

# Increases in the evolutionary potential of upper thermal limits under warmer temperatures in two rainforest *Drosophila* species

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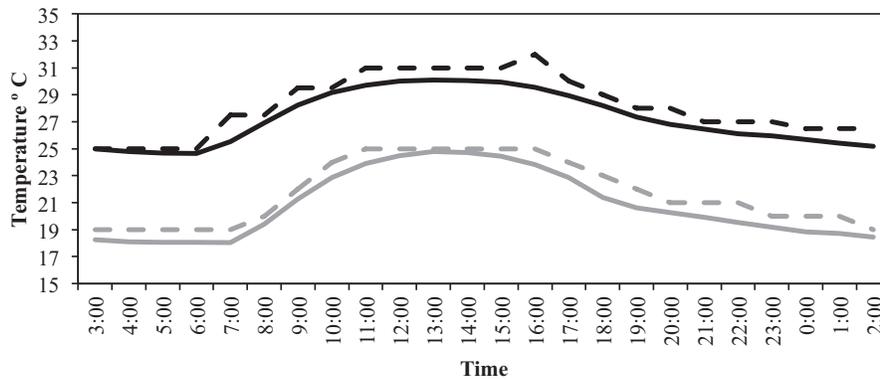
Tropical and subtropical species represent the majority of biodiversity. These species are predicted to lack the capacity to evolve higher thermal limits in response to selection imposed by climatic change. However, these assessments have relied on indirect estimates of adaptive capacity, using conditions that do not reflect environmental changes projected under climate change. Using a paternal half-sib full-sib breeding design, we estimated the additive genetic variance and narrow-sense heritability for adult upper thermal limits in two rainforest-restricted species of *Drosophila* reared under two thermal regimes, reflecting increases in seasonal temperature projected for the Wet Tropics of Australia and under standard laboratory conditions (constant 25°C). Estimates of additive genetic variation and narrow-sense heritability for adult heat tolerance were significantly different from zero in both species under projected summer, but not winter or constant, thermal regimes. In contrast, significant broad-sense genetic variation was apparent in all thermal regimes for egg-to-adult viability. Environment-dependent changes in the expression of genetic variation for adult upper thermal limits suggest that predicting adaptive responses to climate change will be difficult. Estimating adaptive capacity under conditions that do not reflect future environmental conditions may provide limited insight into evolutionary responses to climate change.

**KEY WORDS:** Adaptive potential, genetic variation, heritability, thermotolerance, upper thermal limits.

Temperature is an important factor influencing the physiology, distribution, and abundance of many species (Cossins and Bowler 1987). Increases in average temperatures and the frequency of extreme temperature events over the coming decades (IPCC 2013) are expected to impose increasing selection on upper thermal limits. The close association between environmental and body temperatures in ectotherms suggests that climate change is likely to impact their distribution and abundance (Parmesan and Yohe 2003; Colwell et al. 2008; Chown et al. 2010), metabolism (Dillon et al. 2010), and thus risk of extinction (Addo-Bediako et al. 2000; Deutsch et al. 2008; Huey et al. 2009; Sinervo et al. 2010; Sunday et al. 2011; Diamond et al. 2012).

Although behavioral thermoregulation may buffer body temperatures from changes in air temperature (Bogert 1949; Kearny et al. 2009; Sunday et al. 2014), it will likely have a limited ability to ameliorate the effects of climate warming in many ectotherms (Huey and Pascual 2009; Huey and Tewksbury 2009), particularly tropical species. This is because such species already rely on shady habitats to buffer body temperatures from current air temperatures (Sunday et al. 2014). Thus, temperature is expected to impose significant selection pressures on upper thermal limits in many species (Huey et al. 2012). Whether organisms, particularly tropical ectotherms, are able to modify upper thermal limits via evolutionary responses and reduce their extinction risk remains largely unknown.





**Figure 1.** Temperature regimes (+1°C) projected for Cairns by 2030 in summer (dashed black line: average temperature 28.2°C) and winter (dashed gray line: average temperature 21.8°C). Solid black line indicates current averages in January based on data from 2001 to 2008 (average 27.2°C) and the solid gray line indicates current averages in July based on data from 2001 to 2008 (average temperature 20.8°C). Data from Bureau of Meteorology, <http://www.bom.gov.au/climate/>.

Interspecific studies suggest that some organisms will be unlikely to ameliorate the effects of global warming by evolving higher thermal limits. Specifically, it has been suggested that tropical and subtropical species may be particularly vulnerable to climate change because they are already close to their upper thermal limit (Janzen 1967; Somero 2002; Ghalambor et al. 2006; Deutsch et al. 2008; Tewksbury et al. 2008; Huey et al. 2009; Kearney et al. 2009; McCain 2009; Clusella-Trullas et al. 2011; Sunday et al. 2011; Diamond et al. 2012; Kellermann et al. 2012; Araugo et al. 2013). However, these studies have relied on indirect estimates of adaptive capacity. Few studies have directly estimated the adaptive capacity for upper thermal limits (Hoffmann et al. 2013), even though such information provides important insight into contemporary microevolutionary responses to global change. Those that have been performed provide mixed support for evolutionary constraint of upper thermal limits. Although low levels of genetic variation for heat tolerance has been implicated in limiting the evolution of higher thermal limits in copepods (Kelly et al. 2012, 2013), recent work in widespread species of *Drosophila* suggests that the evolution of upper thermal limits may not be similarly constrained (van Heerwaarden and Sgrò 2013; Blackburn et al. 2014; Hangartner and Hoffmann 2015). However, these studies estimated adaptive capacity for upper thermal limits in widespread species maintained and tested under constant temperature laboratory environments.

There is increasing recognition of the fact that additive genetic variance (and thus heritability and adaptive capacity) may change with environmental conditions (e.g., Sgrò and Hoffmann 1998a,b; Hoffmann and Merila 1999; McGuigan and Sgrò 2009; Husby et al. 2011; Chirgwin et al. 2015). The environmental dependency of the expression of additive genetic variance may complicate predictions of evolutionary constraint under climate change when based on data collected under laboratory conditions

that do not reflect environmental conditions experienced in nature (van Heerwaarden and Sgrò 2014). Importantly in this respect, the role that changing environmental conditions might have on the evolution of upper thermal limits has not yet been considered.

The focus of this current study was to examine the extent to which the expression of additive genetic variance for upper thermal limits in rainforest restricted species of *Drosophila* is environment-dependent. To do this, we performed a half-sib full-sib breeding design in adults of two species of rainforest-restricted *Drosophila*, *D. birchii* and *D. bunnanda*, reared under developmental temperatures projected for Cairns, a site central to their distribution in the Wet Tropics of Australia under moderate–high carbon emissions (IPCC 2013; CSIRO, <http://www.climatechangeaustralia.gov.au>) for summer and winter seasons. This translates into a 1°C increase in average temperature by 2030 in both summer and winter (CSIRO, <http://www.climatechangeaustralia.gov.au>; Fig. 1). This allowed us to first, determine the extent to which additive genetic variance for upper thermal limits changes with thermal environment, and second, to determine whether evolution of heat tolerance might also be influenced by genetic covariance across environments (seasons). Furthermore, to relate these results to typical laboratory studies (Mitchell and Hoffmann 2010; Williams et al. 2012; van Heerwaarden and Sgrò 2013; Blackburn et al. 2014), we also examined additive genetic variance for upper thermal limits in *D. birchii* reared under a constant temperature of 25°C.

Additionally, as preadult and adult life stages of an organism may often occupy different habitats, experience different microclimates, and vary in their thermal sensitivities and evolutionary responses (Krebs and Loeschcke 1995; Loeschcke and Krebs 1996; Kingsolver et al. 2011; Briscoe et al. 2012; van Heerwaarden et al. 2014; Pincebourde and Casas 2015), we also examined egg–adult viability using isofemale lines of *D. birchii* reared under

all three thermal regimes. This allowed us to assess the extent to which preadult life stages have the capacity to evolve in response to changing thermal regimes.

## Methods

### FIELD COLLECTIONS

#### *Summer versus winter experiment*

Field inseminated females of *D. birchii* and *D. bunnanda* were collected from a site central to both species' distribution in the Wet Tropics of Australia near Cairns in Queensland, Australia (latitude 16.522° S) in February 2010 and used to establish mass-bred populations in the laboratory (see Supporting Information for details). These mass-bred populations were then used to investigate the evolutionary potential of heat tolerance under summer and winter temperatures projected in 2030 (see below) after six (*D. bunnanda*) or 11 (*D. birchii*) generations of laboratory culture.

#### *Constant 25°C experiment*

Field inseminated females of *D. birchii* were collected from Finch Hatton National Park (latitude 21.08° S) in a second field trip in late March 2013 and used to examine the evolutionary potential of heat tolerance under a constant (control) developmental temperature of 25°C (Supporting Information).

#### *Isofemale line experiment*

Field inseminated females were collected in a third field trip in May 2014 from Finch Hatton National Park (latitude 21.08° S) and used to examine isofemale line (broad-sense) heritability for egg-to-adult viability under different temperature regimes. The broad-sense genetic covariance across thermal regimes was also examined (Supporting Information).

#### *Summer and winter thermal regimes*

Average temperature for Cairns, a site central to the distribution of both species in the Wet Tropics of Australia, is projected to increase by 1°C by 2030 under all carbon emission scenarios (IPCC 2013; CSIRO, <http://www.climatechangeinaustralia.gov.au>; Supporting Information). To reflect this projected increase in temperature in a more ecologically realistic context, we took daily temperatures experienced in January (summer/dry season) in Cairns (hourly averages from 2001 to 2008; Bureau of Meteorology, <http://www.bom.gov.au/climate/>), and added 1°C to these values to reflect a 1°C increase in temperature encompassing natural fluctuations throughout a 24-h cycle (Fig. 1) to generate a summer/wet season experimental thermal regime. A winter/dry season thermal regime was generated by adding 1°C to daily temperatures experienced in July at Cairns (hourly averages from 2001 to 2008; Bureau of Meteorology, <http://www.bom.gov.au/climate/>; Fig. 1).

#### *Estimating additive genetic variances and covariances for adult upper thermal limits*

We used a half-sib full-sib breeding design to estimate additive genetic variances and covariances for heat knockdown time under the summer, winter (summer vs. winter experiment), and constant 25°C (constant 25°C experiment for *D. birchii* only) thermal regimes. For the summer and winter regimes, each species was examined in separate generations (*D. bunnanda* was examined after six generations of mass breeding, while *D. birchii* was examined after 11 generations of mass breeding), due to the logistical constraints of performing such large experiments. *Drosophila birchii* was assessed after two generations of mass breeding for the constant 25°C experiment (see Supporting Information for full details).

#### *Adult heat tolerance assays*

We used a static heat knockdown assay to examine upper thermal limits in all experiments since we have shown (van Heerwaarden and Sgrò 2013; Blackburn et al. 2014) that this measure provides consistent insight into the adaptive capacity of upper thermal limits in *Drosophila*. Heat knockdown time was measured by placing individual five- to seven-day-old flies individually in 5 mL glass vials and submerging them into a recirculating water bath heated to 38°C (summer vs. winter experiment) or 37.5°C (25°C experiment) by a digital thermoregulator (Ratek, Model: TH5). The 0.5°C difference in heat knockdown test temperatures was due to experimental error; however, our experience (Sgrò and van Heerwaarden unpubl. data) is that this difference will not affect the results or inferences drawn. Heat resistance was scored as the time taken for all flies to be knocked down and immobilized (Sgrò et al. 2010; Williams et al. 2012). A total of eight (five, 25°C) runs were performed over two (one, 25°C) days. Offspring from different dams were randomized across runs. The same two people (observers) performed all of the heat knockdown assays across all runs.

#### *Egg-to-adult viability: summer versus winter experiment*

We quantified the effects that the summer and winter thermal regimes had on egg–adult viability compared to control (constant 25°C) conditions in both species using the mass bred populations collected in 2010 (winter vs. summer). After 12 generations of mass breeding, adult flies of each species eclosing from low density vials (<60 flies) were allowed to mate and aged for four days before allowing them to lay on the standard media (see above) colored with food dye for 12 h. Following this, eggs and the laying medium beneath were picked with a flat-edged spatula and placed into new vials with potato, yeast, and sucrose media to complete development (see Supporting Information for details).

*Isofemale line experiment: Egg-to-adult viability*

In order to obtain an estimate of the extent to which preadult life stages are able to adapt in response to projected changes in thermal environments, we compared isofemale lines (2014 collection) to estimate broad-sense genetic variation and heritability for egg-to-adult viability under the winter, summer, and constant 25°C thermal regimes in *D. birchii* (see Supporting Information for details).

**ANALYSES***Heat knockdown time: Summer versus winter experiment*

The effect of thermal regime on mean adult heat knockdown resistance was assessed using mixed linear models using the MIXED procedure of SAS (SAS Institute, Cary, NC). Thermal regime was considered a fixed effect, while run and scorer were treated as random effects. Analyses were performed separately on each species.

*Half-sib full-sib analyses*

We took a character state approach (Falconer 1952) to the genetic analysis of the data for the summer versus winter experiment, whereby heat knockdown time measured in both environments (summer and winter) was considered to be two traits. The following mixed linear model was then used to represent the half-sib full-sib breeding design following van Heerwaarden and Sgrò (2013):

$$Y_{ijklm} = \mu + X_i + Y_j + S_k + D_{l(k)} + W_{m(kl)},$$

where  $Y_{ijklm}$  is the trait value of an individual,  $\mu$  is the population mean,  $X_i$  is effect of the  $i$ th run,  $Y_j$  is the effect of  $j$ th sex,  $S_k$  is the effect of the  $k$ th sire,  $D_{l(k)}$  is the effect of the  $l$ th dam nested within the  $k$ th sire, and  $W_{m(kl)}$  is the effect of the  $m$ th progeny of the  $l$ th dam nested with the  $k$ th sire. Run and sex were modeled as a fixed effect, whereas all other terms were considered random. The total phenotypic variance for the breeding design for the purpose of estimating genetic parameters was represented by:

$$\sigma_p^2 = \sigma_s^2 + \sigma_D^2 + \sigma_W^2.$$

As observer had a significant effect on the variance in both species at both temperatures, we variance standardized all of the heat knockdown time data by scorer prior to the analyses described below to ensure that this did not bias estimates of the variance and covariance components (van Heerwaarden and Sgrò 2013; Blackburn et al. 2014). The results were qualitatively the same with and without this correction.

Variance and covariance components and additive genetic correlations were estimated using restricted maximum likelihood implemented via the MIXED procedure of SAS (SAS Institute).

Log-likelihood ration tests were performed to test for significance (see Supporting Information for full details).

The narrow-sense heritability for each trait, calculated as the additive genetic variance (VA) divided by the total phenotypic variance (VP; Falconer and Mackay 1996; Lynch and Walsh 1998), was also estimated. The half-sib full-sib data from the constant 25°C experiment (*D. birchii*) were analyzed as described above, but for females only.

*Egg-to-adult viability: Summer versus winter experiment*

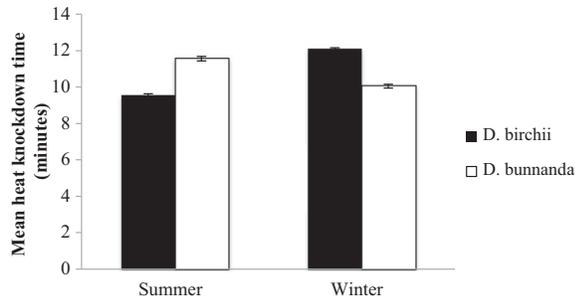
To examine the effects of the thermal regimes on egg-to-adult viability using the populations from the summer versus winter experiment (2010 collection), we used a two-way analysis of variance (ANOVA) on arcsine square root transformed data, with treatment and species as fixed effects. Alternative data transformations were considered (logit transformed (Warton et al. 2011)) but the results were qualitatively the same and did not alter the outcomes or conclusions.

*Isofemale line experiment: broad-sense heritability for egg-adult viability across thermal regimes*

We used an ANOVA to estimate broad-sense genetic variance for egg-to-adult viability in *D. birchii* developing under the three thermal regimes (constant 25°C, summer and winter). To estimate isofemale line heritability, we first calculated the intraclass correlation ( $t$ ) as:  $t = V_b / (V_b + V_w)$ , where  $V_b$  equals the variance between isofemale line and  $V_w$  equals the variance within isofemale line.  $V_b$  and  $V_w$  were calculated by restricted maximum likelihood using the VARCOMP procedure in SPSS (Hoffmann et al. 2001). We then used  $t$  to calculate isofemale heritability (Osborne and Paterson 1952; Parsons 1983; Hoffmann and Parsons 1988) using the following equation:  $h^2 = 2/(1/t - 1/2)$ . We also calculated correlations between isofemale line egg-to-adult viability across the three thermal regimes to obtain estimates of cross-environment broad-sense genetic correlations for egg-to-adult viability.

**Results****EFFECT OF THERMAL REGIME ON ADULT HEAT KNOCKDOWN RESISTANCE: SUMMER VERSUS WINTER EXPERIMENT**

Developmental temperature had a significant effect on heat knockdown time in *D. bunnanda* ( $F_{1,4108} = 85.746$ ,  $P < 0.001$ ); flies developing under the summer regime were more heat tolerant than flies developing under the winter regime (Fig. 2). There was no effect of sex ( $F_{1,4108} = 0.853$ ,  $P = 0.356$ ), but run ( $F_{17,4108} = 3.126$ ,  $P < 0.001$ ) and scorer ( $F_{1,4108} = 19.596$ ,  $P < 0.001$ ) were both significant.



**Figure 2.** Mean heat knockdown time for *Drosophila birchii* (filled bars) and *Drosophila bunnanda* (open bars) reared under the summer and winter thermal regimes. Error bars are 1 SE.

Developmental temperature also had a significant effect on heat knockdown in *D. birchii* ( $F_{1,3857} = 218.743$ ,  $P < 0.001$ ). However, development under the winter regime resulted in flies that were more heat tolerant compared to the summer flies (Fig. 2). There was no effect of sex ( $F_{1,3857} = 0.345$ ,  $P = 0.557$ ), but run ( $F_{16,3857} = 8.311$ ,  $P = < 0.001$ ) and scorer ( $F_{1,3857} = 61.463$ ,  $P = < 0.001$ ) were both significant.

#### Effect of thermal regime on additive genetic variation and covariation for heat tolerance: Summer versus winter experiment

We found evidence for environment-specific adaptive capacity for heat tolerance in both species. Significant additive genetic variance for heat tolerance was only apparent under the summer developmental thermal regime (Table 1), in both species. Further, the additive genetic variance for heat tolerance was significantly higher in the summer developmental thermal regime compared to the winter developmental thermal regime for both species (*D. birchii*:  $\chi^2_1 = 5.6$ ,  $P = 0.018$ ; *D. bunnanda*:  $\chi^2_1 = 8.0$ ,  $P = 0.005$ ).

The nonsignificant narrow-sense heritability estimates for heat knockdown time under the winter regime in both species were driven by the nonsignificant additive genetic variance in this environment (Table 1).

The additive genetic covariance across the summer and winter thermal regimes was not significantly different from zero (not shown) in either species. The cross-environment genetic correlation was also nonsignificant in both species (not shown).

#### Effect of thermal regime on additive genetic variation for heat tolerance: Constant 25°C experiment

Consistent with the results from the first half-sib full-sib experiment described above, the estimate of additive genetic variance for adult heat knockdown time was nonsignificant under the constant 25°C thermal regime. The narrow-sense heritability for this trait at 25°C was also not significantly different from zero, driven by the nonsignificant  $V_A$  estimates.

#### Effect of thermal regime on egg-to-adult viability:

##### Summer versus winter experiment

Developmental temperature had a significant effect on viability ( $F_{2,144} = 58.77$ ,  $P < 0.001$ ); viability was significantly lower in the summer thermal regime, compared to the control and winter regimes, in both species (Fig. 3). There was no effect of species ( $F_{1,144} = 0.401$ ,  $P = 0.527$ ), but a significant interaction between developmental temperature and species ( $F_{2,144} = 12.262$ ,  $P < 0.001$ ) was evident; *D. bunnanda* had higher egg-to-adult viability than *D. birchii* under summer developmental temperatures, and *D. birchii* had higher egg-to-adult viability than *D. bunnanda* under winter conditions (Fig. 3).

We used these values of egg-to-adult viability to estimate relative fitness (see Supporting Information) across the thermal regimes in order to quantify the strength of viability selection that was operating on both species. For *D. birchii*, the winter regime had a relative fitness of 1, the control environment (25°C) 0.877 and the summer regime had the lowest relative fitness (0.2857). In *D. bunnanda*, the control environment (25°C) had the highest fitness, followed by the winter regime (0.909), the summer regime again having the lowest fitness (0.545). Thus, both species were exposed to strong viability selection under the summer thermal regime.

#### Effect of thermal regime on broad-sense genetic variation for egg-to-adult viability: Isofemale line experiment

In *D. birchii*, there was significant broad-sense genetic variation for egg-to-adult viability at all three thermal regimes, evidenced by the significant effect of isofemale line (Table S1). Broad-sense heritabilities for egg-to-adult viability were 0.601, 0.711, and 0.247 under the winter, summer, and 25°C thermal regimes, respectively, and all were significantly different from zero (based on the significant isofemale line term, Hoffmann and Parsons 1988). A significant correlation between isofemale lines was only detected across the winter and constant 25°C thermal regimes ( $r = 0.776$ ,  $P < 0.001$ ).

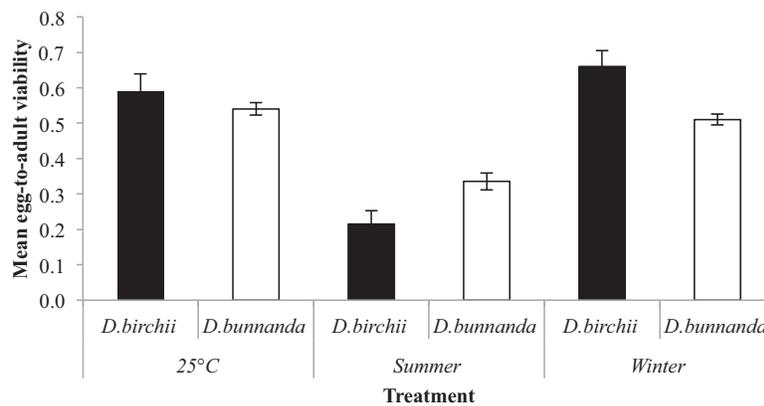
## Discussion

Increases in temperature due to ongoing climatic change are expected to impose significant selection pressures on the upper thermal limits of organisms. The extent to which organisms will be able to evolve in response to these selection pressures will depend on the presence of additive genetic variation for the traits under selection. We show that the expression of additive genetic variation for adult heat tolerance in two rainforest restricted species of *Drosophila* depends on thermal environment; additive genetic variance and narrow-sense heritability for heat tolerance were significantly different from zero in both species when flies

**Table 1.** Mean heat knockdown time (min), additive genetic variance ( $V_A$ ), environmental variance ( $V_E$ ), phenotypic variance ( $V_P$ ), and narrow-sense heritability ( $h^2$ ),  $\pm 1$  SE, for heat knockdown time in *Drosophila bunnanda* and *Drosophila birchii* reared under summer and winter thermal regimes projected for Cairns by 2030 under medium-high emission scenarios, and for *D. birchii* reared at constant 25°C.

Treatment	Mean $\pm$ SE	$V_A \pm$ SE	$V_E \pm$ SE	$V_P$	$h^2 \pm$ SE	$N$
Winter ( <i>D. bunnanda</i> )	10.050 $\pm$ 0.108	1.376 $\pm$ 0.0991	17.879 $\pm$ 0.997	19.255	0.071 $\pm$ 0.053	1676
Summer ( <i>D. bunnanda</i> )	11.560 $\pm$ 0.124	7.033 $\pm$ 2.746*	30.946 $\pm$ 2.50	37.979	0.185 $\pm$ 0.074*	2443
Winter ( <i>D. birchii</i> )	12.078 $\pm$ 0.097	0.553 $\pm$ 0.338	14.917 $\pm$ 1.304	15.470	0.036 $\pm$ 0.096	1687
Summer ( <i>D. birchii</i> )	9.560 $\pm$ 0.088	3.069 $\pm$ 0.077*	13.365 $\pm$ 1.260	16.434	0.187 $\pm$ 0.045*	2192
25°C ( <i>D. birchii</i> )	29.841 $\pm$ 0.248	9.403 $\pm$ 6.431	65.549	74.952	0.125 $\pm$ 0.082	1278

Significance from zero was assessed using log-likelihood ratio tests: \* $P < 0.05$ .



**Figure 3.** Mean egg-to-adult viability for *Drosophila birchii* and *Drosophila bunnanda* reared under control (25°C), summer, and winter thermal regimes.

developed under the summer thermal regime projected under climate change by 2030. Neither the additive genetic variance nor narrow-sense heritability were significant when flies developed at a constant 25°C, or under the winter thermal regime projected under climate change by 2030. The additive genetic covariance in adult heat tolerance across the summer and winter thermal regimes was also nonsignificant, emphasizing the lack of scope for correlated evolutionary shifts in adult heat tolerance during the winter. This suggests that covariances across thermal regimes (seasons) will neither constrain nor facilitate adaptive responses to increasing temperatures in these species (c.f. Chirgwin et al. 2015). These results suggest that the magnitude of the response to thermal selection imposed by climate change will be strongly environment-dependent. Evolutionary gains in adult upper thermal limits under warmer conditions may be counterbalanced by an absence of response under cooler conditions. On the other hand, significant broad-sense genetic variance for egg-to-adult viability in all thermal regimes suggests that pre-adult life stages will have the capacity to evolve in response to warmer developmental temperatures, although dominance and nonadditive genetic effects may also influence these estimates.

The summer thermal regime represented a thermally stressful environment, since viability and relative fitness were reduced

compared to the control (constant 25°C) and winter regimes in both species; thus, viability selection was at play in the summer regime. Despite this, the additive genetic variance and narrow-sense heritability for adult heat knockdown time were significant only under the summer (stressful) regime. The additive genetic variance for this trait under the summer regime was significantly higher in both species (53 and 34% higher in *D. birchii* and *D. bunnanda*, respectively).

Increases in the additive genetic variance in other fitness-related traits under stressful thermal regimes have previously been reported (Sgrò and Hoffmann 1998b; Bublly and Loeschcke 2000; Husby et al. 2011). In addition, the strength of selection acting on additive genetic variance has also been shown to change as the thermal environment changes (Husby et al. 2011). In a wild population of great tits, Husby et al. (2011) showed that in years where spring temperatures were highest, selection was strongest and the additive genetic variance was also highest for laying date, suggesting that the speed of microevolutionary change could in fact be increased by changing environmental conditions. The predicted response to selection was also highly temperature dependent. Indeed, Husby et al. (2011) showed that by not incorporating environmental dependence of the expression of genetic variance and strength of selection, the predicted response to selection may

be underestimated by up to 20%. The findings of Husby et al. (2011) are significant because, if applicable more broadly, they reveal a mechanism that could potentially increase the speed of adaptation to climate change.

One possible explanation for the increase in additive genetic variance for adult heat knockdown time under the summer regime is that the higher temperatures of the summer regime represent not only a stressful but a novel environment. It has been suggested that additive genetic variance could increase in novel environments because selection will act to increase the frequency of rare alleles unselected in the ancestral environment, thereby causing an increase in the standing genetic variance (Holloway et al. 1990; Falconer and Mackay 1996; Guntrip et al. 1997; Holloway et al. 1997). However, other studies have observed no increase in additive genetic variance or heritability under warmer temperatures (e.g., Scheiner and Lyman 1989; Bublly and Loeschcke 2000; Garant et al. 2008), and in some instances, decreases in heritability have been found (e.g., Ketola et al. 2012a,b).

Although the thermal regimes did not impose selection directly on adult heat tolerance, significant decreases in viability under the summer regime in both *D. birchii* and *D. bunnanda* indicate that egg-to-adult viability, which was heritable across all thermal regimes in *D. birchii* and likely so in *D. bunnanda*, was under selection in the summer thermal regime. This may have increased the frequency of rare heat resistant alleles, increasing additive genetic variance for adult heat knockdown resistance in the summer regime. Another possibility is that the increase in additive genetic variance for adult heat tolerance in the summer regime was due to the release of cryptic genetic variation caused by disruption of canalized pathways in response to the stressful environmental conditions experienced during larval development (Waddington 1961; McGuigan and Sgrò 2009). Although we did not directly compare additive genetic variances for upper thermal limits under current and projected winter and summer thermal regimes, our results indicate that evolutionary potential for upper thermal limits in adults is higher under warmer thermal conditions.

Finally, the fact that *D. birchii* was more heat tolerant than *D. bunnanda* when reared under the winter thermal regime suggests that it is more sensitive to thermal stress than *D. bunnanda*. The summer thermal regime imposed stronger viability selection on *D. birchii* compared to *D. bunnanda*, suggesting that this environment is more stressful to *D. birchii*, which is reflected in the reduced heat tolerance (performance) of *D. birchii* under the summer regime. Why these species show different response to the same thermal regimes is unknown, since their distributions overlap.

Our results highlight the importance of considering how the expression of additive genetic variance varies with changing environmental conditions, particularly when those changes give rise

to stressful and/or novel environmental conditions, which are expected with climate change (Charmantier and Grant 2005; Husby et al. 2011; Chirgwin et al. 2015). Estimates of adaptive capacity made under standard laboratory conditions that do not reflect future environmental conditions may provide limited insight into the extent to which evolutionary responses might mediate the impacts of climatic change.

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### Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Supplementary material

**Table S1.** ANOVA examining the effects of isofemale line and vial on egg-to-adult viability in *Drosophila birchii* under the winter and summer thermal regimes and constant temperature, 25°C.