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RESEARCH ARTICLE

Some like it hot: the effects of climate change on reproduction, immune function and disease resistance in the cricket *Gryllus texensis*

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SUMMARY

In many parts of the world, climate change is increasing the frequency and severity of heat waves. How do heat waves impact short-lived poikilotherms such as insects? In the cricket, *Gryllus texensis*, 6 days of elevated temperatures (i.e. 7°C above the average field temperature and 5°C above their preferred temperature) resulted in increased egg laying, faster egg development and greater mass gain. The increased temperature also increased activity of phenoloxidase and lysozyme-like enzymes, two immune-related enzymes, and enhanced resistance to the Gram-negative bacterium *Serratia marcescens*. When given a sublethal *S. marcescens* infection, *G. texensis* maintained increased reproductive output at the elevated temperature (33°C). These data suggest that heat waves could result in more numerous, disease resistant, crickets. However, resistance to the Gram-positive bacterium, *Bacillus cereus* was lower at temperatures above or below the average field temperature (26°C). A sublethal infection with *B. cereus* reduced egg laying at all temperatures and suppressed the increase in egg laying induced by higher temperatures. These results suggest that for some species–pathogen interactions, increased temperatures can induce trade-offs between reproduction and disease resistance. This result may partly explain why *G. texensis* prefers temperatures lower than those that produce maximal reproductive output and enhanced immune function.

Key words: global warming, insect, Orthoptera, phenoloxidase, oviposition.

INTRODUCTION

Global warming is producing increases in both average temperatures and in the frequency and severity of heat waves (Stone et al., 2010). The magnitude of these changes varies across the globe. For example, central Texas, USA, has not experienced any significant warming over the 20th century; however, temperatures are expected to rise gradually during this century (Nielsen-Gammon, 2011). Nevertheless, Austin, TX, has experienced an increase in the frequency and severity of heat waves over the last 50 years (Stone et al., 2010), and for this region, the increase in heat waves remains the largest present-day impact of global warming. For poikilotherms such as insects, ambient temperature profoundly affects the rate of biochemical reactions critical to their physiology (Chown and Nicolson, 2004). For short-lived animals such as insects, even one extended heat wave may have significant impacts. Unfortunately, the effects of heat waves on insect biology and behaviour are hard to predict. Increased temperature should increase the rate of enzymatic reactions within individuals (Angilletta et al., 2010); however, the effect of temperature on enzymatic pathways does not necessarily predict what will happen to the whole organism (Chaui-Berlinck et al., 2004). Nonetheless, it is the effect of warmer temperatures on whole organisms that will have important ramifications for ecosystems.

Within physiological limits, increased enzymatic rates in insects should lead to higher metabolic rates, more rapid growth and increased reproduction (Angilletta et al., 2010). Such effects have been demonstrated for a number of insect species, usually under laboratory conditions (Frazier et al., 2006). In many temperate-zone insects, the optimal temperature (i.e. temperature that generates the greatest number of offspring) is higher than the environmental temperature (Frazier et al., 2006; Deutsch et al., 2008). For some temperate-zone insects, temperatures remain below the optimal temperature for population growth, even in summer (Deutsch et al., 2008). In part this is because insect enzymes often show their highest activity at temperatures 10 or 20°C above the environmental temperature [e.g. phenoloxidase in *Heliothis virescens* (Lockey and Ourth, 1992) and *Musca domestica* (Hara et al., 1993)]. These data suggest that many temperate-zone insects should show enhanced performance, including increased reproduction, at higher temperatures.

Although insects lack the complex neural and physiological mechanisms required to maintain a constant body temperature against changes in external temperature, some insects can control their internal temperature behaviourally (Chown and Nicolson, 2004). For example, crickets thermoregulate by moving to microenvironments that allow them to maintain their preferred body temperature (Adamo, 1998). Behavioural thermoregulation can produce body temperatures considerably above ambient temperature (Chown and Nicolson, 2004). Given the expected benefits of elevated temperatures to insect reproduction, and presumably fitness (Deutsch et al., 2008), it might be expected that most temperate-zone insects would have temperature preferences far above their typical environmental temperature. However, many insects that behaviourally thermoregulate prefer temperatures not too far above the environmental temperature [e.g. crickets (Louis et al., 1986; Adamo, 1998)]. This puzzling issue has not been specifically addressed.

One possible reason why many temperate-zone insects do not prefer extremely warm temperatures, despite the thermodynamic advantage they provide, is that warmer temperatures induce physiological trade-offs that may decrease an individual's fitness

(Angiletta et al., 2003). For example, reproduction is an extremely expensive activity in terms of time, energy and resources for female insects (Harshman and Zera, 2007). Enhanced reproduction may take energy and resources away from other metabolically expensive systems [e.g. immune function (Freitak et al., 2003)]. Many studies have shown that reproduction occurs at the expense of immune function and disease resistance, even when food is abundant (Harshman and Zera, 2007; Lawniczak et al., 2007). Therefore, at higher temperatures, insects may increase their rate of reproduction, but may become more susceptible to disease as a result, leading to reduced lifespan and possibly reduced fitness in the field.

Trade-offs between immune function and reproduction could be offset by the positive effects of elevated temperatures on insect immune systems. Individual enzymatic reactions should become faster, leading to an enhanced immune response. Empirical evidence suggests that warmer temperatures do increase a variety of immune responses (e.g. Ouedraogo et al., 2002; Ouedraogo et al., 2003). However, other studies demonstrate that warmer temperatures lead to a decline in some immune functions [e.g. melanisation (Suwanchaichanda and Paskewitz, 1998)]. Determining how increased temperature will influence reproduction and how this will influence immune function and disease resistance requires empirical study. Few studies have examined these interactions (Guinnee and Moore, 2004), yet they may play a crucial role in the response of an insect to climate change. If both immune function and reproduction are simultaneously enhanced, then, at least for some species, climate change will result in the production of more insects, and these insects will be more disease resistant. If higher temperatures induce or exacerbate trade-offs between reproduction and immune function, then this might be a partial explanation as to why species appear to prefer 'suboptimal' temperatures.

The Texan cricket *Gryllus texensis* (Cade and Otte, 2000) is a good model organism for studying this question. Temperature is known to influence cricket physiology [e.g. metabolic rate is highly correlated with temperature (Nespolo et al., 2003)] and behaviour (e.g. Martin et al., 2000). Moreover, crickets behaviourally thermoregulate [*G. texensis* (Adamo, 1998), *Gryllus integer* (Hedrick et al., 2002), *Gryllus bimaculatus* (Vanwyk and Ferguson, 1995)]. *Gryllus texensis* can be maintained at different temperatures, and female reproductive output is easy to quantify (e.g. Shoemaker et al., 2006a; Shoemaker et al., 2006b). It has been used for eco-immunological studies, and some immune functions can be quantified (e.g. Adamo, 2004a). There is also evidence that immune system activation is costly in this cricket (Shoemaker and Adamo, 2007). Immune challenge usually reduces female reproduction in insects (Reaney and Knell, 2010 and references therein), however, under normal temperatures, there is little evidence for trade-offs between reproduction and immune function in female *G. texensis* (Shoemaker et al., 2006a; Shoemaker et al., 2006b; Shoemaker and Adamo, 2007). Therefore, it is possible to use this species to test whether such trade-offs are induced at higher temperatures.

In this study, we examined whether raised temperatures, mimicking a typical heat wave, increase reproduction in *G. texensis*. We then determined whether increased temperature also enhances immune function as well as disease resistance to two different pathogens. Finally, we examined the effects of infection on reproduction at different temperatures. These experiments will help us to determine whether increased temperatures are likely to result in trade-offs between immune function and reproduction, even if temperature enhances each trait individually. Such trade-offs will influence how this species responds to climate change.

MATERIALS AND METHODS

Animals

Crickets (long-winged *G. texensis*) were collected near Austin, TX, USA, and have been maintained as a laboratory colony for many generations with occasional additions of fresh animals from the field. Crickets were reared at 26±3°C on a 12 h:12 h light:dark cycle. This temperature represents the average temperature crickets experience during their adult life (National Weather Service Climate Report; http://www.weather.gov/climate/index.php?wfo=ewx). *G. texensis* become sexually mature in Austin, Texas in the spring (May) or fall (September–October) (Adamo et al., 1995; Bertram, 2002).

Pellets of dry cat food and water were provided *ad libitum*. Crickets were used 2 weeks (±3 days) after the moult to adulthood. At this age female *G. texensis* have mated (Solymar and Cade, 1990) and are within their lifespan in the field (Murray and Cade, 1995). It is also well before the immune system begins to decline as a result of senescence (~4 weeks) (Adamo et al., 2001). No cricket was used in more than one experiment. All experiments included at least three independent replicates spanning multiple generations within the insect colony.

All studies were approved by the Animal Care Committee of Dalhousie University (#I9-026) and are in accordance with the Canadian Council on Animal Care.

Chemicals were obtained from Sigma Chemical Co. (St Louis, MO, USA) unless otherwise noted.

Temperature preference of *G. texensis*

Crickets were placed individually in opaque tubs (10×10 cm; diameter × depth) for 1 day prior to trials. A temperature gradient was created by placing one end of a glass aquarium (26×48×28 cm) onto a hot plate. The glass aquarium was divided into five lanes, each 5 cm wide. Crickets were unable to see their neighbours through the metal section dividers. The floor of the aquarium was covered with 2 cm of sand. Once the aquarium temperature had reached equilibrium (~3 h), one cricket was placed in the centre of each lane, males and females in alternate lanes. The temperature ranged from 26°C at the cool end to 38°C at the warm end. Crickets were given 30 min to acclimatize to the aquarium, and then the temperature at their position was measured with an electronic thermometer at 30 and 60 min and the average of the two temperatures was calculated. A high proportion of the crickets (38/45, 84%) were at the same temperature for both time points. An earlier study (Adamo, 1998) showed that these time points give an accurate estimate of an individual's temperature preference. A cricket's internal body temperature is identical to the external temperature when crickets are in a temperature gradient (Louis et al., 1986). The aquarium was rotated between trials so that the heated end of the aquarium was changed each trial. The sand was also mixed thoroughly between trials.

Effects of temperature on reproduction

Females were weighed and isolated in containers (15×17×9.5 cm) with food and water *ad libitum*. They were assigned to one of three temperature groups: 18, 26 or 33°C for 6 days, which is 28% of an adult's lifespan (Murray and Cade, 1995). Heat wave temperatures were represented by the 33°C treatment, as experienced in Austin, TX (National Weather Service; http://www.weather.gov/climate/index.php?wfo=ewx; e.g. 14–24 July 2010, average daily temperature, 33°C). A cold snap during the breeding season (e.g. 9–12 October 2009) was represented by the 18°C treatment. Average temperatures during the times *G. texensis* are actively seeking mates (morning and evening) in the field are typically in the range of 17–23°C (Souroukis et al., 1992).

Females were placed in one of two incubators at 18 or 33°C, with a 12 h:12 h light:dark cycle, or left in the rearing room at 26°C. An oxygen monitor placed in the incubators demonstrated that oxygen levels remained normal throughout the incubation period. After 6 days, crickets were removed and the number of eggs laid was counted and females were re-weighed. A random subset of eggs (five eggs from each female) was set aside. These eggs were returned to their previous temperature regime to determine hatching success. Eggs were left in cotton and buried in vermiculite. Hatchlings were collected and counted every day for 50 days.

Eight eggs randomly chosen from eight females per temperature were tested for their total protein content using a Bradford total protein assay (Bradford, 1976). Eggs were sonicated in distilled water and the supernatant was added to the Bradford reagent. The change in absorbance at 595 nm was measured 10 min later and compared with values obtained using a protein standard (bovine albumen) run on the same day.

Effect of temperature on immune function

Females were isolated as previously described. After 6 days at one of the three temperatures (18, 26 or 33°C), 2 µl of haemolymph was removed to assess either phenoloxidase activity or lysozyme-like activity at different temperatures.

Phenoloxidase activity was measured using a method modified from that of Bidochka et al. (Bidochka et al., 1989). Haemolymph (2 µl) was removed through the membrane below the pronotum using a chilled Hamilton syringe. The haemolymph was added to 50 µl of phosphate-buffered saline (PBS) and immediately vortexed for 5 s. The PBS contained bovine pancreas α-chymotrypsin (2 mg 1.5 ml^{-1} PBS), and was left to incubate for 20 min at room temperature. The mixture was then transferred to a cuvette containing 0.9 ml of 0.02 $mol\,l^{-1}$ L-DOPA and the change in absorbance was measured for 60 min at 490 nm (Novaspec II spectrophotometer; Pharmacia, Peapack, NJ, USA). During this 60 min period, the cuvettes were maintained at the cricket's incubation temperature (i.e. 18, 26 or 33°C).

Lysozyme-like enzymatic activity was estimated using a turbidity assay (Adamo, 2004a). Haemolymph (2 µl) was removed from crickets after 6 days at 18, 26 or 33°C and added to 100 µl of PBS and vortexed. The haemolymph–PBS mixture was added to 0.9 ml of a *Micrococcus luteus* cell wall suspension (12.5 mg 30 ml^{-1} double distilled water). The change in absorbance at 450 nm was measured over 45 min. Lysozyme standards were run daily at room temperature and were used to normalize results across days. During the assay, cuvettes were maintained at the crickets' incubation temperature.

Effect of temperature on disease resistance

Insect immunity has many different components (see Beckage, 2008), and temperature may not have a positive effect on all of them. Moreover, the effect of temperature on a pathogen is difficult to predict. Therefore, whether disease resistance increases or decreases with temperature will be at least partly dependent on the pathogen. To examine this issue, we tested the disease resistance of *G. texensis* to two different classes of pathogens, a Gram-negative bacterium (*Serratia marcescens*, MicroKwik culture, Carolina Biological Supply Co., Burlington, NC, USA) and a Gram-positive bacterium (*Bacillus cereus*, MicroKwik culture, Carolina Biological Supply Co.). Both are natural pathogens of insects (Krieg et al., 1987).

To test the effect of temperature on pathogen growth, 1 ml of nutrient broth (Oxoid, Nepean, ON, Canada) was added to 100 µl of *S. marcescens* (~1×10^5 cells μl^{-1}) in a 1.5 ml cuvette. Absorption was measured at 600 nm. Cuvettes were incubated at 18, 26 and 33°C and the absorption was read prior to incubation and at 6, 12 and 24 h. Similarly, 1 ml of nutrient broth was added to 100 µl of *B. cereus* (~1×10^5 cells μl^{-1}) in a 1.5 ml cuvette. Absorption was measured at 600 nm. Cuvettes were incubated at 18, 26 and 33°C and the absorption was read prior to incubation and at 8, 18 and 24 h.

To test resistance, females were isolated and assigned to the three different temperature regimes as previously described. After 6 days, females were injected with the LD_{50} dose for one of the two bacteria [*S. marcescens*, ~1×10^4 cells (Adamo et al., 2010); *B. cereus* ~6×0^4 cells (S.A.A., unpublished data)]. Mortality was recorded for the next 6 days. At room temperature, mortality as a result of these pathogens occurs within 4 days with *S. marcescens* (Adamo et al., 2001) or 5 days with *B. cereus* (S.A.A., unpublished data).

Effect of infection on egg laying

Females were isolated and assigned to one of the three temperature regimes as described above. Crickets were then injected with an LD_{01} dose (i.e. only 1 cricket in 100 kept at 26°C was expected to die from the treatment) of either *S. marcescens* or *B. cereus*, or left uninjected as controls. After 4 days (*S. marcescens*) or 5 days (*B. cereus*), the number of eggs laid by surviving crickets was counted.

Statistics

Data were tested for normality. Nonparametric data were analyzed following the method of Meddis (Meddis, 1984). In cases where multiple tests were performed on the same data set the alpha criterion was adjusted accordingly (i.e. *post hoc* tests). Data are given as means ± 1 standard deviation unless otherwise noted.

RESULTS

Temperature preference of *G. texensis*

There was no significant difference between males and females in their temperature preference (*t*-test: t=0.044, d.f.=43, P=0.97; 22 males, 23 females). Crickets preferred 28.1±1.1°C (N=45), a significantly warmer temperature than the rearing temperature of 26°C (one-sample *t*-test: t_{44}=13, P<0.001, N=45).

Effect of temperature on reproduction

The number of eggs laid increased with increasing temperature (Fig. 1; one-way ANOVA, $F_{2,105}$=14.5, P<0.0001; *post hoc* test for linear trend, r^2=0.22, P<0.0001, N=35 crickets per temperature, except 33°C, N=36). There was no significant difference in the amount of total protein per egg across temperatures (one-way ANOVA, $F_{2,23}$=2.34, P=0.12, N=8 eggs per temperature). The average total protein was 114.3±14.1 µg protein per egg. Hatching success (% hatching: 18°C, 2%; 26°C, 78%; 33°C, 72%; N=135 eggs per temperature) was lower at 18°C (G_2=195, P<0.0001), but was not significantly different between 26 and 33°C (*post hoc G*-test=0.84, P=0.36). The unhatched eggs from the 18°C group showed no signs of development, suggesting that these eggs were not viable (Shoemaker and Adamo, 2007). Development time decreased with increasing temperature [mean incubation period: 18°C, 46.3±1.5 days; 2°C, 16.8±1.3 days; 33°C, 8.0±1.5 days; nonparametric test for linear trend (Meddis, 1984), Z=11.8, P<0.0001].

Female mass gain over the 6 days increased with increasing temperature (Fig. 2; one-way ANOVA, $F_{2,104}$=3.99, P=0.02; *post hoc* test for linear trend, r^2=0.17, P=0.006, N=35 crickets per temperature).

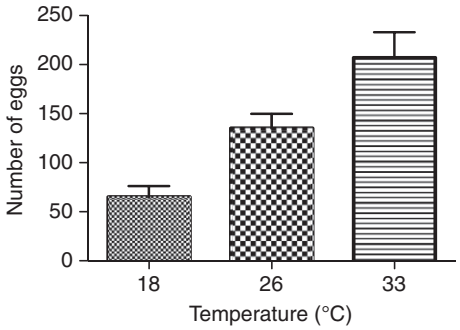

Fig. 1. The effect of temperature on egg-laying of *Gryllus texensis*. Bars represent means and error bars denote the standard error of the mean (s.e.m.). *N*=35 crickets for 18 and 26°C; *N*=36 crickets for 33°C.

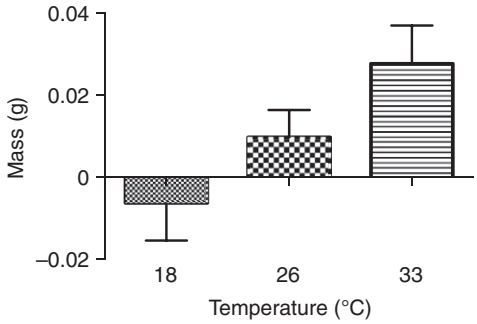

Fig. 2. The effect of temperature on *G. texensis* body mass. Negative values indicate mass loss. Bars represent means and error bars denote s.e.m. *N*=35 crickets per temperature.

Effect of temperature on immune function

Phenoloxidase activity increased with increasing temperature (Fig. 3A; one-way ANOVA, $F_{2,35}$=9.6, P=0.0005; *post hoc* test for linear trend, r^2=0.37, P=0.0001, N=12 samples per temperature).

Lysozyme-like activity increased with increasing temperature (Fig. 3B; one-way ANOVA, $F_{2,41}$=25.6, P<0.0001; *post hoc* test for linear trend, r^2=0.57, P<0.0001, N=14 samples per temperature).

Effect of temperature on disease resistance

S. marcescens growth rate increased with temperature (Fig. 4A; one-way ANOVA, $F_{2,11}$=132.7, P<0.0001; *post hoc* test for linear trend, r^2=0.93, P<0.0001, N=4 samples per temperature). Temperature significantly affected resistance to *S. marcescens* (Fig. 5A; G_2=17.02, P<0.0002, N=51 crickets per temperature). Crickets survived best at 33°C, followed by 26°C with the worst survival at 18°C (*post hoc* test for trends, Z=2.9, P=0.004).

B. cereus growth rate increased with temperature (Fig. 4B; one-way ANOVA, $F_{2,11}$=757.7, P<0.0001; *post hoc* test for linear trend, r^2=0.97, P<0.0001, N=4 samples per temperature). Temperature significantly affected resistance to *B. cereus*. Crickets at 26°C had the highest survival (Fig. 5B; G_2=8.102, P=0.02, N=90 crickets per temperature). There was no significant difference in the number of survivors between 18 and 33°C (*post hoc* test, G=1.67, P=0.20).

For both infections, the colder the temperature, the longer crickets lived before dying of the infection [*S. marcescens*, test for linear trend of frequency data, Z=3.6, P<0.001; *B. cereus*, test for linear trend of frequency data, Z=2.7, P=0.003 (Meddis, 1984)].

Effect of infection on egg laying

Infection with *S. marcescens* had no significant effect on the number of eggs laid by surviving females over 4 days post-infection (Fig. 6A; two-way ANOVA, $F_{1,74}$=0.05, P=0.82). Both infected (18°C, N=17; 26°C, N=15; 33°C, N=18) and non-infected (N=10 per temperature) females laid more eggs at higher temperatures ($F_{2,74}$=27.2, P<0.0001), no significant interaction ($F_{2,74}$=1.2, P=0.31). Infection with *B. cereus* decreased the number of eggs laid over a 5-day period compared with uninfected controls, regardless of temperature (Fig. 6B; two-way ANOVA, $F_{1,188}$=11.9, P=0.0007) and temperature also affected egg laying in infected animals ($F_{2,188}$=21.4, P<0.0001). There was no significant interaction between egg laying and temperature, regardless of infection status ($F_{2,188}$=1.16, P=0.23). Infected crickets at 33°C laid more eggs than at 18°C (Bonferroni *post hoc* test, t=3.8, P<0.001), but did not lay significantly more eggs than females at 26°C (Bonferroni *post hoc* test, t=0.18, P>0.05), in contrast to the healthy females at these temperatures.

DISCUSSION

G. texensis females increased egg laying during the simulated heat wave (Fig. 1). There was no evidence that the eggs laid at 33°C were of inferior quality; they had the same protein content and hatching rate as those laid at 26°C (rearing temperature). Similarly, in a related cricket (*G. bimaculatus*), females also laid more eggs at elevated temperatures, and the mean mass of hatchlings increased at higher temperatures [34°C; see table 1 in Behrens et al. (Behrens et al., 1983)]. These results also suggest that increases in egg laying due to increased temperature do not come at the expense of egg quality. Female crickets store mature eggs in their lateral oviducts, and initially the increase in egg laying could have occurred by laying stored eggs (Shoemaker and Adamo, 2007). However, to maintain increased egg laying for 6 days would require increased egg production (Shoemaker and Adamo, 2007). Therefore, the increase in egg output at 33°C appears to reflect increased reproductive effort. There was also no evidence that the increase in reproduction

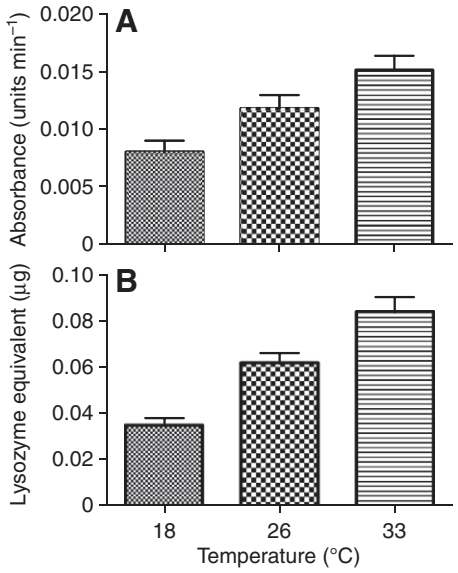

Fig. 3. The effect of temperature on the activities of immune-related enzymes in *G. texensis*. (A) Phenoloxidase activity (N=12 samples per temperature). (B) Lysozyme-like activity. Values were normalized against chicken egg-white lysozyme run at room temperature (N=14 samples per temperature). Bars represent means and error bars denote s.e.m.

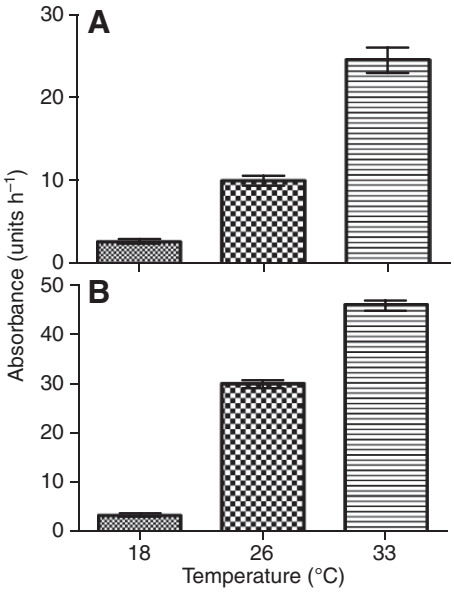

Fig. 4. The effect of temperature on the growth rate of two cricket pathogens: (A) *Serratia marcescens* and (B) *Bacillus cereus*. Bars represent means and error bars denote s.e.m. *N*=4 samples per temperature.

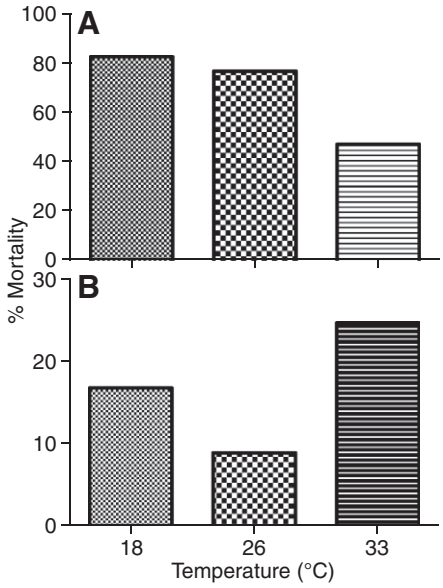

Fig. 5. The effect of temperature on the resistance of *G. texensis* to bacterial infection. Percentage mortality at the three different temperatures when infected with (A) *S. marcescens* (*N*=51 crickets per temperature) and (B) *B. cereus* (*N*=90 crickets per temperature).

occurred at the expense of somatic maintenance. Despite laying more eggs, animals at 33°C also gained the most mass (Fig. 2). This result suggests that female crickets fuelled their higher reproductive rate at 33°C by increased feeding. Moreover, food consumption increases with increasing temperature (between 20 and 38°C) in the related cricket, *G. bimaculatus* (Hoffmann, 1974). In other insects, increased growth at higher temperatures is the result of increased food consumption as opposed to more efficient digestion (Chown and Nicolson, 2004).

Increased temperature also increased the activity of two major components of the immune system, phenoloxidase (Cerenius et al., 2008) and lysozyme-like (Schneider, 1985) enzymes (Fig. 3A,B). Lysozyme-like activity is especially important for immunity in Orthoptera (e.g. crickets and grasshoppers), because they appear to lack many of the antimicrobial proteins found in other insects (Schneider, 1985; Hoffmann et al., 1996). Other arms of the insect immune system are also enhanced at higher temperatures in other species [e.g. nodulation (Mostafa et al., 2005) and haemocyte function (Ouedraogo et al., 2003)]. Therefore, our results demonstrate that reproduction and immune function are both enhanced at temperatures above the environmental mean, and, perhaps more surprisingly, above the cricket's preferred temperature.

However, the effect of temperature on disease resistance was less straightforward. The effect of temperature on disease resistance in other insects varies depending on the species and pathogen. For example, sandflies (*Lutzomyia vexator*) are more susceptible to *Plasmodium mexicanum* at higher temperatures (Fialho and Schall, 1995). *Culex pipiens* is more susceptible to West Nile Virus at higher temperatures (Dohm et al., 2002). Pea aphids (*Acyrthosiphon pisum*) become less susceptible to the fungal pathogen (*Erynia neoaphidis*) as temperature increases (18 *vs* 28°C) (Stacey and Fellowes, 2002), but become more susceptible to a parasitoid (Bensadia et al., 2006). Temperature has complex effects on immune gene expression in *Drosophila melanogaster*, with greater expression of some immune genes at colder temperatures, whereas others have greater expression at warmer temperatures (Linder et al., 2008). In *G. texensis*, resistance to *S. marcescens* increased with temperature, as would be predicted from the lysozyme-like activity results (Adamo, 2004a), but resistance to *B. cereus* was higher at 26 than at 33°C. Both bacteria grow faster at 33 than at 26°C. *B. cereus* grows best at 31°C, but it continues to grow well up to at least 38°C (Choma et al., 2000). *S. marcescens* grows well between 30 and 37°C (Tanaka et al., 2004). Therefore, the difference in resistance cannot be accounted for by one of the bacteria not growing well at 33°C.

However, temperature affects more than growth rates in bacteria. Temperature can also change bacterial biochemistry (e.g. Tanaka et al., 2004). Neither of the bacteria used here is thought to become less virulent with temperature (up to 37°C) [*S. marcescens* (Carbonell et al., 1996) *B. cereus* (Carlin et al., 2010)]. Although *S. marcescens* stops producing the red pigment prodigiosin and the biosurfactant serrawettin at 37°C (Tanaka et al., 2004), it increases its production of cytotoxic molecules from 18°C to a maximum at 37°C (Carbonell et al., 1996). These results highlight the difficulty in predicting the effects of temperature on disease resistance. Despite a general increase in immune function, the activity of the pathogen is often also enhanced. Other insects also show enhanced immune function with temperature, but reduced disease resistance to some pathogens. For example, flour moth (*Ephestia kuehniella*) larvae have an increased nodulation response at higher temperatures, but the caterpillars have lower survival to *Bacillus thuringiensis* at these temperatures (Mostafa et al., 2005). Similarly, warmer temperatures increase immune function in corals (e.g. enhanced phenoloxidase activity); nevertheless, resistance to some pathogens is suppressed at these temperatures (Mydlarz et al., 2010). Linder et al. concluded that temperature effects on disease resistance in insects were due to a combination of temperature effects on both host and pathogen (Linder et al., 2008). These observations underscore the necessity

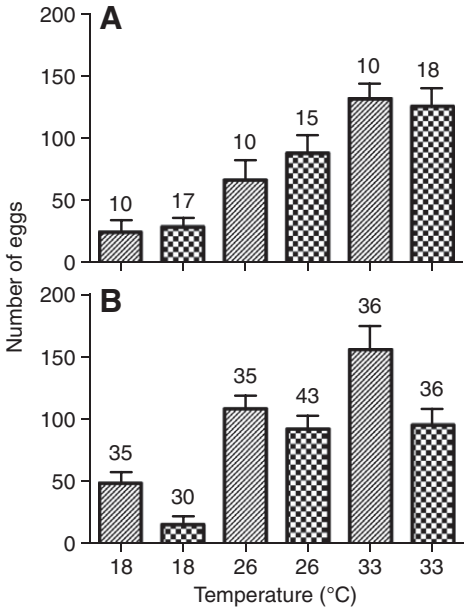

Fig. 6. The effect of infection on egg-laying at different temperatures. (A) Infection with *S. marcescens*, 4 days post-infection. (B) Infection with *B. cereus*, 5 days post-infection. Bars represent means and error bars denote s.e.m. The sample sizes (number of crickets) are given above the bars. Hatched bars, control crickets (i.e. not infected); chequered bars, infected crickets.

of distinguishing between immune function activities and disease resistance (Adamo, 2004b; Adamo, 2009).

At typical environmental temperatures, increased egg production does not suppress immune function (Shoemaker et al., 2006a); nor does a bacterial immune challenge result in a decline in egg output in *G. texensis*, at least in the short term (Shoemaker et al., 2006b; Shoemaker and Adamo, 2007). Similarly, implanting a nylon filament does not reduce egg laying in another female cricket, *Acheta domesticus* (Bascunan-Garcia et al., 2010), for 2 weeks after the implant. In fact, female crickets exhibit terminal reproductive investment, and lay more eggs than controls the day after infection or immune challenge (Adamo, 1999; Shoemaker et al., 2006b). However, this increase is not maintained after the first day post-infection (Adamo, 1999). Consistent with these data, crickets given a sublethal infection with *S. marcescens* laid the same number of eggs as those that were uninfected (Fig. 6A). However, a sublethal infection with *B. cereus* did reduce egg laying compared with uninfected controls, and eliminated the increase in egg laying at 33°C (Fig. 6B). The reason for this difference in response to the two bacteria requires further study. Possibly, mounting an immune response against *B. cereus* that is Gram positive is more costly than defence against *S. marcescens* that is Gram negative, either in terms of the resources needed to counteract the pathogen and/or in terms of toxic molecules produced. Crickets infected with *B. cereus* may be unable to maintain an increased rate of reproduction in the face of these additional costs. There is evidence in other systems (e.g. bacterial resistance to a bacteriophage) that resistance becomes more costly with increasing temperature (Quance and Travisano, 2009), and this may be the case for immune defence against *B. cereus*. Therefore, at least for some pathogens, higher temperatures may result in a trade-off between immune function and reproduction.

There are few studies of the effect of temperature on reproduction in infected hosts (Guinnee and Moore, 2004). In uninfected cockroaches, fecundity is greater at higher temperatures (Guinnee and Moore, 2004). Cockroaches infected with acanthocephalans have the same fecundity as controls at lower temperatures, but have reduced fecundity at higher temperatures (28 and 31°C), similar to the results of *G. texensis* infected with *B. cereus*. Guinnee and Moore suggest that the infected cockroaches maintain normal fecundity because of the reduced rate of parasitic growth at lower temperatures (Guinnee and Moore, 2004). However, it could also reflect the cockroaches' inability to pay both the increased cost of resistance at higher temperatures and maintain an accelerated rate of reproduction. *D. melanogaster* infected with the bacterium *Providencia rettgeri* shows a similar pattern. Increasing temperature leads to increased egg laying (Lazzaro et al., 2008). Infection reduces egg laying, with the largest reduction occurring at the highest temperature (28°C) for both temperate zone populations [see table 4 in Lazzaro et al. (Lazzaro et al., 2008)]. In addition, these effects may be exacerbated by low food availability. In the studies cited above, animals had unlimited access to food. In the field, animals are often food limited [e.g. crickets (Jacot et al., 2004)]. Given that insects (e.g. crickets) maintain their elevated metabolism by consuming more food (Hoffmann, 1974), food limitation may cause greater negative consequences for individuals at higher temperatures than at lower temperatures. In the field, low food availability at higher temperatures may profoundly reduce reproduction and/or increase mortality in infected insects. These effects would reduce the fitness benefits of higher temperatures.

Moreover, higher temperatures reduce lifespan in crickets (Behrens et al., 1983), potentially reducing their fitness benefits. However, this effect is probably of minimal importance in this study. We tested females at middle age (14 days) and most would have been at the end of their natural lifespan in the field by the end of the 'heat wave' (Murray and Cade, 1995). Therefore, effects of the elevated temperature on lifespan may often be of little ecological significance. Even if lifespan is shortened, animals at higher temperatures still produce more progeny. The related cricket *G. bimaculatus* lives longest at 20°C, but has the most offspring at a much higher temperature [34°C (Behrens et al., 1983)]. Therefore, although we did not measure egg output over the entire lifespan, the data strongly suggest that the female's lifetime reproductive output will be increased by a 6 day heat wave.

Colder temperatures increased the life span in infected animals, as has been found in other insects [e.g. bumblebees *Bombus* spp. (Müller and Schmid-Hempel, 1993); *D. melanogaster* (Linder et al., 2008)]. However, the low egg output of females at 18°C means that these females did not lay more eggs prior to death than females at higher temperatures. Crickets continue to lay eggs when heavily infected until they die, even at higher temperatures (Adamo, 1999). The longer survival time at the lower temperature could have a number of causes. For example, it could reflect the increased time required for bacterial numbers to reach the number needed to cause sepsis and death.

One limitation of this study was the use of constant temperatures as opposed to a more natural temperature regime. However, in a related cricket, *G. bimaculatus*, cycling temperatures had the greatest effect at lower temperatures (e.g. 18°C), in which periodic exposure to higher temperatures increased productivity (Behrens et al., 1983). Although oscillating temperatures had effects on both lifespan and fecundity, they followed the same trend of increasing temperature leading to increased reproduction (Behrens et al., 1983). The studies on the effects of oscillating temperature regimes on crickets (Hoffmann, 1974; Behrens et al., 1983) suggest that using oscillating temperatures would produce similar results.

Gryllus texensis preferred temperatures below those required for optimal reproductive and immune performance. A similar mismatch exists in other crickets. For example, *G. bimaculatus* prefer 26.6°C (Louis et al., 1986), but have their best reproductive output at 34°C (Hoffmann, 1974). Other orthoptera prefer temperatures closer to their reproductive optimum. For example, the grasshopper *Melanoplus sanguinipes* prefers 34°C; temperatures a few degrees warmer than this lead to reduced egg laying (Boorstein and Ewald, 1987). Why would some animals prefer temperatures that result in reduced reproduction and immune function? First, thermoregulation can be costly (Chown and Nicolson, 2004). An insect's preferred temperature should be close to the average environmental temperature or energy will be wasted on thermoregulation (see Angilletta et al., 2003; Angilletta et al., 2010). Second, if food is scarce, then higher temperatures may hold few advantages (e.g. see Clarke, 2003). The amount of fat body in *G. texensis* adults in the field can range from abundant in some years to almost none in others (S.A.A., unpublished data), suggesting that *G. texensis* crickets may often be food limited, as is the case in other crickets (e.g. Jacot et al., 2004). Given that the increased reproductive rate at 33°C appears to be fueled by increased food consumption (Fig. 2), thermoregulating to warmer temperatures when food is unavailable may decrease lifespan by exhausting energy reserves. Third, temperature effects on crickets may be complex because they influence the quality of male sexual signalling behaviour (e.g. Martin et al., 2000; Hedrick et al., 2002) and female sexual behaviour (Kindle et al., 2006). Therefore, sexual selection may also act on the preferred temperature, resulting in a reduction from a temperature closer to the physiological optimum. Finally, as discussed above, higher temperatures may result in trade-offs between immune function and reproduction, reducing the fitness benefits of higher temperatures.

How will climate change effect *G. texensis*? The results using *B. cereus* suggest that crickets may become more susceptible to some pathogens at higher temperatures. If insects become more susceptible to their major diseases in the field, then higher temperatures may not necessarily lead to increased population growth, even though both reproductive rate and immune function are enhanced at these temperatures in the laboratory. Therefore, the effects of global warming on insect populations may not be as positive as some studies have suggested (e.g. Deutsch et al., 2008). However, the *S. marcescens* results suggest that climate change could increase the reproductive rate of some temperate zone insects, depending on their disease ecology. In these cases, we may end up not only with more insects, but also with insects that will be more disease resistant (i.e. tougher to kill with biological control agents, e.g. fungi). The effects of climate change on insect reproduction, immune function, disease resistance and their interactions are likely to be complex, and they require further study.

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