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Life history consequences of temperature transients in *Drosophila melanogaster*

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Summary

The physiological and life history consequences of chronic temperatures are well studied in ectotherms. However, little is known about the consequences of short-term exposure to unusually high or low temperatures, as would occur during a weather front. What are the immediate life-history effects of such thermal transients? Can ectotherms recover quickly or do they suffer carry-over effects that persist after weather returns to normal? We measured the impact of thermal transients on egg and progeny production of *Drosophila melanogaster* Meigen from Washington State. We reared flies at 25°C and then transferred 3- to 5-day old adults to one of three transient treatments (1 or 3 days at 18°C, 1 day at 29°C) before returning them to 25°C. We monitored daily egg production and egg-to-adult viability before (as a control), during, and after the transient as well as fecundity and viability of flies held at constant 18°, 25° and 29°C. This population appears particularly heat tolerant as neither constant nor transient exposure to 29°C (usually a stressful temperature for this species) affected female fecundity or the viability of her progeny. However, a 1- or 3-day exposure to 18°C reduced female fecundity by 75–90% relative to controls, and eggs laid during the 3-day exposure had greatly reduced viability. When returned to 25°C after transient exposure to 18°C, females immediately matched the fecundity and viability of females maintained constantly at 25°C. Therefore, these flies do not suffer negative carry-over effects from these moderate thermal transients. Surprisingly, fitness (intrinsic rate of population growth) was not depressed by transient temperature exposure. However, the severity and especially the timing of the transient will probably determine the likelihood of carry-over effects as well as its effect on fitness.

Key words: fitness, fecundity, intrinsic rate of increase, net reproductive rate, weather front.

Introduction

Body temperature profoundly affects the performance and fitness of ectotherms (Huey and Stevenson, 1979; Cossins and Bowler, 1987). Temperature effects are typically studied by maintaining organisms in the laboratory either at different constant temperatures or on different fixed temperature cycles. Nevertheless, ectotherms in nature experience transient weather fronts that may alter their body temperatures for short periods (e.g. Kingsolver, 2000). Weather fronts may not only alter an organism's performance during the front itself, but also induce carry-over effects that persist even after weather returns to normal. Surprisingly little is known about the impact of such thermal transients on the physiology and life history of ectotherms, except in regards to short-term exposure to extreme temperatures (Bubliy and Loeschcke, 2001; David et al., 2003; Gibert et al., 2001; Hercus et al., 2003; Krebs and Loeschcke, 1994a; Krebs and Loeschcke, 1994b; Lee et al., 1987; Maynard Smith, 1958; Rohmer et al., 2004; Sisodia and Singh, 2006; Zani et al., 2005a; Zani et al., 2005b).

Drosophila melanogaster is suitable for an examination of the physiological and fitness impacts of non-stressful thermal transients. These flies are widely distributed in temperate zones (Mueller, 1985) where weather fronts are common: thus studies of thermal transients are ecologically relevant to this species. Moreover, a wealth of information is known about the effects of chronic temperature exposure on performance and life history traits in *D. melanogaster* (David et al., 1983; Hoffmann et al., 2003). For example, fecundity increases gradually with temperature to ~25°C and then decreases rapidly at temperatures above 28° to 30°C (David and Clavel, 1969; Huey et al., 1995; McKenzie, 1975; Schnebel and Grossfield, 1986; Siddiqui and Barlow, 1972).

We studied the impact of thermal transients on fecundity (eggs) and progeny production of *Drosophila melanogaster*. Initially we measured daily fecundity and progeny production of flies living at 25°C for 3 days and then transferred flies to one of three transient treatments (1 day at 18°C, 3 days at 18°C, or 1 day at 29°C) before returning them to 25°C for 3–5 days. The 1- or 3-day transient exposures are equivalent to typical weather front durations (Allen et al., 1996; Bosart et al., 1973; Robb and Forbes, 2006). We selected transient temperatures of 18°C and 29°C because these are displaced from the optimal temperature (~25°C) of these flies (Cohet and David, 1978; Huey et al., 1995; Siddiqui and Barlow, 1972), but are not extreme (David et al., 2005). We also maintained some flies at constant temperatures (18°C, 25°C or 29°C) so we could compare the effects of chronic *versus* transient temperature exposures.

To determine whether transient exposures affected progeny of exposed females, we estimated development time of offspring produced during the experiment. From these data we computed generation time of offspring of experimental flies, net reproductive rate (R_0, that is, total female progeny produced during the experiment) and intrinsic rate of population growth (r). Thus our experimental design enabled us to determine how key life history traits changed during, as well as after, a thermal transient. Although we expected that temperature transients would depress egg production during the exposure, we were uncertain as to whether and how transient exposure would induce carry-over effects once flies were returned to 25°C. Consequently, we considered four competing hypotheses (Fig. 1):

(1) *No carry-over hypothesis*. This hypothesis predicts no carry-over effects of a transient temperature change. If so, then life history parameters will quickly return to baseline levels after the transient.

(2) *Induced damage hypothesis*. If flies are damaged by the thermal transient (Krebs and Loeschcke, 1994a), their fecundity may not return to normal levels for some time, if ever. Because the transient temperatures we selected were intentionally non-extreme (David et al., 1983), this outcome is unlikely in our experiments, but would be likely following more extreme treatments.

(3) *Resource reallocation hypothesis*. If flies interpret a sudden temperature change as a cue (sensu Levins, 1968) that unfavorable weather will persist for some time, they might reallocate resources from reproduction to survival. If so, their fecundity might remain low for some time even following a return to normal temperatures (Mitrovski and Hoffmann, 2001; Schmidt and Conde, 2006). Note that the resulting pattern of fecundity parallels that for the 'induced damage hypothesis' but has a different causal basis.

(4) *Compensation hypothesis*. When transferred back to normal temperatures, flies might actually increase fecundity above baseline levels for a brief period, 'catching up' for fecundity lost during the thermal transient. In effect, compensation could represent a 'make hay while the sun shines' response. Compensation could also occur if flies are able to produce, but not lay, eggs during the thermal transient and then can dump accumulated eggs once conditions improve.

We find little effect of thermal transients on flies once they return to 25°C. This result strongly supports the 'no carry-over hypothesis'. Consequently, *D. melanogaster* seems well buffered against short-term exposure to non-optimal (but non-extreme) temperatures. Parallel studies with exposure to more extreme temperatures (or perhaps with longer durations) will be necessary to set boundaries on the range of permissive temperatures.

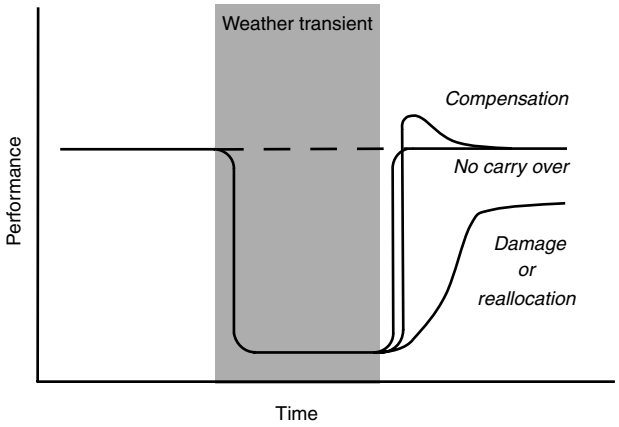

Fig. 1. Hypotheses for the effects of thermal transients on fly fitness. See text for descriptions of hypotheses.

Materials and methods

Source and maintenance of flies

Because natural responses to thermal transients might decay in laboratory stocks that have been evolving at constant temperatures for many years (Quintana and Prevosti, 1990), we chose to work with a relatively fresh stock. Accordingly, we collected 100 isofemale lines of *D. melanogaster* from an apple orchard near Wenatchee, WA, USA (47°30′N, 120°17′W) in June 2005, and established a base stock by pooling about 25 males and 25 females from each isofemale line. This stock was maintained at 25°C (12 h:12 h L:D) until July and early August 2006, when these experiments were run. Thus the flies had been in the laboratory for just over 1 year. To maintain the colony at 1000–3000 flies, eggs were collected approximately every 10 days and reared in controlled conditions (50–100 eggs/vial; 25°C and 12 h:12 h L:D; diet of cornmeal, molasses, yeast, agar, tegosept) before newly emerged adult flies were transferred into the colony. Colony food bottles (150 ml) were replaced every 3–5 days to ensure that all colony flies developed in controlled conditions.

Experimental conditions

Eggs collected from the base stock on July 12, 2006 were reared at low densities of 50–70 eggs/vial (25°C, 12:12 L:D; 2.5×9.5 cm glass vials containing ~10 ml food). Within 12 h after flies emerged (7/21 and 7/22), adults were briefly anesthetized (CO_2) and triads of flies (one female, two males) were placed into individual vials seeded with live yeast. Egg production increases until females are about 3 days of age (Huey et al., 1995), so fly triads were maintained at 25°C until females were 3 to 4 days of age before assigning them to treatment groups. Flies that did not lay eggs during this initial period were eliminated from the experiment. Fly triads were then transferred (without anesthesia) into fresh vials without supplemental yeast (to facilitate egg counting) and then randomly assigned to one of six treatment groups, with about 18 triads per group. One transient group was kept for 3 days at 25°C, 1 day at 18°C and then 5 days back at 25°C. A second was kept for 3 days at 25°C, 3 days at 18°C, and 3 days back at 25°C. The third was kept for 3 days at 25°C, 1 day at 29°C, and 5 days back at 25°C. Three additional sets of fly triads were maintained at constant temperatures of 18°C, 25°C or 29°C for 9 days. Flies were housed in environmental chambers (Percival Scientific, Perry, IA, USA; 12:12 L:D cycle starting at 07:00 h). Chamber temperatures were monitored daily and vials within a chamber were rotated daily to minimize effects of within-chamber temperature variation. Each triad was transferred without anesthesia into a new vial every day at 09:30–10:00 h so as not to interfere with peak oviposition, which occurs in late afternoon

in these flies. Flies were briefly at room temperature (~23°C) during transfers.

After returning triads to environmental chambers, we counted 'transient' eggs laid during the previous 24 h period and then immediately transferred them to 25°C for development. We maintained 'constant' eggs at their respective temperatures. Subsequently, we counted newly emerged flies in late morning every day until no more emerged. Because eggs collected during transient exposure completed most of their development at 25°C, any between-groups difference in progeny viability should be due to effects of the transient on the female or on the eggs themselves.

We estimated egg-to-adult viability as the proportion of eggs that produced adults. Egg counts in vials are often in error, because some eggs either might have hatched by scoring time or might simply have been overlooked. Indeed, 156 of 834 vials had a few more flies emerged than eggs counted (treatment had no effect on the number or magnitude of miscounts). For these vials, viability was taken as the number of emerged flies instead of the number of eggs (thus these females were assumed to have 100% viability). Two vials were excluded from the analysis because they had many fewer eggs than emerged adults. However, the statistical results presented below are robust, even if these vials are included.

Development time and fitness estimates

To determine fitness consequences of the various temperature treatments, we used a life-table analysis (Birch, 1948) and estimated generation time (T_g), net reproductive rate (R_0, the total number of female eggs laid during the experiment), and intrinsic rate of increase (r, computed using an iterative technique; see Appendix A) for each female. Estimates of r require development time, which was not measured for experimental females themselves (because all were reared at 25°C, see above). Instead, the average development times of the females' progeny were used as estimates of their development times. (Thus, our estimate of r is based on fecundity patterns of female parents and development times of their progeny, rather than on data collected within a single generation.) For these calculations we assumed a 50:50 sex ratio of progeny.

Statistical analyses

During the experiment 6 females died (2 at 18°C, 3 at 25°C and 1 at 29°C), 6 escaped during transfers (2 at 18°C and 4 at 25°C), and 12 did not lay any eggs (3 at constant 18°C, 2 at constant 25°C, 1 at constant 29°C, 2 at short 18°C, and 1 at short 29°C). These were excluded from further analysis. Females for which one male escaped or died were included in the analysis. To correct for non-independence of daily measurements within females, we used a repeated-measures MANOVA with daily fecundity, viability (arcsine square root-transformed), or development time as the dependent variable, temperature treatment as the between-subjects effect, and day and treatment×day within-subjects effects. This technique is robust to violations of normality and sphericity (Obrien and Kaiser, 1985). For these analyses, we report several statistics (Pillai's Trace, Hotelling's Trace, Wilks's lambda, and Roy's statistic) because these did not always give similar results. For data not requiring repeated measures, we used multivariate ANOVA analyses with Tukey-HSD *post-hoc* tests when the data were normally distributed. For non-normal data, we used Kruskal–Wallis rank sum tests with non-parametric Behrens–Fisher *post-hoc* multiple comparisons tests. All statistics were done in R (R Development Core Team, 2006), with packages multcomp (Bretz et al., 2004) and npmc (Helms and Munzel, 2006) for parametric and non-parametric *post-hoc* tests, respectively, nlme (Pinheiro et al., 2006) for linear mixed effects modeling, and gregmisc (Warnes, 2006), Hmisc (Harrell, 2006), IDPmisc (Ruckstuhl et al., 2006), and gridBase (Murrell, 2006) for advanced plotting features.

Results

Effects of temperature on fecundity

We analyzed daily fecundity of 94 females over 9 days (total eggs=14,870). Constant temperatures strongly affected female fecundity during the 9 day experiment ('total eggs per female', Fig. 2A, inset; ANOVA, $F_{2,43}$=8.62, P<0.001). Females at 18°C laid significantly fewer total eggs than did females at 25° or 29°C (Tukey HSD, P=0.016, and P<0.001, respectively), but females at 25° and 29°C did not differ significantly in total eggs laid (Tukey HSD, P=0.169).

Females in constant temperature treatments laid fewer eggs as they aged (Fig. 2A; Table 1, MANOVA, within-subjects Day effect, P<0.0001); and the decline in fecundity with age grew steeper with increasing temperature (Fig. 2A). The rapid decline in fecundity with age (Fig. 2A) confounded any simple analysis of effects of transient temperature on egg production, as an observed decline in egg production from day 3 to 4 could be due either to aging or to the transient temperature treatment. Consequently, we standardized mean daily fecundity of transient temperature groups by computing the percent difference between the fecundity of each transient treatment group and that of the constant 25°C group on each day (Fig. 2B).

Prior to exposure to the temperature transient (on days 1–3), females in the constant 25°C treatment and those destined for transient treatments all had similar fecundities (Kruskal–Wallis χ_3^2=2.18, 1.42, 1.42 on days 1, 2 and 3, respectively, all P>0.536; see Fig. 2B). Differences in fecundity among treatment groups on days 4–6 could therefore be attributed to the transient temperature exposure.

A 1-day 'hot' temperature transient had no immediate or carry-over effects on female fecundity. Females at 29°C for 1 day had fecundities similar to those of females kept at 25°C on the transient day (Fig. 2B; day 4 *post-hoc* Behrens–Fisher P=0.700) and on each subsequent day at 25°C (days 5 to 9, Behrens–Fisher, all P>0.52).

Both 1- and 3-day 'cold' temperature transients strongly reduced female fecundity, but these effects disappeared immediately when flies were returned to 25°C. While at 18°C for 1 or 3 days, females laid significantly fewer eggs on those days than did the constant 25°C females (Fig. 2B; Behrens–Fisher, all P<0.001). When returned to 25°C transient cold females had similar fecundities to constant 25°C females (Fig. 2B; Behrens–Fisher P=0.960 for *post-hoc* comparison of 1-day 18°C with constant 25°C on day 5; Kruskal–Wallis χ_3^2=2.44, P=0.486 for day 7). These results strongly support the 'no carry-over hypothesis' (Fig. 1).

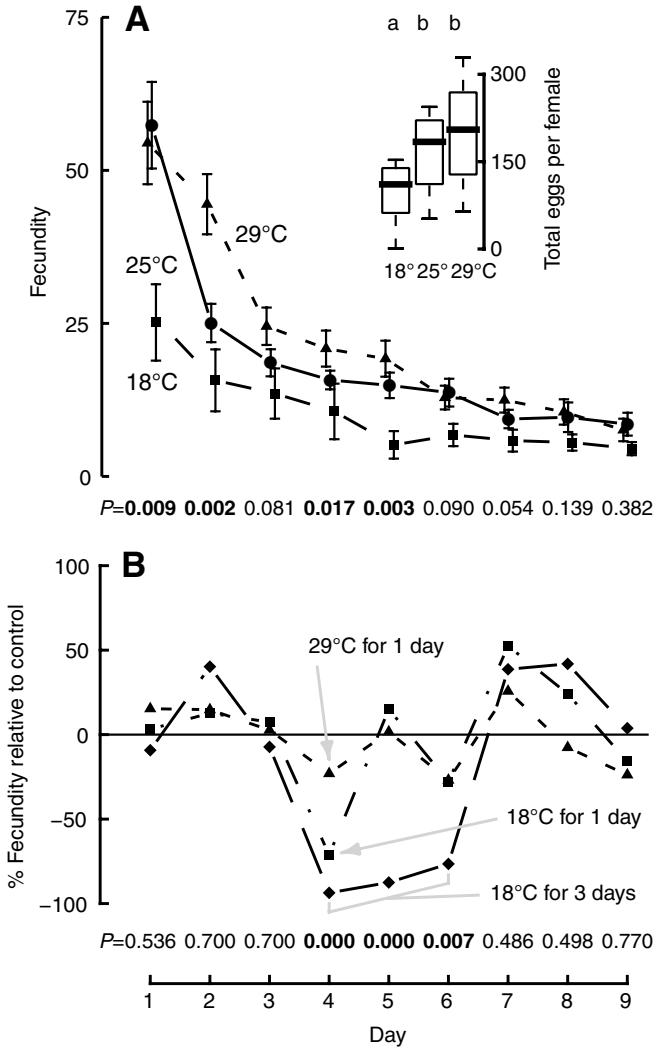

Fig. 2. Daily fecundity for constant (A) and transient (B) temperature treatments. The mean total number of eggs laid by a female varied with constant temperature treatment (A, inset; different lowercase letters indicate significant differences between treatment groups from Tukey HSD *post-hoc* comparisons). For the transient treatment groups (B), flies were kept at 25°C except for either 1 or 3 days at 18°C or 1 day at 29°C. The transient temperature data is presented as the percent difference from the control group maintained at constant 25°C. Each point is the mean ± s.e.m. from ~15 females (range 13–17) per temperature per day, and points are slightly offset to make error bars visible. *P*-values from Kruskal–Wallis rank sum tests for each day are shown below each plot; values in bold type are significant at the $P=0.05$ level. Flies were 3–4 days old on day 1.

Effects of temperature on egg-to-adult viability

Constant low temperatures strongly reduced egg-to-adult viability. Progeny developing at 25°C and 29°C had similar viabilities that were much higher than viabilities of those developing at 18°C (MANOVA, between-subject Temperature effect, $P=0.005$; Table 1; Fig. 3A).

Egg-to-adult viability did not differ among the three transient treatment groups and the 25°C control on days 1 to 3, indicating that these groups were homogeneous prior to temperature transients (Fig. 3B; Kruskal–Wallis $\chi_3^2=6.01, 1.88, 2.55$; and all $P>0.46$). A 1-day exposure of flies to 29°C did not affect egg-to-adult viability of their progeny (Fig. 3B; Behrens–Fisher $P=0.904$ for comparison of 1-day 29°C group with 25°C group on day 4). A 1-day exposure of flies to 18°C reduced egg-to-adult viability of their progeny (Fig. 3B, broken line), but not significantly so (Behrens–Fisher $P=0.110$). However, a 3-day exposure of flies to 18°C significantly reduced egg-to-adult viability of their progeny on the first 2 days (Fig. 3B, broken line; Behrens–Fisher $P=0.002, 0.036$ for days 4 and 5, respectively), and marginally so on day 3 (Behrens–Fisher $P=0.087$ for comparison of 3-day 18°C group and 25°C group on day 6). In no case did temperature transients have carry-over effects on egg-to-adult viability (Fig. 3B; Behrens–Fisher, all $P>0.800$).

Effects of temperature on development time

Development time decreased significantly with increasing constant temperature (Fig. 4A; Table 1, between-subjects Temperature effect, $P<0.0001$). Flies eclosed in 21.8±1.3 days at 18°C, in 9.8±0.8 days at 25°C, and in 8.3±0.7 days at 29°C. Progeny development time did not change significantly with female's age (Fig. 4A; Table 1, within-subjects Day effect, $P=0.480$). Therefore we compared development time of transient treatment groups directly to the 25°C control.

Transient temperature exposure did not affect development time. Progeny of females moved to 18°C or 29°C for 1 day developed in the same time as progeny of females held at constant 25°C (Fig. 4B, day 4, Kruskal-Wallis $\chi_3^2=4.95$, $P=0.175$). Development times of progeny from eggs laid by females during their third day at 18°C were slightly but not significantly longer than eggs laid at 25°C on the same day (Fig. 4B, day 6; Behrens–Fisher $P=0.085$).

Effects of temperature on fitness

Low constant temperatures significantly reduced fitness. Mean generation time decreased significantly, and both net reproductive rate (R_0) and intrinsic rate of increase (r) increased significantly with temperature (Table 2; ANOVA, all $P<0.002$). Both R_0 and r were significantly lower for 18°C flies than for 25°C and 29°C flies (Tukey HSD $P<0.05$), but these traits were not significantly different for 25°C *versus* 29°C flies.

Surprisingly, exposure to transient temperatures did not decrease female fitness. Despite major depression effects of transient exposure to 18°C on fecundity and on egg-to-adult viability (Fig. 2B, Fig. 3B), transient exposure to 18°C or to 29°C temperatures did not affect T_g, R_0, or r relative to the constant 25°C treatment (Table 3; ANOVA all $P>0.257$).

Discussion

Temperature has strong effects on the performance and fitness of flies and other ectotherms (Huey et al., 1995; McCabe and Partridge, 1997; Gilchrist and Huey, 2001). Many ectotherms use behaviors to avoid – or at least reduce – the impact of fluctuations in operative temperatures (Cowles and Bogert, 1944; Stevenson, 1985; Huey et al., 2003). Nevertheless, ectotherms will likely be forced to experience non-optimal temperatures during weather fronts. Two issues are germane. First, how much are performance and fitness depressed during a weather front? Second, are there carry-over effects that persist once temperatures return to normal? Our

Table 1. *Effects of constant temperatures on fecundity, egg-to-adult viability, and development time of Drosophila melanogaster*

	Fecundity		Viability		Development time	
Source (d.f.)	*F*	*P*-value	*F*	*P*-value	*F*	*P*-value
Between-subjects						
Temperature (2,42)	10.20*	**0.002**	5.99*	**0.0052**	490.7*	**<0.0001**
Within-subjects						
Day (8,35)	27.91*	**<0.0001**	1.49*	0.1966	1.10*	0.4801
Temperature × Day						
Pillai's Trace (16,72)	2.32	**0.0082**	1.55	0.1056	1.11	0.4323
Wilk's Lambda (16,70)	2.44	**0.0054**	1.82	**0.0451**	1.47	0.2728
Hotelling–Lawley (16,68)	2.57	**0.0037**	2.09	**0.0188**	1.76	0.2123
Roy's Max Root (8,36)	4.41	**0.0009**	4.32	**0.0100**	4.90	**0.0342**

For details, see Figs 2A, 3A, 4A.
Statistics are from a repeated-measures MANOVA.
*Exact *F* value. All others are approximate. *P*-values in bold are significant at the *P*=0.05 level.

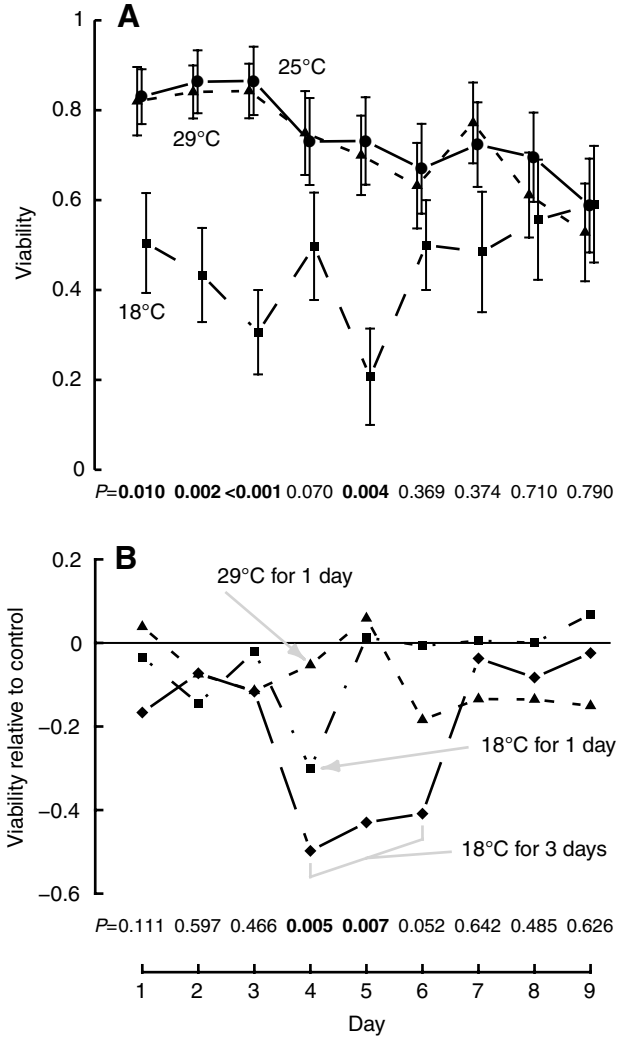

Fig. 3. Daily egg-to-adult viability (proportion of eggs laid that emerged) for flies experiencing constant (A) and transient (B) temperatures. In B, egg-to-adult viability is shown as the difference between treatment viability and viability at constant 25°C on the same day. Flies were kept at 25°C except for 1 day at 18°C or 29°C and 3 days at 18°C. *P* values from Kruskal–Wallis rank sum tests for each day are shown below each plot; values in bold are significant at *P*=0.05 level.

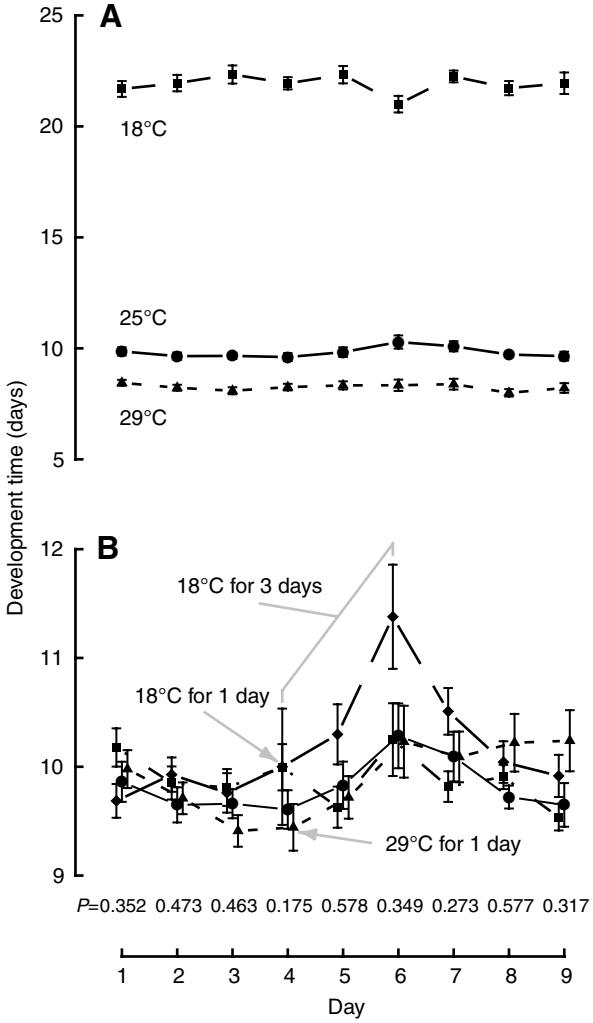

Fig. 4. Development times of progeny from adult flies exposed to constant (A) and transient temperature treatments (B). Included in B are control flies held at constant 25°C for the duration of the experiment (circles, thin solid line), flies that experienced 25°C except for 1 day at 18°C (squares, dot-dash line) or 29°C (triangles, dotted line) or 3 days at 18°C (diamonds, broken line). *P* values from Kruskal–Wallis rank sum tests are shown below plot B; values in bold are significant at *P*=0.05 level.

Table 2. *Effects of constant temperatures on mean generation time, net reproductive rate and intrinsic rate of increase in D. melanogaster*

Temperature (°C)	N	T_g (days)	R_0 (female offspring)	r
18	11	28.6±1.0[a]	37.8±16.4[a]	0.12±0.04[a]
25	15	16.0±1.0[b]	79.2±31.6[b]	0.28±0.04[b]
29	17	14.2±1.1[c]	83.9±41.3[b]	0.30±0.08[b]
$F_{2,40}$		738.59	7.27	32.02
P		**<0.001**	0.002	**<0.001**
r^2		0.97	0.23	0.60

T_g, mean generation time; R_0, net reproductive rate; r, intrinsic rate of increase (calculated using an iterative technique, see Appendix).

Values are mean ± s.d. Results from three ANOVAs testing the effects of temperature treatment on fitness parameters are given below each parameter. Different letters indicate significant differences in response value among treatment groups based on Tukey–HSD *post-hoc* comparisons (P=0.05).

Table 3. *Effects of transient exposure to sub-optimal temperatures on mean generation time, net reproductive rate and intrinsic rate of increase in D. melanogaster*

Temperature (°C)	N	T_g (days)	R_0 (female offspring)	r
25	15	16.0±1.0	79.2±31.6	0.28±0.04
18 for 1 day	16	16.1±1.5	82.8±44.2	0.26±0.08
18 for 3 days	13	15.9±1.3	63.6±30.8	0.25±0.08
29 for 1 day	17	15.3±1.3	73.5±43.7	0.27±0.05
$F_{3,57}$		1.38	0.66	0.51
P		0.257	0.581	0.675
r^2		0.02	0.03	0.03

T_g, mean generation time; R_0, net reproductive rate; r, intrinsic rate of increase (calculated using and iterative technique, see Appendix).

Values are mean ± s.d.

results suggest that female flies experiencing even minor cold fronts (though not minor warming) will have reduced fecundity and egg-to-adult viability during those fronts. Nevertheless, they recover quickly when temperatures return to normal, supporting the 'no carry-over hypothesis'.

The constant temperature treatments provide a necessary baseline for comparison of our fly population with other stocks. Overall, the flies used in this study responded to chronic temperatures as expected from previous work. Chronic exposure to 18°C reduced fecundity and viability in our flies, and increasing chronic temperatures reduced development time and changed the pattern of egg production over time. All of these findings qualitatively match previous work in *Drosophila* (Bochdanovits and de Jong, 2003; Bouletreau-Merle et al., 2003; David and Clavel, 1969; David and Clavel, 1967; Fernandez and Lopez-Fanjul, 1997; Gilchrist and Huey, 2001; Huey et al., 1995; Schnebel and Grossfield, 1986; Siddiqui and Barlow, 1972; Trotta et al., 2006). However, chronic exposure to 29°C did not reduce fecundity, viability or fitness of our flies even though this temperature is stressful for many fly stocks (David and Clavel, 1967; Fernandez and Lopez-Fanjul, 1997; Gilchrist and Huey, 2001; Huey et al., 1995; Schnebel and Grossfield, 1986; Siddiqui and Barlow, 1972; Trotta et al., 2006). Thus our stocks appear heat tolerant relative to previously studied ones. This might reflect geographic variation (Schmidt and Conde, 2006; Hoffmann et al., 2003), or the fact that our flies had been in the laboratory for only a year, whereas prior studies often used lab-adapted stocks; heat tolerance in *D. subobscura* decays quickly in the laboratory (Quintana and Prevosti, 1990).

Immediate impact of thermal transients

We first review changes in life history parameters during transient exposure. Transient exposure to 18°C negatively affected both fecundity and viability. The reduction in fecundity is not surprising, as chronic exposure to 18°C is known to reduce fecundity in many *Drosophila* stocks (Bochdanovits and de Jong, 2003; Bouletreau-Merle et al., 2003; David and Clavel, 1969; David and Clavel, 1967; Fernandez and Lopez-Fanjul, 1997; Gilchrist and Huey, 2001; Huey et al., 1995; Schnebel and Grossfield, 1986; Siddiqui and Barlow, 1972; Trotta et al., 2006), and in our flies (Fig. 2A). However, our finding that viability of progeny is reduced by a 1-day (or less) exposure to 18°C is novel and exacerbates the ecological impact of a weather transient. Eggs were exposed to only 1 day (or less) at 18°C and were at 25°C for the remainder of development (~10 days), suggesting that fly embryos are very sensitive to even brief exposure to moderately cool temperatures. Whether later stages of development are also sensitive will require additional experiments.

Flies experiencing a 1- or 3-day 18°C transient had a larger decline in fecundity during that transient (70–80% decline relative to constant 25°C females, see Fig. 2B) than did same-age females maintained at constant 18°C (32–65% decline on days 4–6; Fig. 2A). Egg viabilities were similar for the two groups (30–50% for 18°C transients, 20–50% for constant 18°C flies). Thus, transient exposure to 18°C depressed fecundity more than did chronic exposure to 18°C; perhaps chronic exposure to 18°C post-eclosion induces a 'beneficial acclimation' effect (Ayrinhac et al., 2004; Nunney and Cheung, 1997).

Transient exposure to 29°C did not significantly affect fecundity or viability relative to flies maintained at 25°C. We were surprised by this result because chronic exposure to 29°C typically reduces the performance and fitness of *D. melanogaster* (David and Clavel, 1967; Fernandez and Lopez-Fanjul, 1997; David et al., 2005; Schnebel and Grossfield, 1986; Siddiqui and Barlow, 1972). However, in our flies, chronic exposure to 29°C did not depress fitness; thus 29°C appears not to be particularly 'hot' for this stock.

Recovery from thermal transients

Although fecundity and progeny viability were markedly reduced during a 1- or 3-day exposure to 18°C, these traits returned to baseline levels immediately after return of females to 25°C (Fig. 2B, Fig. 3B, days 5–9). This pattern strongly supports the 'no carry-over hypothesis' and contradicts alternative hypotheses (Fig. 1).

For our flies, neither fecundity nor progeny viability changed during exposure to 29°C (Figs 2, 3), either chronically or transiently, so it is not surprising that these traits were unchanged (relative to constant 25°C flies) after return to 25°C. Our data document that transient exposures to temperatures as low as 18°C or as high as 29°C have no sustained effects on fecundity or viability. Thus these flies are buffered against temperature fluctuations of more than 10°C. Brief exposures to more extreme temperatures do have sustained negative effects on fitness traits of flies (Krebs and Loeschcke, 1994a; Sisodia and Singh, 2006). In any case, additional experiments will be required to demarcate the range and duration of transient temperatures that inflict sustained damage on flies.

Fitness consequences of thermal transients

Because transient exposure to 29°C did not alter fecundity or progeny viability relative to control flies, we focus only on the fitness consequences of transient exposure to 18°C. Although these transients reduced fecundity and viability, they did not significantly reduce lifetime fitness relative to constant 25°C flies (R_0, r, Table 3). Initially, this seemed paradoxical to us, but on reflection we believe two factors are responsible:

(1) Flies experienced transients for only a fraction of the period for which we obtained life history data (1 or 3 days out of 9), and thus were at 25°C during most of the experimental period. This necessarily dilutes the impact of a transient. Had we terminated the experiment immediately after transient exposure, fitness impacts would be evident.

(2) To allow for measurement of baseline fecundities and viabilities of all groups, we waited until flies were 7–8 days old (as adults) before exposing them to thermal transients. By this age, all flies had fecundities that were 70% lower than on day 1 of the experiment (when flies were 3–4 days old; Fig. 2A), and egg viabilities that were already 10% lower than on days 1–3 (Fig. 3A). Thus relatively heavy reproductive success early in life likely swamped the negative effects of the thermal transient later in life. This is especially likely for r, which is particularly sensitive to reproduction early in life (Birch, 1948). Very likely, exposure of younger flies to a temperature transient would have significantly reduced fitness.

In conclusion, our experiments demonstrate that non-extreme thermal transients can reduce fecundity and progeny viability, but that these transients have no sustained effects on flies. Thus these flies seem relatively well buffered against moderate weather fronts. Future studies should attempt to delimit the range of transients that are tolerable by flies, as well as whether sensitivity to transients varies with age. Our egg viability data suggest that early embryos are very sensitive to brief exposure to temperatures as mild as 18°C.

Appendix

Net reproductive value (R_0) is the total number of eggs laid by the female over the course of the experiment, or, equivalently:

$$R_0 = \sum_{x=3}^{12} l_x m_x \quad , \tag{A1}$$

where x is age in days and l_x is the probability of being alive at age x, and m_x is the number of female offspring produced by the female at age x. Because we analyzed the life table data on a per-female basis and only those females that lived through the experiment were included in the analysis, we set l_x=1 for all x. We used mean development time of a female's progeny to estimate her own development time. We calculated r in two ways. First, we estimated generation time (T_g) as

$$T_g = \frac{\sum_{x=3}^{12} x l_x m_x}{\sum_{x=3}^{12} l_x m_x}, \tag{A2}$$

and used the estimated T_g and R_0 to determine r (Birch, 1948):

$$r = \frac{\ln R_0}{T_g} \ . \tag{A3}$$

This approximation can underestimate r, so we also used an iterative technique to determine the r that satisfied the equation:

$$\sum_{x=3}^{12} e^{-rx} l_x m_x = 1 \ . \tag{A4}$$

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References

Allen, P. E., Goodrich, L. J. and Bildstein, K. L. (1996). Within- and among-year effects of cold fronts on migrating raptors at Hawk Mountain, Pennsylvania, 1934-1991. *Auk* **113**, 329-338.

Ayrinhac, A., Debat, V., Gibert, P., Kister, A., Legout, H., Moreteau, B., Vergilino, R. and David, J. (2004). Cold adaptation in geographical populations of *Drosophila melanogaster*: phenotypic plasticity is more important than genetic variability. *Funct. Ecol.* **18**, 700-706.

Birch, L. C. (1948). The intrinsic rate of natural increase of an insect population. *J. Anim. Ecol.* **17**, 15-26.

Bochdanovits, Z. and de Jong, G. (2003). Temperature dependence of fitness components in geographical populations of *Drosophila melanogaster*: changing the association between size and fitness. *Biol. J. Linn. Soc. Lond.* **80**, 717-725.

Bosart, L. F., Pagnotti, V. and Lettau, B. (1973). Climatological aspects of eastern United States back-door cold frontal passages. *Mon. Weather Rev.* **101**, 627-635.

Bouletreau-Merle, J., Fouillet, P. and Varaldi, J. (2003). Divergent strategies in low temperature environment for the sibling species *Drosophila melanogaster* and *D simulans*: overwintering in extension border areas of France and comparison with African populations. *Evol. Ecol.* **17**, 523-548.

Bretz, F., Hothorn, T. and Westfall, P. (2004). *multcomp: Multiple Tests and Simultaneous Confidence Intervals*. R package version 0.4-8.

Bubliy, O. A. and Loeschcke, V. (2001). High stressful temperature and genetic variation of five quantitative traits in *Drosophila melanogaster*. *Genetica* **110**, 79-85.

Cohet, Y. and David, J. (1978). Control of adult reproductive potential by pre-imaginal thermal conditions in *Drosophila melanogaster*. *Oecologia* **36**, 295-306.

Cossins, A. and Bowler, K. (1987). *Temperature Biology of Animals*. New York: Chapman & Hall.

Cowles, R. B. and Bogert, C. M. (1944). A preliminary study of the thermal requirements of desert reptiles. *Bull. Am. Mus. Nat. Hist.* **83**, 261-295.

David, J. and Clavel, M. (1967). Influence de la température d'élevage sur la mortalité larvo-nymphale et la durée de développement de la *Drosophile*. *Nat. Can.* **94**, 209-219.

David, J. and Clavel, M. (1969). Influence de la température sur le nombre, le pourcentage d'éclosion et la taille des oeufs fondus par *Drosophila melanogaster*. *Ann. Soc. Entomol. Fr.* **5**, 161-177.

David, J., Allemand, R., Van Herrewege, J. and Cohet, Y. (1983).

Ecophysiology: abiotic factors. In *The Genetics and Biology of Drosophila*. Vol. 3d, pp. 106-169. London: Academic Press.

David, J. R., Gibert, P., Moreteau, B., Gilchrist, G. W. and Huey, R. B. (2003). The fly that came in from the cold: geographic variation of recovery time from low-temperature exposure in *Drosophila subobscura*. *Funct. Ecol.* **17**, 425-430.

David, J. R., Araripe, L. O., Chakir, M., Legout, H., Lemos, B., Petavy, G., Rohmer, C., Joly, D. and Moreteau, B. (2005). Male sterility at extreme temperatures: a significant but neglected phenomenon for understanding *Drosophila* climatic adaptations. *J. Evol. Biol.* **18**, 838-846.

Fernandez, J. and Lopez-Fanjul, C. (1997). Spontaneous mutational genotype-environment interaction for fitness-related traits in *Drosophila melanogaster*. *Evolution* **51**, 856-864.

Gibert, P., Moreteau, B., Petavy, G., Karan, D. and David, J. R. (2001). Chill-coma tolerance, a major climatic adaptation among *Drosophila* species. *Evolution* **55**, 1063-1068.

Gilchrist, G. W. and Huey, R. B. (2001). Parental and developmental temperature effects on the thermal dependence of fitness in *Drosophila melanogaster*. *Evolution* **55**, 209-214.

Harrell, F. E., Jr (2006). *Hmisc: Harrell Miscellaneous*. R package version 3.1-1.

Helms, J. and Munzel, U. (2006). *npmc: Nonparametric Multiple Comparisons*. R package version 1.0-3.

Hercus, M. J., Loeschcke, V. and Rattan, S. I. S. (2003). Lifespan extension of *Drosophila melanogaster* through hormesis by repeated mild heat stress. *Biogerontology* **4**, 149-156.

Hoffmann, A. A., Sorensen, J. G. and Loeschcke, V. (2003). Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. *J. Thermal Biol.* **28**, 175-216.

Huey, R. B. and Stevenson, R. D. (1979). Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *Am. Zool.* **19**, 357-366.

Huey, R. B., Wakefield, T., Crill, W. D. and Gilchrist, G. W. (1995). Within- and between-generation effects of temperature on early fecundity of *Drosophila melanogaster*. *Heredity* **74**, 216-223.

Huey, R., Hertz, P. and Sinervo, B. (2003). Behavioral drive versus behavioral inertia in evolution: a null model approach. *Am. Nat.* **161**, 357-366.

Kingsolver, J. G. (2000). Feeding, growth, and the thermal environment of cabbage white caterpillars, *Pieris rapae* l. *Physiol. Biochem. Zool.* **73**, 621-628.

Krebs, R. A. and Loeschcke, V. (1994a). Effects of exposure to short-term heat-stress on fitness components in *Drosophila melanogaster*. *J. Evol. Biol.* **7**, 39-49.

Krebs, R. A. and Loeschcke, V. (1994b). Response to environmental change: genetic variation and fitness in *Drosophila buzzatii* following temperature stress. *EXS* **68**, 309-321.

Lee, R. E., Chen, C. P. and Denlinger, D. L. (1987). A rapid cold-hardening process in insects. *Science* **238**, 1415-1417.

Levins, R. (1968). *Evolution in Changing Environments*. Princeton, NJ: Princeton University Press.

Maynard Smith, J. (1958). Prolongation of the life of *Drosophila subobscura* by a brief exposure of adults to a high temperature. *Nature* **181**, 496-497.

McCabe, J. and Partridge, L. (1997). An interaction between environmental temperature and genetic variation for body size for the fitness of adult female *Drosophila melanogaster*. *Evolution* **51**, 1164-1174.

McKenzie, J. (1975). The influence of low temperature on survival and reproduction in populations of *Drosophila melanogaster*. *Aust. J. Zool.* **23**, 237-247.

Mitrovski, P. and Hoffmann, A. A. (2001). Postponed reproduction as an adaptation to winter conditions in *Drosophila melanogaster*: evidence for clinal variation under semi-natural conditions. *Proc. R. Soc. Lond. B. Biol. Sci.* **268**, 2163-2168.

Mueller, L. (1985). The evolutionary ecology of *Drosophila*. *Evol. Biol.* **19**, 37-98.

Murrell, P. (2006). *gridBase: Integration of Base and Grid Graphics*. R package version 0.4-3.

Nunney, L. and Cheung, W. (1997). The effect of temperature on body size and fecundity in female *Drosophila melanogaster*: evidence for adaptive plasticity. *Evolution* **51**, 1529-1535.

Obrien, R. G. and Kaiser, M. K. (1985). Manova method for analyzing repeated measures designs: an extensive primer. *Psychol. Bull.* **97**, 316-333.

Pinheiro, J., Bates, D., DebRoy, S. and Sarkar, D. (2006). *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-73.

Quintana, A. and Prevosti, A. (1990). Genetic and environmental factors in the resistance of *Drosophila subobscura* adults to high temperature shock. 2. Modification of heat resistance by indirect selection. *Theor. Appl. Genet.* **80**, 847-851.

R Development Core Team (2006). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Robb, T. and Forbes, M. (2006). Age-dependent induction of immunity and subsequent survival costs in males and females of a temperate damselfly. *BMC Ecol.* **6**, 15.

Rohmer, C., David, J. R., Moreteau, B. and Joly, D. (2004). Heat induced male sterility in *Drosophila melanogaster*: adaptive genetic variations among geographic populations and role of the Y chromosome. *J. Exp. Biol.* **207**, 2735-2743.

Ruckstuhl, A., Unternährer, T. and Locher, R. (2006). *IDPmisc: Utilities of Institute of Data Analyses and Process Design*. R package version 0.7.5. (www.idp.zhwin.ch).

Schmidt, P. S. and Conde, D. R. (2006). Environmental heterogeneity and the maintenance of genetic variation for reproductive diapause in *Drosophila melanogaster*. *Evolution* **60**, 1602-1611.

Schnebel, E. M. and Grossfield, J. (1986). Oviposition temperature range in 4 *Drosophila* species triads from different ecological backgrounds. *Am. Midl. Nat.* **116**, 25-35.

Siddiqui, W. and Barlow, C. (1972). Population growth of *Drosophila melanogaster* (Diptera: Drosophilidae) at constant and alternating temperatures. *Ann. Entomol. Soc. Am.* **65**, 993-1001.

Sisodia, S. and Singh, B. N. (2006). Effect of exposure to short-term heat stress on survival and fecundity in *Drosophila ananassae*. *Can. J. Zool.* **84**, 895-899.

Stevenson, R. D. (1985). The relative importance of behavioral and physiological adjustments controlling body temperature in terrestrial ectotherms. *Am. Nat.* **126**, 362-386.

Trotta, V., Calboli, F. C. F., Ziosi, M., Guerra, D., Pezzoli, M. C., David, J. R. and Cavicchi, S. (2006). Thermal plasticity in *Drosophila melanogaster*: a comparison of geographic populations. *BMC Evol. Biol.* **6**, 67.

Warnes, G. R. (2006). *gregmisc: Backword Compatibility Package for Gregmisc Bundle*. R package version 2.0.8.

Zani, P., Cohnstaedt, L., Corbin, D., Bradshaw, W. and Holzapfel, C. (2005a). Reproductive value in a complex life cycle: heat tolerance of the pitcher-plant mosquito, *Wyeomyia smithii*. *J. Evol. Biol.* **18**, 101-105.

Zani, P. A., Swanson, S. E. T., Corbin, D., Cohnstaedt, L. W., Agotsch, M. D., Bradshaw, W. E. and Holzapfel, C. M. (2005b). Geographic variation in tolerance of transient thermal stress in the mosquito *Wyeomyia smithiii*. *Ecology* **86**, 1206-1211.