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Heritability of *hsp*70 expression in the beetle *Tenebrio molitor*: Ontogenetic and environmental effects

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ABSTRACT

Ectotherms constitute the vast majority of terrestrial biodiversity and are especially likely to be vulnerable to climate warming because their basic physiological functions such as locomotion, growth, and reproduction are strongly influenced by environmental temperature. An integrated view about the effects of global warming will be reached not just establishing how the increase in mean temperature impacts the natural populations but also establishing the effects of the increase in temperature variance. One of the molecular responses that are activated in a cell under a temperature stress is the heat shock protein response (HSP). Some studies that have detected consistent differences among thermal treatments and ontogenetic stages in *HSP*70 expression have assumed that these differences had a genetic basis and consequently expression would be heritable. We tested for changes in quantitative genetic parameters of *HSP*70 expression in a half-sib design where individuals of the beetle *Tenebrio molitor* were maintained in constant and varying thermal environments. We estimated heritability of *HSP*70 expression using a linear mixed modelling approach in different ontogenetic stages. Expression levels of *HSP*70 were consistently higher in the variable environment and heritability estimates were low to moderate. The results imply that within each ontogenetic stage additive genetic variance was higher in the variable environment and in adults compared with constant environment and larvae stage, respectively. We found that almost all the genetic correlations across ontogenetic stages and environment were positive. These suggest that directional selection for higher levels of expression in one environment will result in higher expression levels of *HSP70* on the other environment for the same ontogenetic stage.

1. Introduction

The impact of current climate change on biodiversity has been widespread and has involved several types of responses from the molecular to the ecosystem level (Pörtner et al., 2006; Parmesan, 2006; Chown et al., 2010; Hoffmann and Sgrò, 2011). Important determinants of the biological responses to global warming will be the degree of warming itself (Helmuth et al., 2005), the physiological sensitivity of organisms to changes in the temperature (Calosi et al., 2008; Helmuth et al., 2005; Huey et al., 2012) and the effects of the increase in temperature variance and frequency of extreme events (see Seneviratne et al., 2006; Pertoldi and Bach, 2007; Bentz et al., 2010). In addition, it has been shown that the thermal performance of many organisms is proportional to the magnitude of temperature variation they experience (Addo-Bediako et al., 2002; Gilman et al., 2006; Angilletta, 2009) as suggested by Jensen's inequality (Ruel and Ayres, 1999).

One of the molecular responses that is activated in a cell under temperature stress is the heat shock protein response (HSP), an event of genetic activation that occurs in the cells in response to abnormal, stressfully high or low temperatures (Rinehart et al., 2007). The genes that encode for Heat-Shock Proteins (HSPs) are highly conserved and have been found in every studied species (Feder and Hofmann, 1999; Yeh and Hsu, 2002). Among HSP families, the group within the 70-kilo Daltons size range (HSP 70) is the most extensively studied because of its well-known response to stresses (see reviews in Feder and Hofmann, 1999; Sanders, 1993; Halpin et al., 2002; Hofmann and Todgham, 2010). The expression of these proteins could explain differences not only in fitness but also in the geographical distribution of

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organisms (Sorte and Hofmann, 2005; Bernabò et al., 2011; Lyytinen et al., 2012). Several studies that have detected consistent differences among populations and/or thermal treatments in *HSP*70 expression have assumed that these differences had a genetic basis (Roberts and Feder, 2000; Somero, 2010; Tine et al., 2010; Lyytinen et al., 2012). Nevertheless, this assumption has a direct test only in *Drosophila melanogaster* (Krebs et al., 1998; Morgan and Mackay, 2006). Furthermore, it is known that the synthesis, degradation and replacement of these proteins represent an important energetic cost for the organisms (Hartl and Hayer-Hartl, 2002; Sørensen and Loeschcke, 2002). Therefore, trade-offs might be expected between different ontogenic stages if development occurs under different thermal stressful environments (see Krebs et al., 1998; Sørensen et al., 2003).

The speed of evolutionary change in a phenotypic trait is determined by two key components: the amount of additive genetic variance underlying the trait and the strength of selection acting on it. Many studies have shown that both selection and expression of genetic variance may depend on the environmental conditions the population experiences (Husby et al., 2011). It is important to realize that heritability (h^2) may change under different environmental conditions either because of changes in additive genetic variance (V_A) or other variance components (e.g., permanent environmental variance (V_{PE}) or residual variance (V_R). However, changes in V_A are of particular interest because they indicate a change in the "evolvability", or the potential to respond to selection of a trait. On the other hand, cross-environment genetic correlations have important implications for evolution, that is, a trait measured in two environments can be considered as genetically correlated traits (sensu Via and Lande, 1985).

Here, we evaluated whether *HSP*70 expression has a genetic basis and whether that expression is affected by the ontogenetic stage and thermal environment in which an insect develops. We used the yellow mealworm beetle *Tenebrio molitor* (Polyphaga, Tenebrionidae) as our study model, an insect with a complex life-cycle which is a world pest of stored grains and that can be easily captured in field and reared in the laboratory (Worden and Parker, 2001). *T. molitor* has been used previously in molecular and ecological research (Graham et al., 2000; Drnevich et al., 2000; Vainikka et al., 2006), however, we are not aware of any studies examining the evolutionary potential of physiology or life-history traits in this species. In particular, using a quantitative genetic design we evaluated the following questions. First, what is the heritability of *HSP70* under different thermal environments and ontogenetic stages? As stress conditions increase the phenotypic differences between genotypes (Hoffman and Merilä, 1999), we should observe an increase of additive genetic variance (and of heritability) under such conditions. Second, we evaluated the potential evolutionary response of *HSP70* expression across environments and ontogenetic stages by estimating the cross environment genetic correlation. As the magnitude of this correlation reflects the extent to which the same genes control *HSP70* expression, we predict a positive association in each environment and ontogenetic stage.

2. Materials and methods

2.1. Animals and maintenance

We used the common yellow mealworm beetle *T. molitor* Linnaeus 1758 (Coleoptera: Tenebrionidae) as our study model. Animals were captured in San Carlos de Apoquindo in central Chile (http://www.bio.puc.cl/ediem/), transported within the same day and maintained in the laboratory at 18 ± 1 °C with a 12L:12D photoperiod and fed *ad libitum* with a mix of 60% wheat flour, 20% oats, 10% wheat bran and 10% brewer's yeast. All animals were maintained in those conditions for nearly 5 months before the start of the experiments.

2.2. Thermal environments

Animals were reared in thermal environments with identical mean temperature (18 °C) but different variance (Fig. 1). The constant thermal environment had a variance of 1 °C while the variable thermal environment had a variance of 6.8 °C (2.5 and 43 °C for lower and upper temperature, respectively) and with a 12 h temperature fluctuation (Fig. 1). For the variable environment the environmental chamber was programmed weekly using random temperatures following a normal distribution from daily temperatures observed in the last 5 years (minimum and maximum mean temperatures) in central Chile (for more details see Arias et al., 2011). Temperature data were obtained from the weather station located at the same station where the beetles were collected (see Jaksic, 2001). Humidity and photoperiod were the same in both experimental conditions: 85% relative humidity and 12L:12D photoperiod cycle. In all cases, food was provided *ad libitum*.

2.3. Breeding design

In order to eliminate potential maternal effects we used experimental individuals from the third laboratory generation. We carried out a paternal half-sib breeding design in which 30 males were placed with three females in a Petri dish (70 mm diameter; with a base layer of bran) during 5 days. After that time, the females were put in individual Petri dishes to allow egg extrusion. Eggs from each mother were divided, individualized and randomly assigned to a thermal environment and maintained during ontogeny (40 days and 140 days for larvae and adult, respectively). *HSP70* gene expression was evaluated in larvae and adults; roughly a quarter of each female's progeny in each combination of ontogenic stage and thermal environment.

2.4. RNA extraction and real time RT-PCR

We measured induced *HSP70* expression for all individuals (i.e. larvae and adults) in the different thermal regimes and ontogenetic stages. The induction temperature for *HSP70* expression of beetles was 38 °C for 1 h (preliminary tests revealed this temperature induced the highest levels of expression) in a waterbath. After this treatment, beetles were kept at 20 °C for 1 h and immediately snap frozen in liquid nitrogen and then stored at −70 °C. For RNA extraction, larvae and adults were collected and grinded individually with a pestle in an Eppendorf tube. Then, RNA was obtained using the Trizol® (Invitrogen™, USA) method following the manufacturer's instructions, RNA quantities and 260/280 ratios were calculated with an Eon Microplate Spectrophotometer (BioTek®) and the Gen5 Data Analysis Software. For cDNA synthesis, 1 μg of total RNA treated with DNAse I (RQ1, Promega, USA) was reverse transcribed with random hexamer primers using the Improm II reverse transcriptase (Promega, USA), according to the manufacturer's instructions. Real time (RT)-PCR was performed using the Green Master Mix (Quantace, UK) and the Gene-Mx3000P detection system (Stratagene, USA) as described in the manufacturer's manual and described by Arias et al. (2011). Briefly, the PCR mixture (25 ml) contained 2 μl of cDNA and 140 nM of each primer. Amplification was performed under the following conditions: 95 °C for 10 min, then 35 cycles of 94 °C, 30 s; 56 or 60 °C for 30 s; and 72 °C, 40 s, followed by a melting cycle from 55 °C to 95 °C. Relative gene expression calculations were conducted as described in the software manufacturer's instructions: an accurate ratio between the expression of the gene of interest (GOI) and the housekeeping

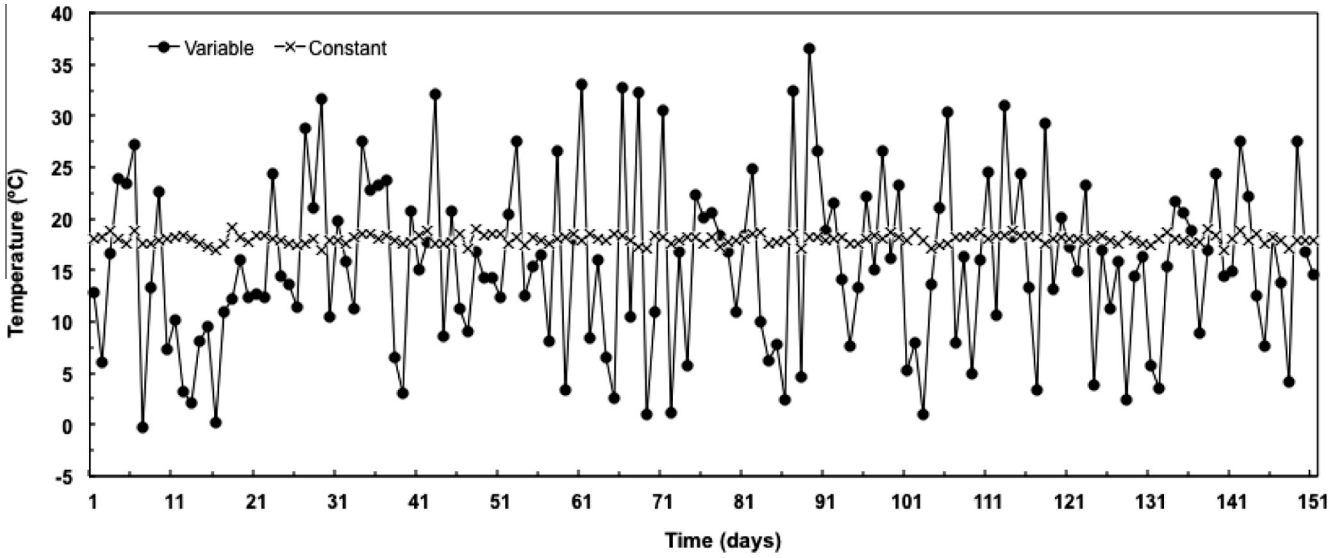

Fig. 1. Time series of temperature in constant and variable thermal environmental during rearing experiment (151 days).

(HK) gene was calculated according to equation: $2^{-(\Delta CtGOI\text{-}HK)}$. Then, gene expression levels were normalized to the average value of expression in the treatment/stage of lowest expression. 18S gene was used as the housekeeping gene because its expression was stable in different samples and stages (see Arias et al., 2011). Melting temperatures were 56 and 60 °C for HSP70-like and 18S gene, respectively. Primers used are described in Arias et al. (2011) and in all cases the reaction specificities were tested with melt gradient dissociation curves and electrophoresis gels (agarose 2%). Individuals were analysed separately (i.e. independent replicates) for each stage and environment ($N = 463$). All samples were analysed with two technical replicates.

2.5. Statistical analyses

We used a linear mixed modelling approach to evaluate the effect of ontogenetic stage (i.e. adult vs. larvae) and thermal environment (i.e. constant vs. variable) on *HSP70* expression levels, while taking into account the nested structure of our design and some unbalance. Hypothesis testing for fixed effects was based on marginal *F* tests (Pinheiro and Bates, 2000). *HSP70* was $\log_{10}$ transformed to meet normality assumptions. Statistical analyses were performed using the nlme package (Pinheiro et al., 2009) for the software R 2.10.1 (R Development Core Team, 2009).

We estimated genetic variances using a Bayesian Monte Carlo Markov Chain mixed modeling approach (Hadeld, 2010). We used uninformative, parameter expanded priors and collected 29,000 samples of the joint posterior from 3,000,000 iterations after a burn-in interval of 100,000. Posterior modes (i.e., parameter estimates) and their 95% credible intervals were based on sampling 29,000 times the posterior parameter distribution. Sires and dams were included as random effects. We fit one model to each combination of thermal environment and ontogenetic stage.

We calculated cross-environment correlations based on sire family means, first by taking the mean of individuals within dams and then the mean of dams within sires. We are aware that estimates of genetic correlations across environments (or ontogenetic stages) from family means might be downwardly biased (Astles et al., 2006). However, given the low sample sizes and large errors of our genetic variance estimates (see below) that would prevent a proper estimation of the correlation, we decided just to evaluate if the correlation was different from zero (i.e., the presence of a pattern, not the significance of an estimate).

3. Results

Descriptive statistics for each thermal environment and ontogenic stage are presented in Table 1. The variable thermal environment represented an important stress for the beetles. Mortality was higher in the variable Ta environment (adult = 29.1%, larvae = 10.4%) in comparison to the constant one (adult = 8.6%, larvae = 6.2%). *HSP70* expression levels were not affected by the interaction between thermal environment and ontogenic stage ($F_{1,80} = 0.565$, $P = 0.455$, Fig. 2). Expression levels were consistently higher in the variable environment ($F_{1,56} = 7.335$, $P = 0.009$, Fig. 2) and in adults ($F_{1,81} = 62.283$, $P < 0.001$, Fig. 2).

Although our results suggest that within each ontogenetic stage V_A was higher in the variable environment and in adults (Table 2) heritability estimates were low to moderate (mean: 0.6, range: 0.03–0.92, Table 2) and estimates had wide credible intervals that went from almost zero to almost one. It is likely that these results reflect low statistical power due to the small number of sire families and offspring used in each combination of thermal environment and ontogenetic stage (Table 1). Nevertheless, the adult expression in the variable environment had a narrow credible interval suggesting that the estimate is significantly different from zero. In overall, our results suggest that there might be little scope for evolution at the molecular level for this trait, with the exception of adults in the variable environment.

We evaluated four of the six possible combinations of correlations. The correlation was positive within each ontogenetic stage across environments (larvae variable – larvae constant: $r_P = 0.51$, t_{23}, $P = 0.01$; adult variable – adult constant: $r_P = 0.42$, t_{22}, $P = 0.04$). It was positive although marginally significant across ontogenetic stages within the constant environment ($r_P = 0.41$, t_{22}, $P = 0.050$) and was not significant across ontogenetic stages within the variable thermal environment ($r_P = 0.16$, t_{20}, $P = 0.464$).

4. Discussion

To the best of our knowledge, this is the first study that has obtained h^2 estimates for *HSP*70 levels of expression and also estimates of genetic correlations of expression across ontogenetic

Table 1
Sample size, number of recorded animals and descriptive data for expression levels of *HSP70*.

Thermal environment	Ontogenic stage	Sires	Dams	Offspring	Descriptive statistics				
					Mean	SD	Min	Max	CV
Constant	Larvae	26	58	101	2.15	2.70	0.04	13.85	138.39
Constant	Adult	24	53	162	7.13	10.31	0.15	52.05	144.51
Variable	Larvae	27	57	103	3.95	7.00	0.07	54.64	177.32
Variable	Adult	26	42	97	13.66	19.13	0.09	91.89	140.10

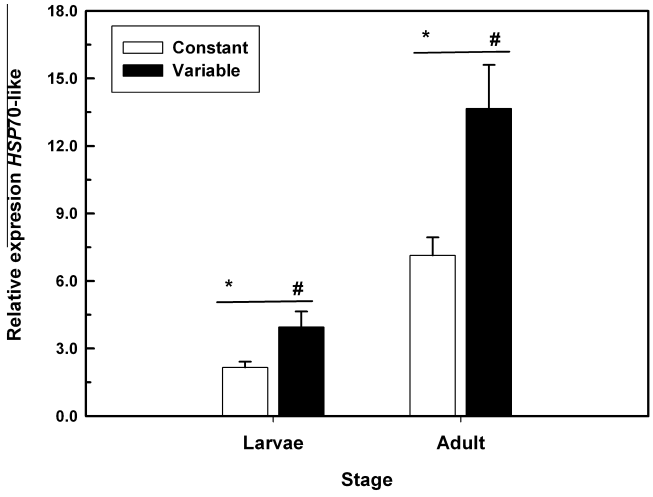

Fig. 2. HSP70-like gene expression relative to 18s expression in larvae and adult of *Tenebrio molitor*. (*) Represents significant differences between stage and (#) shows significant differences between environments (mean ± S.E.M.) in HSP70/18 s expression.

Table 2
Additive genetic variance (V_A), narrow-sense heritability (h^2) for *HSP70* expression levels at each thermal environment and ontogenic stage. Asymptotic standard errors are presented in parenthesis. Estimates are based on $\log_{10}$-transformed data.

Thermal environment	Ontogenic stage	V_A	h^2 (95% CI)
Constant	Larvae	6.24	0.74 (0.005–0.999)
Constant	Adult	67.71	0.75 (0.001–0.999)
Variable	Larvae	1.39	0.03 (0.001–0.523)
Variable	Adult	311.45	0.92 (0.389–0.999)

stages and thermal environments in a non-*Drosophila* species. Heritability estimates were moderate but because of the small sample sizes used their concomitant wide credible intervals (Table 2). In overall, our results suggest the evolvability for *HSP70* expression might be restricted. While previous studies have used isofemale lines (Krebs et al., 1998; Bahrndorff et al., 2010) or specific crosses (Wang and Kang, 2005) to evaluate the genetic basis of *HSP70* expression in different insect species, it is possible to highlight some shared results. First, there is genetic variation underlying *HSP70* expression. Second, the amount of genetic variation usually changes between ontogenetic stages, being higher in adults. Finally, although we have not evaluated this statistically, our results are also in agreement with the general observation that the expression of additive genetic variation increases under adverse conditions, which in this study corresponds to the variable environment (see Hoffmann and Merila, 1999; Carter et al., 2004). Correlations across ontogenetic stages within the same environment and correlations within the same stage and across environment were almost all positive (see Section 3). These suggest that directional selection for higher levels of expression in one environment will result in higher expression levels of *HSP70* on the other environment for the same ontogenetic stage. For sure this will depend on the relationship of the *HSP70* expression with fitness across environments: if the relationship is opposite on both environments then the positive correlation is a constraint. A similar argument can be made for the correlation between larvae and adult expression within the constant thermal environment.

There is ample evidence that acclimation temperature affects both heat and cold resistance in many insects (see Chown and Nicholson, 2004; Chown and Terblanche, 2007; Bowler and Terblanche, 2008). Nevertheless, little evidence is available to support the role for developmental acclimation as an important factor in the thermal tolerance of adult insects (Hoffmann et al., 2003; Terblanche et al., 2005; Arias et al., 2011; Ketola et al., 2012). Clearly, for both larvae and adults, the higher *HSP70* expression observed in the variable environment can be explained by the fluctuations in temperature (from 2.5 to 43 °C) that exposed the organisms to extreme events and thus forced the physiological system to be continuously ready to respond. Similar results were observed in *Drosophila HSP70* genes, which are tightly regulated and not expressed unless induced by thermal stress (DiDomenico et al., 1982; Velazquez et al., 1983; Sørensen and Loeschcke, 2002). Interestingly, the higher expression of *HSP70* genes in adults does not translate into better performance under temperature shocks (see Arias et al., 2011). How can this be explained? For both the larval and adult stages, thermal tolerance could be achieved by several methods (see Hochachka and Somero, 2002; Strom et al., 2005) and therefore it is possible that many different genes could be involved. In fact, several species have shown the presence of more than one heat-inducible *HSP70* gene within the same organism (Lindquist, 1986; Ueda and Boettcher, 2009) that can be switched during the life-cycle of an individual. We cannot discard that other members of the HSP gene family could be acting in the observed responses, and future experiments should analyse a broad spectrum of gene expression under temperature variable environments. This is also partially suggested by what we found for genetic correlations across ontogenetic stages within the same environment. A perfect correlation would suggest that expression is under the same genetic control in both stages. However, although in the constant environment this association was positive (but marginally significant) it was not closer to 1. In addition, in the variable environment the association was rather weak, suggesting maybe that different genes might be involved in the different stages.

Stressful conditions (i.e. variable environment) can directly increase heritability in traits by increasing rates of mutation and recombination, by a strong directional selection against low fitness allelles, and by increase phenotypic differences between genotypes as resources become limiting (for reviews see Hoffmann and Merilä, 1999; Charmantier and Garant, 2005). The first two sources of variation proposed might be important for understanding adaptation and long-term evolution, but they cannot explain heritability differences between environments in our laboratory estimations. It is known that under the favourable conditions commonly encountered by organisms, there is a rapid decrease of heritable variation in traits associated with fitness (see Hoffmann and

Merilä, 1999). Conversely, the increase in the expression of genetic effects on stressful conditions is expected because differences in physiological functions may favour traits that directly affect survival, such as *HSP70* expression, and therefore involve changes in additive genetic effects. However, results in heritability estimations in stressful environments are not consistent neither among taxa studied and the stress condition evaluated (Charmantier and Garant, 2005).

Although, the expression of *HSP*70 has been shown to vary during development in different directions (e.g. Chang, 2005; Mahroof et al., 2005; Ueda and Boettcher, 2009), in general for holometabolous insects, *HSP*70 expression in adults is down regulated probably because they can thermoregulate behaviourally while pupae and larvae are more restricted to move (for review see Bowler and Terblanche, 2008). Contrary to this, our results show that expression was higher in adults, a pattern that has been reported before for this (Arias et al., 2011) as well as other tenebrionid species (Mahroof et al., 2005). We did not provide any thermal shelter to adults, and thus, we cannot completely rule out that this species would have had a lower expression provided they would have been able to thermoregulate using behaviour. At this point, we argue that ontogeny related changes in *HSP*70 in insects are sufficiently widespread for concern as they could strongly influence the outcomes of experimental research, and hence, conclusions of evolutionary potential by natural selection.

Conflict of interest

The authors have not conflicts of interest to declare.

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References

Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2002. Metabolic cold adaptation in insects: a large-scale perspective. Funct. Ecol. 16, 332–338.
Angilletta, M.J., 2009. Thermal Adaptation: A Theoretical and Empirical Synthesis. Oxford University Press, Oxford.
Arias, M.B., Poupin, M.J., Lardies, M.A., 2011. Plasticity of life-cycle, physiological thermal traits and Hsp70 gene expression in an insect along the ontogeny: effect of temperature variability. J. Therm. Biol. 36, 355–362.
Astles, P.A., Moore, A.J., Preziosi, R.F., 2006. A comparison of methods to estimate cross-environment genetic correlations. J. Evol. Biol. 19, 114–122.
Bahrndorff, S., Mariën, J., Loeschcke, V., Ellers, J., 2010. Genetic variation in heat resistance and HSP70 expression in inbred isofemale lines of the springtail *Orchesella cincta*. Clim. Res. 43, 41–47.
Bentz, B.J., Régniere, J., Fettig, C.J., Hansen, M., Hayes, J.L., Hicke, J.A., Kelsey, R.G., Negrón, J.F., Seybold, S.J., 2010. Climate change and bark beetles of the Western United States and Canada: direct and indirect effects. BioScience 60, 602–613.
Bernabò, P., Rebecchi, L., Jousson, O., Martínez-Guitarte, J.L., Lencioni, V., 2011. Thermotolerance and HSP70 heat shock response in the cold-stenothermal chironomid *Pseudodiamesa branickii* (NE Italy). Cell Stress Chaperones 16, 403–410.
Bowler, K., Terblanche, J.S., 2008. Insect thermal tolerance: what is the role of ontogeny, ageing and senescence? Biol. Rev. 83, 339–355.
Calosi, P., Bilton, D.T., Spicer, J.I., 2008. Thermal tolerance, acclimatory capacity and vulnerability to global climate change. Biol. Lett. 4, 99–102.
Carter, M.J., Lardies, M.A., Nespolo, R.F., Bozinovic, F., 2004. Heritability and maternal effects on progeny size: transgenerational environmental effects on a life history trait. Heredity 93, 455–459.
Chang, E.S., 2005. Stressed-out lobsters: crustacean hyperglycemic hormone and stress proteins. Integr. Comp. Biol. 45, 43–50.
Charmantier, A., Garant, D., 2005. Environmental quality and evolutionary potential: lessons from wild populations. Proc. R. Soc. B 272, 1415–1425.
Chown, S.L., Terblanche, J.S., 2007. Physiological diversity in insects: ecological and evolutionary contexts. Adv. Insect Physiol. 33, 50–152.
Chown, S.L., Nicholson, S.W., 2004. Insect Physiological Ecology: Mechanisms and Patterns. Oxford University Press, Oxford, United Kingdom.
Chown, S.L., Hoffmann, A.A., Kristensen, T.N., Angilletta Jr, M.J., Stenseth, N.C., Pertoldi, C., 2010. Adapting to climate change: a perspective from evolutionary physiology. Clim. Res. 43, 3–15.
DiDomenico, B.J., Bugaisky, G.E., Lindquist, S., 1982. Heat shock and recovery are mediated by different translational mechanisms. Proc. Nat. Acad. Sci. U.S.A. 79, 6181–6185.
Drnevich, J.M., Hayes, E.F., Rutowski, R.L., 2000. Sperm precedence, mating interval, and a novel mechanism of paternity bias in a beetle (*Tenebrio molitor* L.). Behav. Ecol. Sociobiol. 48, 447–451.
Feder, M.E., Hofmann, G.E., 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Ann. Rev. Physiol. 61, 243–282.
Gilman, S., Wethey, D.S., Helmuth, B., 2006. Variation in the sensitivity of organismal body temperature to climate change over local and geographic scales. Proc. Natl. Acad. Sci. U.S.A. 103, 9560–9565.
Graham, L.A., Walker, V.K., Davies, P.L., 2000. Developmental and environmental regulation of antifreeze proteins in the mealworm beetle *Tenebrio molitor*. Eur. J. Biochem. 267, 6452–6458.
Hadeld, J.D., 2010. MCMC methods for multi-response generalised linear mixed models: the MCMCglmm R package. J. Stat. Softw. 33, 1–22.
Halpin, P.M., Sorte, C.J., Hofmann, G.E., Menge, B.A., 2002. Patterns of variation in levels of HSP70 in natural rocky shore populations from microscales to mesoscales. Integr. Comp. Biol. 42, 815–824.
Hartl, F.U., Hayer-Hartl, M., 2002. Molecular chaperones in the cytosol: from nascent chain to folded protein. Science 295, 1852–1858.
Helmuth, B., Carrington, E., Kingsolver, J.G., 2005. Biophysics, physiological ecology, and climate change: does mechanism matter? Annu. Rev. Physiol. 67, 177–201.
Hochachka, P.W., Somero, G.N., 2002. Biochemical Adaptation: Mechanism and Process in Physiological Evolution. Oxford University Press, Oxford.
Hoffmann, A.A., Merila, J., 1999. Heritable variation and evolution under favourable and unfavourable conditions. Trends Ecol. Evol. 14, 96–101.
Hoffmann, A.A., Sgrò, C.M., 2011. Climate change and evolutionary adaptation. Nature 470, 479–485.
Hofmann, G.E., Todgham, A.E., 2010. Living in the now: physiological mechanisms to tolerate a rapidly changing environment. Ann. Rev. Physiol. 72, 127–145.
Hoffmann, A.A., Hallas, R.J., Dean, J.A., Schiffer, M., 2003. Low potential for climatic stress adaptation in a rainforest *Drosophila* species. Science 301, 100–102.
Huey, R.B., Kearney, M.R., Krockenberger, A., Holtum, J.A.M., Jess, M., Williams, S.E., 2012. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. Philos. Trans. R. Soc. B: Biol. Sci. 367, 1665–1679.
Husby, A., Visser, M.E., Kruuk, L.E.B., 2011. Speeding up microevolution: the effects of increasing temperature on selection and genetic variance in a wild bird population. PLoS Biol. 9 (2), e1000585. http://dx.doi.org/10.1371/journal.pbio.1000585.
Jaksic, F., 2001. Spatiotemporal variation patterns of plants and animals in San Carlos de Apoquindo, central Chile. Rev. Chil. Hist. Nat. 74, 459–484.
Ketola, T., Kristensen, T.N., Kellermann, V.M., Loeschcke, V., 2012. Can evolution of sexual dimorphism be triggered by developmental temperatures? J. Evol. Biol. 25, 847–855.
Krebs, R.A., Feder, M.E., Lee, J., 1998. Heritability of expression of the 70KD heat-shock protein in *Drosophila melanogaster* and its relevance to the evolution of thermotolerance. Evolution 52, 841–847.
Lindquist, S., 1986. The heat shock response. Annu. Rev. Biochem. 55, 1151–1191.
Lyytinen, A., Mappes, J., Lindström, L., 2012. Variation in HSP70 levels after cold shock: signs of evolutionary responses to thermal selection among *Leptinotarsa decemlineata* populations. PLoS One 7 (2), e31446. http://dx.doi.org/10.1371/journal.pone.0031446.
Mahroof, R., Zhu, K.Y., Subramanyam, B., 2005. Changes in expression of heat shock protein in *Tribolium castaneum* (Coleoptera: Tenebrionidae) in relation to developmental stage, exposure time, and temperature. Ann. Entomol. Soc. Am. 98, 100–107.
Morgan, T.J., Mackay, T.F.C., 2006. Quantitative trait loci for thermotolerance phenotypes in *Drosophila melanogaster*. Heredity 96, 232–242.
Parmesan, C., 2006. Ecological and evolutionary responses to recent climate change. Ann. Rev. Ecol. Evol. Syst. 37, 637–669.
Pertoldi, C., Bach, L.A., 2007. Evolutionary aspects of climate-induced changes and the need for multidisciplinarity. J. Therm. Biol. 32, 118–124.
Pinheiro, J.C., Bates, D.M., 2000. Mixed-Effects Models in S and S- PLUS. Springer, New York, USA.
Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., the R Core team, 2009. nlme: linear and nonlinear mixed effects models. R package version 3.1-96.
Pörtner, H.O., Bennett, A.F., Bozinovic, F., Clarke, A., Lardies, M.A., Lucassen, M., Pelster, B., Schiemer, F., Stillman, J.H., 2006. Trade-offs in thermal adaptation: the need for a molecular to ecological integration. Physiol. Biochem. Zool. 79, 295–313.
R Development Core Team, 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0, URL http://www.R-project.org.
Rinehart, J.P., Li, A., Yocum, G.D., Robich, R.M., Hayward, S.A., Denlinger, D.L., 2007. Up-regulation of heat shock proteins is essential for cold survival during insect diapause. Proc. Natl. Acad. Sci. U.S.A. 104, 11130–11137.

Roberts, S.P., Feder, M.E., 2000. Changing fitness consequences of hsp70 copy number in transgenic *Drosophila* larvae undergoing natural thermal stress. Funct. Ecol. 14, 353–357.
Ruel, J.J., Ayres, M.P., 1999. Jensen's inequality predicts direct effects of environmental variation. Trends Ecol. Evol. 14, 361–366.
Sanders, B.M., 1993. Stress proteins in aquatic organisms: an environmental perspective. Crit. Rev. Toxicol. 23, 49–75.
Seneviratne, S.I., Lüthi, D., Litschi, M., Schär, C., 2006. Land-atmosphere coupling and climate change in Europe. Nature 443, 205–209.
Somero, G.N., 2010. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine "winners" and "losers". J. Exp. Biol. 213, 912–920.
Sørensen, J.G., Loeschcke, V., 2002. Decreased heat-shock resistance and down-regulation of Hsp70 expression with increasing age in adult *Drosophila melanogaster*. Funct. Ecol. 16, 379–384.
Sørensen, J.G., Kristensen, T.N., Loeschcke, V., 2003. The evolutionary and ecological role of heat shock proteins. Ecol. Lett. 6, 1025–1037.
Sorte, C.J.B., Hofmann, G.E., 2005. Thermotolerance and heat-shock protein expression in Northeastern Pacific *Nucella* species with different biogeographical ranges. Mar. Biol. 146, 985–993.
Strom, C.S., Liu, X.Y., Jia, Z., 2005. Why does insect antifreeze protein from *Tenebrio molitor* produce pyramidal ice crystallites? Biophys. J. 89, 2618–2627.
Terblanche, J.S., Klok, C.J., Chown, S.L., 2005. Temperature-dependence of metabolic rate in *Glossina morsitans morsitans* (Diptera, Glossinidae) does not vary with gender, age, feeding, pregnancy or acclimation. J. Insect Physiol. 51, 681–870.
Tine, M., Bonhomme, F., McKenzie, D.J., Durand, J.-D., 2010. Differential expression of the heat shock protein 70 in natural populations of the tilapia, *Sarotherodon melanotheron*, acclimatised to a range of environmental salinities. BMC Ecol. 10, 11. http://dx.doi.org/10.1186/1472-6785-10-11.
Ueda, N., Boettcher, A., 2009. Differences in heat shock protein 70 expression during larval and early spat development in the Eastern oyster, *Crassostrea virginica* (Gmelin, 1791). Cell Stress Chaperones 14, 439–443.
Vainikka, A., Seppälä, O., Löytynoja, K., Rantala, M.J., 2006. Fitness consequences of female preference for male pheromones in *Tenebrio molitor*. Evol. Ecol. Res. 8, 943–957.
Velazquez, J.M., Sonoda, S., Bugaisky, G., Lindquist, S., 1983. Is the major *Drosophila* heat shock protein present in cells that have not been heat shocked? J. Cell Biol. 96, 286–290.
Via, S., Lande, R., 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. Evolution 39, 505–522.
Worden, B.D., Parker, P.G., 2001. Polyandry in grain beetles, *Tenebrio molitor*, leads to greater reproductive success: material or genetic benefits? Behav. Ecol. 12, 761–767.
Yeh, F.L., Hsu, T., 2002. Differential regulation of spontaneous and heat-induced HSP 70 expression in developing zebrafish (Danio rerio). J. Exp. Zool. 293, 349–359.
Wang, X.H., Kang, L., 2005. Differences in egg thermotolerance between tropical and temperate populations of the migratory locust *Locusta migratoria* (Orthoptera: Acridiidae). J. Insect Physiol. 51, 1277–1285.