia to Baja California, Mexico (Abbott and Haderlie, 1980; Sagarin and Gaines, 2002). Previous genetic work using the mitochondrial marker cytochrome oxidase subunit I (COI) found no evidence of differentiation among populations sampled from Oregon to Santa Barbara (Kelly and Palumbi, 2010; Kelly et al., 2010), suggesting that this species has extensive dispersal and may be panmictic across its range. However, *C. funebralis* has a relatively short larval duration of roughly five days (Moran, 1997) and high temperatures, common in the southern portion of the species range, can further reduce developmental times (Hahn, 1989). Hence, *C. funebralis*' short larval duration and broad, environmentally diverse geographic range combine to make adaptive differentiation of populations feasible in this species. Previous experimental studies have shown that local adaptation often occurs in marine invertebrates in response to strong gradients in selective forces such as wave action, temperature, and predation (Sanford and Kelly, 2010). For instance, Kuo and Sanford (2009) found evidence for

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genetically based differences in upper thermal limits in various geographic populations of the intertidal snail *Nucella canaliculata*. We hypothesize that *C. funebralis* may also be locally adapted to the unique temperature environment each population experiences.

The climate across the latitudinal range of *C. funebralis* differs significantly; the maximum, minimum, and average air temperatures along the Pacific coast of North America vary widely (National Oceanographic Data Center (NODC), National Oceanic and Atmospheric Administration and United States Department of Commerce). For instance the maximum air temperature *C. funebralis* experience in the intertidal at Hopkins Marine Station in Monterey (central California) is ~35 °C (Tomanek and Somero, 1999), while the maximum temperature of other intertidal mollusks such as mytilids and littorinids in southern California (i.e. La Jolla) can reach 40 °C (Helmuth et al., 2006; Miller and Denny, 2011). Thus, different populations of *C. funebralis* along the coast likely cope with considerably different temperature maxima.

In this study, we quantified thermally dependent phenotypes to test the hypothesis that northern and southern populations of *C. funebralis* show evidence for local adaptation to emersion-associated heat stress. Such tests elucidate the balance between the selective forces favoring population differentiation versus the homogenizing effects of larval dispersal. We first acclimate individuals from each of six populations to common-garden laboratory conditions and then employ three phenotypic assays to test for differences in thermal tolerance, heat stress mortality, and recovery following heat stress. Our findings suggest that local adaptation can occur despite moderate potential for pelagic larval dispersal. These results help inform predictions regarding potential local extinctions and geographic range shifts resulting from climate change for *C. funebralis*.

2. Materials & methods

2.1. Collection, animal maintenance, and assay preparation

Small to medium sized *C. funebralis* adults (15–20 mm in shell diameter) were collected in the winter of 2011 and the spring of 2012 from three northern California sites: Slide Ranch, Marin Co. (37°52′N, 122°35′W); Pescadero (37°15′N, 122°24′W) and Pigeon Point (37°11′N, 122°23′W), San Mateo Co. and from three southern California sites: Aliso Beach, Orange Co. (33°30′N, 117°45′W); La Jolla (32°52′N, 117°15′W) and Bird Rock (32°48′N, 117°15′W), San Diego Co. (Fig. 1). Snails were transported to Scripps Institution of Oceanography (SIO) within 24 h of collection.

Once at SIO, snails were regularly fed freshly collected *Macrocystis pyrifera*. To eliminate confounding effects due to previous environmental differences, snails were common-garden acclimated for 3–20 weeks in ambient temperature seawater (~15 °C). The entire range of acclimation times was equally represented in all three phenotypic assays. Preliminary trials indicated that variation in acclimation time within this range did not affect population differences in heat stress response (data not shown), so a narrower acclimation time period was not necessary. Acclimation periods did not differ among populations in a single phenotypic assay, and an equal number of individuals from each population collected in both the winter and the spring was used for each of the three assays. Twenty-four hours prior to all assays, individuals from each population were put in weighted "underwater cages" and kept constantly immersed in seawater without food to normalize aerial exposure and feeding status. (Animals in the laboratory feed roughly every day; therefore, a twenty-four hour period is sufficient to normalize feeding status.)

2.2. Heat stress conditions

Because *C. funebralis* inhabit the low to mid intertidal zone (Riedman et al., 1981), they can potentially experience both elevated water temperatures during immersion at high tide and elevated air temperatures during emersion at low tide. However, air temperature varies much more than water temperature (Raffaelli and Hawkins, 1996), and thus severe thermal stress primarily affects *C. funebralis* in the rocky intertidal during emersion, when body temperatures can significantly increase (Sharp et al., 1994). Therefore all heat stress assays were performed in air to mimic the conditions animals experience during low tide in the field.

Fig. 1. *C. funebralis* collecting sites along the California coastline.

2.3. Drop-down assay

A modified knock-down assay (Huey et al., 1992) was developed that could be performed on marine mollusks such as *C. funebralis* (see also Lee and Boulding, 2010). Immediately before experimentation, *C. funebralis* individuals were taken out of their underwater holding cages and at room temperature the foot of each animal was briefly blotted dry with a paper towel. Each individual was then placed on an 8 × 10 cm glass plate until it extended its foot and securely attached to the horizontal glass plate substrate. Excess seawater was blotted dry to prevent individuals from sliding off the glass plates. Each plate with the individual snail attached was then vertically suspended in a Fisher Scientific Isotemp Incubator using large binder clips. Snails were exposed to an air temperature of 35 °C in the incubator, and the time it took for each individual to detach from the suspended glass plate and fall to the bottom of the incubator was recorded. The inability to remain attached to the glass plate suggests that the animal has entered into heat coma (McMahon, 1990); thus, time until "drop-down" was used as a putative measure of thermal tolerance. This phenotype is ecologically relevant because the ability to stay attached to the substrate at a high temperature reduces the chances of a snail falling down into the water, where numerous predators such as starfish, crabs, and/or octopi reside (Fawcett, 1984). For this particular assay 35 °C was chosen as the target temperature since this is the maximum temperature recorded in the field at Hopkins Marine Station (Tomanek, 2002), a site whose climate is representative of the three northern collection sites. Snails that dropped from the glass plates before a minute had elapsed were excluded from the analysis, since this short drop-down time could indicate that the individual did not have a secure initial attachment to the plate. Groups of 10 snails were used in each drop-down assay, and each assay was replicated four times ($n = 40$).

2.4. Heat stress mortality

Dry Petri dishes were equilibrated to 15 °C for 30 min in a temperature-programmable incubator (Thermo Precision Model 818) prior to the start of the assay; high humidity was maintained throughout the test by including a small seawater-saturated sponge in each dish. Snails were removed from their underwater holding cages and a single individual was placed in each Petri dish. At the start of each experiment, air temperature was gradually increased by 3 °C every half hour (starting at 15 °C) to simulate a natural rate of heating snails would experience in the intertidal (Tomanek and Somero, 1999). This gradual increase was continued until the target temperature of 37, 38, 39, 40, or 41 °C was reached, and then the incubator remained at this target temperature for the duration of the experiment. Different individuals from each population were tested at the various target temperatures; no single individual was exposed to multiple heat stresses. The temperature during each experiment was monitored with a HOBO Pendant Water-Resistant Temperature and Light Data Logger (Onset HOBO Data Loggers, Massachusetts). Each heat stress trial lasted a total of 5.5 h (including the ramp time), which is an estimate of a typical low tide period for *C. funebralis* in the intertidal. Because the total ramp time varied for individual trials due to the different target temperatures, the exposure time to each target temperature also varied, with animals in the 37 °C trials experiencing the longest total time at the target temperature, and animals in the 41 °C trials experiencing the shortest total time at the target temperature.

At the end of each heat stress exposure, each dish was filled with 15 °C seawater and dishes were maintained in a 15 °C incubator. Survivorship of each *C. funebralis* individual was assessed six days following the heat stress. Individuals that were not attached to the substrate and that did not retract their foot in response to poking and/or pulling their foot with tweezers were considered dead. Groups of 10 snails were used in each mortality assay, and each assay was replicated two (37, 40 and 41 °C, $n = 20$) or three (38, 39 °C, $n = 30$) times.

2.5. Reattachment during recovery following heat stress

When a *C. funebralis* individual experiences extreme heat stress, it curls the lateral edges of its foot and detaches from the substrate (McMahon, 1990). The time it took each snail to reattach to the Petri dish substrate following heat stress was used as a proxy for recovery time (all individuals were detached from the substrate following heat stress trials). After each 5.5 h heat stress at each temperature described above (37–41 °C), the seawater-saturated sponge was removed from each Petri dish and 15 °C seawater was added, taking care to disturb each animal as little as possible. Animals were kept in these same Petri dishes, and all surviving snails from each experiment were scored as either attached or detached from the Petri dish substrate at 20 min, at 1, 4, 18, 21, and 24 h, and then every 24 h thereafter during recovery. Groups of 10 snails were used in each recovery assay, and each assay was replicated twice. Due to differential mortality following heat stress, between 17 and 20 individuals were monitored for reattachment from each population ($n = 18$ for Slide Ranch, $n = 17$ for Pescadero, $n = 19$ for Pigeon Point, $n = 20$ for Aliso Beach, $n = 19$ for La Jolla, and $n = 20$ for Bird Rock).

2.6. Statistical analyses

All statistical analyses were conducted in R (R Development Core Team, 2008) using a significance value of 0.05. A Shapiro–Wilk normality test revealed that the drop-down data were not normally distributed. Therefore a nonparametric test and associated post hoc analyses were used to compare the northern group of populations (Slide Ranch, Pescadero, and Pigeon Point) to the southern group of populations (Aliso Beach, La Jolla, and Bird Rock) and to examine pairwise differences among the six individual populations, respectively. For the mortality assays, data at each temperature were examined separately, with the northern group of populations and the southern group of populations compared to each other using a Pearson's Chi-square test. To test for differences among the six individual populations at each temperature, a contingency table was used. The Marascuilo procedure, a multiple comparisons approach that is conceptually similar to a Tukey–Kramer posthoc test (Levine et al., 2013), was then employed to test for pairwise differences in the proportion of surviving animals among populations. Like the drop-down assay, the data from the heat stress recovery assay were not normally distributed (Shapiro–Wilk normality test). The data from each temperature trial were treated independently, and a nonparametric test and associated post hoc analyses were used to test for significance between the northern group of populations and the southern group of populations and among the six populations, respectively.

We also performed a cluster analysis using the data from all three phenotypic assays combined (including all target temperatures tested for the survival and reattachment assays) for all six populations. With the pvclust library in R (Suzuki and Shimodaira, 2006), the average linkage method was used to perform bottom-up hierarchical clustering to identify groups in the data. One thousand bootstrap replications were then used to construct a dendrogram, and groups that were strongly supported (based on approximately unbiased (au) p-values greater than 95) were identified. The au p value, which is calculated by multiscale bootstrap re-sampling, is a better approximation to unbiased p value than the bootstrap probability value calculated by ordinary bootstrap re-sampling (Suzuki and Shimodaira, 2006).

3. Results

3.1. Drop-down assay

Although snails from the La Jolla site have the highest median knockdown time of all populations (8.7 min), data from this assay do not differentiate northern (Slide Ranch, Pescadero, and Pigeon Point) and southern (Aliso Beach, La Jolla, and Bird Rock) populations (Wilcoxon rank sum test, $p = 0.162$). Individuals from La Jolla had a significantly higher drop-down time than individuals from neighboring Bird Rock (median 4.8 min, Studentized range Kruskal–Wallis post hoc test, $p = 0.0003$) as well as from the distant Slide Ranch (median 5.7 min, $p = 0.019$), and Pigeon Point (median 4.0 min, $p = 0.005$) sites. Pescadero (median 5.2 min) and Aliso Beach (median 4.9 min) individuals were not statistically different from any of the other populations (Fig. 2).

3.2. Heat stress mortality

Southern populations show significantly higher survival than northern populations at 38, 39, and 40 °C (Pearson's Chi-squared test, $p = 0.005$, $p < 0.001$, $p = 0.008$, respectively). The largest differences between northern and southern populations occurred at 39 °C (Fig. 3). Following this heat stress the southern populations show 90% survival, while the northern populations only show 61% survival. Furthermore, although all populations show a dramatic decline in survival when the heat stress temperature is increased from 39 to 40 °C, the decline for the southern populations is less severe. While survivorship drops to an average of 1.7% for the northern populations at 40 °C, that for the southern populations is only reduced to 15% survival. Significant differences among all six populations were only found at 39 °C (2×6 contingency table using the Chi-square distribution, $p < 0.001$). Significant pairwise differences exist between Pigeon Point (50% survival) and Aliso Beach (90% survival, Chi-square test statistic = 0.4, critical value = 0.35) and between Pigeon Point and Bird Rock (97% survival, Chi-square test statistic = 0.47, critical value 0.32).

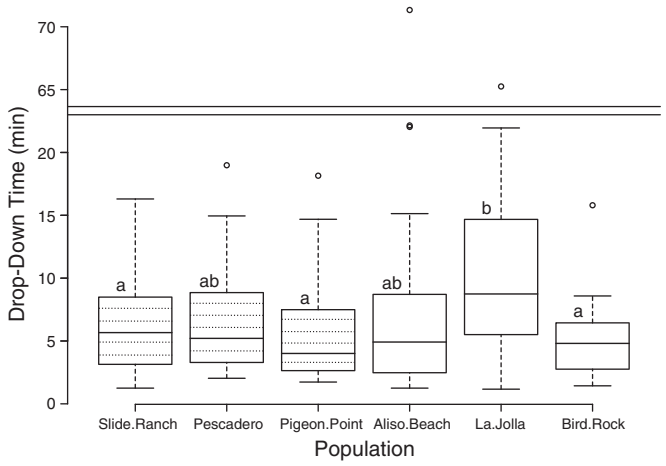

Fig. 2. Boxplot showing median drop-down times of each population. Boxes filled with dotted lines indicate northern populations, and open boxes indicate southern populations. La Jolla individuals have a significantly higher drop down time than individuals from Slide Ranch, Pigeon Point, and Bird Rock. The solid black line within each box represents the median, the upper and lower limits of each box indicate the third and first quartiles respectively, the lines above and below each box represent the high and low values of each dataset respectively, and small circles represent outliers ($n = 40$ for each population). Different letters over each bar indicate significant differences ($p < 0.05$).

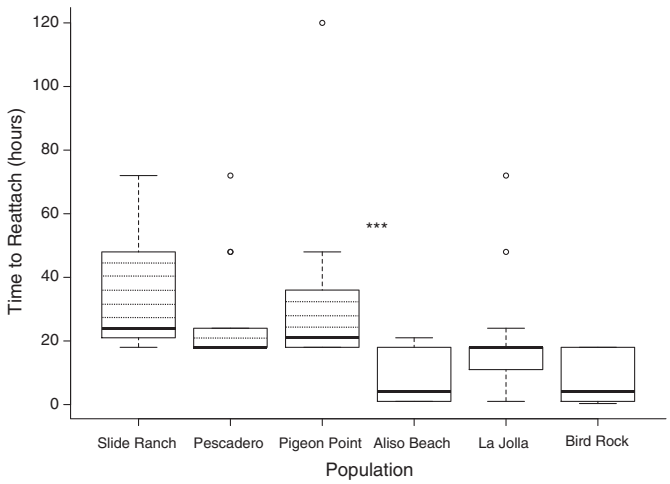

Fig. 4. Boxplot showing median time until reattachment at 38 °C. Boxes filled with dotted lines indicate northern populations, and open boxes indicate southern populations. The three northern populations as a group take significantly longer to recover and reattach to the substrate compared to the three southern populations as a group (*** = $p < 0.01$). The solid black line within each box represents the median, the upper and lower limits of each box indicate the third and first quartiles respectively, the lines above and below each box represent the high and low values of each dataset respectively, and small circles represent outliers ($n = 18$ for Slide Ranch, $n = 17$ for Pescadero, $n = 19$ for Pigeon Point, $n = 20$ for Aliso Beach, $n = 19$ for La Jolla, and $n = 20$ for Bird Rock. Even sample sizes were difficult to obtain due to differential mortality following heat stress, see Section 2.5).

3.3. Heat stress reattachment during recovery

Although there were no significant differences in reattachment between northern and southern populations at 37 or 40 °C, individuals from northern and southern populations did significantly differ in their recovery times following 38 and 39 °C heat stress (Wilcoxon rank sum test, $p < 0.001$, $p = 0.003$ respectively). This difference was most pronounced after a 38 °C heat stress (Fig. 4). Under these conditions northern populations took significantly longer to reattach (median 21 h) than southern populations (median 4 h). There were also significant differences among all six individual populations at 38 and 39 °C (Kruskal–Wallis test, $p = < 0.001$, $p = 0.02$, respectively). At 38 °C eight significant pairwise differences were observed (Table 1); Slide Ranch and Pigeon Point were both significantly different from each of the three southern populations. No northern populations were significantly different from each other, and neither were any southern populations. At 39 °C Slide Ranch animals took significantly longer to reattach than Bird Rock animals (Studentized range Kruskal–Wallis post hoc test, $p = 0.001$) and Aliso Beach and Bird Rock animals also showed significant differences in reattachment times ($p = 0.04$).

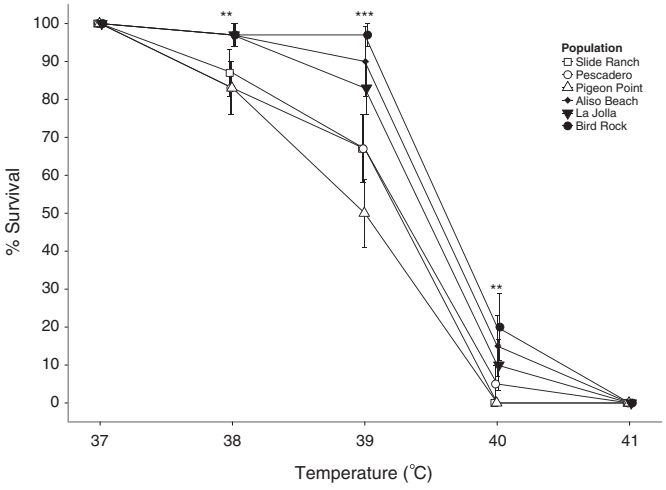

Fig. 3. Percent survival for each population following 37–41 °C heat stress. Open symbols indicate northern populations, and filled symbols indicate southern populations. Data are means ± 1 SE ($n = 20$ for each 37, 40 and 41 °C data point, and $n = 30$ for all other data points). Asterisks indicate a significant difference in survival between the three northern populations as a group compared to the three southern populations as a group at a given temperature. ** = $p < 0.01$; *** = $p < 0.001$.

3.4. Cluster analysis

The six populations group into two distinct clusters, with one group containing the three northern populations and the other group containing the three southern populations (Fig. 5). Within these two groups, Slide Ranch and Pigeon Point formed an additional subgroup, as did Aliso Beach and Bird Rock. Two out of the four approximately unbiased (au) p-values for the cluster analysis were greater than 90; the au value for the general northern clade was 91, and the au value for the northern clade subgroup was 94.

4. Discussion

Two of three experimental tests of thermal response showed clear evidence for enhanced thermal tolerance in southern versus northern populations. Following common garden acclimation, southern populations show significantly higher survival and reattach to the substrate following heat stress significantly faster than northern populations. These results suggest that southern populations possess genetic adaptations to tolerate the extreme heat stress they experience, whereas northern populations are less adapted to such severe conditions.

Table 1
38 °C heat stress recovery assay p-value results from Studentized range Kruskal–Wallis post hoc pairwise comparisons. Values in bold are significant ($p < 0.05$).

	Slide Ranch	Pescadero	Pigeon Point	Aliso Beach	La Jolla	Bird Rock
Slide Ranch						
Pescadero	0.351					
Pigeon Point	0.876	0.862				
Aliso Beach	<**0.001**	<**0.001**	<**0.001**			
La Jolla	**0.007**	0.337	**0.038**	0.115		
Bird Rock	<**0.001**	<**0.001**	<**0.001**	0.074	0.100	

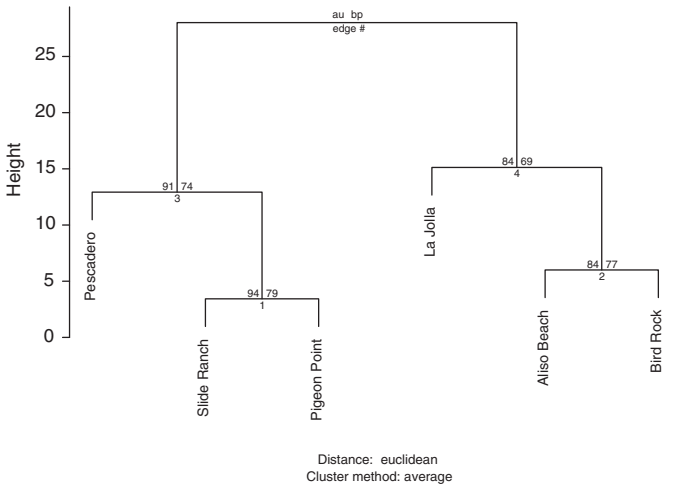

Fig. 5. Cluster dendrogram showing a northern and a southern clade based on the combined data from the phenotypic assays. Values on the left side of each node are the approximately unbiased (au) p-values, and values on the right side are the bootstrap probability (bp) values. The vertical height axis refers to a distance measure between the clusters, which was calculated during the hierarchical clustering procedure used to construct the dendrogram.

It is worth noting a fundamental assumption of our common-garden approach is that the different phenotypic responses to heat stress among *C. funebralis* populations are genetically based (Ballentine and Greenberg, 2010; Franssen et al., 2011). We have utilized a relatively long acclimation period comparable to previous marine mollusk local adaptation studies (Daka and Hawkins, 2004; Sokolova and Pörtner, 2001; Yee and Murray, 2004) to minimize the chances that our common-garden design identifies residual effects from the previous environments of the animals. However, developmental plasticity, persistent acclimation, and other environmental and epigenetic influences during the lifespan of the experimental animals cannot be completely ruled out (Kawecki and Ebert, 2004; Kinne, 1962; Zamer and Mangum, 1979).

In addition to our observations of differential responses to heat stress in northern and southern *C. funebralis* populations, previous work suggests that other differences between northern and southern populations are also genetically based. Frank (1975) found that warm and cold-water populations of *C. funebralis* display differences in shell growth rates. Moreover, Fawcett (1984) concluded that northern and southern populations of *C. funebralis* are genetically differentiated after observing that in order to avoid predation, transplanted southern snails climb to higher shores more quickly and ultimately reach higher heights compared to transplanted northern snails. More recently, Yee and Murray reported that northern and southern *C. funebralis* populations separated by more than 300 km display differences in both activity and feeding response to temperature (Yee and Murray, 2004). These different temperature responses of northern and southern snails led Yee and Murray (2004) to suggest that *C. funebralis* populations are locally adapted to regional conditions. Overall these results, combined with our data in this current study, suggest that widely separated populations of *C. funebralis* experience varying habitats and environmental stresses, and they may genetically adapt to these different environments in multiple ways.

Although we expected the drop-down assay would also show a distinction between northern and southern populations, patterns between the geographic regions were unclear. At least two confounding factors may have influenced this assay. First, since individuals of northern populations have been suggested to occur lower in the vertical intertidal zonation (Fawcett, 1984) and hence experience more wave action, they could have unknown adaptations for stronger substrate attachment than southern populations. This has been observed in other marine mollusks such as *Littorina saxatilis* (Martínez-Fernández et al., 2010). Although preliminary results showed no difference in drop-down time between Pigeon Point and La Jolla snails at room temperature, this could be investigated further. Second, *C. funebralis* individuals, like other marine gastropods, may use mucous threads to help them adhere to substrates (Denny, 1984; Grenon and Walker, 1981; Smith et al., 1999). If this were the case, the ability of a single animal to stay attached to a substrate during heat stress would not be solely dependent on the individual's physical status.

4.1. Thermal stress in the Chlorostoma genus

Although prior work has demonstrated differences in thermal tolerances in *Chlorostoma* congeners found at varying tidal heights, this study is the first to investigate differences in thermal tolerance, mortality, and recovery following heat stress across a geographic range of *C. funebralis* populations. Tomanek and Somero (1999, 2000) have shown that *Chlorostoma brunnea* occupying lower regions of the intertidal exhibit lower thermal tolerance and suffer higher mortality than species such as *C. funebralis* that occupy higher intertidal zones. The current work, which finds that southern populations are more thermally tolerant than northern populations, adds valuable information to the growing body of empirical knowledge about the varying thermal tolerances in the genus *Chlorostoma*.

4.2. Local adaptation and gene flow

Previous work found no genetic structure in the mtDNA marker cytochrome oxidase subunit I (COI) in *C. funebralis* populations along the Pacific coastline (Kelly and Palumbi, 2010; Kelly et al., 2010), presumably due to gene flow via larval dispersal. Our data suggest that local adaptation has evolved in *C. funebralis* despite this apparent lack of genetic differentiation. Several possible explanations (not necessarily mutually exclusive) can be offered to reconcile the current study with previous findings. First, the genetic loci responsible for thermal tolerance in *C. funebralis* may be under selective pressures that do not affect the marker COI. If this were the case, some loci may show little geographic variation while others can show extreme differentiation (Slatkin, 1985). Provided that habitat-specific selection is strong enough to overcome migration, apparent gene flow at one locus such as COI does not preclude differentiation at other regions of the *C. funebralis* genome that are relevant to local ecology (Brown et al., 2001).

Significant self-recruitment combined with high levels of effective selection could also facilitate local adaptation in *C. funebralis* despite gene flow. Only a few migrants per generation are necessary to maintain genetic homogeneity among populations (Wright, 1931); thus, a lack of population structure as indicated by COI does not necessarily indicate a lack of local recruitment. Self-recruitment could increase the chances for local adaptation at alleles with habitat-specific fitness by reducing genetic exchange among populations at these loci (Strathmann et al., 2002). However, local recruitment can only facilitate adaptive differentiation if selection provides a barrier to gene flow at ecologically relevant loci; effective selection, $N_e s$, must be greater than effective migration, $N_e m$ (Brown et al., 2001; Slatkin, 1985). As described in the hypothetical example above, for *C. funebralis* substantial local recruitment could allow selection to act on local populations, adapting each to better cope with its unique environmental stressors, such as heat, and causing ecotypic genetic differentiation.

Another explanation for apparent local adaptation amidst a lack of genetic structure is differential post-settlement survival, or immigrant inviability (Hendry, 2004; Marshall et al., 2010; Nosil et al., 2005; Strathmann et al., 2002). This scenario could result in no genetic difference in genes such as COI, but genes that may confer survival advantages, such as heat shock proteins, could show habitat-specific differences in alleles over time. This has been seen in terrestrial organisms such as the California serpentine sunflower (Sambatti and Rice, 2006), and also in marine invertebrates such as the blue mussel (Hilbish, 1985; Koehn et al., 1980) and the northern acorn barnacle, *Semibalanus balanoides* (Schmidt and Rand,

1999, 2001). For example in *S. balanoides*, certain genotypes of the *Mpi* locus, which metabolizes the mannose found in the barnacles' algal food, experience a pulse of genotype-specific mortality before the larvae metamorphose (Schmidt and Rand, 2001). To address this hypothesis of low immigrant survival in *C. funebralis*, thermal tolerance assays (and genetic studies) could be performed on new recruits and not just on sexually mature adults such as those used in the current study.

4.3. Coping with climate change

Our finding that populations of *C. funebralis* show different thermal tolerances is important to consider in the context of global warming (Sorte et al., 2011). Previous work has demonstrated that, somewhat unexpectedly, more warm-adapted animals may be less able to respond to climate change than more cold-adapted animals because the warm-adapted animals are already close to their upper thermal limit (Somero, 2010; Stillman, 2003; Tomanek, 2010). Our study compares thermal tolerances of northern California *C. funebralis* populations that experience maximum temperatures of 35 °C upon emergence from the intertidal and of southern California populations that can experience maximum temperatures around 40 °C. Our data demonstrate that northern California populations have a relatively large thermal buffer. At least 50% of individuals can survive at 39 °C, 4 °C higher than the maximum temperature that they are likely to experience in the field. Conversely, the southern populations already appear to be at their upper thermal limit; they demonstrated 100% mortality at 41 °C, a temperature they could experience in the field. This result is consistent with a previous study that investigated the thermal limits of heart function in *Chlorostoma* congeners; Stenseng et al. (2005) found that *C. funebralis* can encounter body temperatures in the field in southern California that exceed its flatline temperature, the temperature at which the heart stops beating upon heating. Thus, although southern populations show higher survival than northern populations at 38–40 °C, it appears that southern populations will not be able to cope with temperature increases without suffering complete mortality. These population-specific responses to thermal stress could have a large effect on future local extinctions and geographic range shifts for *C. funebralis*.

Finally, it is worth noting that the assays employed here identify clear differences between populations, but only over a relatively narrow range of temperatures. We suspect that this is partly an artifact of the crude nature of the assays themselves (end points of mortality or recovery time). The extent to which populations differ in unmeasured and potentially more subtle responses remains to be determined. For example, although all populations survived the 37 °C stress, we do not know if they incurred similar costs in terms of cellular damage and potentially reduced future fecundity; such tests represent important challenges for future work.

4.4. Conclusions

This study found phenotypic evidence for local adaptation to heat stress in *C. funebralis*, a marine gastropod with planktonic larvae and no previously identified population structure. Two of the three phenotype assays performed indicate that southern California populations have a higher thermal tolerance than northern California populations. Our results suggest different *C. funebralis* populations possess unique adaptations to tolerate emersion-associated heat stress, and hence will allow more informed predictions of how populations will respond to future environmental changes. Further studies are needed to uncover the genetic basis of this local adaptation to heat stress in *C. funebralis*.

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