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Capacity for protein synthesis following heat stimulus of *Drosophila* associates with heat tolerance but does not underlie the latitudinal tolerance cline

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ABSTRACT

Across populations of *Drosophila melanogaster* along the Australian eastern coastline latitudinal clines occur in both heat-knockdown tolerance and hardened heat-knockdown tolerance – low latitude tropical populations being more tolerant. A latitudinal cline also occurs for rates of total protein synthesis following a mild heat stress, with tropical populations having higher rates. Since the control of protein synthesis following heat stress is an important component of the cellular heat-shock response, we hypothesised that the higher rates of synthesis that follow a heat stimulus lead to higher knockdown tolerance and underpins the cline. However, levels of heat-stimulated total protein synthesis have been negatively related to heat-hardening capacity, a somewhat conflicting result. Here we examine the relationship between these physiological and adaptive traits in a set of 40 family lines derived from a hybrid laboratory population established by crossing populations from either end of the latitudinal transect. Among these lines high levels of heat-stimulated total protein synthesis were associated with both low basal and low heat-hardened adult knockdown time, confirming the importance of a negative relationship between protein synthesis and thermal tolerance. This result, when considered along with the directions of the latitudinal clines in protein synthesis and tolerance, suggests that variation in rates of heat-stimulated total protein synthesis is not a factor contributing to the latitudinal cline in heat tolerance. Given the robustness of this negative relationship we discuss possible explanations and future experiments to elucidate how the cellular heat stress response might facilitate increased knockdown tolerance.

1. Introduction

Heat stress affects many organisms across the globe, with changing climates posing a real threat to species' distributions and abundance. Now that we are overtly aware of global warming increased effort has been put towards understanding adaptation to warmer environments (Dillon et al., 2010; Hoffmann and Sgrò, 2012). Intra-specific thermal tolerance variation among populations and strains has been a focus for investigation of the selective and underlying physiological and genetic basis for such adaptive change, especially in ectothermic species such as fish and insects (Deutsch et al., 2008; Healy and Schulte, 2012). In particular, adaptive geographic variation in heat tolerance in numerous species shows how populations evolve to suit the thermal environment (Kuo and Sanford, 2009; Bahrndorff et al., 2006; Sarup et al., 2006).

The thermal tolerance clines across latitudinal gradients in *Drosophila melanogaster* have been well characterised (Guerra et al., 1997; Hoffmann et al., 2002; Fallis et al., 2011). Several other traits also cline incrementally with latitude along the Australian eastern coast in this species (Hoffmann and Weeks, 2007), and they are underpinned by genetic differentiation established by differential selective processes in divergent climatic regions (Kolaczkowski et al., 2011). The three heat tolerance traits that cline are adult heat knockdown time, heat-hardened knockdown time, and heat-hardening capacity (Hoffmann et al., 2005; Sgrò et al., 2010; Cockerell et al., submitted for publication). Testing conditions are important however as not all measures of heat tolerance show clinal variation (Sgrò et al., 2010; Terblanche et al., 2011).

Recent progress towards understanding the physiological basis of heat tolerance variation in *D. melanogaster* suggests that heritable variation in levels of protein synthesis may be important. First, recent application of genomic technologies to understanding the genetic basis of the response to heat stress indicate that many candidate genes fall into the 'GO'-groupings of *translation* and *regulation of transcription* (Leemans et al., 2000; Sørensen et al., 2005; Laayouni et al., 2007),

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suggesting that protein synthesis may be a relevant underlying process. A change in the control of protein synthesis following heat stress has been recognised for a number of years – it is an important component of the cellular heat-shock response (Storti et al., 1980; Ballinger and Pardue, 1983) in which new heat shock proteins are synthesised and the synthesis of vulnerable housekeeping proteins is curtailed (Lindquist, 1980; Sørensen et al., 2005). The association between *Drosophila* heat tolerance and enhanced synthesis of heat-shock proteins was first reported in the 1980s (Stephanou et al., 1983). The heat-shock proteins are a group of highly conserved proteins that act as molecular chaperones with numerous diverse functions that protect and stabilise other proteins, assisting with protein folding, re-naturation, transport and degradation (Parsell and Lindquist, 1993). Numerous studies suggest that higher levels of the heat-inducible heat-shock proteins associate with higher thermal tolerance (Parsell and Lindquist, 1993), however not all data are in agreement (Feder and Hofmann, 1999; Jensen et al., 2010).

The simplest hypothesis that connects protein synthesis with heat tolerance is that faster synthesis of proteins that are up-regulated in the cellular heat-shock response would increase heat knockdown tolerance. The possibility that variation in protein synthesis may contribute to the Australian latitudinal clines in thermal tolerance is highlighted by the recent detection of clinal variation in levels of heat-stimulated protein synthesis along the same latitudinal transect (Cockerell et al., submitted for publication). This parallel clinal variation represents an intriguing possibility for a causal connection between a physiological mechanism and an adaptive trait since higher rates of heat-stimulated protein synthesis were detected in low-latitude tropical populations that are associated with higher heat tolerance. However in this latter study only a low level of direct association was detected between rates of protein synthesis and adult heat knockdown tolerance.

In contrast, in studies of multiple family lines isolated from a single population, a negative association was detected between the rate of heat-stimulated protein synthesis and the extent of increase in knockdown time following a heat-hardening stress (Johnson et al., 2009). The measure of protein synthesis used in both of these studies detects total new protein synthesised in the 60 min recovery period following a 60 min heat stimulus (37 °C). The cellular heat-shock response quickly activates during such a mild heat pulse (Lindquist, 1980), rapidly up-regulating synthesis of heat shock proteins (Hsps) and curtailing synthesis of normal cellular proteins (25 °C proteins). During the second recovery hour, when total protein synthesis was measured, synthesis of Hsps (one of which is Hsp70) continues, diminishing slowly, and resynthesis of the normal 25 °C proteins begins to be restored (Storti et al., 1980; DiDomenico et al., 1982a; Stephanou et al., 1983). This measure of protein synthesise is therefore a complex one, since it confounds both of these protein synthesis processes.

The above two studies therefore provide somewhat conflicting information – the clinal data being consistent with the hypothesis that faster protein synthesis is positively related to higher heat tolerance, that may help explain the latitudinal clines in tolerance, and the family line data that suggests that a faster rate of heat-stimulated protein synthesis is negatively related to heat hardening capacity. Here we test the hypothesis of a positive protein synthesis – tolerance relationship and re-examine whether or not heat-stimulated protein synthesis is negatively related to *Drosophila* heat knockdown tolerance. The approach is similar to one previously used to maximise trait variation and to test for the robustness of correlations between stress resistance and other traits (Hoffmann et al., 2001). We perform a family association study that incorporates the broadest spectrum of genetic variants from across climatic regions. We derive a hybrid population by crossing two populations, one from the tropical end and one from the temperate end of the latitudinal cline. After mass rearing in the laboratory for twelve generations, to facilitate genome mixing and to minimise latitude specific linkage disequilibrium, we establish a set of 40 family lines and characterised them for variation in both adult heat knockdown tolerance and rates of protein synthesis. The associations we detect provide clear answers to our questions and strongly indicate that latitudinal variation in rates of protein synthesis do not contribute to clinal variation in thermal tolerance.

2. Materials and methods

2.1. Collection and maintenance of lines

Protein synthesis rate and heat knockdown time were measured on a set of 40 single-family lines of *D. melanogaster* established by crossing northern and southern latitude populations. This set of hybrid lines contained gene combinations from the entire latitudinal cline (latitudinal populations used in Sgrò et al., 2010 and Cockerell et al., submitted for publication) that were generated by disrupting gene combinations from the cline ends. First, one southern and one northern population were set up with equal numbers of adult flies from each of three southern populations (latitudes 43.15°S, 37.77°S and 36.92°S) and three northern populations (19.97°S, 18.20°S, 17.52°S), respectively. More than a hundred northern males and an equivalent number of southern virgin females, and the reciprocal cross, were mated for two days and then combined to form one large mass-bred population. After twelve generations as a single mass-bred population (maintained in four culture bottles each containing 80 ml medium-flies being mixed each generation and approximately 300 flies contributing to egg production for four hours per bottle, per generation) 240 lines were initiated using virgin females in single-pair crosses. These 240 lines were reduced to forty lines by performing heat tolerance tests on 10 males and 10 females from each line, using the protocol for heat tolerance testing described below, and selecting the 10 most and 10 least heat tolerant lines, from each of the heat hardened and basal tests, as a strategy to maximise the chances of finding an important physiological or genetic association. Lines were maintained in large numbers (as above) in single bottle culture on potato-yeast-dextrose-agar medium and experiments were performed on F_7 individuals. Density was controlled one generation prior to testing for both heat knockdown tolerance and rates of protein synthesis by having flies lay on medium on small spoons, and counting and transferring 50 eggs to single-vial cultures.

2.2. Rate of protein synthesis

Protein synthesis rate was measured in mature female ovaries by quantifying the amount of ^{35}S-labelled methionine incorporated into newly synthesised proteins in 1 h at 25 °C as described in detail by Johnson et al. (2009). This method was basically reported by Stephanou et al. (1983) except that live females, rather than excised ovaries, were heat stimulated. Briefly, for the heat stimulus, females were placed in a 1.7 ml microcentrifuge tube with a pierced lid for ventilation and incubated in a water bath at 37 °C for 60 min. Immediately flies were anaesthetised with CO_2 and ovaries dissected out for labelling. Samples (ovaries from 4 females) were incubated for 1 h in 6 μl Grace's Insect Medium (Invitrogen) and 6 μCi of ^{35}S-methionine (Amersham Biosciences). Following thorough washing to remove residual unincorporated label and subsequent extraction of proteins, counts per minute (CPM) of radioactive emissions from a supernatant aliquot of each sample were recorded. Three replicate assays using untreated females and three replicate assays using heat-stimulated females were performed for each of the forty lines (a total of 240 samples

were measured). Females for each assay were from different rearing vials. One untreated and one heat-stimulated biological replicate from each line was completed on each day. Within each block/day ten assays occurred per session (five lines per session, each non-treated assay being followed by a heat-stimulated assay of the same line). Lines were selected at random in any session within a day until all three heat-stimulated and three untreated assays were completed for each line.

2.3. Heat tolerance

Adult basal and hardened heat knockdown tolerance was assessed in parallel for 5–7 day old females reared under constant 25 °C conditions (as in Cockerell et al., submitted for publication). Hardening involved exposing flies to 37 °C for 1 h, with 6 h recovery at 25 °C prior to testing. To assess knockdown time, individual flies were placed into 5 ml glass vials that were sealed water-tight with a plastic cap (many being held in a rack) and submerged into a recirculating water bath held at 39 °C. Heat knockdown time was measured as the time taken for each fly to be rendered immobile from the heat. For each treatment (hardened and basal) 12 females per line were tested randomly over 10 runs over 2 days, with all runs containing equal numbers of each treatment.

2.4. Data analysis

Protein synthesis data (CPM values) were corrected for session effects by multiplying each by grand mean/session mean. We also calculated for each line the change in rate of protein synthesis following heat stimulus by subtracting the heat-stimulated rate from the basal rate. Heat knockdown time data were corrected for run by multiplying each knockdown time by grand mean/run mean. Hardening capacity was also determined for these data, calculated as hardened heat knockdown time minus basal heat knockdown time. To test for protein synthesis and knockdown time differences among lines, and interactions between heat treatments and line, two-way analyses of variance were performed. Treatment and line were treated as fixed effects. Linear regression analyses, Pearson correlation coefficients and partial correlations were used to look for relationship between protein synthesis and heat tolerance. All analyses were performed using SPSS for Windows (IBM PASW Statistics 18.0.1).

3. Results

Significant variation in protein synthesis rate was detected among lines (Fig. 1, Table 1). The heat stimulus reduced protein synthesis rate, which was on average 27% lower in flies that were heat-stimulated compared to those that were not. While not significant, a positive correlation occurred between basal and heat-stimulated protein synthesis ($r=0.26$, $p=0.058$). The presence of a significant interaction indicated that the extent of reduction in protein synthesis following heat stimulus was quite different for different lines (Fig. 1).

Significant variation for both basal and hardened heat knockdown time was detected among lines, as indicated by two-way Anova (Table 1). No interaction was detected between line and hardening treatment suggesting that the heat hardening treatment had a similar effect on all lines, increasing knockdown time from an average of 20.9 min to 26.5 min (Fig. 2).

Heat-stimulated protein synthesis rates showed significant negative associations with both basal heat knockdown time and heat-hardened knockdown time (Table 2, Fig. 2), and basal rates of protein synthesis were negatively associated with hardened knockdown time. The partial correlation of basal rate of protein synthesis with hardened knockdown time, controlling for rate of heat-stimulated protein synthesis, reduced the association to a non-significant value ($r_p=-0.297$, $p=0.078$), whereas the relationship of heat-stimulated protein synthesis to hardened knockdown time, controlling for basal rate of protein synthesis, remained significant ($r_p=-0.469$, $p=0.004$). This suggests that the more robust association was between heat-stimulated protein synthesis and hardened knockdown time. No associations were detected

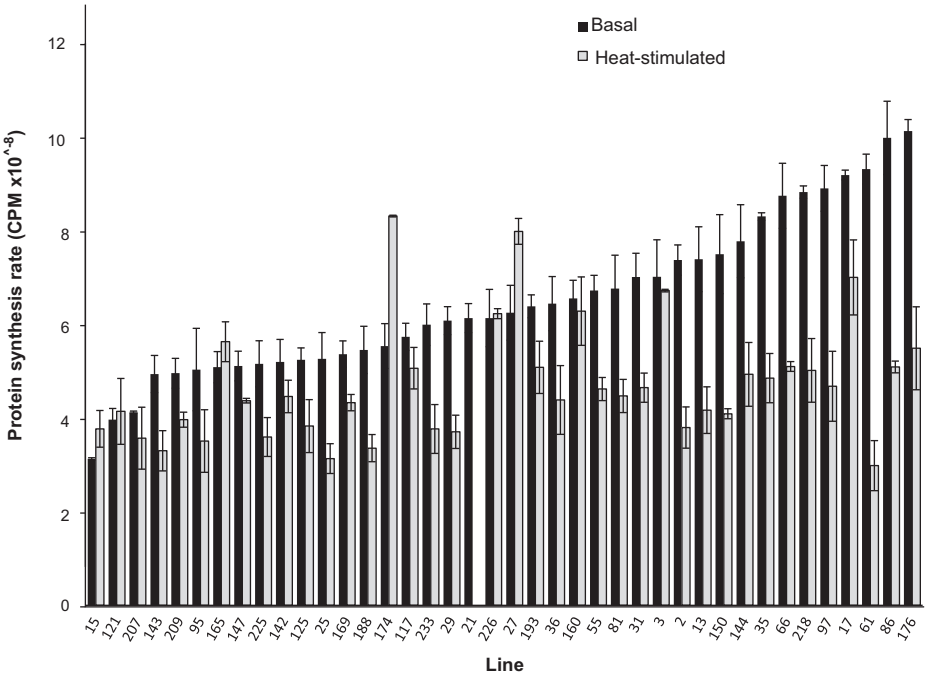

Fig. 1. Rates of basal and heat-stimulated total protein synthesis for the 40 lines from the hybrid population, ranked in order of ascending basal rates. Error bars ± standard error ($n=3$ for each bar).

Table 1
Two-way analyses of variance testing for effects of line and heat stimulus on rates of ovarian protein synthesis and for effects of line and heat hardening on heat knockdown time across forty lines from the hybrid population.

Trait	Term	df	SS	*F*	*p*
Protein synthesis	Line	39	321.87	11.803	< 0.0001
	Heat-stimulated treatment	1	174.22	249.163	< 0.0001
	Line × heat-stimulated treatment	39	201.20	7.378	< 0.0001
	Error	158	110.48		
Heat knockdown	Line	40	7602.56	4.17	< 0.0001
	Hardening treatment	1	7225.14	158.43	< 0.0001
	Line × hardening treatment	37	1825.98	1.08	0.3417
	Error	819	37351		

between basal or heat-stimulated protein synthesis and heat hardening capacity (Table 2).

Given the marked variation among lines in the extent of difference between heat-stimulated and basal rates of protein synthesis we looked for associations between the rate differences and the heat tolerance measures, but none were indicated (data not shown).

4. Discussion

For technical expediency we have measured basal and heat-stimulated total protein synthesis in ovarian tissue as this seemed a reasonable proxy for rates of synthesis in the whole fly – in mature females the ovarian tissue constitutes a high proportion of total body mass. We therefore need to keep in mind that, while the cellular heat stress response occurs in most if not all tissues (Kültz, 2005), this measure may not reflect exact patterns of protein synthesis in other body tissues.

In an earlier study, that used a set of lines derived from a single field population, a negative association was detected between heat-stimulated protein synthesis and hardening capacity (Johnson et al., 2009). In the current set of lines this specific association was not detected. These hybrid lines did not show significant differences in heat hardening capacity which may be one reason why we did not detect a hardening capacity/protein synthesis association. However, among these hybrid lines negative relationships were detected between rates of protein synthesis, particularly after a heat stimulus, and adult heat knockdown time. Although basal levels of protein synthesis were negatively associated with hardened knockdown time it was heat-stimulated protein synthesis that was the more robustly negatively associated, with both basal and hardened knockdown time, since partial correlation analyses suggested that the association between basal rate of protein synthesis with knockdown time was a consequence of the relationship between basal and heat-stimulated rates of protein synthesis.

Consistent between the current study and that of Johnson et al. (2009) is that the associations in both studies are negative in direction and both involve heat-stimulated protein synthesis. This negative relationship however does not help explain the latitudinal clines in knockdown time since *both* protein synthesis rate and knockdown time increase at tropical latitudes, and a positive relationship would be required. Therefore it seems likely that other physiological processes have an over-riding effect on any influence that protein-synthesis might have on the latitudinal cline in adult heat knockdown time. While clinal latitudinal variation in protein synthesis in tissues of *D. melanogaster* does not obviously relate to clinal variation in adult heat knockdown tolerance, clines in rates of both heat-stimulated protein synthesis and basal protein synthesis (when cultured at 18 °C – Cockerell et al., submitted for publication) might differentially affect cellular processes at different latitudes. This variation may influence other quantitative traits involved with climatic adaptation. Further investigation into the role of protein synthesis in climatic adaptation is therefore warranted - in particular asking how rates of heat-stimulated protein synthesis measured in ovaries relate to female reproductive fitness traits measured following periods of thermal stress.

The extent to which total protein synthesis was reduced following the heat stimulus was notably different among the lines – a few lines even appeared to increase protein synthesis following the heat treatment. This suggested the idea that the more extreme changes are needed in some lines to maintain tolerance, i.e. that these are the less tolerant lines. However the absence of any association between the extent of heat-stimulated change in protein synthesis and our two measures of heat tolerance did not support the idea.

Can our measure of heat-stimulated protein synthesis, that is not taken during the first 30 mins of heat stress – the time during which the adult succumbs and is immobilised in a tolerance test – tell us anything about the processes that affect heat knockdown time? Our measure of protein synthesis is that which occurred during the 1 h of recovery that followed a mild 1 h 36 °C heat stimulus. Considerable *in vitro* and *in vivo* research on this type of exposure of *D. melanogaster* or their cells to heat stress provides pertinent understanding of the underlying cellular heat stress response. While the RNA expression of many hundreds of genes are up- or down-regulated within this two hour period (Sørensen et al., 2005; Kültz, 2005) the level of total protein synthesised in the recovery hour, as visualised by radio-labelled protein on one- and two-dimensional electrophoretic gels, can be partitioned into that due to the conserved Hsps and that due to synthesis of normal cellular proteins (Storti et al., 1980; DiDomenico et al., 1982b; Stephanou et al., 1983) – the functional mRNAs of these 25 °C proteins having been preserved during the mild stress (Yost et al., 1990). In general, immediately after the return to 25 °C cells continue to produce Hsps, with production of 25 °C proteins re-occurring slowly (Yost et al., 1990). Gel labelling intensity suggests that a major part of the recovery-hour synthesis is of Hsps, mostly Hsp70. Therefore this measure could reflect either line differences in the capacity to synthesise Hsps or line differences in the capacity to shutdown 25 °C proteins, two processes that may not be independent (DiDomenico et al., 1982a).

In general, faster production of Hsps has been associated with increased thermal tolerance (Parsell and Lindquist, 1993). Since a major component of our heat-stimulated rate measurement can be attributed to synthesis of Hsps, and this rate only related negatively to knockdown tolerance, our data do not support the idea that faster production of Hsps in general increases heat tolerance. In fact this result is consistent with a recent finding that variation among lines of adult heat knockdown tolerance did not relate to line variation in their rate of Hsp70 production (Jensen et al., 2010). However, since the different Hsps have individual tissue and timing profiles of expression (Lindquist, 1980; Palter et al., 1986) our results do not exclude the possibility that faster synthesis of a specific Hsp might not directly influence adult knockdown time.

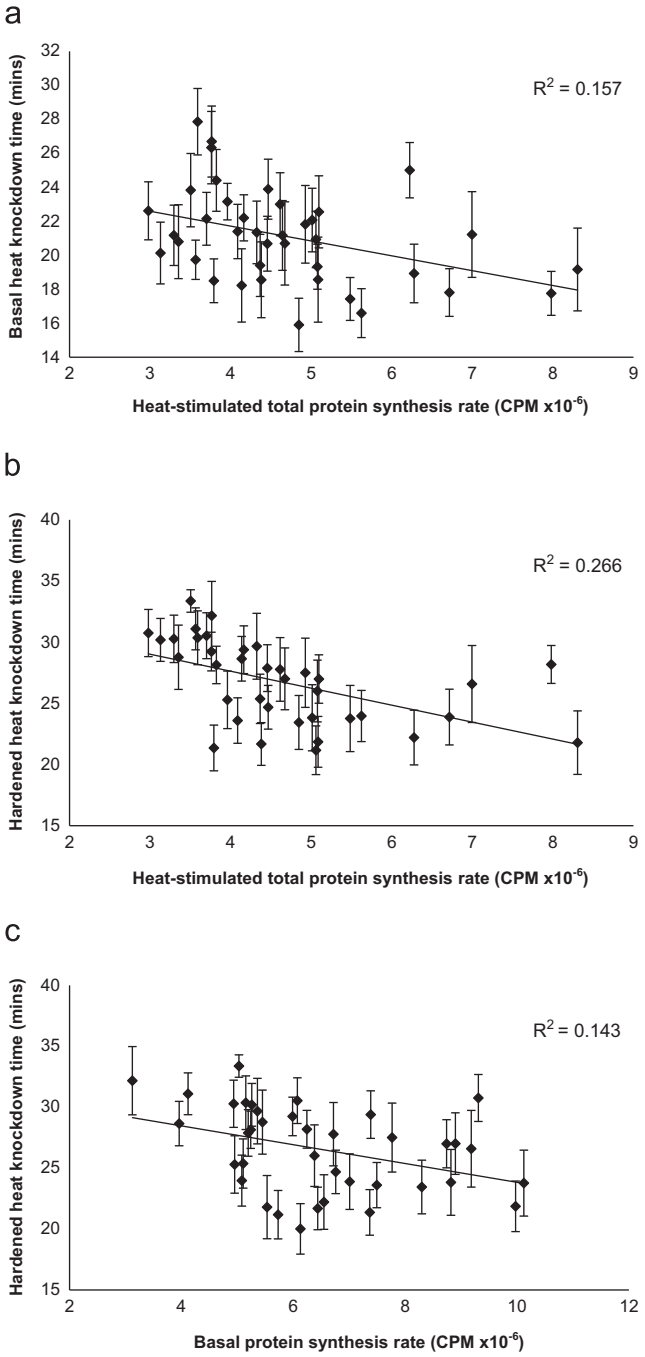

Fig. 2. Relationships between protein synthesis rate and heat tolerance in lines from the hybrid population. (a) Basal knockdown time associated with heat-stimulated total protein synthesis rate ($p=0.013$, slope $=-0.873\pm0.333$, $R^2=0.157$). (b) Hardened heat knockdown time associated with heat-stimulated total protein synthesis rate ($p=0.001$, slope $=-1.388\pm0.389$, $R^2=0.266$). (c) Hardened heat knockdown time associates with basal protein synthesis rate ($p=0.019$, slope $=-0.771\pm0.315$, $R^2=0.143$). Error bars $\pm$ standard error (based on 12 females per line).

While numerous explanations for a negative association are possible, two possibilities can be suggested. If slower production of heat-shock proteins is responsible for higher tolerance the heat-inducible Hsp90 could be implicated. Hsp90 is constitutively present in all cells and binds with and stabilises a large number of client, cell-signal proteins (Chiosis et al., 2013). If, early in the response to heat, reduced levels of Hsp90 were to occur this could potentially increase heat knockdown time for two reasons. First, Hsp90 is a negative regulator of the cellular heat-shock response (Zou et al., 1998; Bharadwaj, et al., 1999) and lower levels of Hsp90 would be expected to enhance the response, and second Hsp90 contributes directly to pausing the expression of many stress response genes (Sawark et al., 2012), many of which help protect cells from heat damage, so lower levels could lead to earlier activation of these genes. Also note that Hsp90 has been postulated to down regulate global protein synthesis during stress (Pal, 1998). Alternatively, if faster shutdown in synthesis of 25 °C proteins is responsible for the negative association this could, early in the heat stress, result in less heat-denatured and aggregated proteins that might otherwise interfere with normal cell physiology and decrease knockdown time. If curtailing synthesis of 25 °C proteins proved to be a key factor, investigating the role of the heat stress RNA gene *hsr-omega* that is up-regulated by heat shock in concert with the Hsps, and by the same heat-shock-factor mechanism, would be worthwhile. *Hsr-omega* helps to down-regulate general protein synthesis (Johnson et al., 2011) and its expression and genotype variation have been associated with adult heat knockdown time in this species (McKechnie et al., 1998). These speculative ideas could be considered in future research. For example, it would be of interest to look for associations of knockdown tolerance specifically with, (a) rates of production of individual Hsps, and *hsr-omega* transcripts, during the first 30 min of heat stimulus (as is used to measure knockdown time), and (b) rates of shutdown of normal 25 °C proteins in this 30 min period, and compare these with heat knockdown time across a set of lines similar to those used in the current study. Both specific Hsp synthesis and 25 °C protein synthesis could be quantified from radio-labelled gels in the same experiment using established techniques (Storti et al., 1980; Stephanou et al., 1983). Of course the negative association might be indirect and some common factor that influences both knockdown time and heat-stimulated protein synthesis may be responsible for the association.

Table 2
Associations between protein synthesis rate and adult heat knockdown tolerance among *D. melanogaster* lines from the hybrid population (Pearson's correlation coefficient, n = sample size).

Heat knockdown time	Protein synthesis rate			
	Basal	n	Heat-stimulated	n
Basal	−0.196 ($p=0.112$)	40	−0.396 ($p=0.006$)	39
Hardened	−0.378 ($p=0.010$)	38	−0.516 ($p=0.001$)	37
Hardening capacity	−0.226 ($p=0.081$)	40	−0.112 ($p=0.249$)	39

In summary, we have used a fairly course measurement of total protein synthesis to see if it relates to levels of heat knockdown time measured in adult female *D. melanogaster*. While the evidence is only correlative it re-affirms, since it largely repeats two similar previous observations, the significance in this species of a negative relationship between levels of heat-stimulated total protein synthesis and heat tolerance. We discuss possible mechanisms that might be responsible for this negative association, in terms of components of the cellular heat stress response, and we suggest a future experiment to narrow down possible explanations. We found no evidence for a positive association between rates of general protein synthesis following heat stress and thermal tolerance, although this result does not exclude the possibility that up-regulation of any particular heat stress gene might be directly and positively related to knockdown tolerance. Our data clearly do not support the idea that the reported clinal variation in protein synthesis in this species contributes to the latitudinal clines in thermal tolerance.

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References

Bahrndorff, S., Holmstrup, M., Petersen, H., Loeschcke, V., 2006. Geographic variation for climatic stress resistance traits in the springtail *Orchesella cincta*. J. Insect Physiol. 52, 951–959.

Ballinger, D.G., Pardue, M.L., 1983. The control of protein synthesis during heat shock in *Drosophila* cells involves altered polypeptide elongation rates. Cell 33, 103–114.

Bharadwaj, S., Ali, A., Ovsenek, N., 1999. Multiple components of the hsp90 chaperone complex function in regulation of heat shock factor 1 *in vivo*. Mol. Cell. Biol. 19, 8033–8041.

Chiosis, G., Dickey, C.A., Johnson, J.L., 2013. A global view of Hsp90 functions. Meeting report. Nat. Struct. Mol. Biol. 20 (1), 1–4.

Cockerell, F.E., Sgrò, C.M., McKechnie, S.W., 2013. Latitudinal clines in heat tolerance, protein synthesis rates and transcripts of a candidate gene in *Drosophila melanogaster*. J Insect Physiol. (Submitted for publication).

Deutsch, C.A., Tewskbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C., Martin, P.R., 2008. Impacts of climate warming on terrestrial ectotherms acoss latitude. Proc. Natl. Acad. Sci. USA 105, 6668–6672.

DiDomenico, B.J., Bugaisky, G.E., Lindquist, S., 1982a. Heat shock and recovery are mediated by different translational mechanisms. Proc. Natl. Acad. Sci. USA 79, 6181–6185.

DiDomenico, B.J., Bugaisky, G.E., Lindquist, S., 1982b. The heat shock reponse is self regulated at both the transcriptional and post-transcriptional levels. Cell 31, 593–603.

Dillon, M.E., Wang, G., Huey, R.B., 2010. Global metabolic impacts of recent climate warming. Nature 467, 704–706.

Fallis, L.C., Fanara, J.J., Morgan, T.J., 2011. Genetic variation in heat-stress tolerance among South American *Drosophila* populations. Genetica 139, 1331–1337.

Feder, M.E., Hofmann, G.E., 1999. Heat-shock proteins, molecular chaperones, and the stress response. Annu. Rev. Physiol. 61, 243–282.

Guerra, D., Cavicchi, S., Krebs, R.A., Loeschcke, V., 1997. Resistance to heat and cold stress in *Drosophila melanogaster*: intra and inter population variation in relation to climate. Genet. Sel. Evol. 29, 497–510.

Healy, T.M., Schulte, P.M., 2012. Factors affecting plasticity in whole-organism thermal tolerance in common killfish (*Fundulus heteroclitus*). J. Comp. Physiol. B, Biochem. Syst. Environ. Physiol. 182, 49–62.

Hoffmann, A.A., Anderson, A.R., Hallas, R., 2002. Opposing clines for high and low temperature resistance in *Drosophila melanogaster*. Ecol. Lett. 5, 614–618.

Hoffmann, A.A., Hallas, R., Sinclair, C., Mitrovski, P., 2001. Levels of variation in stress resistance in *Drosophila* among strains, local populations, and geographic regions: patterns for desiccation, starvation, cold resistance, and associated traits. Evolution 55, 1621–1630.

Hoffmann, A.A., Sgrò, C.M., 2012. Climate change and evolutionary adaptation. Nature 470, 479–485.

Hoffmann, A.A., Shirriffs, J., Scott, M., 2005. Relative importance of plastic vs genetic factors in adaptive differentiation: geographical variation for stress resistance in *Drosophila melanogaster* from eastern Australia. Funct. Ecol. 19, 222–227.

Hoffmann, A.A., Weeks, A., 2007. Climatic selection on genes and traits after a 100 year-old invasion: a critical look at the temperate-tropical clines in *Drosophila melanogaster* from eastern Australia. Genetica 129, 133–147.

Jensen, L.T., Cockerell, F.E., Kristensen, T.N., Rako, L., Loeschcke, V., McKechnie, S.W., Hoffmann, A.A., 2010. Adult heat tolerance variation in *Drosophila melanogaster* is not related to Hsp70 expression. J. Exp. Zool. Part A Comp. Exp. Biol. 313A, 35–44.

Johnson, T.K., Cockerell, F.E., Carrington, L.B., Rako, L., Hoffmann, A.A., McKechnie, S.W., 2009. The capacity of *Drosophila* to heat harden associates with low rates of heat-shocked protein synthesis. J. Therm. Biol. 34, 327–331.

Johnson, T.K., Cockerell, F.E., McKechnie, S.W., 2011. Transcripts from the *Drosophila* heat-shock gene *hsr-omega* influence rates of protein synthesis but hardly affect resistance to heat knockdown. Mol. Genet. Genomics 285, 313–323.

Kolaczkowski, B., Kern, A.D., Holloway, A.K., Begun, D.J., 2011. Genomic differentiation between temperate and tropical Australian populations of *Drosophila melanogaster*. Genetics 187, 245–260.

Kültz, D., 2005. Molecular and evolutionary basis of the cellular stress response. Annu. Rev. Physiol. 67, 225–257.

Kuo, E.S., Sanford, E., 2009. Geographic variation in the upper thermal limits of an intertidal snail: implications for climate envelope models. Mar. Ecol. Prog. Ser. 388, 137–146.

Laayouni, H., Garcia-Franco, F., Chavez-Sandoval, B.E., Trotta, V., Beltran, S., Corominas, M., Santos, M., 2007. Thermal evolution of gene expression profiles in *Drosophila subobscura*. BMC Evol. Biol. **7**, 42–56.

Leemans, R., Egger, B., Loop, T., Kammermeier, L., Haiqiong, H., Hartmann, B., Certa, U., Hirth, F., Reichert, H., 2000. Quantitative transcript imaging in normal and heat-shocked *Drosophila* embryos by using high density oligonucleotide attays. Proc. Natl. Acad. Sci. USA 97, 12138–12143.

Lindquist, S., 1980. Varying patterns of protein synthesis in *Drosophila* during heat shock: Implications for regulation. Dev. Biol. 77, 463–479.

McKechnie, S.W., Halford, M.M., McColl, G., Hoffmann, A.A., 1998. Both allelic variation and expression of nuclear and cytoplasmic transcripts of *hsr-omega* are closely associated with thermal phenotype in *Drosophila*. Proc. Natl. Acad. Sci. USA 95, 2423–2428.

Pal, J.K., 1998. Hsp90 regulates protein synthesis by activating the heme-regulated eukaryotic initiation factor 2α (eIF-2α) kinase in rabbit reticulocyte lysates. J. Biosci. 23, 353–360.

Palter, K.B., Watanabe, M., Stinson, L., Mahowald, A.P., Craig, E.A., 1986. Expression and localisation of *Drosophila melanogaster* hsp70 cognate proteins. Mol. Cell. Biol. 6, 1187–1203.

Parsell, D.A., Lindquist, S., 1993. The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. Annu. Rev. Genet. 27, 437–496.

Sarup, P., Sorensen, J.G., Dimitrov, K., Barker, J.S.F., Loeschcke, V., 2006. Climatic adaptation of *Drosophila buzzatii* populations in southeast Australia. Heredity 96, 479–486.

Sawark, R., Sievers, C., Paro, R., 2012. Hsp90 globally targets paused RNA polymerase to regulate gene expression in response to environmental stimuli. Cell 149, 807–818.

Sgrò, C.M., Overgaard, J., Kristensen, K.V., Mitchell, K.A., Cockerell, F.E., Hoffmann, A.A., 2010. A comprehensive assessment of geographic variation in heat tolerance and hardening capacity in populations of *Drosophila melanogaster* from eastern Australia. J. Evol. Biol. 23, 2484–2493.

Sørensen, J., Nielsen, M., Kruhøffer, M., Justesen, J., Loeschcke, V., 2005. Full genome gene expression analysis of the heat stress response in *Drosophila melanogaster,* Cell Stress and Chaperones 10, 312–328.

Stephanou, G., Alahiotis, S.N., Christodoulou, C., Marmaras, V.J., 1983. Adaptation of *Drosophila* to temperature: heat-shock proteins and survival in *Drosophila melanogaster*. Dev. Genet., 299–308.

Storti, R.V., Scott, M.P., Rich, A., Pardue, M.L., 1980. Translational control of protein synthesis in response to heat shock in *Drosophila melanogaster* cells. Cell 22, 825–834.

Terblanche, J.S., Hoffmann, A.A., Mitchell, H.K., Rako, L., le Roux, P.C., Chown, S.L., 2011. Ecologically relevant measures of tolerance to potentially lethal temperatures. J. Exp. Biol. 214, 3713–3725.

Yost, H.J., Petersen, R.B., Lindquist, S., 1990. Post-transcriptional regulation of heat shock protein synthesis in *Drosophila* Stress Proteins. In: Morimoto R.I., Tissiêres A. and Georgopoulos C. (Eds.). Biology and Medicine. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., pp. 379–409.

Zou, J., Guo, Y., Guettouche, T., Smith, D.F., Voellmy, R., 1998. Repression of heat shock transcription factor HSF1 activation by HSP90 (Hsp90 complex) that forms a stress-sensitive complex with HSF1. Cell 94, 471–480.