Modulation of the heat shock response is associated with acclimation to novel temperatures but not adaptation to climatic variation in the ants *A ae ga e cea* and *A. d*

Sara Helms Cahan

and Angert, 2014). In more extreme environments, populations are likely to frequently face conditions at or beyond their physiological limits, selecting for increased constitutive expression or enhanced inducibility of chaperone molecules (Barshis et al., 2013; Bettencourt and Feder, 2001). Under milder conditions, however, the cost of maintaining a response mechanism may lead to reduction or loss of the capacity to re-

Library size range and quality were verified on a Bioanalyzer and with kapa qPCR. Each library was single-end sequenced in a single HiSeq 2000 rapid-run lane at the University of Vermont Advanced Genome Technologies Core facility, yielding approximately 2.5 million reads per sample.

Sequences were demultiplexed using the program sabre (https://github.com/najoshi/sabre), allowing for up to a single base pair mismatch in the barcoding sequence, and the restriction site sequence was trimmed. The total length of all sequences was trimmed to 90 bp with the fastx_trimmer tool in the FASTX Toolkit v. 0.0.14, and low-quality reads, defined as those whose quality score dropped below 10 at any point along the sequence, were excluded from downstream analysis with the fastq_trimmer tool.

Because there is no sequenced genome available for the genus

Gene expression was quantified using the ΔΔCT method (Livak and Schmittgen, 2001). For basal expression, we used the mean CT values across all samples for each Hsp and housekeeping gene treated at 25 °C as the reference to calculate relative quantity for each sample. To quantify induction of each gene, we calculated fold-increase as the CT values at 37 °C relative to CT values at 25 °C for each colony.

2.5. S a ca a a e

We determined the effects of shared ancestry, thermal niche, and acclimation temperature on baseline expression and fold-induction of each of the three heat shock protein genes with linear regressions performed in R. Phylogenetic relationships were estimated with RaxML 8 (Stamatakis, 2014) following a GTR + Gamma substitution model; group support was evaluated with 100 fast bootstrap replicates. To convert phylogenetic relationships into continuous variables suitable for a regression model, we decomposed the phylogenetic distances with a principal coordinate analysis with the *a e* statistical package (Paradis et al., 2004), which produces orthogonal eigenvectors that capture different nodes across the tree (Fig. S1; Diniz et al., 1998). The first three eigenvectors corresponded to the three deepest nodes with 100% bootstrap support (Fig. 1); all three were included in the full models, but because Axis 1 was highly correlated with Tmax (correlation coefficient = 0.836), and therefore collinearity could have prevented detection of an effect of environment, we also conducted the analyses without Axis 1.

It is also possible that the heat shock response may not be the most

Mountains is already moving upwards (Warren and Chick, 2013), while *A. d* is being displaced in warmer areas by a more thermally tolerant species, *C e a ga e e a a* (Resasco et al., 2014). Understanding how stress-related costs affect growth rates and competitive ability will be important for predicting their ability to persist in a changing landscape.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.cbpa.2016.11.017.

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