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Evolutionary trade-offs between baseline and plastic gene expression in two congeneric oyster species

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Organismal responses to environmental stresses are a determinant of the effect of climate change. These can occur through the regulation of gene expression, involving genetic adaptation and plastic changes as evolutionary strategy. *Heat shock protein* (*hsp*) family genes are extensively expanded and play important roles in thermal adaptation in oysters. We investigated expression of all heat-responsive *hsp*s in two allopatric congeneric oyster species, *Crassostrea gigas* and *C. angulata*, which are respectively distributed along the northern and southern coasts of China, using common garden and reciprocal transplant experiments. Our results showed that *hsp*s in *C. gigas* have evolved higher basal levels of expression under ambient conditions at each field site, with lower expression plasticity in response to heat stress in comparison to *C. angulata*, which exhibited lower baseline expression but higher expression plasticity. This pattern was fixed regardless of environmental disturbance, potentially implying genetic assimilation. Our findings indicate divergent adaptive strategies with underlying evolutionary trade-offs between genetic adaptation and plasticity at the molecular level in two oyster congeners in the face of rapid climate change.

1. Introduction

Understanding individual responses to environmental stresses, a central goal of evolutionary ecology, is an urgent task in the face of rapid climate change. Genetic adaptation and phenotypic plasticity represent two forms of evolutionary strategy to respond to environmental variability [1,2]. The choice between these strategies is species specific and often involves evolutionary trade-offs between genetic variation and plasticity, which depend on interactions between genes and the environment [3,4]. The regulation of gene expression is a common factor reflecting both genetic and environmental variations. Many constitutive divergences have a heritable genetic basis, indicating evolutionary adaptation under normal conditions [5], whereas environmentally induced regulatory patterns indicate plastic change. In most cases, expression patterns of gene regulation after stressful stimuli indicate divergence among related species with different thermotolerances [4].

Oysters inhabiting highly variable intertidal zones provide an ideal model to investigate such adaptive strategies, and the destruction of oyster reefs
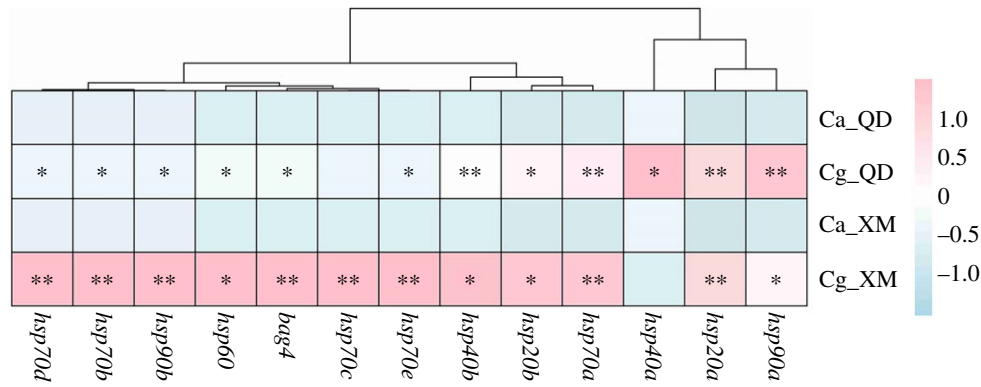
Figure 1. Heatmap for the basal level of *hsp*s in two congeneric oyster species under ambient field conditions. Asterisks indicate significant difference between species at each habitat (Ca_QD versus Cg_QD, Ca_XM versus Cg_XM; *0.05 < *p* < 0.01, **p < 0.01). Ca, *C. angulata*; Cg, *C. gigas*; QD, Qingdao; XM, Xiamen. (Online version in colour.)

worldwide necessitates assessment of their adaptive potential [6,7]. Oysters have evolved a series of genetic and physiological adaptations that confer resilience against harsh environmental conditions, especially in *heat shock protein* (*hsp*) genes. *Hsp* family genes are extensively expanded in oysters and are strongly upregulated as a stress response to changes in ambient conditions [8,9], which is a common feature in other marine invertebrates such as amphipods and corals [4,10]. Meanwhile, the *Bcl-2 associated athanogene* (*bag*) genes are highly conserved across wide evolutionary distances and have been found to be involved in stress tolerance by positively or negatively regulating the function of *hsp* genes [11]. Analysing these genes should provide insights into adaptive mechanisms and enable assessment of evolutionary potential.

Two allopatric congeneric oyster species, *Crassostrea gigas* and *C. angulata*, are the dominant oyster species, respectively, distributed along the northern and southern coasts of China, which are separated by the Yangtze River [12]. Phylogenetic analysis has shown that they diverged approximately 2.7 Ma [13]. Comparative laboratory and field studies have demonstrated their adaptive divergence in detail at morphological, physiological, molecular and genomic levels [3,14,15]. However, the evolutionary relationship between the two oysters is controversial and some authors have considered them as a single species [16], while other authors concluded that they are genetically closely related species [12,14,17]. Few studies have been reported regarding the evolutionary responses of these two oyster congeners in response to climate change. To investigate these strategies, we collected wild oysters of both species, conducted common garden and reciprocal transplant experiments and analysed the patterns of baseline and plastic expression between northern and southern oysters to reveal their adaptive mechanisms in response to climate change.

2. Material and methods

(a) Common garden and reciprocal transplant experiments

The experimental design is described in detail in a previous study by Li *et al.* [3]. Briefly, two oyster congeners, *C. gigas* and *C. angulata*, were collected from their respective northern and southern coastline habitats in Qingdao (QD, 35°44′ N) and Xiamen (XM, 24°33′ N). Mean air and sea surface temperatures at the southern site were 8.7°C and 6.6°C higher than those at

the northern site during the past 2 years, respectively [3]. We conducted a one-generation common garden experiment under identical conditions for both oyster congeners to alleviate maternal effects on gene expression [18]. For each species, eggs from 30 mature females were mixed and divided among 30 beakers. Sperm from 30 mature males were crossed individually with each beaker of eggs. Culture of zygotes and larvae was conducted under farm conditions, and spat and juvenile oysters were cultured in the sea [3]. Eight-month-old F_1 progenies were transported to their origin sampling sites as a reciprocal transplant experiment to test the effects of the local environment. After two months of acclimatization, adult progeny oysters were collected for gene expression measurements at each site. These were cleaned to remove epifauna and reared in aquaria for 15 days with aerated and sand-filtered seawater at 20°C, changed daily. Commercial spirulina powder was added once per day as a food source. No mortality was detected during laboratory acclimation.

(b) Gene expression measurements

Oysters reared as described above were exposed to 35°C, the sublethal temperature of *C. gigas* and the maximum air temperature of the southern site [3], for 48 h. Gills were sampled at 0, 3, 6, 12, 24 and 48 h and immediately frozen in liquid nitrogen and stored at −80°C for subsequent RNA extraction (*n* = 15 per time point). RNA extraction, cDNA synthesis using 1 µg mixed RNA from each of five oysters, and qRT-PCR using two technical replicates for each cDNA were performed as described in previous studies, with six replicates for each time point [3,7]. All strongly thermally induced *hsp* family genes (including the *hsp*-regulating gene *bag4* and collectively called *hsp*s) were selected as candidate genes [8,19]. Primers are shown with their amplification efficiency for the *hsp*s and the reference gene elongation factor alpha (*ef1α*) in electronic supplementary material, table S1. Relative expression under heat shock was determined by the method of $2^{-\Delta\Delta C_T}$ [20]. Adult oysters of both species were collected from ambient conditions of each field site, and basal expression of *hsp*s in the gills was determined in the same manner (*n* = 15 for each site). To compare basal levels of *hsp*s between *C. gigas* and *C. angulata* under ambient conditions, we first calculated the mean basal level in *C. angulate* ($\overline{E_a}$). Relative basal level of each replicate *hsp* in *C. gigas* (E_{gi}) was individually contrasted with $\overline{E_a}$, using the following formula:

$$\overline{E_a} = \frac{\sum_n^{i=1} 2^{-(C_{Tic,a} - C_{Tir,a})}}{n}$$

$$\text{and} \quad E_{gi} = \frac{2^{-(C_{Tic,g} - C_{Tir,g})}}{\overline{E_a}},$$

where C_{Ti} indicates the C_T value of *i*th replicate, *c* indicates each
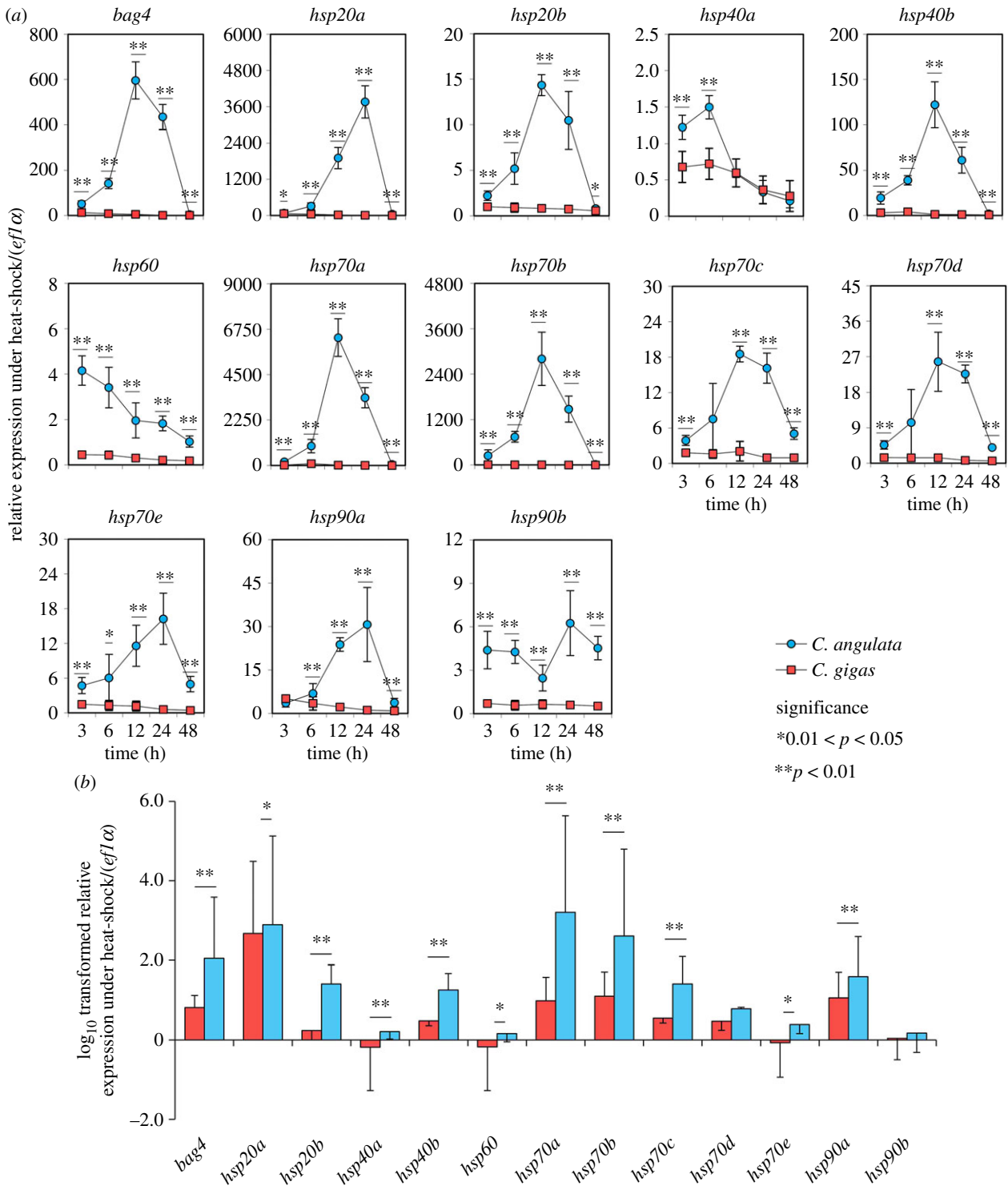
Figure 2. Relative expression of *hsps* in *C. gigas* in comparison to *C. angulata* under heat shock at 35°C. Oysters were sampled at (*a*) Qingdao and (*b*) Xiamen (log$_{10}$ transformed). Asterisks indicate significant differences between species at each sampling time, and error bars represent 1 s.d.

candidate gene, *r* indicates reference gene (*ef1α*), *a* indicates *C. angulata*, *g* indicates *C. gigas*.

(c) Statistical analyses

All statistical analyses were carried out using R v. 3.5.0. The comparison of basal expression between *C. angulata* ($\overline{E_a}/\overline{E_a} = 1$) and the relative basal level for *C. gigas* ($E_{gi}/\overline{E_a}$) was made using a one-sample Wilcoxon single-rank test. The comparison for relative expression under heat shock for each *hsp* between two oyster congeners was conducted using a *t*-test after confirming normality of distribution using a Kolmogorov–Smirnov test and homogeneity of variance using Bartlett's test. Species, *hsp* genes, time of sampling and their interactions were modelled as fixed factors. The significance of fixed factors was evaluated

using likelihood ratio tests. If an effect of species, gene or time was found to be significant at *p* < 0.05 following false discovery rate correction using the Bonferroni method, a post hoc Tukey test using the *ghlt* function of the *multcomp* package [21] was used to evaluate the significance of each pairwise comparison.

3. Results

Both basal expression of *hsps* under ambient conditions and relative expression under heat shock were strongly affected by species, gene, time point and their interactions at both sites (*p* < 0.05, electronic supplementary material, table S2). The relative basal expression of 12 of the 13 *hsps* in *C. gigas* was significantly higher than in *C. angulata* at both sampling

sites (1.3–4.2-fold higher at Qingdao and 1.6–8.6-fold higher at Xiamen; $p < 0.05$; figure 1).

All genes in *C. angulata* showed dramatic fluctuations in expression from 1.2-fold (*hsp40a* at 3 h) to 6.3×10^3-fold (*hsp70a* at 12 h) at the northern site, as well as a 1.6×10^3-fold (*hsp70a* at 6 h) change at the southern site when the oysters were exposed to high temperature (figure 2*a*). However, 11 of 13 genes in *C. gigas* showed no more than 10-fold changes, and the greatest thermally induced change was only 85-fold (*hsp70a* at 6 h) at the northern site (electronic supplementary material, figure S1). Similarly, all *hsps* in *C. gigas* showed more moderate expression patterns than in *C. angulata* at the southern site (figure 2*b*). Furthermore, highly increased expression of most *hsps* in *C. angulata* occurred at later stages (12–24 h) during heat shock, but occurred at earlier stages (3–6 h) in *C. gigas*.

Expression of most genes (11 of 13) in *C. angulata* obviously increased at 6 h and sharply decreased at 24 h, while those in *C. gigas* continuously decreased after 3 h during heat stress at QD. We chose 6 h as a time point to show the magnitude of divergence between the two species cultured at XM.

4. Discussion

The basal levels of *hsp* expression under ambient conditions at each field site and relative expression under heat shock showed divergent expression patterns between the two species. Southern oysters (*C. angulata*) showed greater expression plasticity than the northern species (*C. gigas*) in response to thermal stress. Other marine invertebrates, such as corals, show a similar pattern, where inshore warm-adapted populations require a greater degree of plastic changes to be resistant to a thermally stressful environment [22]. Considering previous findings [3,6,7,23], we propose that oysters inhabiting thermally stressful environments may preferentially evolve high expression plasticity. However, a concern should be raised that this sublethal temperature may have led to cellular damage in the northern oysters, resulting in lower expression plasticity. Furthermore, warm-adapted *C. angulata* exhibited higher expression plasticity of *hsps* at a later stage of heat shock, while this occurred in northern *C. gigas* at an early stage. This delay is consistent with our previous findings that a greater extent of transcriptional plasticity in thermotolerant oyster populations is delayed in comparison to their thermosensitive counterparts [7]. Whereas there is high species specificity in transcriptional responses to temperature, the fact that cold-adapted species may exhibit a long lag time in upregulating *hsp* synthesis—as seen in this study and in a study of congeneric marine snails [24]—suggests that the speed with which an adaptive response can be elicited may vary with species' adaptation temperatures.

However, *C. gigas* showed higher basal expression under ambient conditions at both field sites. This finding confirms that there are evolutionary trade-offs between the basal and plastic expression levels of *hsps* in these species, implying evolutionary trade-offs between the acquisition of extreme tolerance limits and the retention of highly plastic tolerance limits [25]. Identical expression patterns have been similarly detected in a common fish *Fundulus heteroclitus*, in which *hsc70* mRNA in southern individuals showed higher thermally induced expression plasticity than that in northern individuals [26]. This expression trade-off is species specific, while in contrast, the thermotolerant amphipod *Eulimnogammarus cyaneus* dwelling in the upper littoral zone exhibited higher constitutive expression of HSP70 proteins under normal physiological conditions and lower plastic expression after heat stress than those of its more thermosensitive congener *E. verrucosus* in the sublittoral zone [4]. However, further studies are required to quantify relationships between *hsp* gene and HSP protein expression under these conditions. In addition to the molecular level, evolutionary trade-offs were also demonstrated at the organismal level: oysters inhabiting environments with greater thermal fluctuation evolve higher thermotolerance but slower growth than their counterparts inhabiting moderate environments [7], as well as oysters dwelling in intertidal or subtidal zones [6].

Interestingly, the decreased gene expression plasticity in northern oysters was consistently exhibited at both sites, regardless of the differences in the environment. This potentially indicates that expression plasticity between the two oyster species may be fixed with a genetic basis. This same pattern of high baseline expression plus lower expression plasticity in the heat-sensitive species is in line with the evolutionary mechanism of genetic assimilation, in which derived populations lose plasticity in new environments [27–30]. Our results provide further evidence of evolutionary divergence of the two oysters and indicate that genetic assimilation is likely to have occurred, mediating the adaptive evolution of these two species.

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