



A test of the thermal coadaptation hypothesis with ultimate measures of fitness in flour beetles

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ABSTRACT

Whole-organism performance of ectotherms depends on body temperature, which is tightly linked to environmental temperatures. Individuals attempting to optimize fitness must thus select appropriate temperatures. The thermal coadaptation hypothesis posits that T_o for traits closely linked to fitness should match temperatures selected by a species (T_{set}) and should coevolve with T_{set} . T_o may mismatch T_{set} if the thermal reaction norm for fitness is asymmetric. In this study, we examined six traits related to fitness in red and in confused flour beetles (*Tribolium castaneum* and *T. confusum*, respectively), including longevity, lifetime reproductive success, reproductive rate, and development time at four temperatures between 23 and 32 °C. For reproductive traits, T_o matched T_{set} whereas for longevity T_o was lower than T_{set} . *Tribolium* species have a strongly r-selected life history strategy, therefore reproductive traits are likely more tightly linked to fitness than longevity due to high predation rates at early life stages. We therefore provide support for the thermal coadaptation hypothesis for reproductive traits that are tightly linked to fitness. Our results highlight the importance of knowing the relationships of traits to fitness when studying thermal physiology.

1. Introduction

Ectotherms, by definition, rely largely on environmental temperatures to regulate body temperature (T_b). The body temperature of ectotherms is thus variable due to spatial and temporal variations in environmental temperatures. Variations in body temperature have large impacts on fitness (Angilletta, 2009; Angilletta et al., 2002a, 2002b; Huey and Kingsolver, 1989); fitness increases as body temperature increases from the critical thermal minimum (CT_{min} ; see Table 1 for a list of abbreviations) to the optimal temperature (T_o), after which fitness decreases sharply as temperature increases from T_o to the critical thermal maximum (CT_{max}) (Angilletta, 2006; Bulté and Blouin-Demers, 2006). The relationship between performance and temperature is described by thermal reaction norms (TRNs; also known as thermal performance curves; Angilletta, 2009). Performances that are commonly measured as proximate measures of fitness include righting time (Ben-Ezra et al., 2008; Halliday and Blouin-Demers, 2015), sprint speed (Angilletta et al., 2002a; Bauwens et al., 1995; Ben-Ezra et al., 2008; Halliday and Blouin-Demers, 2015; Row and Blouin-Demers, 2006; Stevenson et al., 1985), jumping distance (Beuchat et al., 1984; Knowles and Weigl, 1990), tongue flicking rate (Blouin-Demers et al., 2003; Stevenson et al., 1985), and digestion rate (Angilletta et al., 2002a; Dorcas et al., 1997; Halliday and Blouin-Demers, 2016; Lelièvre

et al., 2010; Stevenson et al., 1985).

The thermal coadaptation hypothesis posits that T_o for different TRNs related to fitness should coevolve with thermal preference (T_{set}) so that fitness is maximized at temperatures most commonly experienced in the wild (Angilletta, 2009; Bennett, 1980; Hertz et al., 1983; Huey and Bennett, 1987; Huey and Kingsolver, 1989). Therefore, TRNs that affect fitness should have similar T_o values and these should correspond with T_{set} . Martin and Huey (2008) extended the thermal coadaptation hypothesis to account for the asymmetry of TRNs. Ectotherms are generally imprecise thermoregulators, and given that many TRNs are strongly asymmetric, with a fast decrease in fitness over a small change in temperature when T_b rises over T_o , T_{set} should be centred over the portion of the TRN that changes more slowly. Ectotherms with strongly asymmetrical TRNs should therefore have a greater mismatch between T_o and T_{set} than ectotherms with more symmetrical TRNs (Martin and Huey, 2008).

Most tests of the thermal coadaptation hypothesis thus far have focused on performance measures that may only be weakly related to fitness (reviewed in Halliday and Blouin-Demers, 2015, and Martin and Huey, 2008), such as righting time (Ben-Ezra et al., 2008; Halliday and Blouin-Demers, 2015) and sprint speed (Angilletta et al., 2002a; Bauwens et al., 1995; Ben-Ezra et al., 2008; Blouin-Demers et al., 2003; Halliday and Blouin-Demers, 2015; Stevenson et al., 1985). Only two

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Table 1

List of abbreviations used.

Term	Abbreviation
Body temperature	T_b
Optimal temperature for performance	T_o
Preferred body temperature range	T_{set}
Temperature range at 80% of maximum performance	B_{80}
Critical thermal maximum	CT_{max}
Critical thermal minimum	CT_{min}
Thermal reaction norm	TRN

tests of the coadaptation hypothesis have focused on more ultimate measures of fitness (Anderson et al., 2011; Halliday and Blouin-Demers, 2015). Anderson et al. (2011) compared TRNs for lifetime reproductive success and intrinsic growth rate to T_{set} in a nematode (*Caenorhabditis elegans*), whereas Halliday and Blouin-Demers (2015) compared egg laying rate to T_{set} in two species of flour beetle (*Tribolium castaneum* and *T. confusum*). These tests with ultimate measures of fitness provided mixed support for the thermal coadaptation hypothesis. Anderson et al. (2011) found that one strain of nematode had a T_{set} that matched with T_o for lifetime reproductive success, whereas the other strain had a T_{set} that matched with T_o for intrinsic growth rate. Each strain had other TRNs who's T_o did not match T_{set} . Halliday and Blouin-Demers (2015) found that both species of flour beetle had a T_{set} that matched T_o for egg laying rate, but the match between T_o and T_{set} was much stronger for *T. castaneum* than for *T. confusum*, and *T. castaneum* also showed good matches between other TRNs and T_{set} , whereas *T. confusum* did not. While both studies had at least one fitness measure that supported the coadaptation hypothesis, they also both included other fitness measures that did not support the coadaptation hypothesis. Given that only two studies have tested the thermal coadaptation hypothesis with ultimate measures of fitness, and that both of these studies provide mixed support, further work is needed to test the thermal coadaptation hypothesis.

Halliday and Blouin-Demers (2015) measured egg laying rate, righting time, and sprint speed of 20 individuals of each sex for *T. castaneum* and *T. confusum* at eight temperatures between 17 and 38 °C as proxies of fitness. Here, we present an extension of our previous study by measuring fitness directly: we measured traits related to reproduction (lifetime reproductive success, reproductive rate), longevity, and development (development time, development range) at four temperatures. We used the same flour beetle system with a different experimental design to test explicitly the thermal coadaptation hypothesis, and we compared T_o for ultimate measures of fitness to T_{set} . We specifically predicted that T_{set} should overlap with T_o only for ultimate measures of fitness. We also took Martin and Huey's (2008) extension of the thermal coadaptation hypothesis into account, and we predicted that asymmetrical TRNs should exhibit more mismatch between T_o and T_{set} than symmetrical TRNs. *Tribolium* beetles have been studied in the laboratory since the 1920s (Chapman, 1924). The effects of temperature on reproduction (Howe, 1962; King and Dawson, 1973; White, 1987) and longevity (Soliman and Lints, 1982) have been measured, and their temperature preference has also been documented (Langer and Young, 1976). We build upon these previous studies and try to uncover the link between thermal preference and optimal temperature for fitness.

2. Methods

We started our population of *T. castaneum* from 200 individuals obtained from Carolina Biological Supply Company (Burlington, North Carolina, USA) in November 2012, and our population of *T. confusum* the same way in November 2013. We let the populations grow in an *ad lib* mixture of 95% all purpose flour and 5% brewer's yeast (henceforth referred to as flour) with an ambient temperature of 30 °C and a 12:12 h

light:dark cycle to approximately 5000 individuals.

In April 2015, we collected pupa from the culture of each species, identified the sex of each pupae based on the presence or absence of ovipositors (Good, 1936), and allowed both sexes of each species to eclose in separate containers filled with flour. One week following eclosion, we marked each beetle with a dab of non-toxic acrylic paint (colour specific to sex and species) and put one male and one female (both randomly selected) of a species together in a petri dish (75 × 10 mm) with 2.5 ml of flour which had been pre-sifted through a 250 µm sieve to aid in egg collection. We then randomly assigned each breeding pair of each species to a temperature treatment (23, 26, 29, or 32 °C). We set up ten replicates of each temperature treatment for each species, for a total of 80 breeding pairs. We created temperature treatments by setting an environmental chamber to 23 °C, and placing heat tape under the petri dishes to heat them to the appropriate temperature. We monitored the temperature of all dishes periodically, and maintained each dish at set temperature ± 1 °C. Once per week, we sifted the flour and transferred all eggs to a new petri dish with 2.5 ml of flour. This petri dish contained all offspring of each breeding pair and was maintained at the same temperature as the parents. We then replenished the flour in the petri dish of the breeding pair. We repeated this process every week until either both adults had died or the experiment had run for 24 weeks. If the male of a breeding pair died first, we replaced it with a new male to allow the female to continue reproducing, but we only used the age of death of the first male for longevity. We monitored the offspring petri dishes weekly, replenished the flour, and removed new adults as they eclosed to decrease the effects of egg cannibalism.

We calculated six variables related to fitness: total number of adult offspring eclosed (lifetime reproductive success), reproductive rate (total number of adult offspring/number of weeks until the last adult offspring eclosed), number of weeks before the first adult offspring eclosed (development time), number of weeks over which adult offspring eclosed (development range), longevity of males, and longevity of females. We assigned a longevity value of 25 (length of experiment plus one week) to any beetle that did not die during the experiment (13 *T. castaneum*, 19 *T. confusum*). We transformed both of the traits related to development (development time and development range) because they both fit a U-shaped curve, but typical TRNs are inverted Us (they peak at T_o). We transformed these variables by subtracting each value from 24 to make the curve fit the shape of typical TRNs. We used non-linear models in R (package: stats; function: nls; R Core Team, 2016) to fit three curves (quadratic, cubic, and quartic) to each variable for each species. We also fit our data to other non-linear curves like the curve described by Ratkowsky et al. (1983), but the polynomial curves fit our data much better ($\Delta AIC > 100$). For each curve, we included values of zero at 40 °C to represent CT_{max} because beetles die at 40 °C (Halliday et al., 2015). For the TRNs for longevity, we also included values of zero at 5 °C to represent CT_{min} because it is similarly the temperature at which beetles die (W.D. Halliday, *Unpublished results*). For all other fitness measures, we did not include a value for CT_{min} because data for the 23 °C treatment were already at or near zero. These estimates of CT_{max} and CT_{min} were both calculated using beetles that were acclimated to 30 °C, and both estimates represent lethal temperatures after multiple days of exposure; beetles can survive short-term exposure to colder temperatures (Scharf et al., 2016) and short-term exposure to 40 °C (W.D. Halliday, *Personal observation*). The values for CT_{max} and CT_{min} would also likely change with different acclimation temperatures and with different criteria (e.g., lack of righting response instead of death). For the purpose of curve-fitting, however, these values for CT_{max} and CT_{min} had little effect on our estimates of T_o .

We compared the fit of each of the three curves using Akaike's information criteria (package: stats; function: AIC) and considered the curve with the lowest AIC to be the best curve describing the TRN for that variable. If curves were within 2 AIC units of each other, we selected the more parsimonious model (fewest parameters) as the best

model. We then calculated the 95% confidence interval (CI) around each curve (package: propagate; function: predictNLS; Spiess, 2014); this statistical package uses both Taylor expansion and Monte Carlo simulation to estimate CIs around a curve (Spiess, 2014). We calculated the optimal temperature (T_o) and the temperatures at which 80% of the maximum performance could be achieved (B_{80} ; a measure of thermal specialization) for the mean curve of all individuals. We calculated a 95% CI around T_o based on the temperatures at which the 95% CI curve was equal to or greater than the maximal performance measure for the mean curve. Similarly, we calculated a 95% CI around B_{80} based on the temperature at which the 95% CI curve was equal to or greater than 80% of the maximum performance measure for the mean curve.

We compared the 95% CIs around each curve to the T_{set} for each species in a thermal gradient as measured by Halliday and Blouin-Demers (2015), which is 27.5–32.5 °C for *T. castaneum* and 24.1–29.7 °C for *T. confusum*. These values for T_{set} were confirmed in another study (Halliday and Blouin-Demers, 2014).

Finally, to examine patterns in reproductive senescence, we analyzed the number of adult offspring that eclosed per week for the duration of the experiment for females that survived the experiment, excluding individuals that produced no offspring (4 *T. castaneum*, 13 *T. confusum*). We used a linear mixed effects model in R (package: nlme; function: lme; Pinheiro et al., 2016) with the number of offspring as the dependent variable and the number of weeks into the experiment as the independent variable. We included species, temperature, and total offspring per female as control variables. We included the identity of the female beetle as a random effect to control for repeated measures. We compared models with different polynomial terms (linear, quadratic, cubic, and quartic) to account for curvilinear trends and used AIC to select the best model.

3. Results

Sample size for all TRNs was 10 per temperature treatment (N = 40 per species). Total adult offspring, reproductive rate, and development range were highly correlated ($r > 0.8$), but all other variables were not highly correlated ($r < 0.6$).

Male longevity TRNs for both species were fit best by quartic curves, whereas female longevity TRNs for both species were fit best by cubic curves. For both species, longevity TRNs for both sexes had optima below the temperatures measured in our experiment because the highest values for longevity were at our lowest temperature treatment (Tables 2 and 3, Figs. 2 and 3). Thus, T_o and B_{80} for longevity TRNs do not overlap with any values for the other traits (Table 3).

All reproductive traits (lifetime reproductive success, reproductive rate) and development traits (development time, development range)

Table 2

Curve selection for thermal reaction norms examining total offspring, reproductive rate, range of time that offspring were produced, time when first offspring emerged as adult, male longevity, and female longevity for red flour beetles (*Tribolium castaneum*) and confused flour beetles (*Tribolium confusum*). We compared three curves (quadratic, cubic, and quartic) for each reaction norm. The upper value in each cell is the Akaike's information criterion (AIC) value, and the lower value is the difference in AIC between that curve and the curve with the lowest AIC. Bolded cells are considered the best curve for each trait.

Model	<i>Tribolium castaneum</i>			<i>Tribolium confusum</i>		
	Quadratic	Cubic	Quartic	Quadratic	Cubic	Quartic
Total Offspring	– 701.8 30.4	– 706.2 26.0	– 732.2 0.0	– 684.0 43.8	– 689.8 38.0	– 727.8 0.0
Reproductive rate	– 1064.4 26.3	– 1071.6 19.1	– 1090.7 0.0	– 1053.9 37.6	– 1059.6 31.9	– 1091.5 0.0
Offspring range	– 988.1 32.6	– 987.4 33.3	– 1020.7 0.0	– 999.4 26.5	– 1003.8 22.1	– 1025.9 0.0
First offspring	– 946.9 12.8	– 948.4 11.3	– 959.7 0.0	– 963.2 8.9	– 966.6 5.5	– 972.1 0.0
Male longevity	– 2748.6 46.3	– 2783.5 11.4	– 2794.9 0.0	– 2765.9 13.7	– 2776.3 3.6	– 2779.6 0.0
Female longevity	– 2744.9 10.0	– 2754.9 0.0	– 2753.2 1.7	– 2736.1 24.1	– 2860.2 0.0	– 2758.2 2.0

Table 3

Characteristics of thermal reaction norms for six measures of fitness in red flour beetles (*Tribolium castaneum*) and confused flour beetles (*Tribolium confusum*). Under T_o , the first value is the median T_o , and the range beside this is the 95% confidence interval for T_o . T_{set} of *T. castaneum* in a thermal gradient is 27.5–32.5 °C, and T_{set} for *T. confusum* is 24.1–29.7 °C (Halliday and Blouin-Demers, 2015).

Reaction Norm	T_o	Lower B_{80}	Upper B_{80}
<i>Tribolium castaneum</i>			
Total offspring	26.4, 24.8–28.2	24.2–24.8	28.3–29.1
Growth rate	26.7, 24.8–28.7	24.2–24.9	28.7–29.7
Offspring range	26.2, 24.7–28.0	24.0–24.6	28.1–28.9
First offspring	26.9, 24.8–29.4	24.1–24.9	29.3–31.1
Male longevity	< 23	< 23	< 23
Female longevity	< 23	< 23	< 23
<i>Tribolium confusum</i>			
Total offspring	26.4, 24.9–28.0	24.3–24.8	28.3–29.0
Growth rate	26.6, 25.0–28.3	24.3–24.9	28.6–29.4
Offspring range	26.4, 24.7–28.3	23.9–24.5	28.6–29.5
First offspring	27.3, 24.9–30.5	24.0–24.9	30.5–40.0
Male longevity	< 23	< 23	< 23
Female longevity	< 23	< 23	< 23

for both species were fit best by quartic curves (Table 2). Optima for all reproductive traits and for development range for both species, as well as for development time for *T. castaneum*, were around 26 °C, but the optima for development time for *T. confusum* was at 27.3 °C (Table 3, Figs. 1 and 2). The 95% CIs around all reproductive and development traits for both species overlapped (Table 2). B_{80} for all reproductive traits and development range for both species, as well as development time for *T. castaneum*, were approximately 24–30 °C, but the B_{80} for development time for *T. confusum* was 24–40 °C (Table 3).

T_{set} overlapped with the 95% CI around T_o for all reproductive and development traits for both *T. castaneum* and *T. confusum* (Table 3, Figs. 1 and 2). For *T. confusum*, T_{set} was centred on mean T_o for all reproductive and development traits (Fig. 2). Both *T. castaneum* and *T. confusum* therefore matched the thermal coadaptation hypothesis ($T_o = T_{set}$) for reproductive and development traits, but not for longevity.

The number of offspring born per week was best explained by a model with a quadratic term for the number of weeks into the experiment. Species and temperature did not explain significant variance, but unsurprisingly beetles that had more total offspring tended to have more offspring born per week (slope = 0.04 ± 0.003 , $t_{404} = 11.63$, $p < 0.0001$). The number of beetles eclosed per week started increasing at 6 weeks, peaked around 15 weeks, and decreased until the end of the experiment (Fig. 3).

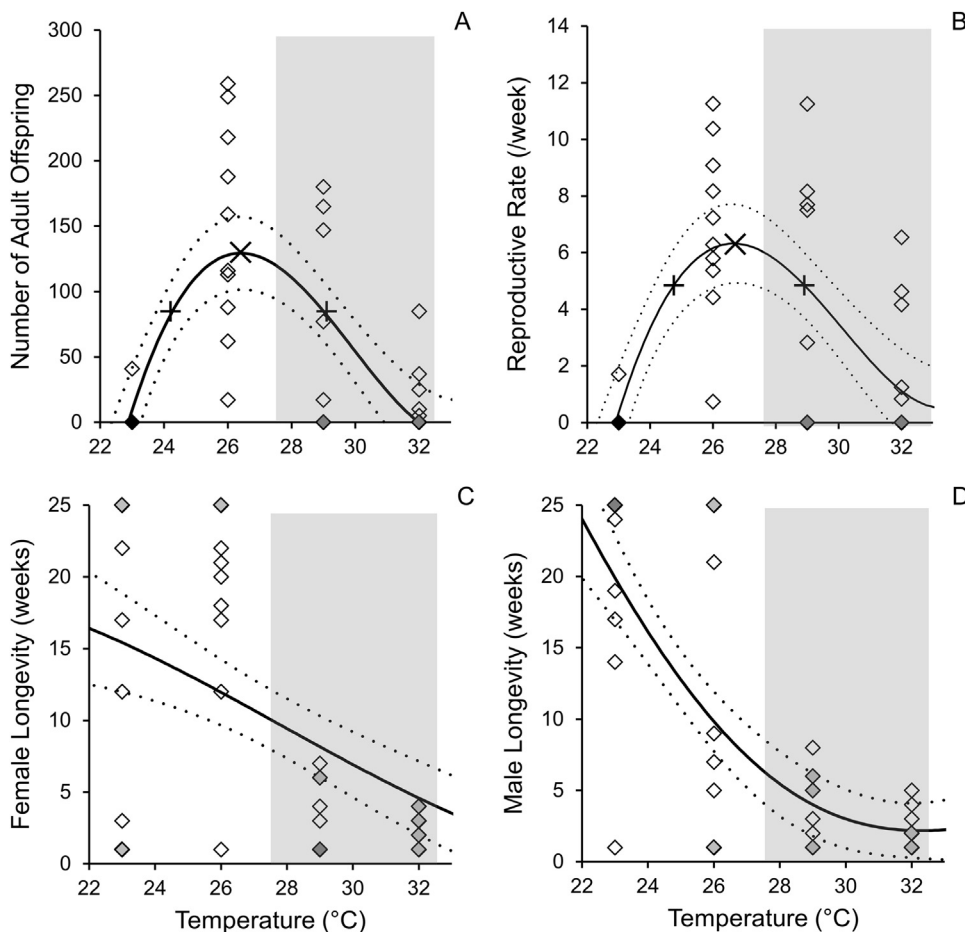


Fig. 1. Thermal reaction norms for red flour beetles (*Tribolium castaneum*) for four measures of fitness: total number of offspring (A), reproductive rate (B), female longevity (C), and male longevity (D). $n = 10$ per temperature treatment. Diamonds represent raw data, the solid curve represents the mean trend, and the dashed curve on either side of the mean curve represents the 95% confidence interval around the mean curve. Points with a solid fill represent multiple points overlapping: light grey represents 2–4 points, dark grey represents 5–7 points, black represents 8–10 points. + on the mean curve represents 80% of maximum performance (B_{80}), and × represents the optimal temperature (T_o). The light grey box from 27.5 to 32.5 °C represents T_{set} (data from Halliday and Blouin-Demers, 2015).

4. Discussion

The TRNs for reproductive and development traits of both species supported the thermal coadaptation hypothesis: T_o for all metrics of reproduction and development were within T_{set} . For *T. confusum*, mean T_o for all metrics of reproduction and development were within T_{set} , whereas for *T. castaneum*, the 95% CI around T_o overlapped with T_{set} . While T_o for the traits related to reproduction and development matched T_{set} , T_o for longevity was not close to T_{set} . Unlike all other traits measured for these species, T_o for longevity was much cooler than T_{set} . Despite the different optima for longevity, we still believe that our study provides strong support for the thermal coadaptation hypothesis. The thermal coadaptation hypothesis applies specifically to TRNs that are tightly linked to fitness. *Tribolium* species mature quickly and immediately begin reproducing. Having a match between T_o for reproductive TRNs and T_{set} is therefore logical since so much of *Tribolium* life history is focussed on rapid reproduction. Having a high longevity is not necessarily linked to reproductive output of these species because reproductive senescence occurs. Species that mature and reproduce quickly (like MacArthur and Wilson's (1967) r-selected species) typically have high mortality rates from predators at a young age, thus a long life is unlikely in nature. We demonstrated that reproductive senescence occurred within our 24-week experiment, where peak reproduction was reached around 15 weeks for individuals that survived the experiment. Increased longevity with lower reproductive rates at lower temperatures may be linked to a survival strategy, where colder individuals are simply surviving until more favourable conditions occur, at which point they can reproduce efficiently. More work is needed to test if beetles that live long in cold conditions can then move to warm conditions and still effectively reproduce. Conversely, theory from metabolic ecology predicts that high longevity at low

temperatures is simply a function of low metabolic rate, and that natural selection is acting on measures of fitness related to reproduction, which are maximized at higher metabolic rates (Savage et al., 2004).

Martin and Huey (2008) posited that TRNs that are strongly asymmetrical should have T_o that mismatch T_{set} . While Martin and Huey (2008) specifically predicted that T_o should be higher than T_{set} because TRNs are generally left-skewed, their model predicts that T_{set} should be shifted away from the steepest side of the TRN to avoid the negative fitness consequences associated with imprecise thermoregulation. The TRNs for reproduction and development that we measured in this study all had a steep increase from 23 to 26 °C, followed by a shallow decrease from 26 to 32 °C (Figs. 1 and 2). According to Martin and Huey's (2008) model, T_o should thus be lower than T_{set} . T_{set} for *T. castaneum* was higher than T_o , as predicted; however, T_{set} for *T. confusum* was centred around T_o and, for both species, T_o overlapped statistically with T_{set} . We therefore did not find good support for Martin and Huey's (2008) extension of the thermal coadaptation hypothesis.

Both species of beetles exhibited similar TRNs, including the measures of locomotor performance and egg laying rate from our previous study (Halliday and Blouin-Demers, 2015), yet all measures of reproduction and development from this study had lower T_o than the measures from our previous study, and longevity had an extremely low T_o . In our previous study, egg laying rate was measured over 24 h, whereas the present study lasted 24 weeks. It is possible that long-term exposure to high temperatures can have deleterious effects on fitness, which can lead to a TRN that is shifted towards colder temperatures (Kingsolver and Woods, 2016). According to this hypothesis, TRNs measured over shorter periods should have higher T_o than TRNs measured over longer periods. If this hypothesis is correct, then the actual T_o for fitness in these beetles should be closer to the T_o for egg laying. However, this hypothesis assumes that TRNs are highly left-skewed and

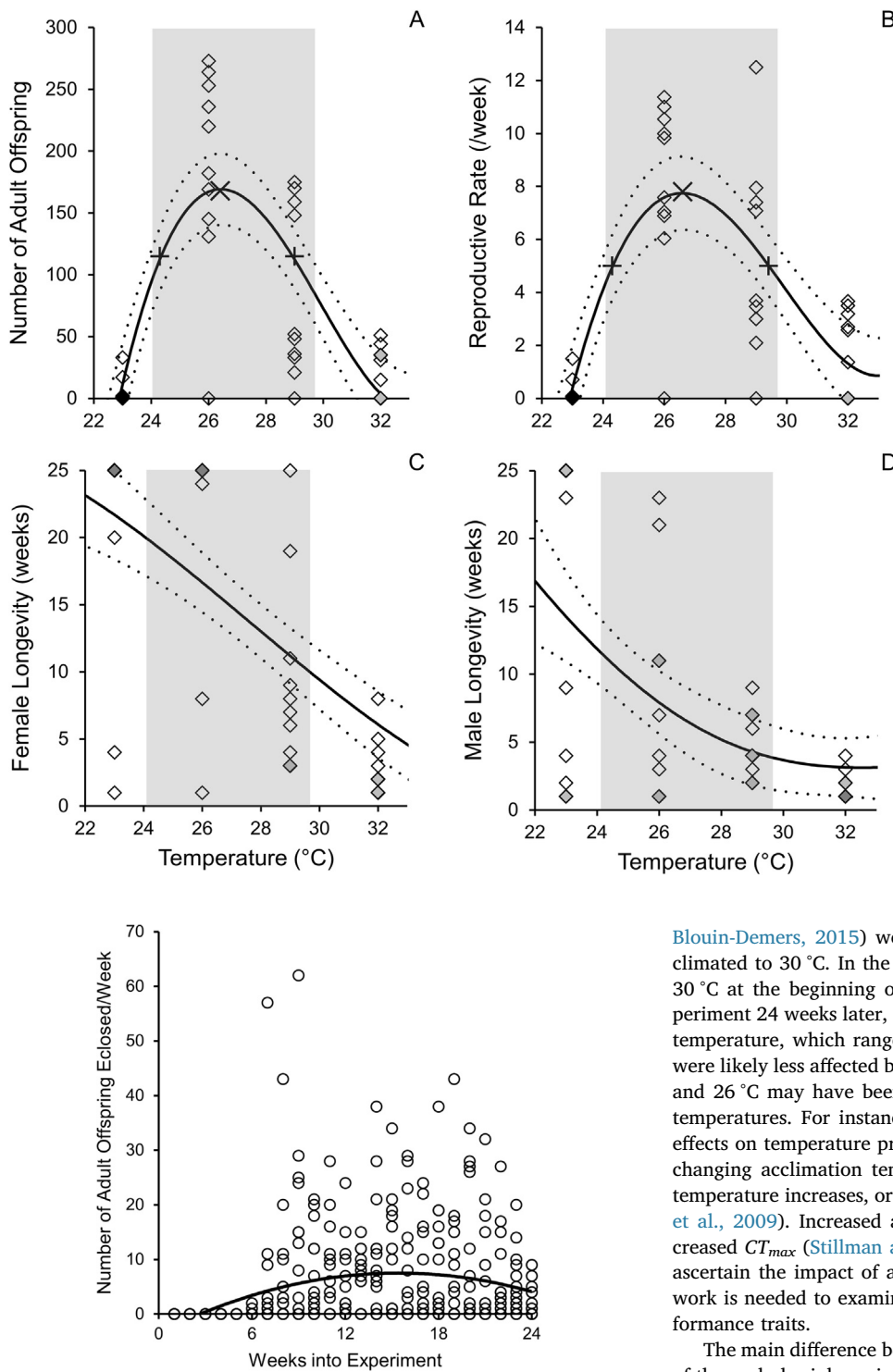


Fig. 2. Thermal reaction norms for confused flour beetles (*Tribolium confusum*) for four measures of fitness: total number of offspring (A), reproductive rate (B), female longevity (C), and male longevity (D). $n = 10$ per temperature treatment. See Fig. 1 for explanation of symbols. The light grey box from 24.1 to 29.7 °C represents T_{set} (data from Halliday and Blouin-Demers, 2015).

Fig. 3. Reproductive senescence for *Tribolium castaneum* and *T. confusum* combined based on the number of adult offspring eclosed per female per week into the experiment.

that T_o is very close to CT_{max} . Yet, T_o for egg laying was 10 °C colder than CT_{max} and the T_o for reproduction and development that we measured in this study were 14 °C colder than CT_{max} . We therefore suggest that the long duration of our study, which included chronic exposure to one temperature, likely did not influence the shape of the TRNs because the temperatures that we used were far from CT_{max} .

Acclimation temperature likely had important implications for our results. Our stocks of beetles were acclimated to 30 °C and all measures of T_{set} , CT_{max} , CT_{min} , and TRNs from our previous study (Halliday and

Blouin-Demers, 2015) were all conducted with beetles that were acclimated to 30 °C. In the present study, beetles were all acclimated to 30 °C at the beginning of the experiment, but by the end of the experiment 24 weeks later, they were likely acclimated to their treatment temperature, which ranged from 23 to 32 °C. Beetles at 29 and 32 °C were likely less affected by this changing temperature, but beetles at 23 and 26 °C may have been more affected by the different acclimation temperatures. For instance, acclimation temperature can have mixed effects on temperature preference, where individuals may not react to changing acclimation temperatures, may increase T_{set} as acclimation temperature increases, or may exhibit a more complex response (Dillon et al., 2009). Increased acclimation temperature can also lead to increased CT_{max} (Stillman and Somero, 2000). It is therefore difficult to ascertain the impact of acclimation temperature on our results. More work is needed to examine the impact of acclimation on TRNs of performance traits.

The main difference between *T. castaneum* and *T. confusum*, in terms of thermal physiology, is their T_{set} . This suggests that the species could have evolved different T_{set} in response to available temperatures in their historical geographic ranges, yet apparently their thermal physiologies did not evolve in a similar fashion. For example, Good (1936) suggested that *T. castaneum* is more common in the warmer parts of the USA, whereas *T. confusum* is more common in cooler parts of the USA, which may reflect their respective thermal preferences. Despite this potential historical difference in biogeography, the stocks that we used were essentially domesticated, as is the case for the majority of laboratory studies on these species. Unlike wild stocks, these domesticated stocks often live in over-crowded conditions at constant temperature. These laboratory living conditions likely have large implications for thermal physiology, and likely mean that our results cannot be extrapolated to

wild populations of either species. Comparative data on laboratory colonies and on wild beetles are needed to document what effects, if any, artificial conditions have had on the thermal physiology of these beetles.

Although all of our reproductive and development TRNs had similar values for T_o , they may not all be linked to ultimate fitness, and a perfect test of the thermal coadaptation hypothesis requires fitness metrics that are closely related to ultimate fitness. The two most commonly used metrics of ultimate fitness are intrinsic rate of increase (r : rate of population increase in a closed population) and net reproductive rate (R_o : number of female offspring born to a female over her lifetime) (Huey and Berrigan, 2001). R_o is the appropriate ultimate fitness measure for stable populations, whereas r is the appropriate ultimate fitness measure for expanding populations. Huey and Berrigan (2001) found that T_o for R_o was usually cooler than T_o for r because generation time (i.e. development time) tends to be shorter at warmer temperatures, which biases T_o for r to warmer temperatures. These two metrics of ultimate fitness are therefore not equivalent and interchangeable based on both demography and on thermal physiology. The beetles that we used for this experiment were in a stable laboratory stock (i.e. at an equilibrium density), therefore R_o should be the most appropriate metric of ultimate fitness. Our TRNs for lifetime reproductive success and reproductive rate should both be strongly related to R_o given that lifetime reproductive success is equal to $R_o \times 2$, assuming a 1:1 sex ratio. Huey and Berrigan (2001) suggested that development time should also be related to r ; however, our metrics of development time both had similar T_o values to lifetime reproductive success and reproductive rate. We therefore expect that our fitness metrics were closely linked to the appropriate metric of ultimate fitness, R_o . Also, given that development time had a similar T_o as our reproductive TRNs, we also expect that r would have had a similar T_o to the reproductive and development traits that we measured.

In conclusion, our study provided support for the thermal coadaptation hypothesis based on ultimate measures of fitness related to reproduction and development. While reproductive and development traits appear coevolved with T_{set} , longevity traits are not related to T_{set} in these *Tribolium* species, which is likely due to their life history.

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