Received Date : 19-Nov-2015
Revised Date : 14-Jun-2016
Accepted Date : 14-Jun-2016
Article type : Standard Paper

Section: Evolutionary Ecology
Editor: Brent Sinclair

Adaptation to heat stress reduces phenotypic and transcriptional plasticity in a marine copepod

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Running head: Adaptation reduces plasticity

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1365-2435.12725

Summary

1. Organisms may respond to changing environments through phenotypic plasticity or adaptive evolution. These two processes are not mutually exclusive, and may either dampen or strengthen each other's effects, depending on the genetic correlation between trait values and the slopes of their norms of reaction.
2. To examine the effect of adaptation to heat stress on the plasticity of heat tolerance we hybridized populations of the crustacean *Tigriopus californicus* that show divergent phenotypes for heat tolerance. We then selected for increased heat tolerance in hybrids and measured heat tolerance and the phenotypic plasticity of heat tolerance in both selected lines and unselected controls.
3. To test whether changes in phenotypic plasticity were associated with changes in the plasticity of gene expression, we also sequenced transcriptomes of selected and unselected lines, both under heat shock and at ambient temperatures.
4. We observed increased heat tolerance in selected lines, but also lower phenotypic and transcriptional plasticity in response to heat stress. The plastic response to heat stress was highly enriched for hydrolytic and catalytic activities, suggesting a prominent role for degradation of mis-folded proteins.
5. Our findings have important implications for biological responses to climate change: if adaptation to environmental stress reduces plasticity, then plasticity and adaptive evolution will make overlapping, rather than additive contributions to buffering populations from environmental change.

Introduction

Phenotypic plasticity and adaptive evolution are two possible organismal responses to environmental change. These processes act at different scales: plasticity is expressed in the lifetime of a single organism, while adaptation occurs in populations, over multiple generations. As a result, plasticity has historically been viewed as separate from adaptive evolution (Simpson 1953), and some theoretical models predict that plasticity will shield genotypes from the effects of selection (Falconer 1981; Ancel 2000; Price, Qvarnström & Irwin 2003). However, there is also a growing appreciation that phenotypic plasticity could play a key role in adaptation to new and changing environments (West-Eberhard 2003; Badyaev 2005; Chevin, Lande & Mace 2010; Chevin & Lande 2010; Pfennig *et al.* 2010). Plasticity may increase the likelihood of persistence in a new

environment, and thus "buy time" for adaptation, a process known as the Baldwin effect (Baldwin 1896, 1902; Simpson 1953; Lande 2009; Chevin, Lande & Mace 2010; Chevin & Lande 2011). Plasticity may also provide new targets for evolutionary processes, via genetic assimilation (Waddington 1956, 1959; West-Eberhard 2003; Badyaev 2005; Pigliucci, Murren & Schlichting 2006; Crispo 2007; Lande 2009; Schlichting & Wund 2014).

But while it is clear that plasticity and adaptive evolution are likely to interact in some way to influence phenotypic trajectories during periods of environmental change, it is less clear whether in most cases they will dampen or strengthen each other's effects (Chevin *et al.* 2013; Samani & Bell 2016). The nature of their interaction will depend on the genetic correlation between trait values in the new environment and the slopes of their norms of reaction, the curve that describes the relationship between trait values across different environments for a particular genotype (Scheiner 1993)(Fig 1). However, this relationship is unknown for most traits. Selection for increased trait values may lead to a correlated increase in plasticity if only the most plastic genotypes are able to reach the most extreme phenotypes (Fig 1a). Alternately, there may be a trade-off between plasticity and increased trait values, with the genotypes producing the most extreme phenotypes also having the lowest plasticity. In this case selection for increased trait values is expected to lead to a loss of plasticity (Fig 1b). This is especially likely in cases where plastic and evolved responses have the same underlying physiological mechanism: if adaptive evolution occurs through converting the plastic response into a fixed trait value, then adaptive evolution will tend to lead to a loss of plasticity. Finally, in cases where variation in trait means and variation in plasticity have a separate mechanistic basis, there may be no genetic correlation between plasticity and trait values, so that evolution of increased trait values produces no correlated change in plasticity (Fig 1c).

There are several possible tests of the three scenarios described above. One possibility is to compare ancestral and derived trait values in natural systems; for example, many phylogenetically controlled comparisons indicate a fixed trait value has evolved from a plastic ancestor (Janzen & Paukstis 1991; Aubret, Shine & Bonnet 2004; Otaki *et al.* 2010). In other cases, comparisons of contemporary populations indicate a loss of plasticity in response to recent environmental change or range expansions (Otaki *et al.* 2010; Scoville & Pfrender 2010; Grossman & Rice 2014). However, as with any pair of traits, phenotypic correlation does not necessarily imply an underlying genetic correlation, because trait correlations may also arise from correlated selection pressures (e.g. simultaneous selection for increased trait mean and for decreased plasticity)(Stebbins 1950; Armbruster & Schwaegerle 1996).

Interactions between adaptation and plasticity can also be tested through experimental evolution, which can provide some of the most direct evidence for a linkage between two traits because sources of selection and population histories can be controlled (Fry 2003). Indeed, several experimental evolution studies have shown a change plasticity as a correlated response to selection on mean trait values (Scheiner 2002; Garland 2006; Macke *et al.* 2011; Sikkink *et al.* 2014). However the response to selection in these experiments depends on standing genetic variation in the source population or new mutations that occur over the time frame of the experiment, and so they may provide less information about longer term evolutionary processes.

A final strategy, when seeking to disentangle genetic correlations among traits, is to hybridize naturally divergent populations, and then impose selection for the trait of interest (Earley & Jones 2011; Parts *et al.* 2011; Arnegard *et al.* 2014). This strategy allows for the quantification of effects of loci involved in adaptations that have already occurred in nature, while separating the effects of multiple sources of selection, drift, and population history, which can complicate comparisons of natural populations. Here, we employed this third strategy, hybridizing populations of the copepod *Tigriopus californicus* that were divergent for heat tolerance, and then subjecting F2 hybrids to laboratory selection for increased tolerance. This allowed us to directly test whether selection for increased heat tolerance leads to a correlated change in the plasticity of heat tolerance.

T. californicus, a small (~1mm) crustacean abundant in rocky splashpools along the Pacific coast of North America, is an emerging model system for the genetics of adaptive and nonadaptive divergence among allopatric populations (Pereira *et al.* 2016). *Tigriopus* exhibits both evolved and plastic responses to heat stress: individuals from southern populations have higher thermal optima and higher upper lethal limits, and exposure to prior heat stress leads to increased upper lethal limits within a single generation (Willett 2010; Kelly *et al.* 2012).

We tested for changes in plasticity as a correlated response to selection in in two ways: we measured upper lethal limits (LT_{50}'s) and the phenotypic plasticity of upper lethal limits in selected lines and un-selected controls. We also measured the transcriptomic response to heat shock in both sets of lines, to test for an effect of selection on the 'genomic reaction norm' (Aubin-Horth & Renn 2009). This approach allowed us to examine the genetic basis of the plastic responses to heat stress. It is often difficult to draw causal linkages between changes in gene expression and downstream changes in other traits (Toth *et al.* 2007; Becks *et al.* 2012). By testing for an effect of selection on both the plasticity of heat tolerance phenotypes, and the transcriptomic response to heat stress, we were able to test for an association between changes in the plasticity of a

macroscopic trait (heat tolerance) and changes in the plasticity of gene expression. We observed increased upper lethal temperatures in selected lines, but also lower phenotypic and transcriptional plasticity in response to heat stress. Our results suggest that adaptive evolution and plasticity will make overlapping rather than additive contributions to phenotypic changes in the heat tolerance of this species in the context of climate change.

Materials and methods

Field collection, crosses, and selection for increased heat tolerance

Field collection and copepod culture are described in detail in Kelly *et al*. (2012). Briefly, we established laboratory cultures of *T. californicus* from two sites, Salt Point in northern California, USA, (SA, 39˚20'N, 123˚33'W) and Bird Rock in southern California (BD, 32˚49'N, 117˚16'W). At each site we collected individuals from 3–4 tidepools. These pools included both the deepest/most shaded and the shallowest/most exposed pools we could find, encompassing the range of thermal conditions present at a site. We initiated one laboratory culture for each pool, with 50 gravid females per culture, and maintained cultures at 19˚C under 12h light/12h dark conditions. We maintained cultures in the laboratory for >10 generations before establishing crosses. We kept generations separate and established each new generation with 40 haphazardly selected mate-guarding pairs.

Previous work has established that copepods from northern and southern sites differ in their thermal tolerances, with LT_{50} temperatures of 34.8˚C and 36.5˚C for SA and BD respectively, and also that genetic variation for thermal tolerance within sites is limited, with 99% of genetic variation for thermal tolerance partitioned between sites (Kelly *et al.* 2012). We initiated the cross between SA and BD with ten males and ten females from each source population, teasing apart mate guarding pairs (males + virgin females) with a fine probe, and then pairing each individual with a partner from the opposite population (Fig 2). To ensure successful mating had occurred, each pair was held separately in a 24-well tissue culture plate until the female had produced her first brood. After that, all females and broods were combined into a single 250 mL culture, and maintained as described above. We could be sure that each female mated only with the intended male because female *Tigriopus* mate once (after the final moult) and produce all subsequent broods from stored sperm (Burton 1985). Given that most of the genetic variation for thermal tolerance is partitioned between populations, we expected 20 individuals from each population to be sufficient

to sample the majority of the genetic variation for thermal tolerance segregating between populations.

When the F1 generation reached adulthood, we created three replicate cultures, each established with 30 mate guarding pairs from the F1 generation. Replicate cultures were also maintained at 19˚C as described above. Starting in the F2 generation we selected for increased heat tolerance by exposing >80 mate guarding pairs to the temperature that produced 50-90% mortality (36.0-36.5 ˚C for 1h). We then established a "selected" line from 25 of the surviving mate guarding pairs. For each selected line we also established three "unselected" lines with 25 haphazardly chosen pairs from each F2 culture. We repeated the selection procedure in the F3 generation and also propagated each of the unselected lines, again with 25 haphazardly selected mate-guarding pairs from each unselected line.

Measurement of heat tolerance and plasticity of heat tolerance

After two generations of selection, we measured the effect of selection and on upper lethal limits following Kelly *et al.* (2012). For each of the three selected lines and each of the three unselected controls, we exposed sets of nine mate-guarding pairs to a target temperature for one hour, allowed 48 hours for recovery, and then assessed survival. We did this for a series of 5-10 temperatures at 0.2 ˚C intervals, spanning from the temperature that produced 100% survival to the temperature that produced 100% mortality, and then used the mortality at each temperature to estimate LT_{50} for each line.

Phenotypic plasticity is the ability of one genotype to produce more than one phenotype when exposed to different environments(West-Eberhard 2003). Here, we defined the phenotypic plasticity of thermal tolerance as the degree to which copepods in particular line (≈ genotype) exhibited increased thermal tolerance in response to prior heat shock. We tested for an effect of heat shock on upper lethal limits in all selected and unselected lines by repeating the same procedure above for copepods that had first been exposed to 1 hour at 34˚C (a stressful but non-lethal temperature) and then allowed 24 hours to recover. We tested for an effect of selection treatment (selected vs. control) and heatshock treatment (heat-shocked vs. ambient temperature) on the probability of survival via logistic regression in the statistical program R (R Development Core Team 2014).

Transcriptome sequencing

We measured the effect of selection on the transcriptomic response to heat shock using RNA sequencing (Fig 2). In the F4 generation, we created 12 sequencing libraries, each from the pooled RNA of 30 copepods per treatment combination. For each of three selected lines and three control lines, we created two libraries: one from copepods that had been held at ambient temperatures and then flash-frozen, and one from copepods that had been heat shocked for 1 hr at 34˚C, allowed 1 hr to recover, and then flash-frozen. We sequenced three additional cDNA libraries from each of the two "pure" parental populations on a separate lane of sequencing (six libraries total). Each of the parental cDNA libraries was created from the pooled RNA of 40 adult copepods: 20 that had been heat shocked, and 20 that had been held at ambient temperatures.

We extracted total RNA using guanidinium thiocyanate (Chomczynski & Sacchi 1987). Following extraction, we processed RNA to remove tRNA and degraded fragments using an RNeasy Mini Kit according to manufacturer's instructions (Cat. no. 74104, Qiagen, Valencia, CA, USA). RNA yield and purity were assessed by measuring A260/A230 and A260/A280 ratios with a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), and then using a Bioanalyzer (Agilent, Santa Clara, CA). We constructed cDNA libraries using the Illumina TruSeq RNA sample prep kit (Version 2; Illumina, San Diego, CA), following the manufacturer's instructions. Each library was given an individual barcode adapter, then all twelve libraries from the hybrid populations were pooled and sequenced using paired end, 100 base pair reads on a single lane of Illumina HiSeq 2500 (Illumina) at the University of Davis Genome Center Core Facility. We sequenced three libraries from each of the two pure parental populations on a separate lane, once again using paired end, 100 base pair reads.

Transcriptome assembly and gene expression analysis

Transcriptome assemblies were conducted using Trinity v. 20013-8-25 (Grabherr *et al.* 2011) with default parameter settings. Prior to assembly, adapters were removed using cutadapt (Martin 2011), the first eight positions of each read were removed using fastx_trimmer, and we retained only those positions that had a quality score >28 using fastx_quality_trimmer (http://hannonlab.cshl.edu/fastx_toolkit/). In addition, transcriptomes were subjected to CD-Hit to collapse the assembly to transcripts with <98% similarity (Fu *et al.* 2012). Raw reads are available at at NCBI bioproject PRJNA316512. We initially assembled a hybrid transcriptome using all BD and

SA reads, resulting in a transcriptome with 118,501 genomic elements. However, we noticed that assemblies of pure parental population data contained roughly half as many genomic elements (see below). The size of the hybrid assembly suggested that a majority of the BD and SA transcripts were assembling into population-specific contigs (i.e., alleles), which could negatively impact our ability to call differential gene expression between experiments. Because of this, we conducted all differential gene expression analyses on parental transcriptome assemblies. The BD and SA assemblies and other data are deposited at the Dryad link associated with this paper (doi:10.5061/dryad.3v71f).

To measure gene expression, reads from each of 12 experimental samples were mapped back to the SA and BD parental transcriptome assemblies separately using the default settings of RSEM v. 1.2.7 (Li & Dewey 2011). Comparisons of percentage of reads mapped for each library to either the SA and BD assemblies revealed comparable mapping efficiencies with between 79.84 and 90.47 % of reads from each library mapping to BD and between 79.68 and 88.35 % of reads from each library mapping to SA.

We investigated patterns of gene expression using the R Bioconductor package, limma (Smyth 2005; Law *et al.* 2014; Ritchie *et al.* 2015). Limma employs an empirical Bayes method to estimate log-fold changes in expression, and has recently been shown to be more robust to false positives than methods that rely heavily on fitting a negative binomial distribution to the data (Rocke *et al.* 2015). In limma, we fit a generalized linear model to the data, testing for differences in gene expression between heat selected lines and controls and for differences in gene expression between heat-shocked and ambient temperature treatments, setting the threshold for false discovery in all analyses to FDR<0.05 (Benjamini & Hochberg 1995).

We assigned functional annotations and tested for enrichment in gene ontology (GO) terms in differentially expressed genes using BLAST2GO v. 3.1.3 (Conesa & Götz 2008). We tested for significantly enriched GOs in lists of differentially expressed genes using Fisher's exact test after correcting for multiple comparisons using an FDR of 0.05 (Benjamini & Hochberg 1995) .

Results

Thermal tolerance

After two generations of selection, we measured the effect of selection and prior heat shock on upper lethal limits following Kelly *et al.* (2012). A logistic regression revealed an effect of selection ($P < 0.0001$) and heat shock ($P < 0.0001$) on heat tolerance, but also a significant interaction between selection and heat shock ($P = 0.0009$) with a smaller effect of heat shock on heat tolerance in selected lines, indicating lower plasticity of heat tolerance (Table 1, Fig 3).

Transcriptome assembly

We assembled 44,617,800 and 23,285,289 pairs of reads to obtain transcriptome sequences for the BD and SA populations respectively. The final collapsed and filtered assembly for the BD population contained 59,519 contigs, with an N_{50} of 2969 bp and a GC content of 47.3%. The collapsed and filtered assembly for the SA population contained 48,525 contigs, with an N_{50} of 2750 bp and a GC content of 48.1%. After filtering contigs that did not have an average expression support of at least one read per million for at least four libraries, the final mapping transcriptome contained 15,256 unique transcripts for the BD transcriptome and 15,838 unique transcripts for the SA transcriptome. We blasted these assemblies to the nr database (e-value cutoff 1e-5) and annotated transcripts in BLAST2GO. We obtained significant blast hits for 71% and 70% of contigs, for the BD and SA transcriptomes respectively, with 73% and 72% of top 50 blast hits going to other arthropod sequences.

Gene expression

Gene expression results were qualitatively very similar for mapping results to each of the parental population transcriptome assemblies. As a result, we will report only the analyses based on mapping to the SA transcriptome here. Analyses based on mapping to the BD transcriptome are reported in the supplement (S1). Across all lines, overall patterns of gene expression differed by heat shock treatment (506 differentially expressed transcripts, FDR=0.05), but did not indicate any baseline differences in expression between selected lines vs. controls (0 differentially expressed transcripts, FDR=0.05). The effect of heat shock on gene expression was smaller in selected lines, with only 93 transcripts differentially expressed in response to heat shock as compared to 462 differentially expressed in response to heat shock within the unselected lines. Of the 93 transcripts that were differentially expressed in selected lines in response to heat shock, 49 of these were also

differentially expressed in response to heat shock in the un-selected control lines. Of the 506 transcripts responding to heat shock in either unselected or selected lines, the majority had greater fold-change in expression in response to heat shock in controls, as compared to heat-selected lines (429 out of 506, Fisher's exact test, $p<0.0001$, Fig 4).

The transcriptional response to heat shock was enriched for catalytic, hydrolase, and exopeptidase activities (Fisher's exact test, $p<0.05$, Fig 5). As expected, the plastic response to heat stress included up-regulation of HSP 70, 40, and 67b, and an activator of HSP90. We validated the finding of elevated HSP 70 expression in thermally stressed individuals wild caught from the BD population using qPCR (N=26; Wilcoxon P=0.03).

Discussion

Both phenotypic plasticity and adaptive evolution may facilitate persistence in a changing environment, but their reciprocal interactions during periods of environmental change are poorly understood (Chevin *et al.* 2013). In this study, we sought to test whether the evolution of increased heat tolerance would lead to a change in the plasticity of this trait in the copepod *Tigriopus californicus.* When we hybridized populations that were divergent for heat tolerance, and then subjected F2 hybrids to selection for increased upper lethal limits, we observed increased tolerance in selected lines, but also lower phenotypic and gene expression plasticity in response to heat stress.

Our results are in direct contrast to our previous findings from among-population comparisons of *Tigriopus*, where we observed that populations with greater heat tolerance also had greater plasticity of heat tolerance (Kelly *et al.* 2012). In that case, the positive correlation between tolerance and plasticity could have been driven by correlated selection pressures among environments, highlighting the utility of selection experiments for identifying true genetic correlations among traits. Other empirical studies have observed that directional selection on trait means resulted in increased plasticity (reviewed in Scheiner 2002, Garland and Kelly 2006). However, a few recent studies focusing on physiological traits have found a decrease in plasticity in response to selection for increased mean trait values (Sikkink *et al.* 2014; Grossman & Rice 2014), suggesting that in contrast to other types of traits, the evolution of increased physiological tolerance tends to produce correlated reductions in plasticity. Consistent with this prediction, the same pattern has been observed in several recent studies comparing natural populations that vary

in stress tolerance traits (reviewed in DeBiasse and Kelly 2015). For example, lower plasticity of heat tolerance has been observed in more thermally tolerant populations of corals (Barshis *et al.* 2013), and tropical lizards (Phillips *et al.* 2015) and in more thermally tolerant species of *Petrolisthes* crabs (Stillman 2003). Given the interaction we observed between changes in fixed trait values and changes in acclimation capacity, our results support recent calls to integrate models of developmental and reversible plasticity which have previously been treated separately (Beaman, White & Seebacher 2016).

Observed reductions in the phenotypic plasticity of heat tolerance in selected lines were mirrored by reductions in transcriptional plasticity. Recent work in a variety of taxa provides evidence for substantial heritable variation in patterns of gene expression (Telonis-Scott *et al.* 2014; Leder *et al.* 2015), so it is not surprising that transcriptional plasticity can respond to selection. Recent work in *Arabidopsis* has also demonstrated that genes with genetically variable expression responses to environmental stress play an important role in adaptation to local environments (Lasky *et al.* 2014), providing an important link between the evolution of transcriptional plasticity and adaptation to abiotic stress. However, while our results suggest that evolution of increased tolerance is associated with decreased phenotypic and transcriptional plasticity, it is also possible that increased heat tolerance caused the selected lines to not experience 34˚C as stressful, and that exposure to even higher temperatures would have produced a response of similar magnitude to the one exhibited by the unselected lines. This pattern was observed by Sikkink et al. (2014), who experimentally evolved increased heat tolerance in nematodes and found that an apparent loss of plasticity was actually due to a shift in the threshold for induction of the plastic response.

An important challenge in interpreting our results is the extensive hybrid breakdown observed in inter-population crosses of *Tigriopus* (Burton 1990; Edmands 1999; Ellison & Burton 2008). Hybrids typically show a dramatic decline in fitness in the F2 generation, followed by generations of inter-locus selection that purges incompatible alleles, so that hybrid populations may eventually exceed the fitness of either parental population (Pereira, Barreto & Burton 2014). This underscores the importance of comparing heat-selected hybrid lines with hybrid controls from the same cross, at the same generation. Because we expect the average effects of post-hybridization intra-locus selection to be the same for both heat selected lines and controls, we feel confident that we can attribute the increase in heat tolerance and the decrease in plasticity (relative to the hybrid controls) to the effects of our heat tolerance selection regime, rather to the side effects of hybrid

breakdown. Further, recent work has shown that heat tolerance is one of the few fitness traits that does not show hybrid breakdown effects in *T. californicus* (Willett 2012).

It is important to note that most, if not all, of the response to selection observed in our experiment is likely based on standing variation (divergence between the SA and BD populations) rather than *de novo* mutations over the course of our experiment. We performed only two generations of laboratory selection, but the timescale of divergence between the SA and BD populations is on the order of millions of generations (Burton & Lee 1994). By hybridizing divergent populations and selecting for increased heat tolerance, we were effectively selecting for increased frequencies of the alleles involved in local adaptation to temperature.

Our gene enrichment analyses demonstrated strong involvement of hydrolytic and catalytic activities in the transcripts responding to heat shock, a finding that is consistent both with the biology of heat shock, and previously observed transcriptomic responses to heat stress in *Tigriopus* (Barreto, Moy & Burton 2011; Schoville *et al.* 2012). Many of the physiological effects of heat shock are driven by the aggregation of mis-folded proteins (Richter, Haslbeck & Buchner 2010). Thus, two major components of the heat shock response machinery are molecules involved in protein stabilization (classical heat shock proteins) and proteolytic molecules involved in "cleanup." It seems that the observed enrichment for hydrolytic and catalytic activities is driven by the latter response. Previous work on gene expression responses to heat shock has suggested that multicellular organisms (human cell lines, *Arabadopsis, C. elegans*) may rely on mainly protein stabilization (classic heat shock proteins) while bacteria appear to rely more heavily on mechanisms for protein degradation (Richter *et al.* 2010). The prominent overexpression of hydrolytic and catalytic gene ontologies in response to heat shock in *Tigriopus* would seem to counter this generalization. It may be that, as an abiotic stress specialist, *Tigriopus* must rely on both mechanisms rather than on protein stabilization alone.

Our approach (crossing of populations that are diverged for a trait of interest followed by artificial selection) allowed for the separation of loci involved in divergence of an ecologically relevant trait from background divergence among populations. Combining this experiment with next generation sequencing (NGS) technology allowed us to identify specific gene expression differences involved in the phenotypic response to heat tolerance. As NGS technologies become increasingly tractable in non-model systems, the marriage of natural history, experimental biology and NGS data holds great promise for improved understanding of the evolution of ecologically relevant traits in natural populations.

Although there is a growing appreciation that plasticity plays an important role in evolutionary diversification and innovation, little is known, at a mechanistic level, about the relationship between plastic responses and evolutionary changes in trait means (Pfennig & Ehrenreich 2014). In this context, our results have important implications for anticipating biological responses to climate change. Some theoretical models assume that the buffering effects of plasticity and adaptation will be additive, and that the slopes of reaction norms will not, themselves, evolve (Chevin *et al.* 2010). However if adaptation to heat occurs through conversion of a plastic response to a fixed trait value, it will not tend to confer additional protections to threatened populations above and beyond those already conferred by plasticity.

Acknowledgments

We thank Craig White and three anonymous reviewers for insightful comments on earlier versions of this manuscript, and we thank Raymond Huey for conversations that improved the Discussion. Heat tolerance data were collected by Colleen Cecola and Hope Roberts. Transcriptomic data were analyzed using high performance computing resources provided by Louisiana State University (http://www.hpc.lsu.edu), and sequencing was funded by NSF DDIG 09-09788 to MWK.

Data Accessibility

Transcriptome assemblies, analysis scripts, and other data are deposited at the Dryad link associated with this paper (doi:10.5061/dryad.3v71f). Raw sequencing reads are available at at NCBI bioproject PRJNA316512.

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Table 1. Results of logistic regression testing for effects of heat shock, selection regime, and interaction between heat shock and selection on upper lethal limits in *Tigriopus californicus.* β is the regression coefficient, OR is the odds ratio (the proportional change in mortality risk for a unit change in the variable of interest), z is the z-statistic, the regression coefficient divided by its standard error, and P is the probability that β=0.

Treatment	β	**OR**	z	**P**
temperature	-3.35	0.035	-22.19	<.0001
heat shock	3.24	25.5	14.80	<.0001
selection	1.48	4.39	8.46	<.0001
heat shock * selection	-0.80	0.45	-3.32	0.0009

Figure Legends

Figure 1. Three scenarios for the relationship between phenotypic plasticity and trait means. Each line represents a reaction norm, trait values of an individual genotype in two or more environments. Reaction norms with steeper slopes indicate greater phenotypic plasticity. Dashed lines indicate the population mean. In scenario A, genotypes with the greatest trait mean in environment I also have the greatest plasticity, so that selection for increased trait values will lead to an increase in plasticity as a correlated response to selection. In scenario B, genotypes with the greatest trait values in environment II also have the lowest plasticity, leading to a decrease in plasticity as a correlated response to selection. Finally, scenario C posits no correlation between trait means and plasticities.

Figure 2. Study design: testing for changes in the phenotypic and transcriptional plasticity in response to selection for increased heat tolerance in the copepod *Tigriopus californicus.*

Figure 3. Thermal tolerance (LT_{50}) in hybrid populations of *Tigriopus californicus* either selected for increased heat tolerance, or held under the same conditions as un-selected control lines. Figure shows heat tolerance for both sets of lines held at ambient temperature, and after being subjected to heat shock at 34˚C and allowed a 24 hour recovery. A logistic regression revealed an effect of selection ($p<0.001$) and prior heat shock ($p<0.001$) on thermal tolerance, but also a significant interaction between selection and heat shock ($p<0.001$) with a smaller effect of heat shock on thermal tolerance in selected lines.

Figure 4. Log-fold change in expression for transcripts differentially expressed in response to heat shock. One-to-one line divides transcripts with greater fold change in expression in selected vs. control lines. The majority of transcripts had greater fold-change in expression in response to heat shock in control, as compared to heat-selected lines (429 out of 506, Fisher's exact test, $p<0.0001$).

Figure 5. Gene ontologies that were functionally enriched among transcripts responding to heat shock (Fisher's exact test, FDR=0.05). Light bars show the percent representation of ontologies in the transcriptome as a whole; dark bars show percent representation within the differentially expressed transcripts.