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Transcriptional profiles of plasticity for desiccation stress in *Drosophila*

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ABSTRACT

We examined the transcriptional responses of desiccation resistance candidate genes in populations of *Drosophila melanogaster* divergent for desiccation resistance and in capacity to improve resistance *via* phenotypic plasticity. Adult females from temperate and tropical eastern Australian populations were exposed to a rapid desiccation hardening (RDH) treatment, and groups without RDH to acute desiccation stress, and the transcript expression of 12 candidate desiccation genes were temporally profiled during, and in recovery from stress. We found that desiccation exposure resulted in largely transitory, stress-specific transcriptional changes in all but one gene. However linking the expression profiles to the population-level phenotypic divergence was difficult given subtle, and time-point specific population expression variation. Nonetheless, rapid desiccation hardening had the largest effect on gene expression, resulting in distinct molecular profiles. We report a hitherto uncharacterised desiccation molecular hardening response where prior exposure essentially 'primes' genes to respond to subsequent stress without discernible transcript changes prior to stress. This, taken together with some population gene expression variation of several *bona fide* desiccation candidates associated with different water balance strategies speaks of the complexity of natural desiccation resistance and plasticity and provides new avenues for understanding the molecular basis of a trait of ecological significance.

1. Introduction

Water availability impacts the behavior, abundance and range distribution of terrestrial ectotherms (Chown and Nicolson, 2004; Chown et al., 2011). For small insects highly sensitive to environmental change like *Drosophila*, spatial distribution is limited by genetic variation for desiccation resistance, where more resistant species and populations inhabit drier habitats compared to their less resistant counterparts (Parsons, 1982; van Herrewege and David, 1997). The physiological adaptations underpinning the desiccation resistance spectrum can arise through multiple mechanisms including increased water retention, bulk water storage, and greater water loss tolerance (Gibbs et al., 2003; Gibbs and Matzkin, 2001). Water budgeting mainly occurs *via* the epicuticle barrier, through respiratory control across the spiracles or gut epithelia and by excretion and osmoregulation *via* the Malpighian tubules (MTs) (Hadley, 1994). Intra- and inter-specific data in *Drosophila* demonstrate that desiccation resistance is achieved by remarkably diverse, genotype specific combinations of the different water balance mechanisms (Chippindale et al., 1998; Folk et al., 2001; Gibbs and Matzkin, 2001; Marron et al., 2003; Telonis-Scott et al., 2006).

The environment also impacts patterns of desiccation resistance; female *Drosophila* increased their desiccation resistance following a non-lethal exposure to desiccation ('hardening') (Hoffmann, 1990; Hoffmann, 1991). Flexibility to improve desiccation resistance *via* hardening is of interest to biologists studying insects in a changing climate, although the contribution of phenotypic plasticity to fitness under naturally fluctuating humidity remains unclear. In the laboratory plastic resistance physiology appears to be as variable as genetic resistance, although there may be some shared mechanisms (Hoffmann, 1990). Hardening may cause changes in cuticular permeability to stem water loss in *D. melanogaster* (Bazinet et al., 2010), including changes in cuticular hydrocarbon (CHC) abundances (Stinziano et al., 2015). Fixed cuticle structure may also influence hardening. Specifically, melanic species of four Indian *Drosophila* subgenera exhibited hardening responses despite lack of changes in cuticular traits or cuticular water loss, while non-melanic species lacked resistance plasticity (Kalra et al., 2014). Alternatively, Parkash et al. (2014) associated increases in desiccation resistance in hardened *D. melanogaster* to changes in osmolyte concentration, owing to increases in bulk water storage.

Despite the well characterised physiology, key knowledge gaps remain in linking the desiccation resistant/susceptible phenotype-to-genotype, and it is unknown if genetic (innate/basal) and plastic tolerances share similar molecular architectures or present as separate traits. Candidate genes for desiccation resistance to date derive from approaches focussed on either gene/s of large effect or from complex trait analyses spanning a range of water balance mechanisms and other

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plausible functions including initial hygrosensing and activation of stress response pathways, stress sensing and excretory/osmoregulatory balance in the gut and Malpighian tubules (MTs), insulin signalling, the cuticular water loss barrier (CHCs) and tissue protectants including trehalose (reviewed in Chown et al., 2011; Telonis-Scott et al., 2016). Surprisingly few of these genes have been examined in detail in natural *Drosophila* varying in desiccation resistance, and none in the context of desiccation hardening. To address this, we examined patterns of genetic ('basal') desiccation resistance and hardening ('plastic') responses in tropical and temperate *D. melanogaster* females and profiled the expression of key candidate genes with and without a non-lethal pre-exposure over a comprehensive stress/recovery time-course.

We sampled populations from the ends of the east Australian latitudinal gradient given previous evidence for plasticity and genetic differentiation for desiccation resistance in tropical and temperate *D. melanogaster* and *D. simulans* (Hoffmann, 1991). We profiled candidate genes linked either directly or indirectly to desiccation resistance encompassing several potential desiccation and defence mechanisms. Desiccation specific genes include fluid transporting diuretic neuropeptides *capa*, *capaR*, and *klu* (Kean et al., 2002; Terhzaz et al., 2014) and antidiuretic peptides *ITP* and *sNPF* (Kahsai et al., 2010). We also examined *Treh*, essential to trehalose metabolism and a marker of larval desiccation resistance (Matsuda et al., 2015; Thorat et al., 2012), insulin receptor *InR* associated with metabolic homeostasis and survival of desiccation (Söderberg et al., 2011), and *FASN2* encoding precursor fatty acids required for the synthesis of cuticle constituents methyl-branched CHC (mbCHCs) (Wicker-Thomas et al., 2015). Less directly associated with *Drosophila* desiccation resistance but expressed in the MTs include hygrosensing Transient Potential Receptors (TRPs) *trp* and *trpl*, (MacPherson et al., 2005), and *CG7084*, the most highly expressed gene in the MTs (Wang et al., 2004). Finally we examined the expression of *Mtl*, a modulator of the MAPK stress signalling network, shown to be elicited by desiccation stress in human cell lines (Huang and Tunnacliffe, 2005). While gene expression during desiccation has not been examined in east Australian populations, previous work mapping allelic polymorphisms associated with desiccation resistance in temperate females revealed variation in candidate genes *trpl*, *klu*, *ITP*, *sNPF*, *InR*, *Treh* and *Mtl* (Telonis-Scott et al., 2012; Telonis-Scott et al., 2016). Whether the expression of these genes is in fact induced by desiccation in adult females and linked to field-based divergence in basal desiccation resistance or desiccation plasticity has not been assessed.

Utilizing a natural study system and candidate gene framework, we addressed the following questions: 1) Do the temperate and tropical populations differ in their genetic and plastic responses to desiccation? 2) Are the desiccation candidate genes examined desiccation responsive? 3) Do any transcriptional profiles correspond to phenotypic differences in desiccation resistance between the populations? 4) Are there differences in the expression profiles of hardened and unhardened flies? The latter question is of interest given the well-known plastic effect of non-lethal exposure to heat stress, or heat hardening, which essentially 'primes' the fly to respond to subsequent stress largely due to the induction and sustained elevation of heat shock genes (*Hsps*) (Yost et al., 1990). We address these knowledge gaps with a particular focus on the molecular responses to desiccation hardening which remain largely unexplored in wild flies.

2. Materials and methods

2.1. Fly collection and maintenance

Flies were collected from eastern Australia and maintained as described in (Clemson et al., 2016). Briefly, mass-bred *D. melanogaster* populations from Melbourne ('temperate population', 37.8136° S, 144.9631° E) and Innisfail ('tropical population' 17.5236° S, 146.0292° E) were initiated from 30 field inseminated females. Progeny were mixed into one pool and redistributed into three 250 mL bottles with media each generation to control density. Populations were maintained this way at a census size of approximately 1000 individuals at 25 °C under 12:12 light:dark conditions.

2.2. Desiccation hardening and mortality assays

Experimental flies (generation F_8 and F_6, temperate and tropical populations respectively) were reared under common garden conditions in 250 mL bottles with media and density controlled by restricting parental oviposition to 2 h. Progeny were collected at one-two days post-eclosion into mixed sex cohorts and allowed to mate for 48 h. At three-four days post-eclosion females were sorted into groups of 10 using aspiration without CO_2. Flies from each population were then randomly assigned into two test groups: (i) 'basal' (genetic) resistance or (ii) 'hardened' (plastic) resistance and maintained in their groups in vials with media until the assays.

First, flies allocated to the hardening treatment group were subjected to a 10 hour non-lethal pre-treatment, where the groups of 20 flies were placed into empty vials t topped with gauze and put into as sealed glass desiccator containing silica gel (25 °C, < 10% relative humidity (RH)). After 10 hour exposure the flies were removed and allowed to recover on media at 25 °C for 10 h, where repeated measures show RH consistently reaches > 90% within the culture vials (Fig. 1). During this time, flies allocated to the basal treatment group were maintained in culture vials (25 °C, RH > 90%, Fig. 1). Next, the desiccation resistance of both treatment groups was measured using a desiccation mortality assay. Briefly, five-six day old individual females were transferred into 5 mL glass vials covered by gauze, and placed into the desiccator (25 °C, RH > 10%, Fig. 1). The time taken for each fly to die was recorded hourly until 100% of flies were dead (lethal time 100%, LT_{100}). Approximately 30 flies from each treatment/population were assessed for a total of 120 flies.

2.3. Time-series sampling

While population level desiccation resistance was assessed using a desiccation mortality assay, we aimed to assess the temporal expression dynamics of the candidate genes *prior* to death to avoid signatures of apoptosis. To best determine the sampling time-points during desiccation stress, flies were incrementally subjected to < 10% RH (3, 5, 6, 7, 8, 9, 10 and 11 hour stress exposure) in 10 groups of 10 females from each population, and scored for mortality after 48 hour recovery (media, 25 °C, RH > 90%). Mortality began to occur at 7 h of stress

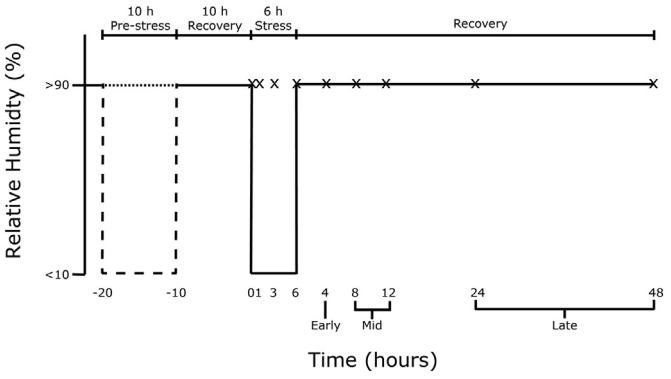

Fig. 1. Sampling schematic for two desiccation stress treatment profiles. At pre-stress, flies either underwent hardening (dashed line) at < 10% RH or remained at > 90% RH on media (basal, dotted line). The first time-point (time zero) represents 20 h following the commencement of hardening (10 hour stress, 10 hour recovery) or constant RH (basal flies). Flies sampled during stress were exposed to < 10% RH for 1, 3 or 6 h. Recovery flies were exposed to < 10% RH for 6 h before returning to > 90% RH for 4, 8, 12, 24 or 48 h. For analysis, the five 'recovery' time-points were combined to form the early, mid and late recovery time-points. Crosses represent time-points where samples were snap-frozen for gene expression analysis. All assays were performed at 25 °C.

exposure therefore the duration of stress for the transcript analysis was limited to 6 h (data not shown).

For the time-series sampling, flies were reared in controlled densities by transferring groups of 50–70 eggs into food vials then maintained as above with flies randomly assigned into the two treatment groups and subjected to the same pre-stress regime. Groups of 20 female flies were then randomly allocated into time-point groups (for a total of 60 flies per population/treatment/time-point) in the following time-series: 'pre-stress' (time-zero control group, > 90% RH, media); 'stress' at 1, 3 and 6 h (< 10% RH, no media); 'recovery' from 6 hour exposure (< 10% RH, no media) at 4, 8, 12, 24, 48 hour post stress (> 90% RH, media; Fig. 1). Flies in the 'pre-stress' group were placed into 1.7 mL Eppendorf tubes and snap frozen in liquid N_2 immediately at the start of the stress exposure. The 'stress' groups were placed into empty gauze-covered 15 mL Bunzel cryotubes, sampled immediately at the appropriate time and snap frozen in liquid N_2. The 'recovery' groups were placed into empty gauze-covered vials and stressed for 6 h, removed and recovered as above for the appropriate recovery duration, then transferred to 1.7 mL Eppendorf tubes and snap frozen in liquid N_2. All assays were conducted at 25 °C.

2.4. Quantification of transcript abundance

Total RNA was extracted using a combination of TRIzol® Reagent coupled with the Zymo Direct-zolTM RNA kit (Zymo Research Corporation, Orange, CA), following the manufacturer's instructions. This resulted in 50 μL of purified RNA per sample, which was quantified using a Nanodrop Spectrophotometer (NanoDrop Technologies, Wilmington, DE). RNA integrity was assessed with 1% agarose gel electrophoresis. Complimentary DNA (cDNA) was synthesized from 1 μg of RNA using the Roche Transcriptor First Strand cDNA Synthesis Kit (Roche Applied Science, Indianapolis, IN), following the manufacturer's instructions. The final volume was then diluted 1:10 with Diethylpyrocarbonate (DEPC) water before quantitative real-time PCR (qRT-PCR).

PCR was performed using a Roche Lightcycler® 480 and SYBR Green chemistry in 384-well plates assembled using an Eppendorf epMotion® 5075 automated pipetting system. Transcripts were amplified in a 10 μL reaction, where each well contained 5 μL Lightcycler® 480 SYBR Green 1 master-mix, 4 μL 1 μM primer mix and 1 μL 1:9 diluted cDNA. Samples were quantified in duplicate, for a total of three biological replicates per population/treatment/time-point (108 samples in total). As all samples could not fit onto a single 384 well plate, samples were blocked on plates by replicate. Transcript abundance was quantified relative to the geometric mean (GM) of three stable 'housekeeping' genes (*RpL11*, *Gapdh2* and *CycK*) using the formula: transcript of interest (TOI) = $2^{(GM - TOI)}$. To confirm that each housekeeper was not affected by desiccation stress and population differences, expression was assayed using one-way analysis of variance (ANOVA; data not shown). Primer sequences were designed using PRIMER-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/; Ye et al., 2012, Supp. Table 1).

2.5. Statistics

2.5.1. Phenotypic responses to desiccation

Mean desiccation resistance (in hours) and the effect of RDH in both populations was analysed using a two-way mixed model analysis of variance (ANOVA) with population (temperate or tropical) and treatment (basal or hardened) as fixed factors, individual fly as a random factor and the interaction between population and treatment. Residual diagnostics showed a slight departure from normality (Shapiro-Wilk test $P = 0.0157$) and this was resolved by fitting a mixed model to log transformed data with REPEATED/SUBJECT = individual (population) and the GROUP = treatment statements (PROC MIXED, SAS V9.4) to account for heterogeneity of treatment variances (Telonis-Scott et al., 2014). The interaction term was further examined by Tukey's honestly significant difference (HSD) *post hoc* tests.

2.5.2. Transcript level responses to desiccation

The effect of population (temperate or tropical) and treatment regime (basal or hardened) on the mean raw temporal expression (time-point) of each gene was examined using a three-way fixed-effects ANOVA. Residual diagnostics were performed using Shapiro-Wilk test and Levene's tests and several genes showed departures from normality, therefore the data were log transformed to improve linearity. The significant population or treatment effects were explored using planned contrasts, where treatment contrasts were performed within a population. The time-series included sampling during the recovery period at 8, 12, 24 and 48 h, however first pass analyses revealed negligible variation between 8 and 12 h and 24 and 48 h, therefore the data were pooled to create mid- (8–12 h) and late- (24–48 h) recovery time-points respectively. For consistency, we refer to the 4 h time-point as 'early-recovery'. Planned contrast *P*-values were corrected for multiple tests using a false discovery rate (FDR) approach (Benjamini and Hochberg, 1995).

We also examined differences in the temporal dynamics across the time-series within each population/treatment by comparing pre-stress (time-zero) expression to each stress/recovery time-point using Dunnett's multiple comparison tests with time-zero as the control. This resulted in a total of 20 comparisons per gene (5 for each population/treatment group combination). The average fold-changes scaled to time-zero for each time-point for each population/treatment group were calculated using the mean time-zero expression values compared to each time-point by biological replicate and shown for illustrative purposes with error bars to represent ± SE.

3. Results

3.1. Temperate and tropical females differ in their genetic and plastic phenotypic responses to desiccation

Analyses revealed significant effects of population and treatment on desiccation resistance (Table 1). The temperate females had higher basal resistance, surviving on average a further 3.2 h than tropical females (Fig. 2, Tukey's HSD, $P < 0.001$). Hardening resulted in a plastic increase in desiccation resistance of 1.2 h for temperate females and 2.8 h for tropical females (7% and 19.5% increase, temperate and tropical populations respectively, (Fig. 2); within population comparisons: Tukey's HSD, $P < 0.001$).

The significant population-by-treatment interaction term (Table 2) was largely driven by the differences in hardening responses, which were larger in the tropical population. (Fig. 2, Tukey's HSD, $P < 0.001$). Despite the tropical females' larger plastic response, the plastic increase was comparable to the basal desiccation resistance of unhardened temperate females (Fig. 2, Tukey's HSD, $P = 0.55$).

3.2. Transcript level responses to desiccation

3.2.1. Desiccation elicits expression of the candidate genes

Analyses revealed that all 12 genes exhibited significant temporal

Table 1
Two-way mixed model analyses of variance (ANOVA) with fixed effects of population and treatment (basal or hardened), and the interaction term for mean desiccation resistance. Significant terms are shown in bold.

Source	*d.f.*	F	*P*
Population	1	21.1	**4.13E − 07**
Treatment	1	28.4	**1.01E − 05**
Population × treatment	1	4.82	**0.0298**
Error	131		

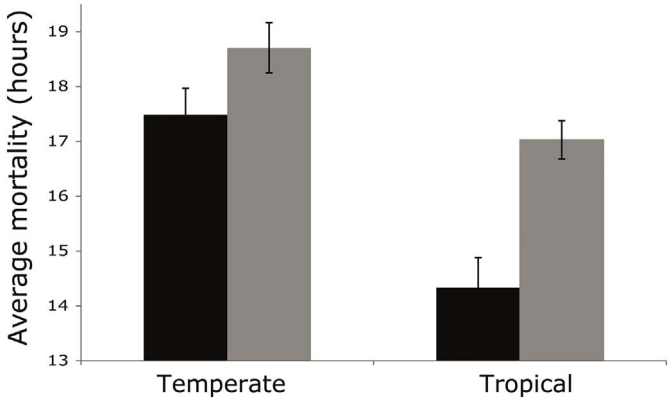

Fig. 2. Average desiccation resistance of individual female *D. melanogaster* from two populations from eastern Australia. Black bars represent unhardened treatment groups ('basal' resistance) and grey bars represent hardened treatment groups. Average desiccation resistance was significantly different within treatments and between the populations (Tukey's HSD $P < 0.001$) except for the 'basal' temperate group compared to the 'hardened' tropical group where resistance was similar (Tukey's HSD $P = 0.5452$). Error bars represent ± SE of the mean.

Table 2
Simple summary of between treatment significant contrasts for pre-stress, stress exposure and early recovery treatments*. Arrows indicate the change in expression at each time-point of the hardened treatment group (H) *vs* basal treatment group. Changes in both/and or at least one population are shown for brevity.

Gene	Pre-stress	1 hour des	3 hour des	6 hour stress	Early rec
trp	–	–	H↑	–	–
InR	H↓**	–	–	–	–
Treh	–	–		–	H↑
CG7084	–	–	H↑	H↑	H↑
klu	H↓	H↑	H↑	H↑	H↑
capa	–	H↑	H↑	H↑	–
capaR	–	H↑	H↑	H↑	–
ITP	–	H↑	H↑	H↑	–
sNPF	–	H↑	–	H↑	–
Mtl	H↓	H↓	H↓	H↓	H↓
FASN2	–	H↑	H↑	H↑	H↑

* The FDR corrected contrast *P* values for each gene, population, and time-point are given in Supp. Table 3 and expression values are shown in Supp. Fig. 1.

** Significant *P* value lost after FDR correction but trend suggests this is not a type I error.

variation in transcript abundance following desiccation exposure (Supp. Table 2). We discuss the effects of population, treatment and time-series in detail below.

3.2.2. Low levels of population-level differentiation

3.2.2.1. Population comparisons. The population term was significant for only one gene encoding cuticle constituents methyl-branched CHC (mbCHCs), *FASN2* (Three-way ANOVA, Supp. Table 2). Between population planned contrasts were performed for each treatment (Supp. Table 3), and the log2 expression profiles showing the direction of expression for each gene and population separately are in Supp. Fig. 1. Tropical females consistently overexpressed *FASN2* transcripts compared to temperate females ('basal' temperate *vs* tropical time-zero, 6 hour stress, early, mid and late recovery; FDR < 0.01, Supp. Table 3) and similarly following hardening ('hardened' temperate *vs* tropical 1 h and 6 h stress; FDR < 0.001 and < 0.05 respectively, and during mid- and late-recovery FDR < 0.01, Supp. Table 3).

The significant population-by-time-point interaction for one gene, *ITP*, encoding an antidiuretic peptide was driven by temperate females significantly down-regulating *ITP* transcripts at 6 hour stress ('basal' temperate *vs* tropical 6 h stress; FDR < 0.01 Supp. Tables 2 and 3). There were subtle differences between unhardened temperate and tropical females during stress; 6 hour stress elicited significantly higher expression of the MAPK kinase *Mtl* in tropical females compared to temperate females, and at 3 hour stress, higher expression of *Treh* in temperate females ('basal' temperate *vs* tropical FDR < 0.01 and 0.05 *Mtl* and *treh* respectively, Supp. Table 3).

3.2.3. Hardening elicits distinct molecular profiles

3.2.3.1. Treatment comparisons. The treatment term was significant for all genes barring *trpl*, *trp* and *InR* (Supp. Table 2). The effect of treatment varied temporally however, where all genes except *trpl* exhibited significant treatment-by-time-point interactions (Supp. Table 2). For each gene and population separately, we therefore used between treatment planned contrasts to examine expression differences pre-stress, during stress and in recovery. For clarity, we restricted contrasts to within each time-point for a total of six comparisons for each gene and population.

First, we assessed the effect of hardening on gene expression prior to subsequent desiccation stress, *i.e.* pre-stress basal expression *vs* pre-stress hardened expression. Hardening impacted pre-stress levels for two genes, *Mtl* and *klu* (*Mtl*: basal *vs* hardened time-zero temperate and tropical females respectively; FDR < 0.05, FDR 0.06; *klu*: FDR < 0.05, Supp. Table 3, Supp. Fig. 1) and weakly for *InR*. Rather than elevated expression following hardening, transcription of all three genes was down-regulated prior to subsequent stress and recovery, (Supp. Table 3, Supp. Fig. 1).

The effects of treatment regime were most evident during stress exposure and early recovery (11/12 genes) in at least one population. Given the large number of significant contrasts and slight population variations (Supp. Table 3,), for brevity we summarised the direction of expression change (hardening *vs* basal expression) at each time-point *across* the two populations (Table 2). Flies exposed to hardening exhibited higher expression compared to unhardened flies at 1, 3 and 6 h desiccation stress for *capa*, *capaR*, *ITP*, also extending to early recovery for *klu* and *FASN2* (Table 2). After hardening *sNPF* transcripts where higher than basal expression after 1 and 6 hour desiccation exposure, and *CG7084* transcripts were higher during mid and late stress and early recovery (Table 2). *trp* expression was higher at 3 hour stress and *Treh* was higher at early recovery (Table 2). Only *mtl* expression was lower in hardened females compared to unhardened females from the onset of stress through to early recovery (Table 2).

3.2.3.2. Temporal profiling: fold-changes relative to pre-stress levels. The between treatment contrasts revealed time-point specific treatment effects, but did not reflect the temporal dynamics of the gene/population/treatment stress and recovery expression profiles. We therefore more finely dissected the significant time-point and treatment expression variation by comparing pre-stress expression (time-zero control) to each stress and recovery time-point separately for each population and treatment using Dunnett's multiple comparison test (Fig. 3A–L, Supp. Table 4).

Temporal profiling compared to the control for each gene revealed two predominant and opposing patterns:

1) *basal treatment group*; no significant expression changes across the entire time-series in both populations *vs hardened treatment group*; significant up-regulation in stress and/early/mid-recovery (*trpl*, *trp*, *InR*, *Treh*, *CG7084*, Fig. 3A–E, Table 3).
2) *basal treatment group*; significant down-regulation of genes mostly in late stress/mid-recovery *vs hardened treatment* no significant expression changes across the entire time-series in both populations (*capa*, *capaR*, *ITP*, *sNPF* and *Mtl*, Fig. 3G–K, Table 3). Only one gene *klu*, exhibited both significant patterns during stress exposure and recovery; (basal expression down, hardened expression up, Fig. 3F, Table 3). For *FASN2*, despite significant population, and treatment differences, there were no within-population treatment differences

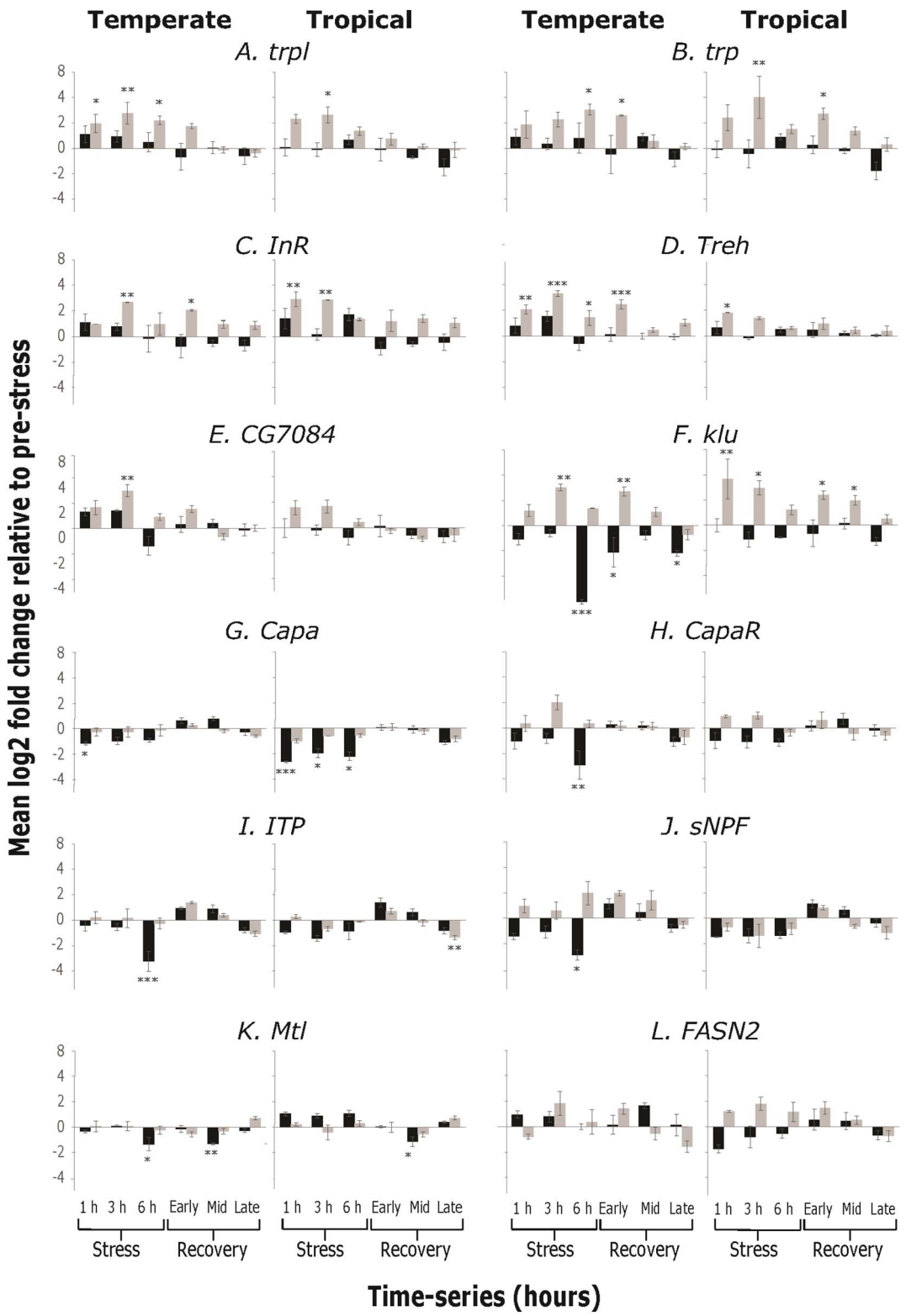

Fig. 3. Average fold changes relative to the pre-stress control for 12 desiccation candidate genes during exposure to, and recovery from desiccation. Black bars represent basal treatment groups and grey bars represent hardened treatment groups. The recovery time points were pooled into 'early', 'mid' and 'late' stages due to negligible variation (see Methods). Asterisks indicate values that are significantly different from the pre-stress control using Dunnett's tests for each population/treatment/time-point (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Error bars represent ± SE of the mean, n = 3.

Table 3
Summary of the temporal expression profiles of the 12 desiccation resistance candidate genes relative to pre-stress (time-zero) for each time-point, population and treatment. NC indicates non-significant fold changes compared to pre-stress*, arrows indicate the direction of expression for the significant time-point fold-changes relative to pre-stress (FDR 0.05). Range fold-change shows the minimum and maximum expression relative to pre-stress across the time-series.

Population	Basal expression	Hardened expression	Range fold-change
trpl			
Temperate	NC	↑Throughout stress	− 0.19–2.59
Tropical	NC	↑Early and mid-stress	− 0.06–2.49
trp			
Temperate	NC	↑Late stress & early recovery	0.07–2.86
Tropical	NC	↑Mid-stress & early recovery	0.14–4.01
InR			
Temperate	NC	↑Mid-stress & mid-recovery	1.3–2.5
Tropical	NC	↑Early-stress & mid-stress	1.45–2.74
Treh			
Temperate	NC	↑Throughout stress & early recovery	1.18–3.12
Tropical	NC	↑Early-stress	0.20–1.86
CG7084			
Temperate	NC	↑Early-stress	− 0.31–2.81
Tropical	NC	NC	
klu			
Temperate	↓Late stress, early recovery, late recovery	↑Mid-stress & early recovery	− 5.25 to − 0.31/ − 0.36–2.83
Tropical	NC	↑Early & mid-stress, early & mid-recovery	0.25–3.56
Capa			
Temperate	↓Early stress	NC	− 1.48–0.39
Tropical	↓Throughout stress	NC	− 2.49–0.03
CapaR			
Temperate	↓Late stress	NC	− 2.75–0.13
Tropical	NC	NC	
ITP			
Temperate	↓Late stress	NC	− 3.06–1.39
Tropical	NC	↓Late recovery	− 1.60–0.35
sNPF			
Temperate	↓Late stress	NC	− 2.70–1.49
Tropical	NC	NC	
Mtl			
Temperate	↓Late stress, mid-recovery	NC	− 1.59–0.03
Tropical	↓Mid-recovery	NC	− 1.49–0.69
FASN2			
Temperate	NC	NC	
Tropical	NC	NC	

* *P*-values derived from Dunnett's tests to control for multiple comparisons using time zero as the control (Supp. Table 4).

over the time-course compared to the pre-stress control, albeit there were some slight trends in the tropical females showing a similar expression pattern to *klu* (Fig. 3L, Table 3).

4. Discussion

4.1. Phenotypic responses to desiccation stress and rapid desiccation hardening

Over twenty years after the first study revealed differences in desiccation resistance and plasticity between wild temperate and tropical *Drosophila* (c.f. Hoffmann, 1990; Hoffmann, 1991), our results confirm that temperate (Melbourne) females exhibited higher basal desiccation resistance than tropical (Innisfail) females (Hoffmann, 1991). Higher desiccation resistance in temperate populations fits with 'the desiccation adaptation hypothesis' (summarised in Bujan et al., 2016), which predicts increased desiccation resistance as habitats become more arid. Such patterns are observed in *Drosophila* species (Gibbs and Matzkin, 2001; Karan et al., 1998; Kellermann et al., 2006), although less consistently across latitudinal gradients at the intraspecific level (Hallas et al., 2002; Hoffmann et al., 2005; Stanley and Parsons, 1981), while altitudinal gradients reflect more robust population-level associations (Parkash et al., 2008).

Non-lethal pre-exposure to low humidity or 'rapid desiccation hardening' (RDH) induces phenotypic plasticity to improve *Drosophila* desiccation resistance (Bazinet et al., 2010; Hoffmann, 1990; Hoffmann, 1991). We observed this plasticity in both populations, although tropical females showed greater capacity to increase their desiccation resistance *via* plasticity in contrast to previous data (Hoffmann, 1991). Tropical organisms inhabiting relatively stable habitats are predicted to have limited scope for plasticity compared to organisms exposed to fluctuating and extreme conditions in temperate zones (Janzen, 1967; Tewksbury et al., 2008). Alternatively, at least for thermal plasticity, some ectotherms may be constrained to increase heat resistance *via* plasticity when basal resistance is higher (Gerken et al., 2015; Stillman, 2003), but see (Kellett et al., 2005). While desiccation plasticity trade-offs are yet to be reported in *Drosophila*, it is difficult to generalise here from two populations. It is possible that the lower hardening response in temperate flies may simply be an artefact of experiencing less stress during the assays than tropical females due to their higher innate tolerance, similar to observations from *D. melanogaster* selected for increased desiccation resistance (Hoffmann, 1990).

4.2. Transcript level responses to rapid desiccation hardening and desiccation stress

Desiccation exposure impacted transcription of all 12 genes, confirming previous observations for desiccation responsive genes *klu*, *capa*, *capaR*, *ITP*, *sNPF* (Kahsai et al., 2010; Terhzaz et al., 2014; Terhzaz et al., 2015). We also provide transcript data for genes encoding products linked to desiccation survival *Treh* and *InR* (Söderberg et al., 2011; Yoshida et al., 2016), and for genes with indirect associations and/or lacking functional phenotypes, *trpl*, *trp*, *CG7084*, *Mtl*, *FASN2* (Huang and Tunnacliffe, 2005; Telonis-Scott et al., 2012; Telonis-Scott et al., 2016).

The transcriptional activity of most genes was temporary, and largely specific to stress exposure, and the populations differed in overall expression of only one gene, *FASN2*. *FASN2* products are required for the synthesis of precursor branched/methylated long chain fatty acids of methylated cuticular hydrocarbons (Chung et al., 2014). Adult *D. melanogaster* produce cuticular hydrocarbons required for waterproofing and pheromones in oenocyte cells (reviewed Wicker-Thomas et al., 2015). The tropical population consistently overexpressed *FASN2* compared to the temperate population, and while *within* population fold-changes relative to pre-stress during stress and recovery were not significantly altered, the hardening treatment elevated expression in both populations, and this was stable across the time-course. Stinziano et al. (2015) found that rapid desiccation hardening impacted unsaturated and methylated hydrocarbon profiles, in turn reducing water loss in *D. melanogaster* females *via* decreased cuticular permeability. Bulk water loss occurs across epicuticle, and it is plausible that *D. melanogaster* females in the current study are similarly altering cuticular permeability following hardening. CHC profiling in these populations would provide further research focus to test if this is a conserved water loss strategy among different *D. melanogaster* genotypes.

Finer dissection of each time-point compared to the pre-stress control yielded some subtle population variation in stress responses of key genes including *Treh*, and *klu*, and neuropeptide encoding *capa*, *capaR*,

ITP and *sNPF* (discussed below). The effect of treatment however was the most striking outcome, resulting in 'discrete' gene-specific expression profiles in unhardened flies (stress down regulation genes) *vs* stress up-regulation genes in flies exposed to RDH.

4.3. A novel molecular hardening response

Prior exposure to desiccation or thermal stress confers greater resistance with effects lasting beyond recovery and subsequent stress in a stress specific way. Elevated resistance following rapid desiccation hardening in *D. melanogaster* is known to persist for over 24 h (Hoffmann, 1990). Mechanistically the heat hardening response is at least in part underpinned by the rapid turnover of key heat shock genes (*Hsps*), where pre-stress transcripts remain highly elevated following hardening and recovery, remaining thus into acute subsequent heat stress (Lindquist, 1986). Previous work in *D. melanogaster* using a similar desiccation stress regime as used here showed that exposure to desiccation stress did not induce key *Hsps* under basal conditions, but transcriptional response to desiccation hardening was not examined (Sinclair et al., 2007). We observed an intriguingly different transcriptional response to rapid desiccation hardening; for the genes with elevated transcripts during downstream acute stress, pre-stress expression levels were not different compared to the unhardened treatment group; rather *klu* and *Mtl* exhibited lower pre-stress expression levels. This suggests an alternative and yet to be explored mechanism of stress hardening; namely, prior desiccation exposure could effectively prime key genes to respond to subsequent stress without transcriptional activity at the onset of stress. Whether these genes are expressed during the 10 h each of hardening and recovery remains to be determined by additional time-course sampling; yet the lack of pre-stress expression variation between the hardened and basal treatment groups suggests that any effects are likely transitory yet sill impart an unknown means of 'stress memory' in flies with enhanced desiccation resistance.

4.3.1. Hardening treatment genes - stress elevated transcription

Recent data for invertebrate sensory physiology mediated by the Transient Receptor Potential (TRP) channels extends the functional repertoire of the TRP channels to include both dry and humid air detection in *D. melanogaster* (reveiwed in Chown et al., 2011). Hygrosensation in adult flies has been most clearly linked to TRP channels *wtrw* and *nan* (Liu et al., 2007) and *painless* in larvae (Johnson and Carder, 2012), although given the high relatedness between TRPs, it is possible that other unknown TRP channels work in concert with *nan* in particular. We previously found genomic differentiation at the TRP like encoding locus *trpl* after strong directional selection for desiccation resistance (Telonis-Scott et al., 2012), and in the current study profiled the expression of both *trpl* and *trp* which are known to be expressed in the MTs with a role in fluid transport (MacPherson et al., 2005) as well as forming unitary channel currents essential for *trpl* and *trp* photoreceptors (Delgado and Bacigalupo, 2009). We have shown that prolonged desiccation exposure switched these genes on to a fairly high degree (from around 6-fold up to 16-fold), suggesting a role for additional TRP channel activity during desiccation stress.

We previously found SNP variation at the *Treh* locus in temperate desiccation resistant *D. melanogaster* (Telonis-Scott et al., 2016). While we have shown in the current study that *Treh* expression was elicited in both populations after 1 h of desiccation stress, only temperate females continued expressing moderate levels of *Treh* transcripts throughout stress and into early recovery (up to 8-fold in early stress). Elevated transcription of the trehalose hydrolysing enzyme *Treh* during desiccation in wild adult *D. melanogaster* fits with data from knockdown studies on the sole *de novo* trehalose synthesis gene *Tps1* and for *Treh* (Yoshida et al., 2016). These genes are essential for trehalose metabolism and result in high desiccation mortality when perturbed in developing flies: trehalose presence and metabolism in *D. melanogaster* larvae is essential to mitigate desiccation stress, and recruitment of high levels of trehalose in the larval MTs likely functions in water homeostasis (Yoshida et al., 2016). The high metabolic activity in the MTs also extends to multiple processes including detoxification (Söderberg et al., 2011), where trehalose is implicated in scavenging of desiccation induced reactive oxygen species in *D. melanogaster* (Thorat et al., 2016).

We have also shown that SNPs in the sole *D. melanogaster* insulin receptor, *InR*, are associated with temperate population desiccation resistance (Telonis-Scott et al., 2016); this study confirms that *InR* is transcriptionally a desiccation responsive receptor in wild *D. melanogaster*. The MTs are also a novel site of insulin signalling associated with stress resistance, where the tubule principal cells produce insulin-like peptide DILP5 and InR, the ubiquitous receptor of the insulin-like peptides, under the regulation of the stress induced release of tachykinin peptide (DTK) and receptor (DTKR) during desiccation, starvation and oxidative stress (Söderberg et al., 2011). Given the wide number of *D. melanogaster* tissues expressing *InR* (Chintapalli et al., 2007) expression during desiccation could be related to another aspect of insulin signalling, but the DTK desiccation stress response MT pathways present an alternative desiccation response mechanism that should be further explored.

Finally, while *CG7084* is one of the most transcriptionally active genes in the MTs (Wang et al., 2004), no direct evidence links this locus to desiccation exposure, besides an indirect association from genomic differentiation in desiccation resistant temperate flies (Telonis-Scott et al., 2012). The current study reveals that *CG7084* was desiccation responsive in temperate females but only weakly in tropical females. The limited functional data for this gene suggests a role in neurogenesis and transmembrane transport, and that transcripts are upregulated in response to thermal, abiotic and biotic stressors (Graveley et al., 2011). Perhaps this gene plays a yet undefined role in broad-spectrum stress responses associated with the multifunctional fly renal tissues (Beyenbach et al., 2010).

4.3.2. Basal treatment genes - transcriptional down-regulation

Transcripts of fluid balance neuropeptide encoding genes were consistently down-regulated during desiccation stress in basal treatment flies. While these transcripts showed the largest differences between the temperature and tropical populations, they did not differ from pre-stress basal levels following the hardening treatment. Detailed functional studies demonstrate the role of capa diuretic peptides and the capa cognate G-protein coupled receptor capaR in mitigating desiccation stress from the tubules, the site of the highest known fluid movement with the primary role of osmoregulation and water balance (Beyenbach et al., 2010). Repression of both *capa* and *capaR* expression consistently results in a more desiccation resistant phenotype with reduced wet weight loss, suggesting an important role for excretory water balance in desiccation resistance (Terhzaz et al., 2015). The current model suggests that when capa signalling is elevated, fluid excretion is increased resulting in diuretic water loss and lower defence to desiccation (Terhzaz et al., 2012; Terhzaz et al., 2014; Terhzaz et al., 2015).

The transcript data from the current study suggests that capa signalling is decreased, supported by low levels of *capaR* transcripts as well as the EGR like transcription factor *klu* which responds to capa signalling during desiccation stress (Terhzaz personal communication, Terhzaz et al., 2014). Desiccation stress had a greater impact on *capa* transcripts in the less resistant tropical females, which downregulated *capa* throughout stress, suggesting that capa signalling requires greater repression in tropical females, resulting in less change in *capaR* and *klu* transcripts compared to the more resistant temperate females. The direction of *capa* expression depends in part on the desiccation exposure; prolonged but less acute stress in a *D. melanogaster* laboratory strain and other dipterans resulted in elevated *capa* transcripts (Terhzaz et al., 2015), but it is also possible that our wild flies naturally express higher basal *capa* levels. Capa peptides are localised exclusively to the tubule principal cells (Terhzaz et al., 2012) and are not released until early

recovery and quickly return to basal levels (Terhzaz et al., 2015). We also found that *capa* and *capaR* transcripts were restored to basal levels by 4 h of recovery. We detected some slight but non-significant stress elevation of *capaR* following hardening; perhaps hardened flies of both populations may be primed to rely less on rapidly regulating diuretic water balance in the MTs during desiccation stress, *i.e.* hardening and recovery pre-equips the fly to better balance water under acute stress. *Klu* was the only gene to show robust contrasting effects of treatment on gene expression. While strongly down-regulated in unhardened flies by six hours stress (27.5-fold), *klu* expression was elevated following hardening in both populations and into recovery; rather than suppressing expression the hardening treatment activated this transcription factor, but it remains unknown if this indicates a different role for capa peptides in stress activated pathways such as NF-κB (Terhzaz et al., 2014), or some other stress response.

Osmotic and water homeostasis is largely dependent on MT fluid secretion and hindgut reabsorption mediated by complex neuropeptide signalling including diuretic and antidiuretic peptides. While the transcript data suggests a role for diuretic water balance in wild flies, expression of the antidiuretic neurosecretory peptides *sNPF* and *ITP* were highly down-regulated in temperate females by 6 hour severe stress (but weakly in tropical females) rather than up-regulated as expected from previous work. *sNPF* encodes complimentary peptides to DTK (encoded by *tachykinin*) and ITP with roles in regulation of osmotic homeostasis and ITP acts on water reabsorption in the hindgut (Nassel and Winther, 2010). Silencing both *tachykinin* and *sNPF* expression resulted in water loss and reduced desiccation resistance (Kahsai et al., 2010). While we observed co-expression of *ITP* and *sNPF* supporting the concerted action of the neuropeptides (Kahsai et al., 2010), it is unclear if the temperate females are limiting hindgut water absorption, or if the pleiotropic hormone encoding genes are involved in some other stress-activated function. Finally, in contrast to human cells under desiccation stress (Huang and Tunnacliffe, 2005), unhardened flies down-regulated *Mtl* during stress, and expression was still repressed following hardening and recovery, suggesting that while desiccation responsive, *Mtl* products may play a different role in *Drosophila* responses to desiccation.

5. Conclusion

D. melanogaster desiccation resistance can arise from multiple mechanisms. In the current study we have revealed that desiccation elicited transcriptional responses from an array of genes either directly shown to impact water balance during desiccation and/or recovery, or genes indirectly linked to temperate female resistance through genomic screens. While the cline end populations exhibited phenotypic divergence for both basal and hardened desiccation resistance, molecular level signatures underpinning this variation in desiccation resistance and hardening were less clear. Nonetheless, subtle population differences were observed for several well elucidated desiccation candidate genes spanning different water balance strategies available to flies under desiccation. The treatment regime revealed more distinct transcriptional profiles; flies without a desiccation pre-treatment tended to repress gene expression, while flies exposed to hardening either returned these genes to basal levels or for other genes up-regulated expression. In addition, there were some subtle but not significant co-expression trends between the hardened and basal treatments for some genes, perhaps confounded by the difficulty in detecting shifts in expression from whole bodies when gene expression is often tissue specific. In conclusion, we found a novel and yet to be explored hardening mechanism for desiccation candidate genes, providing scope to further understand the molecular basis of desiccation plasticity.

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Appendix A. Supplementary data

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References

Bazinet, A.L., Marshall, K.E., MacMillan, H.A., Williams, C.M., Sinclair, B.J., 2010. Rapid changes in desiccation resistance in *Drosophila melanogaster* are facilitated by changes in cuticular permeability. J. Insect Physiol. 56, 2006–2012.
Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B Methodol. 289–300.
Beyenbach, K.W., Skaer, H., Dow, J.A., 2010. The developmental, molecular, and transport biology of Malpighian tubules. Annu. Rev. Entomol. 55, 351–374.
Bujan, J., Yanoviak, S.P., Kaspari, M., 2016. Desiccation resistance in tropical insects: causes and mechanisms underlying variability in a Panama ant community. Ecol. Evol. 6, 6282–6291.
Chintapalli, V.R., Wang, J., Dow, J.A., 2007. Using FlyAtlas to identify better *Drosophila melanogaster* models of human disease. Nat. Genet. 39, 715–720.
Chippindale, A.K., Gibbs, A.G., Sheik, M., Yee, K.J., Djawdan, M., Bradley, T.J., Rose, M.R., 1998. Resource acquisition and the evolution of stress resistance in *Drosophila melanogaster*. Evolution 52, 1342–1352.
Chown, S.L., Nicolson, S., 2004. Insect Physiological Ecology: Mechanisms and Patterns. Oxford University Press.
Chown, S.L., Sorensen, J.G., Terblanche, J.S., 2011. Water loss in insects: an environmental change perspective. J. Insect Physiol. 57, 1070–1084.
Chung, H., Loehlin, D.W., Dufour, H.D., Vaccarro, K., Millar, J.G., Carroll, S.B., 2014. A single gene affects both ecological divergence and mate choice in Drosophila. Science 343, 1148–1151.
Clemson, A.S., Sgro, C.M., Telonis-Scott, M., 2016. Thermal plasticity in *Drosophila melanogaster* populations from eastern Australia: quantitative traits to transcripts. J. Evol. Biol. 29, 2447–2463.
Delgado, R., Bacigalupo, J., 2009. Unitary recordings of TRP and TRPL channels from isolated Drosophila retinal photoreceptor rhabdomeres: activation by light and lipids. J. Neurophysiol. 101, 2372–2379.
Folk, D.G., Han, C., Bradley, T.J., 2001. Water acquisition and partitioning in *Drosophila melanogaster*: effects of selection for desiccation-resistance. J. Exp. Biol. 204, 3323–3331.
Gerken, A.R., Eller, O.C., Hahn, D.A., Morgan, T.J., 2015. Constraints, independence, and evolution of thermal plasticity: probing genetic architecture of long- and short-term thermal acclimation. Proc. Natl. Acad. Sci. U. S. A. 112, 4399–4404.
Gibbs, A.G., Matzkin, L.M., 2001. Evolution of water balance in the genus Drosophila. J. Exp. Biol. 204, 2331–2338.
Gibbs, A.G., Fukuzato, F., Matzkin, L.M., 2003. Evolution of water conservation mechanisms in Drosophila. J. Exp. Biol. 206, 1183–1192.
Graveley, B.R., May, G., Brooks, A.N., Carlson, J.W., Cherbas, L., Davis, C.A., Duff, M., Eads, B., Landolin, J., Sandler, J., Wan, K.H., Andrews, J., Brenner, S.E., Cherbas, P., Gingeras, T.R., Hoskins, R., Kaufman, T., Celniker, S.E., 2011. The *D. melanogaster* Transcriptome: modENCODE RNA-Seq Data for Differing Treatment Conditions.
Hadley, N.F., 1994. Water Relations of Terrestrial Arthropods. Academic Press, San Diego.
Hallas, R., Schiffer, M., Hoffmann, A.A., 2002. Clinal variation in *Drosophila serrata* for stress resistance and body size. Genet. Res. 79, 141–148.
van Herrewege, J., David, J.R., 1997. Starvation and desiccation tolerances in Drosophila: comparison of species from different climatic origins. Ecoscience 151–157.
Hoffmann, A.A., 1990. Acclimation for desiccation resistance in *Drosophila melanogaster* and the association between acclimation responses and genetic variation. J. Insect Physiol. 36, 885–891.
Hoffmann, A.A., 1991. Acclimation for desiccation resistance in Drosophila - species and population comparisons. J. Insect Physiol. 37, 757–762.
Hoffmann, 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.
Huang, Z., Tunnacliffe, A., 2005. Gene induction by desiccation stress in human cell cultures. FEBS Lett. 579, 4973–4977.
Janzen, D.H., 1967. Why mountain passes are higher in the tropics. Am. Nat. 101, 233–249.
Johnson, W.A., Carder, J.W., 2012. Drosophila nociceptors mediate larval aversion to dry surface environments utilizing both the painless TRP channel and the DEG/ENaC subunit, PPK1. PLoS One 7, e32878.
Kahsai, L., Kapan, N., Dircksen, H., Winther, A., Nässel, D.R., 2010. Metabolic stress responses in Drosophila are modulated by brain neurosecretory cells that produce multiple neuropeptides. PLoS One 5, e11480.
Kalra, B., Parkash, R., Aggarwal, D.D., 2014. Divergent mechanisms for water conservation in Drosophila species. Entomol. Exp. Appl. 151, 43–56.
Karan, D., Dahiya, N., Munjal, A.K., Gibert, P., Moreteau, B., Parkash, R., David, J.R., 1998. Desiccation and starvation tolerance of adult Drosophila: opposite latitudinal clines in natural populations of three different species. Evolution 52, 825–831.

Kean, L., Cazenave, W., Costes, L., Broderick, K.E., Graham, S., Pollock, V.P., Davies, S.A., Veenstra, J.A., Dow, J.A., 2002. Two nitridergic peptides are encoded by the gene capability in *Drosophila melanogaster*. Am. J. Phys. Regul. Integr. Comp. Phys. 282, R1297–R1307.

Kellermann, V.M., Heerwaarden, B.V., Hoffmann, A.A., Sgrò, C.M., 2006. Very low additive genetic variance and evolutionary potential in multiple populations of two rainforest Drosophila species. Evolution 60, 1104–1108.

Kellett, M., Hoffmann, A.A., McKechnie, S.W., 2005. Hardening capacity in the *Drosophila melanogaster* species group is constrained by basal thermotolerance. Funct. Ecol. 19, 853–858.

Lindquist, S., 1986. The heat shock response. Annu. Rev. Biochem. 55, 1151–1191.

Liu, L., Li, Y., Wang, R., Yin, C., Dong, Q., Hing, H., Kim, C., Welsh, M.J., 2007. Drosophila hygrosensation requires the TRP channels water witch and nanchung. Nature 450, 294–298.

MacPherson, M.R., Pollock, V.P., Kean, L., Southall, T.D., Giannakou, M.E., Broderick, K.E., Dow, J.A.T., Hardie, R.C., Davies, S.A., 2005. Transient receptor potential-like channels are essential for calcium signaling and fluid transport in a Drosophila epithelium. Genetics 169, 1541–1552.

Marron, M., Markow, T., Kain, K., Gibbs, A., 2003. Effects of starvation and desiccation on energy metabolism in desert and mesic Drosophila. J. Insect Physiol. 49, 261–270.

Matsuda, H., Yamada, T., Yoshida, M., Nishimura, T., 2015. Flies without trehalose. J. Biol. Chem. 290, 1244–1255.

Nassel, D.R., Winther, A.M., 2010. Drosophila neuropeptides in regulation of physiology and behavior. Prog. Neurobiol. 92, 42–104.

Parkash, R., Rajpurohit, S., Ramniwas, S., 2008. Changes in body melanisation and desiccation resistance in highland vs. lowland populations of *D. melanogaster*. J. Insect Physiol. 54, 1050–1056.

Parkash, R., Singh, D., Lambhod, C., 2014. Sex-specific differences in desiccation resistance and the use of energy metabolites as osmolytes in *Drosophila melanogaster* flies acclimated to dehydration stress. J. Comp. Physiol. B. 184, 193–204.

Parsons, P.A., 1982. Evolutionary ecology of Australian Drosophila - a species analysis. Evol. Biol. 14, 297–350.

Sinclair, B.J., Gibbs, A.G., Roberts, S.P., 2007. Gene transcription during exposure to, and recovery from, cold and desiccation stress in *Drosophila melanogaster*. Insect Mol. Biol. 16, 435–443.

Söderberg, J., Birse, R.T., Nässel, D.R., 2011. Insulin production and signaling in renal tubules of Drosophila is under control of tachykinin-related peptide and regulates stress resistance. PLoS One 6, e19866.

Stanley, S.M., Parsons, P.A., 1981. The response of the cosmopolitan sepecies, *Drosophila melanogaster*, to ecological gradients. Proc. Ecol. Soc. Aust 11, 121–130.

Stillman, J.H., 2003. Acclimation capacity underlies susceptibility to climate change. Science 301, 65.

Stinziano, J.R., Sové, R.J., Rundle, H.D., Sinclair, B.J., 2015. Rapid desiccation hardening changes the cuticular hydrocarbon profile of *Drosophila melanogaster*. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 180, 38–42.

Telonis-Scott, M., Guthridge, K.M., Hoffmann, A.A., 2006. A new set of laboratory-selected *Drosophila melanogaster* lines for the analysis of desiccation resistance: response to selection, physiology and correlated responses. J. Exp. Biol. 209, 1837–1847.

Telonis-Scott, M., Gane, M., DeGaris, S., Sgro, C.M., Hoffmann, A.A., 2012. High resolution mapping of candidate alleles for desiccation resistance in *Drosophila melanogaster* under selection. Mol. Biol. Evol. 29, 1335–1351.

Telonis-Scott, M., Clemson, A.S., Johnson, T.K., Sgro, C.M., 2014. Spatial analysis of gene regulation reveals new insights into the molecular basis of upper thermal limits. Mol. Ecol. 23, 6135–6151.

Telonis-Scott, M., Sgro, C.M., Hoffmann, A.A., Griffin, P.C., 2016. Cross-study comparison reveals common genomic, network, and functional signatures of desiccation resistance in *Drosophila melanogaster*. Mol. Biol. Evol. 33, 1053–1067.

Terhzaz, S., Cabrero, P., Robben, J.H., Radford, J.C., Hudson, B.D., Milligan, G., Dow, J.A., Davies, S.A., 2012. Mechanism and function of Drosophila capa GPCR: a desiccation stress-responsive receptor with functional homology to human neuromedinU receptor. PLoS One 7, e29897.

Terhzaz, S., Overend, G., Sebastian, S., Dow, J.A., Davies, S.-A., 2014. The *D. melanogaster* capa-1 neuropeptide activates renal NF-kB signaling. Peptides 53, 218–224.

Terhzaz, S., Teets, N.M., Cabrero, P., Henderson, L., Ritchie, M.G., Nachman, R.J., Dow, J.A., Denlinger, D.L., Davies, S.-A., 2015. Insect capa neuropeptides impact desiccation and cold tolerance. Proc. Natl. Acad. Sci. 112, 2882–2887.

Tewksbury, J.J., Huey, R.B., Deutsch, C.A., 2008. Ecology - putting the heat on tropical animals. Science 320, 1296–1297.

Thorat, L.J., Gaikwad, S.M., Nath, B.B., 2012. Trehalose as an indicator of desiccation stress in *Drosophila melanogaster* larvae: a potential marker of anhydrobiosis. Biochem. Biophys. Res. Commun. 419, 638–642.

Thorat, L., Mani, K.-P., Thangaraj, P., Chatterjee, S., Nath, B.B., 2016. Downregulation of dTps1 in *Drosophila melanogaster* larvae confirms involvement of trehalose in redox regulation following desiccation. Cell Stress Chaperones 21 (2), 285–294. http://dx.doi.org/10.1007/s12192-015-0658-0.

Wang, J., Kean, L., Yang, J., Allan, A.K., Davies, S.A., Herzyk, P., Dow, J.A., 2004. Function-informed transcriptome analysis of Drosophila renal tubule. Genome Biol. 5, R69.

Wicker-Thomas, C., Garrido, D., Bontonou, G., Napal, L., Mazuras, N., Denis, B., Rubin, T., Parvy, J.P., Montagne, J., 2015. Flexible origin of hydrocarbon/pheromone precursors in *Drosophila melanogaster*. J. Lipid Res. 56, 2094–2101.

Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., Madden, T.L., 2012. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinf. 13, 134.

Yoshida, M., Matsuda, H., Kubo, H., Nishimura, T., 2016. Molecular characterization of *Tps1* and *Treh* genes in Drosophila and their role in body water homeostasis. Sci. Rep. 6, 30582.

Yost, H.J., Petersen, R.B., Linquist, S., 1990. RNA metabolism: strategies for regulation in the heat shock response. Trends Genet. 6, 223–227.