

# Interactions between developmental and adult acclimation have distinct consequences for heat-tolerance and heat-stress recovery

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## **Keywords**

Insects, thermal biology, acclimation, heat-stress, heat coma, thermal tolerance

## **Summary statement**

We first document that the interaction between developmental and adult acclimation promotes heat-survival in *D. melanogaster* under specific conditions. Further, heat tolerance and heat stress recovery appear partly decoupled processes.

## **Abstract**

Developmental and adult thermal acclimation can have distinct, even opposite, effects on adult heat resistance in ectotherms. Yet, their relative contribution to heat-hardiness of ectotherms remains unclear despite the broad ecological implications thereof. Furthermore, the deterministic relationship between heat-knockdown and recovery from heat stress is poorly understood but significant for establishing causal links between climate variability and population dynamics. Here, using *D. melanogaster* in a full-factorial experimental design, we assess flies heat-tolerance in static stress assays, and document how developmental and adult acclimation interact with a distinct pattern to promote survival to heat-stress in adults.

We show that warmer adult acclimation is the initial factor enhancing survival to constant stressful high temperatures in flies, but also that the interaction between adult and developmental acclimation becomes gradually more important to ensure survival as the stress persists. This provides an important framework revealing the dynamic interplay between these two forms of acclimation, that ultimately enhance thermal tolerance as a function of stress duration. Furthermore, by investigating recovery rates post-stress, we also show that the process of heat-hardening and recovery post heat knockdown are likely to be based on set of (at least partially) divergent mechanisms. This could bear ecological significance as a tradeoff may exist between increasing thermal tolerance and maximizing recovery rates post-stress, constraining population responses when exposed to variable and stressful climatic conditions.

## **Introduction**

In ectotherms, thermal acclimation has long been recognized to occur at different timescales. Transient increase in heat tolerance or heat resistance can be achieved through a heat-hardening response (*i.e.*, a brief exposure to sublethal temperatures enhancing an individual's ability to cope with a subsequent heat-stress; Levins, 1969; Dahlggaard *et al.*, 1998). In turn, developmental acclimation (*i.e.*, temperature of embryonic development or rapidly developing life-stages prior to sexual maturity) also contributes to the plasticity of individuals' thermal limits, through additive or interactive effects with thermal conditions or acclimation occurring later in life (Crill *et al.*, 1996; Angilletta Jr, 2009; Slotsbo *et al.*, 2016, Beaman *et al.*, 2016, Kellermann *et al.*, 2017). In consequence, disentangling the relative contribution of short-term acclimation (*i.e.*, heat-hardening) and long-term developmental and adult acclimation responses to the phenotypic plasticity of thermal limits has proven a complex task in ectotherms. Since thermal tolerance correlates strongly with geographic distribution and population abundance or viability in insects (Sørensen *et al.*, 2005; Mitchell *et al.*, 2011; Kellermann *et al.*, 2012; Vorhees *et al.*, 2013, Overgaard *et al.*, 2014; Andersen *et al.*, 2015, Bush *et al.*, 2016; Liu *et al.*, 2020), modeling the contribution of different forms of acclimation to thermal plasticity might prove pivotal in our ability to predict species' responses to climate change (Allen *et al.*, 2016, Sinclair *et al.*, 2016; González-Tokman *et al.*, 2020, Braschler *et al.*, 2020).

In a variety of insects, and especially *Drosophila melanogaster*, the relationship between thermal acclimation (i.e., the exposure to new thermal conditions), induced phenotypic plasticity (i.e., the response), and the molecular processes responsive to heat-stress has been a subject of intense research for several decades (Sørensen *et al.*, 2005; Overgaard *et al.*, 2005; Malmendal *et al.*, 2006; Colinet *et al.*, 2013; MacMillan *et al.*, 2016; Kristensen *et al.*, 2016; Schou *et al.*, 2017, Somero, 2020). These processes include, among many others, the evolutionary conserved heat-shock response that mitigate the effects of proteotoxic-stresses (Richter and Haslbeck, 2010). However, quantifying the nature and relative contributions that each form of acclimation brings to heat-tolerance has proven challenging at least partly since the outcomes of these stress assays depends on choice of methodology (Mitchell and Hoffmann, 2010; Terblanche *et al.*, 2011). Metrics of heat-tolerance in *D. melanogaster* have historically been recorded using a few major approaches, that include static (constant controlled temperatures) or ramping (increasing controlled temperatures) assays, and the specific assay conditions can greatly impact any tests' outcomes and interpretations thereof. Using acclimation temperatures ranging from 12 to 32°C as well as a variety of ramping rates, a number of studies highlighted CT<sub>max</sub> shifts (CT<sub>max</sub>: often described as the temperature that results in loss of muscle coordination or onset of muscle spasms in a heating assay) of about 1°C due to warmer adult acclimation, and up to 3°C for its developmental counterpart (Crill, 1996; Sejerkilde *et al.*, 2003, Slotsbo *et al.*, 2016; van Heerwaarden *et al.*, 2016; Kellerman *et al.*, 2017; Schou *et al.*, 2017; Salachan *et al.*, 2019). Consistently, all forms of acclimation also increase the time flies could tolerate milder but constant heat-stress in static assays (time to knockdown; Levins, 1969; Castañeda *et al.*, 2015 Salachan *et al.*, 2019). Furthermore, a significant interactive effect between developmental and adult acclimation has been highlighted (Slotsbo *et al.*, 2016; Kellerman *et al.*, 2017), and whose magnitude and contribution to the heat-hardening process (i.e., acquired thermotolerance) remains rather poorly known.

Building on this background, this work primarily aims to further investigate the impact of the interactive effects between the two major forms of thermal acclimation, and how they dynamically contribute to enhancing *D. melanogaster* heat limits. To do so, we explored the combinations of three developmental and three adult acclimation temperatures on time to heat-knockdown (HKD) and recovery post-knockdown of flies in static assays. Interactive effects between developmental and adult acclimation in *D. melanogaster* have been demonstrated through measures of CT<sub>max</sub> using controlled ramping protocols (Slotsbo *et al.*, 2016; Kellerman *et al.*, 2017). The later studies also emphasized a greater impact of developmental acclimation in enhancing flies' upper thermal limits. We therefore hypothesized that here in our static assays, warmer developmental acclimation would be the dominant factor increasing flies' time to knockdown. Second, we predicted that the

magnitude of this interaction would be dynamic and follow a temporal pattern, based on the assumption that the two forms of acclimation may leverage sets of partially different molecular-level processes with distinct timescales of dynamic responses (e.g., Telonis-Scott *et al.*, 2014). Finally, we investigated how these two forms of thermal acclimation may potentially drive faster recoveries post heat knockdown, as this metric has seldom been reported before, but might prove important to survive transient stress exposure under more variable microclimatic conditions (Ma *et al.*, 2020).

## **Materials and methods**

### ***Fly stocks, rearing, and acclimation***

Wild *Drosophila* flies were bait-trapped in April 2019 around the Stellenbosch University campus, Western Cape, South Africa. Single female flies were isolated into 250 ml rearing flask supplied with 50ml of Bloomington cornmeal diet (Lewis, 1960) and left to lay eggs for one day. Between 2 and 5 of the first emerging adults were used for species determination following the key of Markow and Grady (2005). The remaining first-generation individuals from 16 confirmed *Drosophila melanogaster* lines were pooled and mass-reared together for five generations prior to experiments. An overview of the rearing and acclimation procedures used in experiments is given in Fig. S1. Stocks were kept in separated incubators at either 15, 25 or 30°C in the form of three 250ml flask supplied with Bloomington cornmeal diet, at a density of ~100 adult flies per flask. Before the emergence of a new generation (~7 days at 30°C, ~10 days at 25°C, and ~21 days at 15°C), older individuals were discarded. Less than 24h after their emergence, about ~100 newly hatched flies were isolated in a fresh flask to replenish stocks, while ~50 others were isolated for experimental assays. These experimental batches were further left to mature for 10 additional days at 15, 25 or 30°C prior to experiments, and their offspring discarded. This effectively created 9 unique thermal history conditions, in which batches of adult flies emerged within 24 hours under developmental acclimation conditions (15, 25 or 30°C, adults therefore experienced developmental acclimation conditions for max 1 days or  $\leq 10\%$  of their adult life) were subsequently exposed to either one of the three adult acclimation temperatures (15, 25 or 30°C) until they reached the age of 10 days old.

### ***Heat-knockdown dynamic***

For each of our 9 unique thermal history conditions, between 7 and 12 replicates each containing 15-25 flies, with a 1:1 sex ratio, were isolated into glass vials with a moist cotton ball. The vials were then submerged in a digital water bath (GD120 series stirred water bath, Grant Instrument Ltd) and the temperature inside the vials was monitored using 0.075 mm

diameter thermocouples (Type T Thermocouple (Copper/Constantan), OMEGA Engineering, CT, USA) connected to a digital thermometer (Fluke 52-II Dual Input Digital Thermometer, WA, USA). Flies were exposed to a constant 37°C, and the proportion of heat-knocked down flies in each batch was monitored every 15 minutes. HKD was considered positive for an individual fly when it adopted an immobile curled up position on its back, without any further response to external stimulation (vial shaking). Downstream analysis was performed by fitting a logistic regression model on each replicate of our 9 thermal history conditions, through a best fit model approach. This allowed us to extract 5 percentiles per thermal history condition, based on 7-12 replicated curves per thermal history, corresponding respectively to the time needed on average to reach 10% (Lethal time; LT10), 25% (LT25), 50% (LT50), 75% (LT75) and 90% (LT90) of heat-knocked down flies. Finally, we further tested for statistical significance of treatments on LTs values, and quantified the relationship between developmental acclimation, adult acclimation and extracted LT values using an effect size Omega  $\omega^2$  statistic (Olejnik *et al.*, 2003).

### **Recovery experiments**

Single flies were isolated into glass vials containing a moist cotton ball. The vials were then submerged in a programmable water bath and flies continuously exposed to a constant 41°C and until HKD, as described above in the heat-knockdown assay (i.e. each adult fly was exposed to 41°C for a specific amount of time until HKD, resulting in a different exposure time for each fly). Each heat knocked-down fly was immediately placed back at room temperature (21°C) and continuously monitored until recovery, defined as the time at which it could stand on its legs again, without stumbling over from external stimulation (vial shaking). Both times to HKD and recovery were carefully recorded for 30 flies coming from 3 different rearing bottles replicates (10 flies taken from each bottle), tested with a 1:1 sex ratio per thermal history condition (9 conditions; 270 individuals in total). Mortality was typically low in these assays, amounting to two flies (99.23% survival rate) and thus not considered further in subsequent analyses. Kaplan-Meier knockdown curves were drawn from the raw observed data, and the impact of thermal histories on time to HKD and time to recovery was assessed using two complementary analyses. First, a log-rank analysis was performed to test for significant differences between knockdown curves. Second, we used a Cox Proportional Hazard model to test for significance of our treatments on HKD curves (developmental acclimation, adult acclimation, and the interaction between the two).

### ***Relationship between time to heat-knockdown and time to recovery***

Each fly used in HKD experiments at 41°C also had a linked recovery time. These times were plotted against each other's screened for significant ordinary least-square linear regressions both as a function of their thermal history and on the global dataset. Second, since Kaplan-Meier curves do not integrate slope data, we refitted polynomial curves on time to HKD and recovery at 41°C and extracted slope values. These slopes were plotted against time to recovery slopes and also screened for significant linear regressions.

### ***Statistical analysis***

Log-rank tests of Kaplan-Meier curves were performed using GraphPad Prism 6.01. Cox proportional Hazard and best-fit models as well as effect size statistics were performed using R v.3.6.3 (R core team, 2013; Maechler *et al.*, 2013) with the survival and effsize libraries.

## ***Results***

### ***Adult and developmental acclimation contribute in unique ways to heat stress dynamics***

Overall, warmer adult acclimation had a strong and consistent effect in delaying time to HKD in flies (Table 1) at 37°C, shifting HKD curves to the right (Fig. 1, from left to right). Adult acclimation accounted for most of the variation across all LTs, but its statistical significance decreased at later stages of the experiment (Table 1). Warmer developmental acclimation (Fig.1, from top to bottom) had an overall weaker effect in delaying time to HKD. However, as opposed to adult acclimation, its statistical significance and effect size contribution increased as a function of time under heat stress, *i.e.* as knockdown progressed (Table 1). Finally, while we detected no initial effect of the interaction between developmental and adult interaction in delaying time to HKD in flies, its statistical significance and effect size contribution increased proportionally with the duration of the stress (Table 1). To summarize, the two forms of acclimation did not equally contribute to variation in the shape of the HKD curve. Adult acclimation was overall more significant in delaying the onset of HKD at the beginning of the experiment, while developmental acclimation and the interactive effect of developmental and adult acclimation became gradually more significant at later stages.

### ***Impact of developmental and adult acclimation on recovery dynamics***

To further test how developmental and adult acclimations may contribute differently to heat-tolerance and especially HKD recoveries, we performed static knockdown and recovery assays by exposing single *Drosophila* flies to a constant 41°C. Time to HKD was carefully monitored for each individual, and each knocked-down fly was then immediately removed from the experimental setup and placed back at room temperature (21°C) until recovery. Consistent with data from HKD dynamics at 37°C (Table 1, Fig.1), a log-rank analysis of HKD curves at 41°C confirmed that warmer adult acclimation was the dominant effect delaying the onset of knockdown (Fig. 2A, B, C), with developmental acclimation having a milder impact (Fig. 2C, D, E). Concerning recoveries from HKD, we did not detect adult acclimation to have an impact (Fig. 3A, B, C), whereas colder developmental acclimation consistently decreased time to recovery (Fig. 3D, E, F). A second layer of analysis using a Cox Proportional Hazards model confirmed these observations. Both adult ( $p < 0.001$ ) and developmental ( $p < 0.01$ ) acclimation had a strong effect on flies' time to HKD at 41°C, with adult acclimation being the far greater effect of the two. Adult acclimation did not impact recovery times, whereas developmental acclimation had a strong significant effect on time to recovery ( $p < 0.001$ ).

### ***Relationship between heat-hardiness and recovery rates***

Each *Drosophila* used in static assays at 41°C had a single time to HKD linked to its time to recovery. These values were plotted against each other for each individual *Drosophila*, and significant regressions were screened both as a function of flies' thermal history (Fig. 4A) and on the global dataset (Fig. 4B). No significant positive nor negative correlations were found when considering thermal history of individuals, with exception of a weak but significant linear relationship in flies reared at 25°C and adult acclimated at 30°C (Table S1). A negligible but statistically significant linear correlation between time to HKD and recovery was also present at the level of the global dataset (Fig. 3B,  $R^2 = 0.07$ ,  $p < 0.001$ , Table S1). Finally, to test for correlations between time to HKD and time to recovery, we fitted polynomial curves on time to HKD (Fig. S3A) and time to recovery (Fig. S3B) data and extracted curve slopes as a function on thermal histories of individuals (Table S2). No significant correlation between rates to HKD slopes and rates to recovery slopes were found on the global dataset (Fig. S4A). In line with analysis from Kaplan-Meier curves (Fig. 3), recovery rates were comparatively higher for colder developmentally acclimated flies than for warmer ones (Fig. S4B).



## Discussion

Our results first show that warmer adult acclimation was the dominant factor delaying the onset of heat knockdown in static assays, for flies exposed to 37°C (Fig. 1, Fig. 2, Table 1). We measured the effect size of adult acclimation to be at least several fold greater than that of developmental acclimation at lower knockdown percentages (Table 1). This was somewhat unexpected, since it contrasts with both our predictions and results of previous studies (Slotsbo *et al.*, 2016; Kellermann *et al.*, 2017, Schou *et al.*, 2017) that showed that developmental acclimation was found to increase the  $CT_{max}$  of flies to a greater extent than adult acclimation did. Such discrepancies in results could first be the outcome of methodological artefacts. Indeed, milder static assays perhaps can, due to their longer duration, offer a better temporal resolution of a heat-hardening process than a ramping one (Salachan *et al.*, 2019). However, reaching time to heat knockdown in milder conditions could incur other physiological costs or induce other processes (such as dehydration or starvation that could lead to a bias in heat-tolerance metrics, see Terblanche *et al.*, 2011; Manenti *et al.*, 2018; but also see Overgaard *et al.*, 2012 and Mitchell *et al.*, 2017 for evidence to the contrary). The inherent methodological tradeoffs between static and ramping assays have long been argued to induce diverse molecular stress responses and therefore perhaps constitute distinct forms of genetic variation, with different dynamics, in exposed animals (Cooper *et al.*, 2008; Mitchell and Hoffmann, 2010; Sgrò *et al.*, 2010; Santos *et al.*, 2011; Terblanche *et al.*, 2011; Rezende *et al.*, 2014). Thus, stress duration, intensity and potential ramping rates are critical parameters to interpret heat-tolerance metrics in their biologically relevant context (Kovacevic *et al.*, 2018; Kingsolver and Umbanhowar, 2018, Ma *et al.*, 2020; Jørgensen *et al.*, 2021).

From this perspective, our results also highlight that warmer developmental acclimation gained secondary importance in increasing flies' heat-tolerance as the stress persisted (Table 1). In addition, other works have proven warmer developmental acclimation to be dominant to increase  $CT_{max}$  of flies in ramping assays (Slotsbo *et al.*, 2016; Kellermann *et al.*, 2017, Schou *et al.*, 2017). Taking these into account, it could also be that developmental and adult acclimation interact through a two geared system: the first would uplift the potential level of expression of stress-tolerance genes, the second set the expression within the range allowed by the first. This would produce a dynamic response as observed: the potential increased expression of tolerance genes through warmer developmental acclimation would prove critical to survive enduring, or indeed increasing, stress exposure. By contrast, tolerance mechanisms already elevated to higher levels through warmer adult acclimation would explain the delays in the onset of heat knockdown in flies as we observed under milder, static conditions. Such a model may find support in our current understanding of the



molecular level processes related to heat stress acclimation and could be tested directly in future. Indeed, developmental acclimation has been shown to reorganize chromatin structure and modulate the accessibility of stress responsive genes to the transcription machinery (Farkas *et al.*, 2000; Feil and Fraga, 2012; Vihervaara *et al.*, 2018), and thus potentially their levels/rates of expression. Given that different methodological approaches can mask or reveal thermal acclimation effects (Terblanche and Hoffmann, 2020), such hypothesis remains merely speculative at this point and await additional scrutiny. Thermal landscape assays have more recently been proposed as a unifying methodology between static and dynamic assays (Castañeda *et al.*, 2015; Jørgensen *et al.*, 2019) and in this context, testing the impact of developmental/adult acclimation under similar full-factorial designs but through a thermal landscape approach would perhaps be useful to further detail these findings and our interpretations.

Finally, and quite surprisingly, we did not detect adult acclimation to contribute to speeding up recovery post-knockdown in any marked way (Fig. 3, A, B, C). In addition, and in contrast to our predictions, colder developmental acclimation, not warmer, drove faster heat stress recoveries (Fig. 3, D, E, F). The heat-hardiness of flies, that is the time needed for a given fly to enter HKD at 41°C, was a poor predictor of its time to recovery (Fig. 4, Fig. S3, S4, Table S.1). This refutes the intuitive assumption that times to recovery from heat stress should be related to the duration of stress exposure (time needed to reach HKD). Overall, this provides indirect evidence that the mechanisms underlying heat-hardening and recovery from knockdown must be at least partially decoupled. Furthermore, this set of mechanisms related to recovery from heat stress seems to be leveraged specifically by colder developmental acclimation alone. Extending our interpretation by inference, increased thermal tolerance from warmer developmental acclimation may thus come at the cost of decreased recovery capabilities. Such a trade-off between heat knockdown and heat recovery may also prove to be a critical link in understanding trade-offs between basal and plastic stress resistance, an area in urgent need of further investigation (van Heerwarden and Kellermann, 2020). However, caution also needs to be exercised when inferring potential biological tradeoffs, as the interactive acclimation effects have been shown to sometimes remain population-specific (Kellerman *et al.*, 2017), and sometimes display non-linear reaction norms to temperature (Salachan *et al.*, 2019; Sørensen *et al.*, 2020). Thus, it will be pivotal to further document the presence of a dynamic interaction between developmental and adult acclimation as well as a potential tradeoff between increased thermal tolerance and recovery capabilities through different methodological approaches in various *Drosophila melanogaster* strains, and across *Drosophila* species, to better understand the generality of these outcomes.

## Conclusion

In summary, using a full-factorial experimental design and analysis of the effect of developmental and adult acclimation on heat-knockdown resistance and knockdown recovery in *D. melanogaster*, we have shown that these two forms of acclimation occurring at different stages of development contribute in distinct ways to the dynamic of heat-tolerance. Warmer adult acclimation strongly delays the onset of heat-knockdown in flies exposed to constant stressful temperatures. By contrast, the effect of developmental acclimation, and the interaction between the two forms of plasticity, gradually gained importance as a function of the stress duration. Finally, we also show that, as opposed to developmental acclimation, adult acclimation had no detectable impact on the rates of recovery post heat knockdown. Thus, the mechanisms underlying the heat hardening response (that increases the flies' initial heat-tolerance), and the mechanisms underlying recovery (once knockdown has occurred) are likely to be at least partly distinct and could react to acclimation temperature in opposite in different ways. These results therefore provide an important framework to understand the temporal interaction of developmental and adult acclimation to promote stress-tolerance in insects. They also underpin a potential ecological and evolutionary significant acclimation tradeoff between increasing thermal tolerance and maximizing recovery rates post-stress, which could constrain the response of populations exposed to more variable microclimatic conditions.

## List of abbreviations

CT<sub>max</sub>, Critical thermal maximum

HKD, Heat-knockdown

LT(s), Lethal time(s)

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## Competing interests

We declare we have no competing interests.

## Authors' contribution

Q.W., B.L. and J.T. conceived and planned the study. Q.W. collected the data, Q.W. and J.T. analyzed the data, Q.W. prepared the figures. All authors contributed to drafting the article, approved the final published version and agreed to be held accountable for all aspects of the work.

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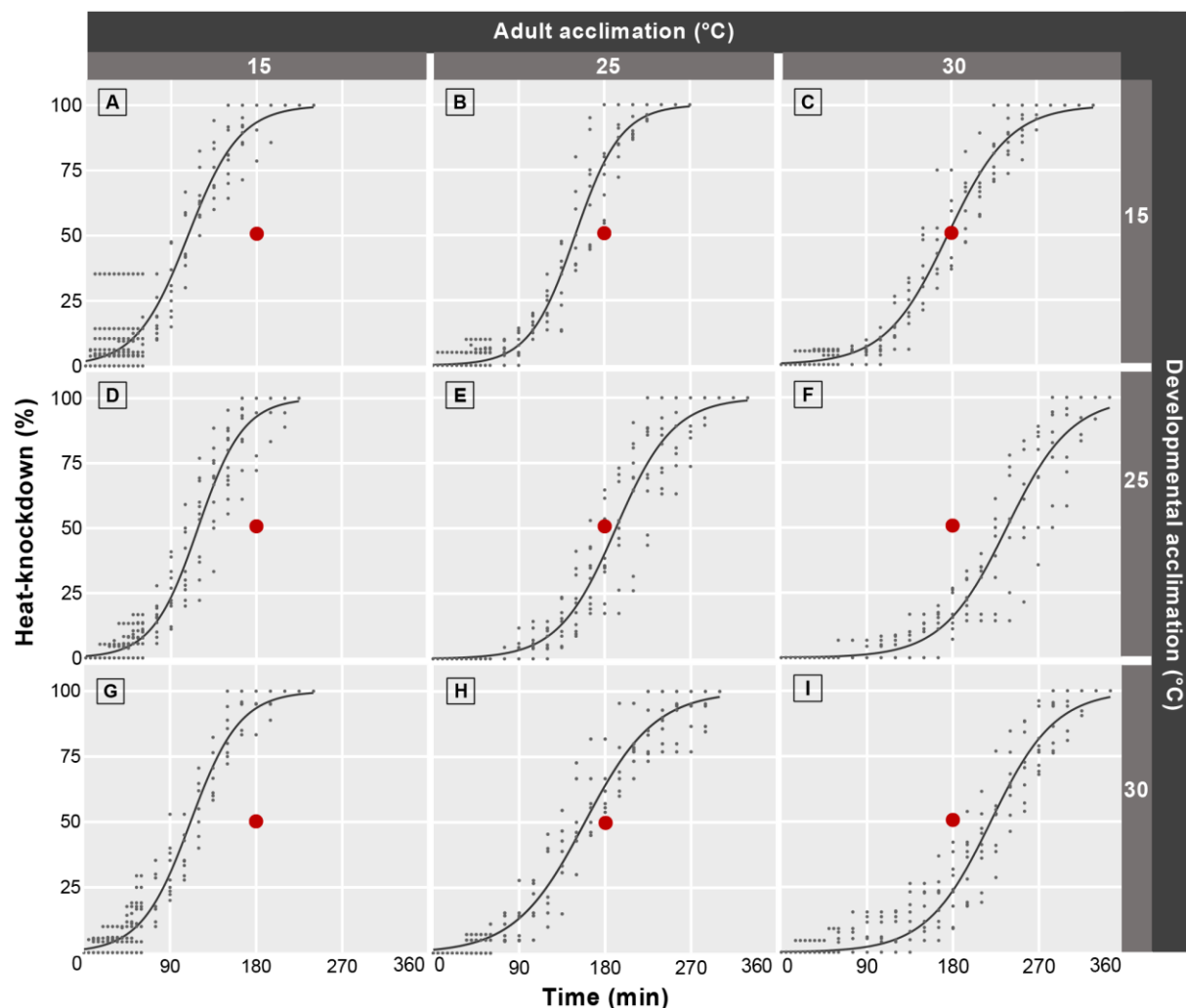


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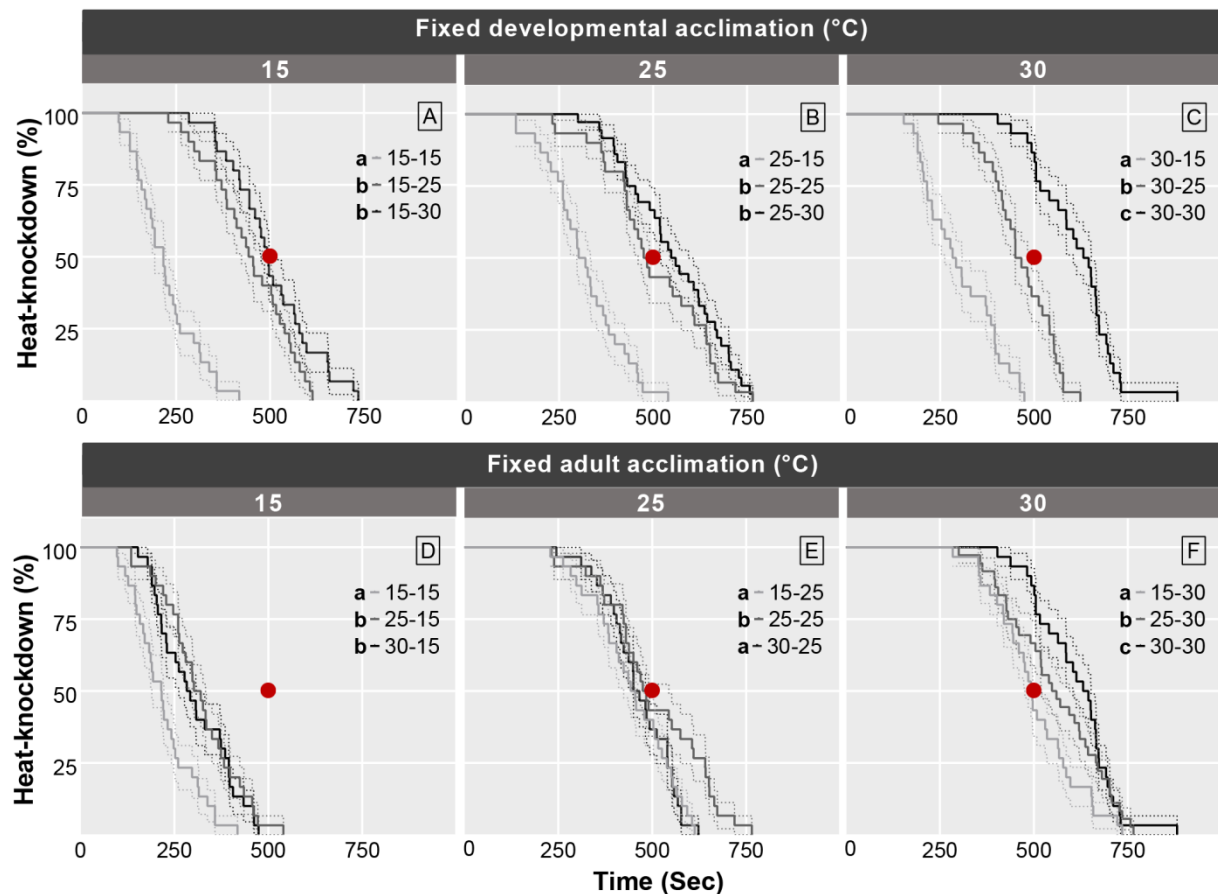
### Supporting information

Additional supporting information may be found in the online version of this article.

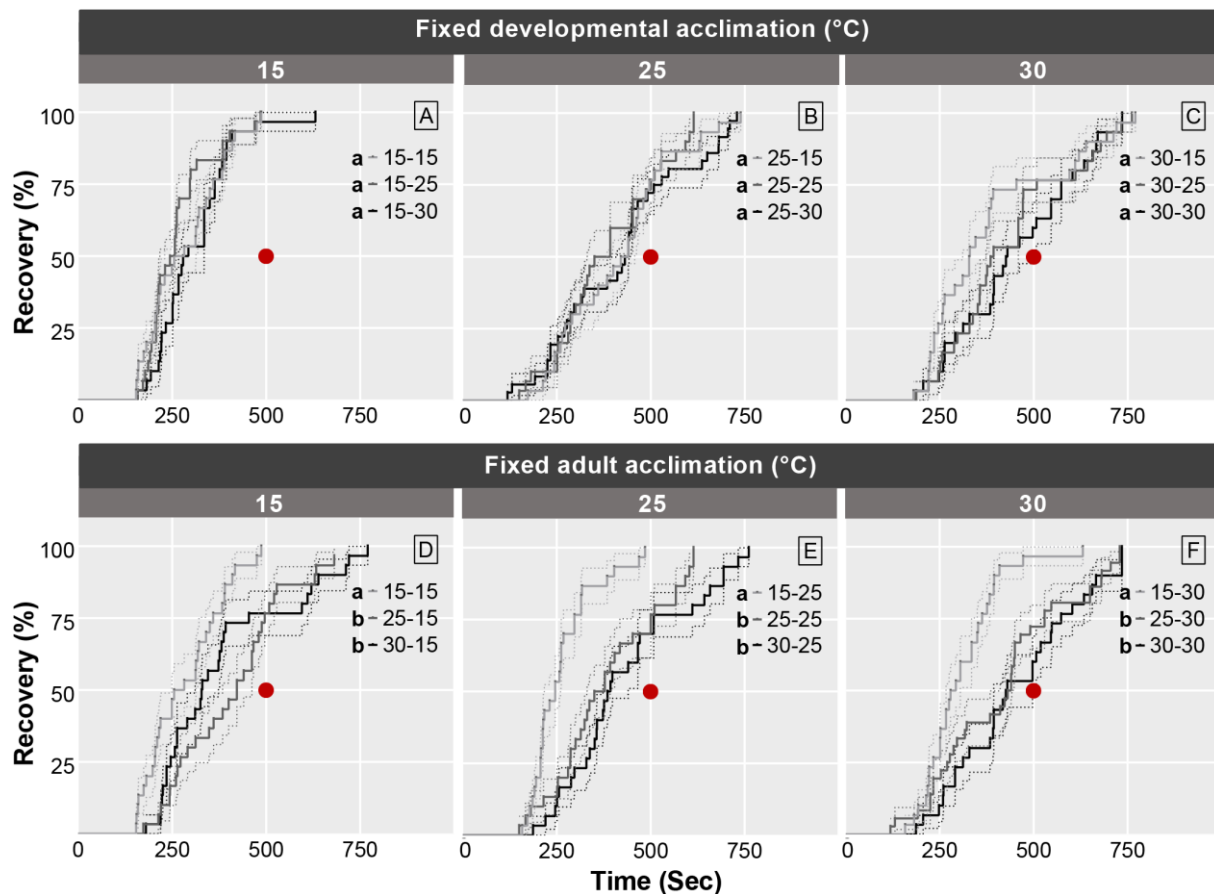
## Figures



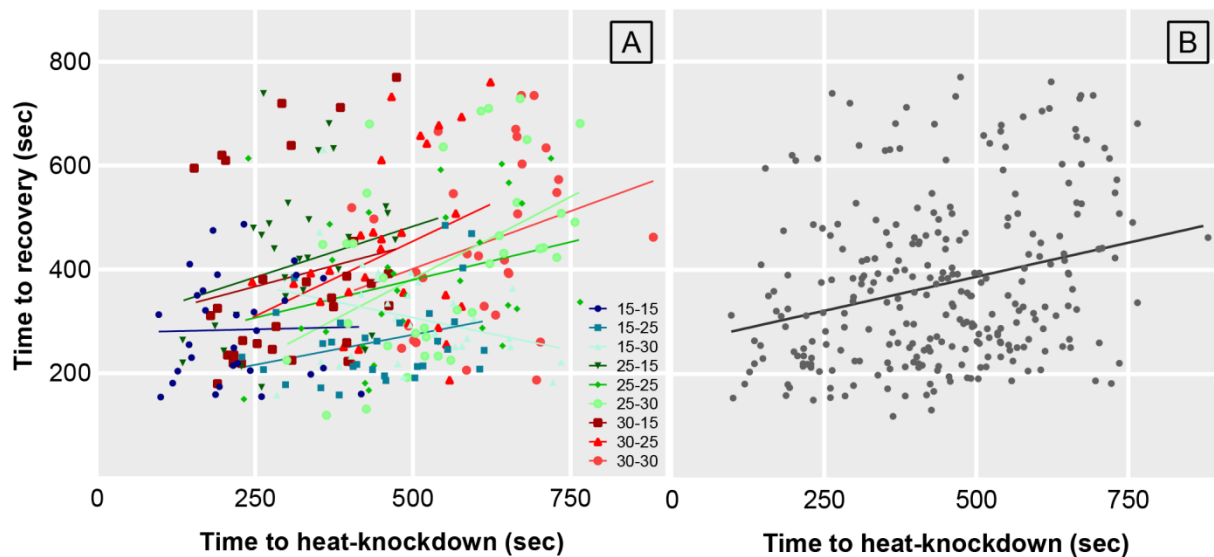
**Fig. 1. Best fit model of HKD curves for flies exposed to a constant 37°C, as a function of their thermal histories.** HKD curves are presented for flies under the 9 conditions of our full-factorial analysis, sorted by the developmental/adult acclimation temperatures. **A:** 15/15°C. **B:** 15/25°C. **C:** 15/30°C. **D:** 25/15°C. **E:** 25/25°C. **F:** 25/30°C. **G:** 30/15°C. **H:** 30/25°C. **I:** 30/30°C. Adult acclimation for a fixed developmental acclimation temperature thus increases from left to right. Developmental acclimation for a fixed adult acclimation thus increases from top to bottom (N=172±32 flies over 7-12 replicates per curve). The central red dots allow for quicker visual comparison of curve shifts as result of treatments. Increased adult acclimation temperatures (from left to right) consistently increased time for flies to enter knockdown and shifted the curves to the right regardless of developmental acclimation. Increased developmental acclimation temperatures (from top to bottom) had a more marginal and less consistent effect on curve shapes. Statistical analysis is presented in Table 1.



**Fig. 2. Knockdown curves of flies as a function of their thermal histories.** Flies were exposed to a constant 41°C until knockdown, and curves are provided with fixed developmental (A, B, C) or adult (D, E, F) acclimation temperatures (N=30 per condition). Time to HKD was measured for 30 individual flies per thermal history conditions, and data was pooled to form a single HKD curve per thermal history. The central red dots allow for quicker visual comparison of curve shifts as result of treatments. Warmer adult acclimation consistently increased times to HKD (A, B, C). Warmer developmental acclimation also had a significant but less consistent effect on times to HKD (D, E, F). Different lowercase letters indicate a significant difference between treatments (log-rank test,  $p < 0.01$ ).



**Fig. 3. Recovery curves of flies as a function of their thermal histories.** Flies were exposed to 41°C until knock-down and immediately put back at 21°C for recovery, as a function of their thermal histories, with either fixed developmental (A, B, C) or adult (D, E, F) acclimation temperatures (N=30 per condition). Time to recovery was measured for 30 individual flies per thermal history conditions, and data was pooled to extract a single HKD curve per thermal history. The central red dots allow for quicker visual comparison of curve shifts as result of treatments. No effect of adult acclimation was found on recovery rates (A, B, C), whereas colder developmental temperatures significantly sped up recoveries (D, E, F). Different lowercase letters indicate a significant difference between treatments (log-rank test,  $p < 0.01$ ).

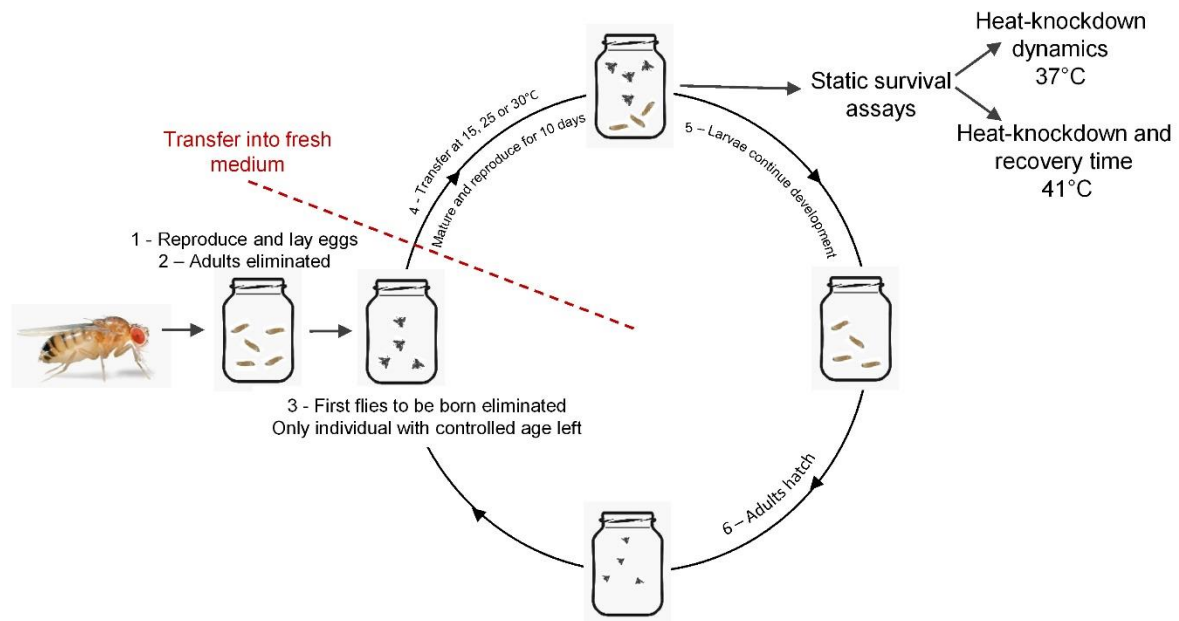


**Fig. 4. Time to recovery as a function of time to HKD in individual *D. melanogaster* flies.** **Fig. 4A:** Linear regression for individuals pooled as a function of their thermal histories (N=30 per thermal history conditions). No significant correlation was observed, with exception of a weak linear relationship in flies reared at 25°C and adult acclimated at 30°C (Table S.1). **Fig.4B:** regression on the global dataset (N=270). A weak but significant linear correlation was present ( $R^2= 0.07$ ,  $p<0.001$ , Table S1), indicating that time to heat knockdown was overall a poor predictor of time to recovery.

## Table

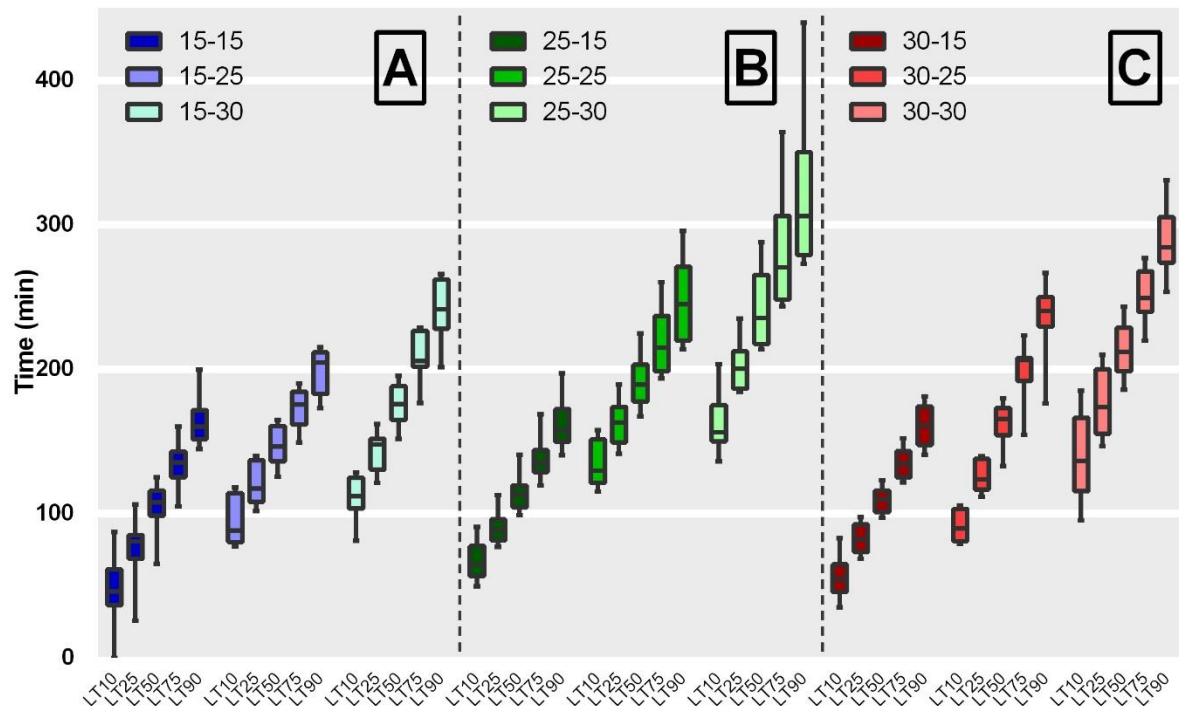
**Table 1. Analysis of the impact of development and adult acclimation on time for flies to reach a set proportion of knocked down individuals.** Overall, adult acclimation accounted for the majority of variation in LTs across thermal histories but became less significant later stage of the experiment (LT90). As opposed, developmental acclimation accounted for an initial minority of variation in LTs, but its significance increased with knockdown proportion. Finally, both the significance and size effect of the interaction between developmental and adult acclimation increased proportionally with stress-duration.

Lethal time (%)	Fixed effects	Effect size	Effect size 90% CI	p value
LT10	Adult acclimation	0.66	0.56 - 0.73	0.032 *
	Developmental acclimation	0.07	0.01 - 0.18	0.841
	Developmental x adult acclimation	6.95 <sup>-04</sup>	0.00 - 0.00	0.331
LT25	Adult acclimation	0.76	0.68 - 0.81	0.001 **
	Developmental acclimation	0.12	0.04 - 0.26	0.551
	Developmental x adult acclimation	0.03	0.00 - 0.12	0.763
LT50	Adult acclimation	0.79	0.72 - 0.83	0.004 **
	Developmental acclimation	0.17	0.06 - 0.29	0.485
	Developmental x adult acclimation	0.07	0.01 - 0.18	0.065
LT75	Adult acclimation	0.77	0.70 - 0.82	0.035 *
	Developmental acclimation	0.17	0.06 - 0.29	0.117
	Developmental x adult acclimation	0.09	0.02 - 0.21	0.006 **
LT90	Adult acclimation	0.73	0.65 - 0.79	0.132
	Developmental acclimation	0.15	0.02 - 0.21	0.036 *
	Developmental x adult acclimation	0.10	0.02 - 0.21	0.001 **

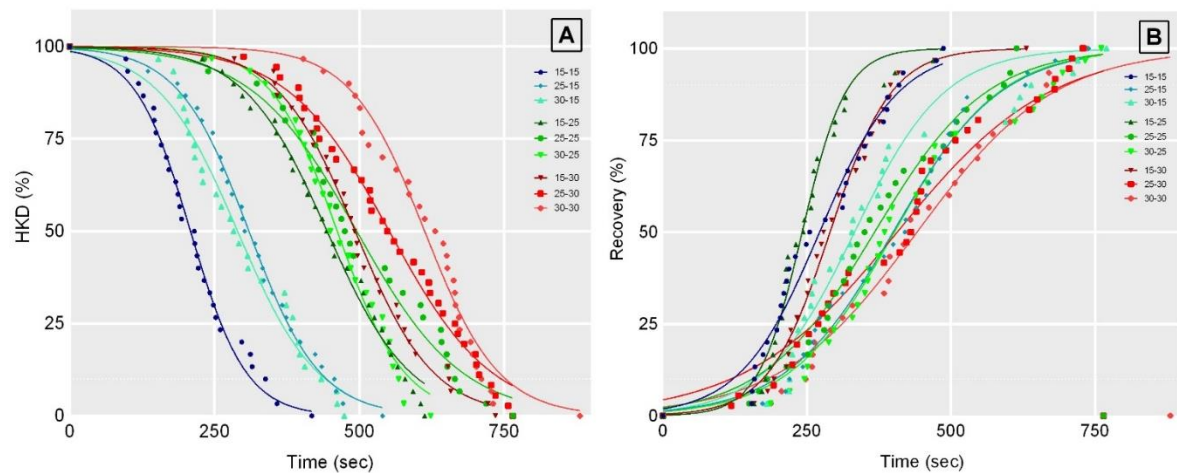


**Fig. S1.** Overview of the rearing and acclimation process performed for the full-factorial analysis. Batches of flies reared at either 15, 25 or 30°C (developmental acclimation) and hatched within 24 hours were transferred again on either 15, 25 or 30°C for 10 days to mature and lay eggs (adult acclimation), creating 9 thermal history conditions. After this 10-day acclimation period, adult flies were removed and use in static knockdown assays experiments aimed at assessing the impact of their thermal histories on heat-knockdown dynamics and recovery times, and a new generation was allowed to grow to start the cycle over.

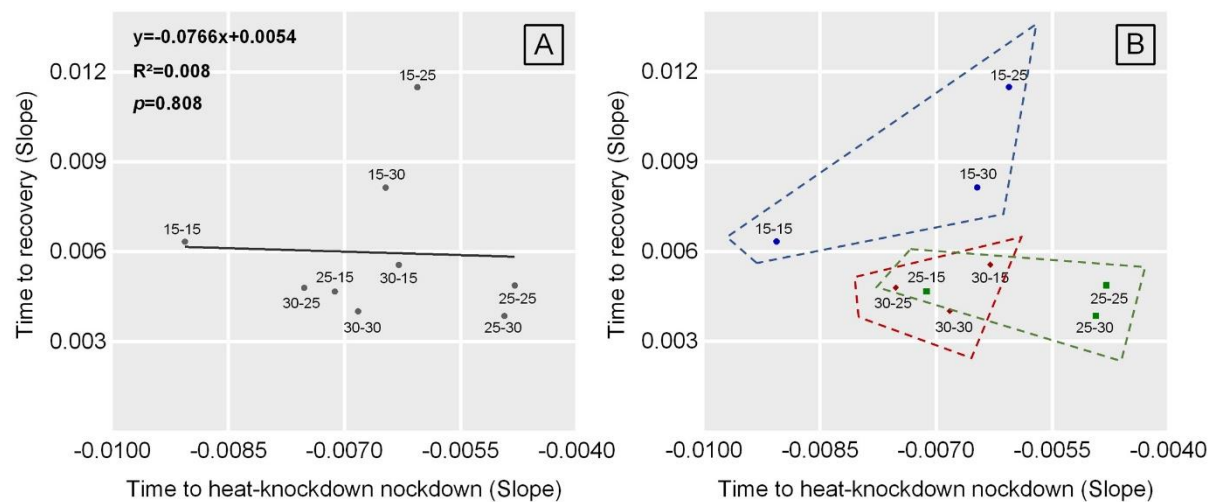




**Fig. S2.** Extracted percentiles from HKD curves of flies exposed to a constant 37°C, as a function of their thermal history ( $N=172\pm32$  flies over 7-12 replicate per condition; mean  $\pm$  s.d). Lethal time (LT) refers to the time needed to reach a set percentage of mortality, namely 10% (LT10), 25% (LT25), 50% (LT50), 75% (LT75), and 90% (LT90) respectively. **Fig S1.A:** percentiles for flies developmentally acclimated at 15°C, and acclimated as adult at either 15, 25 or 30°C. **Fig S1.B:** percentiles for flies developmentally acclimated at 25°C, and acclimated as adult at either 15, 25 or 30°C. **Fig S1.C:** percentiles for flies developmentally acclimated at 30°C, and acclimated as adult at either 15, 25 or 30°C.



**Fig. S3.** Polynomial curve fitted on time to HKD at 41°C and on times to recovery for each thermal condition (N=30 per condition). **A:** polynomial curve fitted on time to HKD data. **B:** polynomial curve fitted on time to recovery data.



**Fig. S4:** Slopes of time to recovery plotted against slopes of time to heat-knockdown (N=30 per condition). **A:** Linear regression between slopes of time to recovery and slopes of time to heat-knockdown, across all developmental acclimation conditions. **B:** Slopes of time to recovery against slopes of time to heat-knockdown for flies developmentally acclimated at 15°C (blue), 25°C (green) and 30°C (red).

**Table S1.** Details of the linear regressions calculated for time to recovery as a function of time to HKD at 41°C in individual flies. No significant relationship was detected as a function of thermal histories of individuals with exception of flies reared at 25°C and adult acclimated at 30°C. A weak but significant relationship was found on the total dataset.

Thermal history (°C)	Equation	Slope	R <sup>2</sup>	p value
15-15	y=0.0284x+277.5	0.028	5*10 <sup>-4</sup>	0.90
15-25	y=0.2317x+158.4	0.231	0.09	0.10
15-30	y=-0.2504x+432.3	-0.250	0.08	0.12
25-15	y=0.3921x+286.9	0.392	0.07	0.15
25-25	y=0.2919x+233.9	0.292	0.08	0.12
25-30	y=0.6319x+66.1	0.632	0.22	<0.01**
30-15	y=0.3232x+286.3	0.323	0.03	0.33
30-25	y=0.5770x+165.9	0.577	0.10	0.08
30-30	y=0.4440x+179.3	0.444	0.08	0.13
Total dataset	y=0.2613x+256.3	0.261	0.07	<0.01***

**Table S2.** Curve slope values and general goodness of the polynomial curve fit of time to HKD at 41°C and time to recovery values as a function of thermal histories of individuals.

Thermal history (°C)	N flies	HKD Slopes	R <sup>2</sup>	Recovery slopes	R <sup>2</sup>
15-15	30	-0,009	0,99	0,006	0,97
15-25	30	-0,006	0,99	0,011	0,98
15-30	30	-0,006	0,99	0,008	0,99
25-15	30	-0,007	1	0,005	0,98
25-25	30	-0,005	0,98	0,005	0,99
25-30	36	-0,005	0,99	0,004	0,98
30-15	30	-0,006	0,97	0,006	0,95
30-25	30	-0,007	0,99	0,005	0,98
30-30	30	-0,007	0,97	0,004	0,99