How does ambient temperature impact the fitness of the red flour beetle (*Tribolium castaneum*)?

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v. ABSTRACT

It is well established that temperature plays a critical role in the life of ectotherms. Unable to control their internal temperature physiologically, these organisms often turn to behavioural thermoregulation as a means of maximizing physiological processes and thereby fitness. In this project I examined the impacts of ambient temperature (15, 20, 25, 30, 35, and 40°C) on several proxies of fitness, namely oviposition rate, number of adult offspring, development rate, survival rate, and morphology. This was done through the creation and maintenance of ten populations per temperature treatment which were monitored weekly until their development was complete. Oviposition rates increased between 15 and 30°C and decreased thereafter. The final number of live adults increased between 25 and 35°C as did survivorship. Development times decreased between 25 and 35°C. No morphometric differences were seen between the adults of any treatment. These results both support and contradict previous findings in this field, all of which will be discussed along with potential paths for future research.

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Fig. 2 The final number of adult *Tribolium castaneum* beetles plotted against the ambient temperature to which the populations were exposed. For each temperature n=10 with each population being maintained in a petri dish within an incubator. The food (2.5 ml of 95% flour and 5% brewer's yeast) was refreshed weekly. The line represents a quadratic line of best fit.

Fig. 3 The number of weeks prior to the appearance of the first (a) and last (b) adult *Tribolium castaneum* plotted against the ambient temperature to which the populations were exposed. For each ambient temperature n=10 with each population being maintained in a petri dish within an incubator. The food (2.5 ml of 95% flour and 5% brewer's yeast) was refreshed weekly. The lines represent linear lines of best fit.

Fig. 4 The percentage of *Tribolium castaneum* individuals that survived (egg to adult) plotted against the ambient temperature to which the populations were exposed. For each temperature n=10 with each population being maintained in a petri dish within an incubator. Final survivorship counts were taken after the population in the dish was finished developing. The line represents a quadratic line of best fit.

I. INTRODUDCTION

Temperature plays a critical role in the life of ectotherms, (Hochachka and Somero, 1984; Cossins and Bowler 1987) determining the speed and quality of physiological processes, (John-Alder *et al.*, 1988; Ben-Ezra *et al.*, 2008) impacting critical processes such as locomotion (Stevenson et al., 1985; Blouin-Demers and Weatherhead, 2008), feeding (Stevenson *et al.*, 1985), and somatic growth (Hofmann and Fisher, 2003), and biochemical constraints such as enzyme performance (such as lactate dehydrogenase and phosphofructokinase), cellular physiology (such as membrane viscosity), protein stability, and the acidity of the body (Hochachka and Somero, 2002). In these processes and other ectotherms perform optimally within a specific range of temperatures, while outside of this range ectotherms experience reduced performance; this relationship is typically illustrated using a performance curve (or rate-temperature curve) (Kingsolver and Gomulkiewicz, 2003). To avoid decreases in performance, ectotherms often use behavioural thermoregulation and other physiological processes to regulate their body temperature (Reinert, 1993; Huey and Kingsolver, 1989).

Given its effect on performance, temperature also plays a role in determining the fitness of ectotherms. Metrics of fitness such as growth rate (Angilletta *et al.*, 2004), maturation rate of both juveniles (Forsythe et al., 2001) and eggs (Berger *et al.*, 2008), generation time (Pearl, 1928), and oviposition rates (Berger *et al.,* 2008) in ectotherms have been shown to be impacted by ambient temperature. Ectotherms may also respond to environmental temperature as a way so as to improve or maintain fitness by altering feeding behaviours and oxygen consumption (Brockington and Clarke, 2001). The metabolic impacts

of temperature may also allow for survival under colder conditions (Brockington and Clarke, 2001) and have been shown to influence life histories so as to maximize survival time (Brockington and Clarke, 2001). The impact of temperature on ectotherm fitness is thoroughly confirmed in the existing literature, yet the specifics of these impacts appear to be largely species or range specific.

For my project, I have examined the effects of temperature on the fitness of the red flour beetle (*Tribolium castaneum*). In order to do this I measured how different metrics of fitness, including oviposition rate, development rate, offspring survival rate, and morphology varied over different ambient temperatures. The red flour beetle is a species that has been studied for many years and is ideal for use in experiments examining development and growth (Brown *et al.*, 2009). This is due in part to the ease of their maintenance, high availability, and moderately short life cycle (Park, 1934), traits that also make this ectotherm an ideal model for my project.

II. MATERIALS AND METHODS

All beetles were originally obtained from Carolina Biological Supply Company (Burlington, North Carolina, USA). Prior to my experiment the beetles were maintained in a culture of 95% wheat flour and 5% brewer's yeast at an ambient temperature of 30 °C. This mixture served as both their habitat and food source and was available in excess.

To begin my experiment I placed adult beetles in six ambient temperature conditions: 15, 20, 25, 30, 35, and 40°C. Each condition had a total of 10 replicates which I maintained in petri dishes within incubators set to the appropriate temperatures. Initially, I placed 20 adult beetles (randomly selected from a large culture of beetles that contained approximately

a 1:1 sex ratio) in petri dishes and left them for a period of four days to breed and lay egg.

During this time the beetles lived in 2.5 mL of a mixture of 95% wheat flour and 5%

brewer's yeast that had been pre-sifted through a 250 μm sieve to ensure uniformity and

prevent contamination.

Following this four day oviposition period, I separated eggs from the flour using a

250 μm sieve. I then counted the number of eggs in each dish, returned them to their petri

dishes with fresh 2.5mL of the flour mixture, and placed the egg in the same conditions in

which they were laid. These eggs were then left to mature into full adults with observations

of their progress/condition being made weekly. At a similar time each week I counted the

number of live adults (if present), recorded the general progress of the replicate, and

refreshed the food matrix.

I examined the number of eggs laid and the number of adult offspring (log10-

transformed) in a treatment temperature using a quartic regression and a quadratic regression

respectively performed in R (package: stats; function: lm; R Core Team 2013). I examined

survival (percentage of eggs that survived to adulthood $= \frac{\text{live adults}}{\text{eggs}}$ x 100) in each treatment

using a quadratic model with a quasi-Poisson distribution (package: stats; function: glm;

family: quasipoisson). The distribution of survival data nearly fit a Poisson distribution, but

it was over-dispersed, leading to the use of a quasi-Poisson distribution. Finally, I examined

development time (time to the emergence of the first and last adult offspring) using a general

linear model with a Poisson distribution (package: stats; function: glm; family: poisson). In

all of these regressions I used temperature as an independent variable, and also included a

combination of temperature^2, temperature^3, and temperature^4 as independent variables in

each polynomial model, this combination being determined by its degree.

I measured the morphometrics of ten randomly selected beetles from each replicate (or all of the beetles if the total number of live adults in the replicate was ten or less) by taking a picture of the ventral side of each beetle using a microscope camera (OptixCam OCS-1.3X, The Microscope Store, LLC, Roanoke, VA), paired with a dissecting microscope (Stemi 2000, Zeiss, Oberkochen, Germany). Measurements were derived from these pictures using OCView version 7.2.1.7 (The Microscope Store, LLC, Roanoke, VA). I measured the length of each body segment (head, thorax, and abdomen), and summed these measurements to calculate the total length of the beetle. This total length was then analyzed using a linear mixed effects model in R (package: nlme; function: lme; Pinheiro *et al.* 2014), with total length as the dependent variable, temperature as a fixed effect, and replicate as a random effect. I also verified this method of measurement for accuracy through repeated measurements of the same beetle. To complete this I randomly selected 20 beetles and took ten pictures of each of them. I then measured their total length in each of the ten pictures, calculated the standard deviation between the measurements for each individual, and then compared these standard deviations among individuals.

III. RESULTS

It was found that oviposition rates increased from 15 to 30°C, and decreased from 35 to 40°C ($df = 55$; $F = 43.9$, $R^2 = 0.744$ $p < 0.0001$, Fig 1) (temperature: coefficient = 8.80×10^1, $t = .712$, $p > 0.1$; temperature^2: coefficient = -5.66, $t = -0.781$, $p > 0.1$; temperature^3: coefficient = 1.77×10^{-1}, $t = 0.974$, $p > 0.1$; temperature^4: coefficient = -2.05×10^{-3}, $t = -1.24$, $p > 0.1$).

The final number of adult beetles (number observed after replicate had fully developed) increased with ambient temperature between 25-35°C ($df = 27$; $F = 22.2$, $R^2 = 0.594$, p < 0.0001, Fig 2) (temperature: coefficient = -1.03, $t = -3.74$, $p < 0.001$; temperature^2: coefficient = 0.0183, $t = 3.99$, $p < 0.001$). No adult offspring developed from any of the other temperature treatments, although low numbers of small larvae were seen in some replicates after two weeks of development at 15 and 20°C. These larvae, however, did not develop any further and these replicates along with those of the 40°C treatment were terminated after five weeks.

Development time decreased linearly with temperature from 25 to 35°C for both the time to the first adult offspring ($df = 28$; $z = 7.49$, $p < 0.0005$, Fig 3 a) and the time to the last adult offspring ($df = 28$; $z = -2.13$, $p < 0.05$, Fig 3 b).

Survivorship increased with temperature ($df = 27$, Fig 4) (temperature: coefficient = -2.16, $t = -3.02$, $p < 0.01$; temperature^2: coefficient = 0.0383, $t = 3.25$, $p < 0.01$) across the range of 25-35°C. Beyond or below this temperature range no adults were seen and so survivorship = 0%.

The total length of beetles did not differ between temperature treatments ($df = 221$; $t = -0.215$, $p > 0.5$). Morphometric measurements were repeatable and had very low error (mean standard deviation ± S.D. = 0.18 mm ± 0.037 mm).

IV. DISCUSSION

Temperature impacts proxies of fitness in ectotherms. Many studies looking at ectothermic species and *Tribolium castaneum* in particular confirm this statement, although the specifics of these impacts vary between studies. By examining my results in relation to

past work in this field we are able to evaluate the methods used and determine where this study sits in the spectrum of results.

In this study I found that oviposition rates, development rates, and survival rates of flour beetles are significantly impacted by the ambient temperature of their environment. While survival, oviposition rates, and development rate increased between the 25 and 35°C treatments, only the latter was the same for the upper two temperatures. In all cases at temperatures above 35 or below 25°C fitness decreased dramatically, indicating that the processes involved in all of these proxies of fitness are severely hindered or arrested outside of this temperature range. It is also important to note that during the four day breeding period all of the adult beetles in the 40°C treatment were killed, implying that this temperature in particular is especially damaging to this species in terms of adult beetle survival and possibly other developmental stages. This was also the only temperature treatment that showed zero development of eggs.

IV. i Oviposition Rates

In this study I found that oviposition rates of red flour beetles are significantly impacted by the ambient temperature of their environment. The findings of this for this proxy of fitness are consistent with evidence from previous studies (Park and Frank, 1948; White, 1987). An optimal temperature of 35°C is also consistent with previous studies (White, 1987). A similar increase in oviposition rates was also seen in a study by Stanley in 1932, although this was limited to a temperature range of 22 to 32°C.

Error may have been introduced into this aspect of the study through the timing of the placement of the parent generation in the petri dishes. That is to say, when parent generation

females were placed in the dishes, some may have been about to lay eggs while others may have just finished laying eggs. This could have resulted in oviposition rate variation that could be attributed to the individual and not the ambient temperature. This potential error can likely be disregarded due to the adequate number of replicates. It is, however, possible that the egg laying ability of each female may have differed as studies on similar species have shown that although oviposition rates are consistent throughout the lifetime of a female, they are dissimilar between individuals (Park, 1934). Again, this variation was likely similar throughout the populations and may be ignored due to sufficiently large sample sizes.

IV. ii Development Rates

It has been suggested that development time may be one of the best indicators of the thermal sensitivity of fitness (Kozlowski, 1993). The optimal range of development indicated by this study (between 30 and 35°C) is partially inconsistent with past research (Howe, 1956) which showed that 35°C allows for faster development than 30°C. Other studies mimic the results of Howe's 1956 study (Park and Frank, 1948), suggesting that the present study may not be as accurate as those done in the past. It is possible that the method by which development was monitored (weekly observation) did not allow for sufficiently precise measurements of development rate and that if the treatments had been checked more frequently (daily, as done in Howe, 1956) a difference in the development times may have been observed between the 30 and 35°C treatments.

As previously mentioned, temperatures that did not show significant development after five weeks were terminated – this occurred in the 15, 20, and 40°C treatments. In this case "significant development" qualifies as development beyond early larval stages, which are characterized by a white colour and small size (<6.0 mm) (Park, 1934). During normal

development this stage is typically passed after as few as 15 days (Park, 1934) with the entire life cycle (egg to adult) being around 30 days (Park, 1934). In previous studies with similar species when larvae were left to develop in lower and higher temperatures (17.5 and 37.5°C) indefinitely they still failed to pupate (Howe, 1962). This suggests that if left for longer, the 20°C treatments may have managed to pupate and produce live adults, but it is unlikely that the 15 and 40°C treatments would have ever developed further.

IV.iii Survivorship

The survivorship data collected from this study is largely consistent with existing literature, with many studies concluding that the optimal temperature for the red flour beetle is around 35°C (Howe, 1956; White, 1987). Others, however, have found it to be closer to 30°C (King and Dawson, 1973).

Although it has been suggested that red flour beetles may be able to withstand temperatures of 40°C or even higher for short periods of time (Johnson *et al*., 2004), as previously mentioned, most studies agree that the fitness of the red flour beetle decreases quickly at temperatures above 35°C. Howe (1956) found that between temperatures of 15-40^{0}C, only the 40^{0}C treatments showed larval mortality rates of above 20%, and the eggs did not hatch below 17.5^{0}C or at 40^{0}C. This suggests that survival rates may be differentially impacted by temperature depending on which developmental stage the beetles are in and could imply that there may be different optimal temperatures for the different developmental stages in terms of overall survival. This could be investigated in future research.

IV. iv Morphometrics

Temperature-size relationships have puzzled biologists for many years (Angilletta *et al.*, 2004). Although many theories have been proposed, none are universally accepted (Angilletta *et al.,* 2004). This may be due to the highly variable results obtained from studies in this area. In this study no morphological differences in the adult beetles were seen between any of the treatment temperatures. This may indicate that body size is not impacted by ambient temperature, though may also indicate that one generation does not provide sufficient time to see any morphological impact temperature may have. Other studies that have attempted to manipulate the morphology of the beetles over multiple generations through artificial selection have failed (Tigreros and Lewis, 2011), suggesting that in this species, either morphology may be controlled by a variety of factors or that there is not a significant amount of morphological variance within the natural population.

Some studies on ectotherms have found that adult size may vary inversely depending on factors like growth rate (Angilletta *et al*., 2004). Others have concluded that for all ectotherms higher ambient temperatures lead to smaller adults (Van Der Have and De Jong, 1996); both of these conclusions are inconsistent with this study. It is also possible that an aspect of morphology that was not measured (such as carapace thickness) could have demonstrated a relationship with ambient temperature, an aspect that might be examined in future studies.

IV. v Future Work

As of right now there is no universally accepted robust methodology for measuring thermal dependents of fitness (Huey and Berrigan, 2001). The methods used in this study suggest that some of the different proxies of fitness are optimized at different ambient

temperatures around 30 and 35^{0}C. These findings are largely consistent with the existing literature, which indicates that this study was effective and its conclusions reliable. Although less prominent, there are past studies that contradict the findings of this paper, which could indicate errors in methodology in this study or in those performed in the past. Research investigating these methods and the methods used to measure thermal dependents of fitness, as well as how effective each of these methods is for demonstrating these fitness effects will be important in the future and moving forward in the field of thermal ecology.

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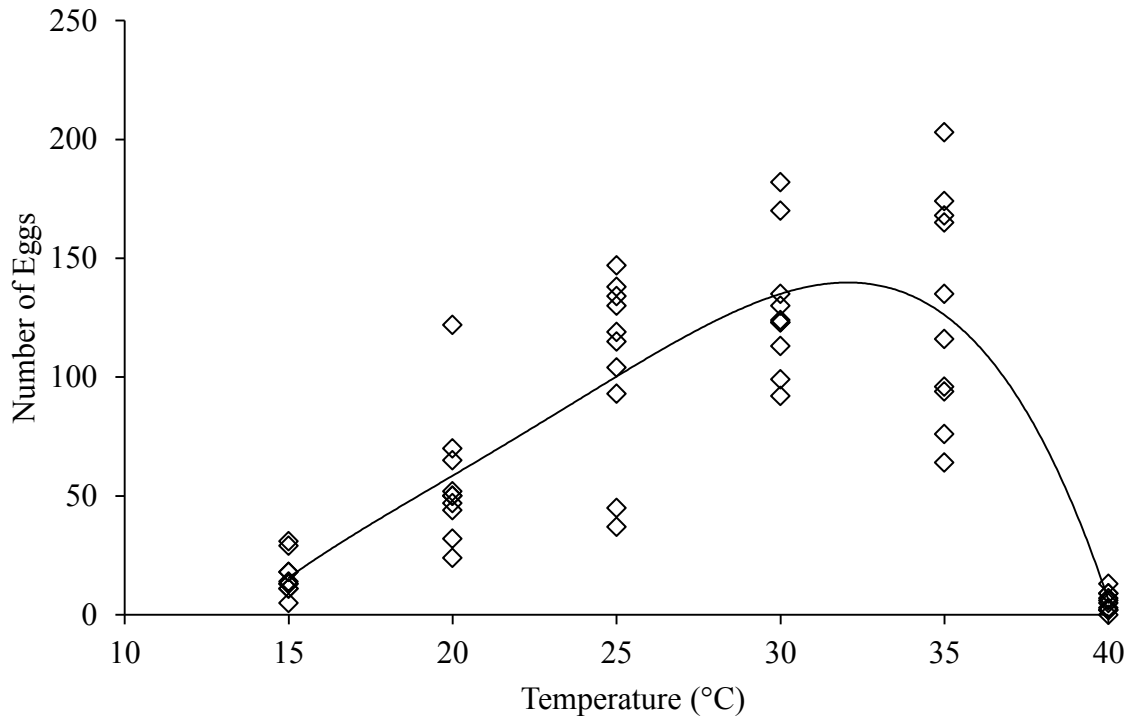
VI. Appendix
<u>VI. i Figure 1</u>

Fig. 1 The number of eggs laid by 20 adult *Tribolium castaneum* during a four day breeding period plotted against the ambient temperature to which the population was exposed. N=10 for each temperature treatment with each population being maintained in a petri dish within an incubator with 2.5 mL of food (95% flour and 5% brewer's yeast). The line represents a quartic line of best fit.
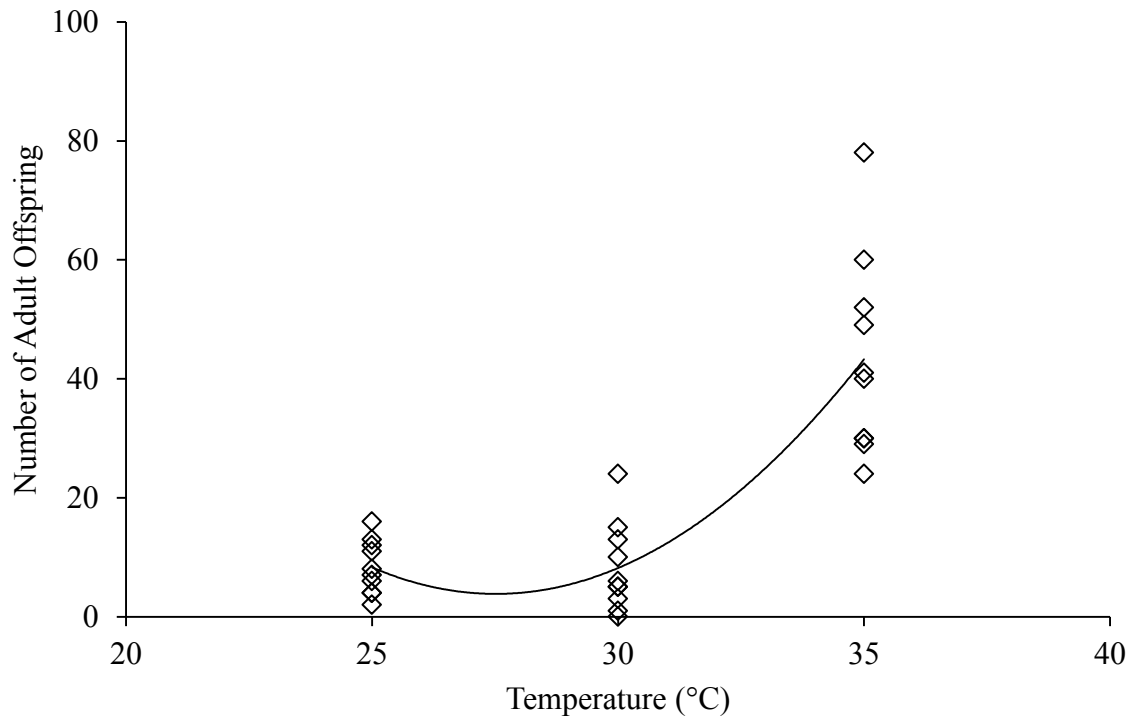
VI. ii Figure 2

Fig. 2 The final number of adult *Tribolium castaneum* beetles plotted against the ambient temperature to which the populations were exposed. For each temperature n=10 with each population being maintained in a petri dish within an incubator. The food (2.5 ml of 95% flour and 5% brewer's yeast) was refreshed weekly. The line represents a quadratic line of best fit.
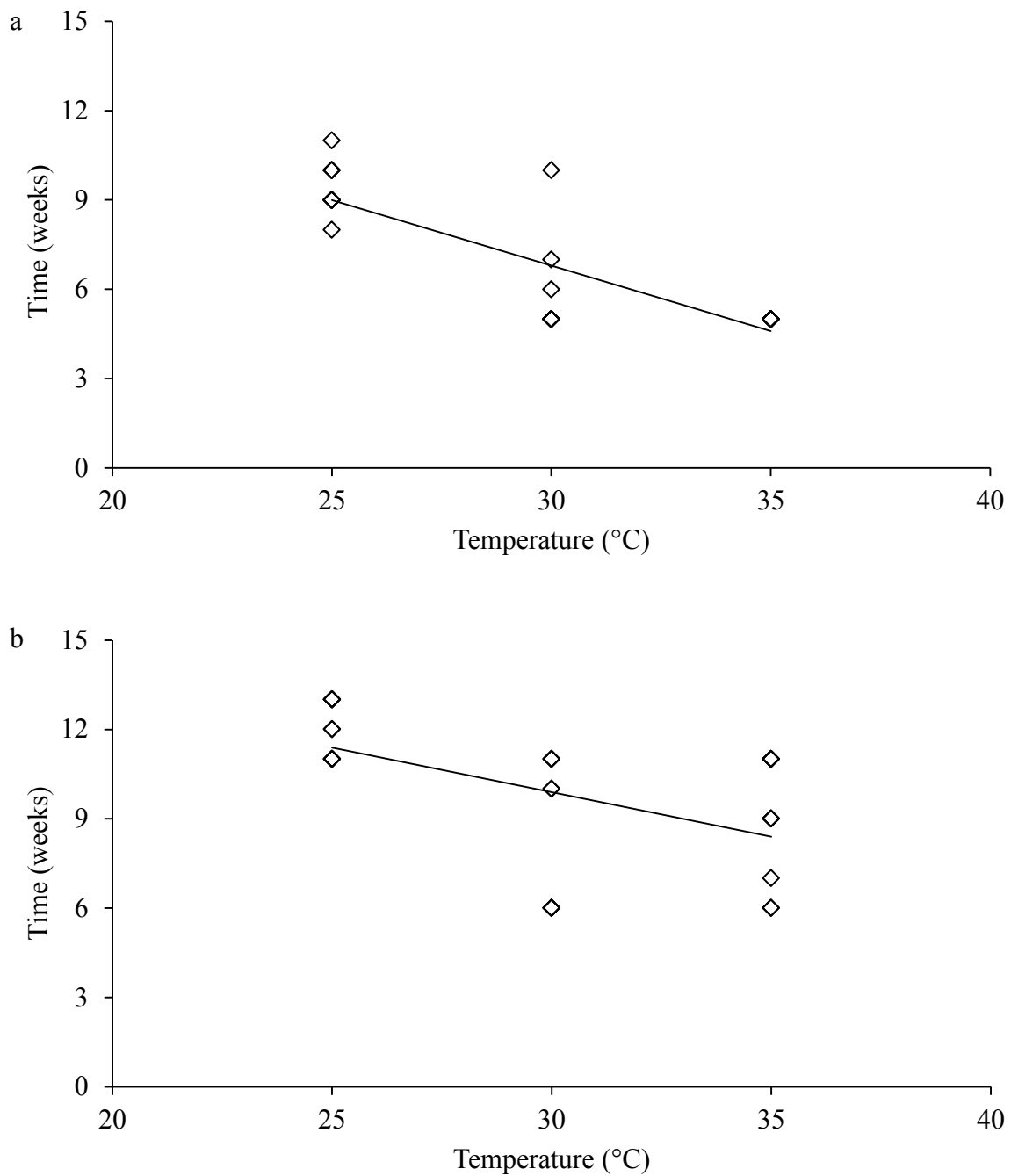
VI. iii Figure 3

Fig. 3 The number of weeks prior to the appearance of the first (a) and last (b) adult *Tribolium castaneum* plotted against the ambient temperature to which the populations were exposed. For each ambient temperature n=10 with each population being maintained in a petri dish within an incubator. The food (2.5 ml of 95% flour and 5% brewer's yeast) was refreshed weekly. The lines represent linear lines of best fit.
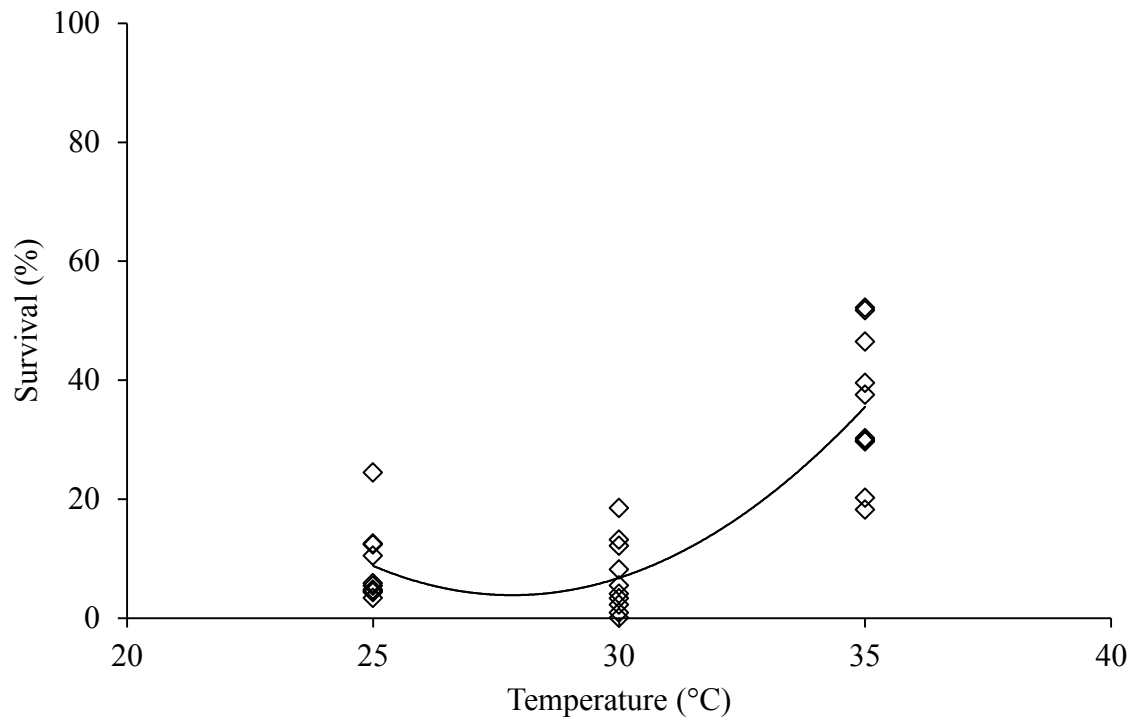
<u>VI. iv Figure 4</u>

Fig. 4 The percentage of *Tribolium castaneum* individuals that survived (egg to adult) plotted against the ambient temperature to which the populations were exposed. For each temperature n=10 with each population being maintained in a petri dish within an incubator. Final survivorship counts were taken after the population in the dish was finished developing. The line represents a quadratic line of best fit.