

Effects of Temperature Variation on Male Behavior and Mating Success in a Montane Beetle

Cynthia A. Dick^{1,2}
 Nathan E. Rank^{2,3}
 Meagan McCarthy^{1,2}
 Stephen McWeeney³
 Daniel Hollis^{2,3}
 Elizabeth P. Dahlhoff^{1,2,*}

¹Department of Biology, Santa Clara University, Santa Clara, California 95053; ²University of California, White Mountain Research Station, Bishop, California 93514; ³Department of Biology, Sonoma State University, Rohnert Park, California 94928

Accepted 4/4/2012; Electronically Published 6/18/2013

ABSTRACT

Locomotion and mating ability are crucial for male reproductive success yet are energetically costly and susceptible to physiological stress. In the Sierra willow beetle *Chrysomela aeneicollis*, male mating success depends on locating and mating with as many females as possible. Variation at the glycolytic enzyme locus *phosphoglucose isomerase* (*Pgi*) is concordant with a latitudinal temperature gradient in these populations, with *Pgi*-1 frequent in the cooler north, *Pgi*-4 in the warmer south, and alleles 1 and 4 in relatively equal frequency in areas intermediate in geography and climate. Beetles experience elevated air temperatures during a mating season that causes differential physiological stress among *Pgi* genotypes, and running speeds of individuals homozygous for *Pgi*-4 are more tolerant of repeated thermal stress than individuals possessing *Pgi*-1. Here the importance of running behavior for male mating activity was examined, and differential effects of thermal stress among *Pgi* genotypes on male mating activity were measured. In nature, males run more than females, and nearly half of males mate or fight for a mate after running. In the laboratory, mating activity was positively correlated with running speed, and repeated mating did not reduce running speed or subsequent mating activity. Males homozygous for *Pgi*-4 mated longer and more frequently after heat treatment than 1-1 and 1-4 males. All heat-treated males had lower mating frequencies and higher heat shock protein expression than control males; however, mating frequency of recovering 4-4 males increased

throughout mating trials, while treated 1-1 and 1-4 males remained low. These results suggest that effects of stress on mating activity differ between *Pgi* genotypes, implying a critical role for energy metabolism in organisms' response to stressful temperatures.

Introduction

Environmental temperature has an important limiting effect on the performance, reproductive success, and distribution of many organisms (Hochachka and Somero 2002). This is especially true in unpredictable environments, where exposure to extreme temperature variation can alter survival, impair physiological performance, and reduce survival or reproductive output (Dillon et al. 2007; Musolin 2007; Lachenicht et al. 2010; Kallioniemi and Hanski 2011; Zizzari and Ellers 2011). Small ectotherms (e.g., insects) may be particularly vulnerable to physiological effects of environmental temperature variation because behavioral thermoregulation is less effective for controlling body temperature than in larger ectotherms (Stevenson 1985; Chown and Nicolson 2004). The ability of insect populations to respond to a changing environment depends in part on the ability of adults to reproduce successfully while facing stressful conditions. Prior studies have documented effects of temperature variation on female fecundity and male mating frequency (Watt et al. 1985; Watt 1992; Mahroof et al. 2005; Dahlhoff et al. 2008) and shown that locomotion plays an important role in reproductive success of some ectotherms (Gibert et al. 2001; Michalak et al. 2001; Watt et al. 2003; Feder 2010; Feder et al. 2010). Few studies have directly quantified the effects of environmental temperature variation on the relationship between mating and locomotion.

In order for natural populations of ectotherms to respond effectively to a changing thermal environment, there must be sufficient genetic variation in traits under natural selection (Huey and Kingsolver 1993; Loeschcke et al. 1997; Hoffmann et al. 2003; Fasolo and Krebs 2004). Multiple studies have identified the gene *phosphoglucose isomerase* (*Pgi*), which codes for a dimeric enzyme (PGI) whose activity is central to glucose metabolism, as one candidate for understanding how natural populations respond to environmental temperature variation (Watt 1992; Mitton 1997; Haag et al. 2005; Karl et al. 2009; Niitpold et al. 2009; Saastamoinen et al. 2009; Niitpold 2010). Variation at *Pgi* has been associated with differences in metabolic rate, dispersal ability, oviposition, life span, and larval development (Watt 1983, 1992; Watt et al. 1983, 1985; Haag

* Corresponding author; e-mail: edahlhoff@scu.edu.

et al. 2005; Karl et al. 2008; Saastamoinen and Hanski 2008; Niitepold et al. 2009; Saastamoinen et al. 2009; Niitepold 2010). The role of *Pgi* variation in flight activity associated with mating has also been reported for several species of butterflies; mating flights occur at specific air temperatures in most species and are impaired by temperatures that are too high or variable (Watt et al. 1983; Boggs and Murphy 1997; Karl et al. 2008; Saastamoinen et al. 2008; Niitepold et al. 2009; Niitepold 2010). To date, differential effects of thermal stress on male mating success among *Pgi* genotypes have not been experimentally measured.

Here we investigate effects of temperature variation on male mating success in Sierra Nevada, California, populations of the willow leaf beetle *Chrysomela aeneicollis* (Brown 1956). The mating system of this beetle is best described as scramble competition (Rank et al. 2006). Fast, active males maximize their reproductive success by encountering more females than slower, less active males. Thus, in scramble mating systems, speed is a critical component of male reproductive success. Furthermore, mating activity (copulation, mate guarding) and the locomotion associated with it (running, flying) are typically the most energetically demanding activities conducted by these males (Partridge and Farquhar 1983; Sartori et al. 1992; Marden and Rollins 1994). In Sierra willow beetles, exposure to potentially stressful temperatures during mating season (Rank and Dahlhoff 2002; Dahlhoff et al. 2008) may disrupt the balance between energetically demanding mating activities and maintenance behaviors (e.g., sitting and eating) and thereby reduce subsequent male mating success.

Prior studies strongly suggest that *Pgi* is under natural selection in Sierra willow beetle populations (Neargarder et al. 2003; McMillan et al. 2005; Dahlhoff and Rank 2007; Rank et al. 2007; Dahlhoff et al. 2008). Two *Pgi* alleles are prevalent in these populations. *Pgi* allele 1 (*Pgi*-1) is present in high frequency at populations living in northern, cooler sites and *Pgi* allele 4 (*Pgi*-4) in southern, warmer sites. *Pgi* alleles 1 and 4 are in relatively equal frequency in populations intermediate in geography and climate (Rank 1992; Dahlhoff and Rank 2000). PGI allozymes differ in effects of temperature on enzyme stability and kinetics, and differences among *Pgi* genotypes in thermal tolerance, heat shock protein (Hsp70) expression, larval growth and survival, metabolic rate, running speed, and fecundity have been documented; generally, *Pgi* 1-1 and 1-4 individuals perform best under mild conditions or after a single stress, 4-4 homozygotes under extreme conditions or after repeated stress (Neargarder et al. 2003; McMillan et al. 2005; Rank et al. 2007; Dahlhoff et al. 2008). It is therefore likely that PGI, a glycolytic enzyme critical for energy metabolism, plays an important role in energetic activities associated with male mating behavior.

This study was executed in three phases. First, mating behavior was observed in nature to examine differences in male and female activity and to assess the role of running in male mating behavior. Variation in ambient temperature was measured to assess typical thermal conditions experienced in nature during mating. Second, to test the hypothesis that both running and mating are energetically demanding behaviors important

for male mating success and that there may be energetic trade-offs between the two behaviors when conditions are stressful, running speed was measured immediately after field collection and after repeated mating with multiple females. Effects of repeated mating and running were then compared between *Pgi* genotypes before and after heat treatment. Third, to quantify differences among *Pgi* genotypes in recovery of mating activity after heat stress, males were first acclimated to mild laboratory conditions and then mating activity was compared between males treated with a temperature known to cause physiological stress and nontreated controls. In this experiment, expression levels of a 70-kD Hsp70 were measured to confirm that experimental temperature treatment was physiologically challenging.

Material and Methods

Study Populations and Sample Collection

Beetle populations were studied in Bishop Creek (BC), Eastern Sierra Nevada, California (Bluff Lake: 37°10'37.6"N, 118°33'04.5"W; 3,200 m). Populations are in Hardy-Weinberg equilibrium with respect to *Pgi* ($P > 0.9$ for all comparisons), and *Pgi* alleles 1 and 4 are found in approximately equal frequency in BC (Rank 1992; Dahlhoff et al. 2008). *Pgi* allele frequencies at these sites are sensitive to seasonal and decadal changes in climate (Dahlhoff et al. 2008) but do not shift throughout the course of the day (N. E. Rank and E. P. Dahlhoff, unpublished observations). Beetles were collected from

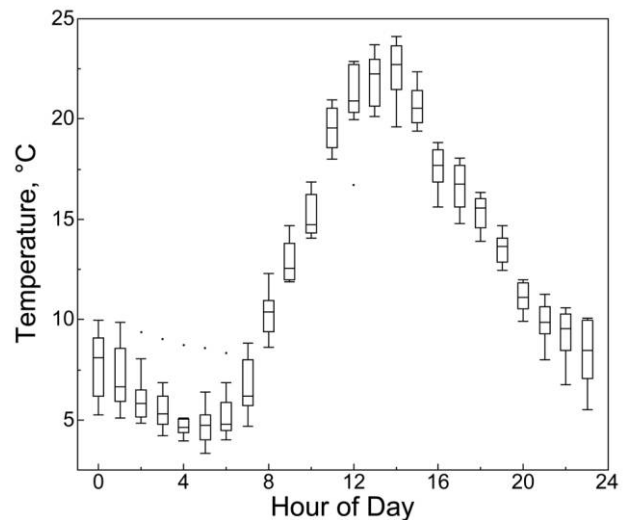


Figure 1. Diurnal temperature variation during peak mating season. Data shown are twenty-fifth- to seventy-fifth-percentile box plots calculated from hourly means of air temperatures measured during field behavioral observations (June 17–26, 2008). Maximum and minimum hourly temperatures during this period are shown as error bars, median hourly temperatures as a horizontal line in each box. Statistical outliers are shown as single data points.

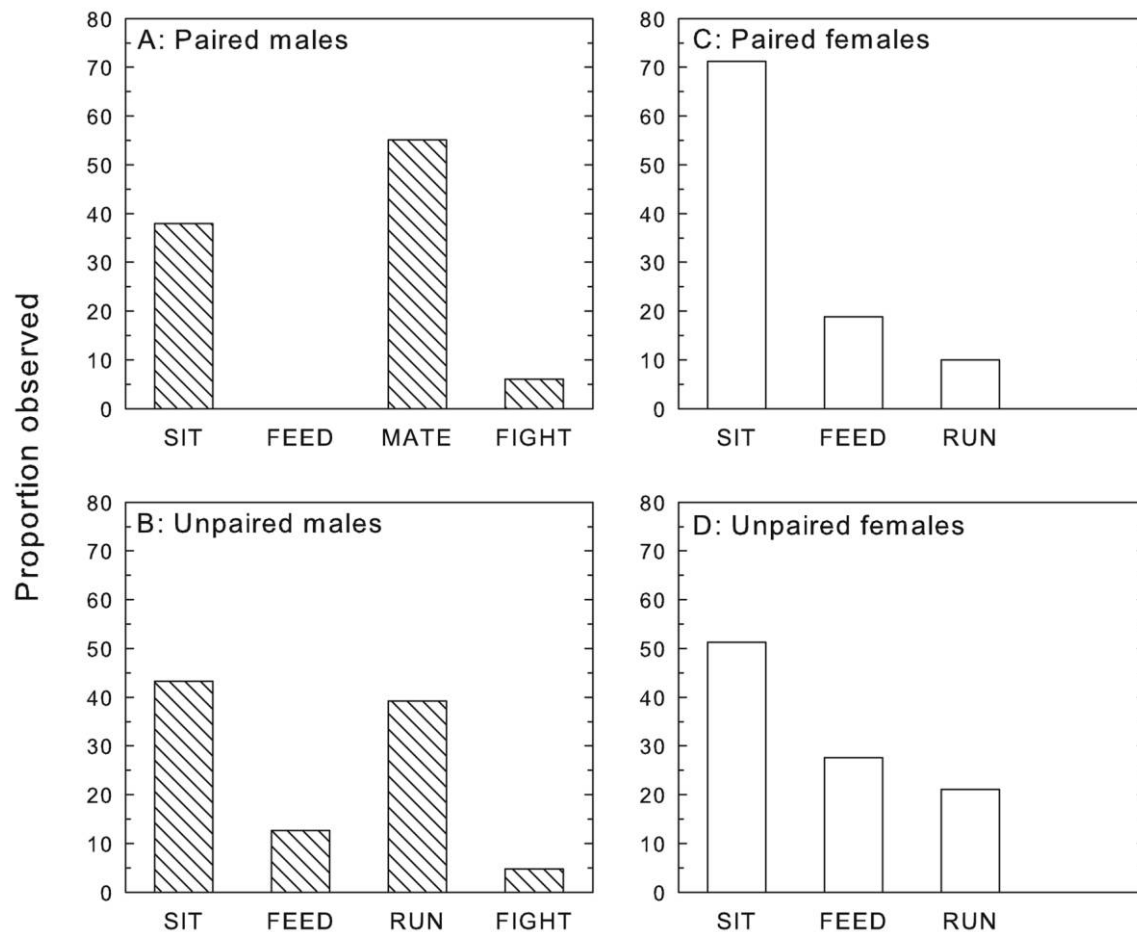


Figure 2. Behavior of unpaired or paired males and females in nature. Observations were made on sunny days in Bishop Creek between 1030 and 1330 hours, June 17–26, 2008. Data show proportion of males (striped bars; A, B; $n = 510$) and females (open bars; C, D; $n = 156$) engaging in common behaviors. Sitting paired males (A) were “mate guarding” and were always sitting on the female’s back. Seventy-two percent of paired females (C) observed sitting, feeding, or running were also copulating with a male.

the field and returned to the Owens Valley laboratory of White Mountain Research Station in Bishop, California. For experiments examining relationships between mating frequency and running speed, beetles were collected on June 24 and 26, 2006, and immediately measured in the laboratory. Daytime air temperatures were measured every 30 min 3 d before collection using automated loggers deployed in thermal shields (Hobo Pendant loggers, Onset Computer, Pocasset, MA) to verify that thermal conditions were similar between collection days. For experiments examining recovery of mating activity after heat stress, beetles were obtained in four separate collections from June 17 to June 24, 2007, and acclimated to laboratory conditions for 3 d. For both experiments, sexes were separated and beetles kept in petri dishes containing fresh leaves from their favored host *Salix orestera*.

Behavior in Nature

Field observations were conducted between 1030 and 1330 hours on June 17–26, 2008, during peak adult abundance,

which was determined using published methods (Dahlhoff et al. 2008). Three separate willow patches located along a creek flowing out of Bluff Lake were surveyed each day (1 h per locality). Observers walked along the creek transect, visually scanning willow foliage, noting paired and unpaired males and females, and recording relevant behavior(s) for each individual: sitting, feeding, running, mating, or fighting. The behavior of 665 individuals was documented. Individual running surveys were conducted in these same three patches. An observer walked up to a willow at random, and the first running beetle observed was followed for 15 min, or until it stopped running; its next behavior was then recorded. If a running male initiated a fight with a resident mating male, duration of the fight was recorded. Behavior after running of more than 100 individuals was recorded; we report activity of beetles that ran continuously for at least 2 min after they were first observed running ($N = 66$). Air temperatures were measured throughout field observations using a shielded temperature logger deployed at the site.

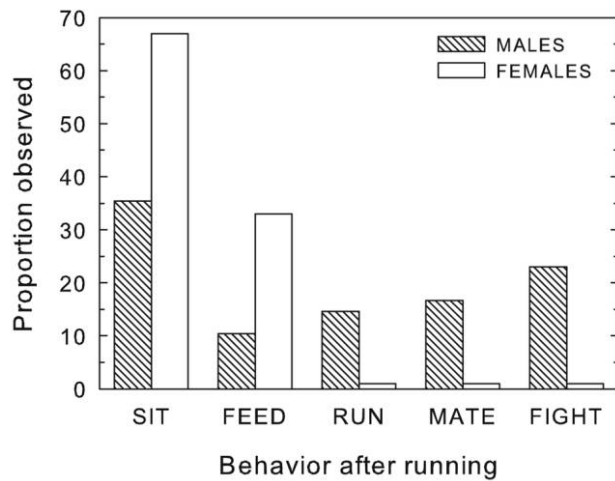


Figure 3. Differences between male and female behavior after running in nature. Data show proportion of males (striped bars, $n = 48$) and females (open bars; $n = 18$) engaging in common behaviors after being observed running for at least 2 min.

Mating Frequency versus Running Speed

This experiment was designed to re-create the experience of males in nature while being exposed to temperatures known to alter running speed among PGI genotypes (Rank et al. 2007). For these and all other laboratory experiments, beetles were given access to fresh *S. orestera* leaves at all times except during temperature treatments (or controls), when males were held in dishes with moist paper towels. Two experimental trials were run, and each trial took 2 d to complete. On day 1, males were collected and running speed immediately measured in a controlled-temperature room ($20 \pm 2^\circ\text{C}$) following published methods (Rank et al. 2007). Briefly, males were placed on a 1-inch wooden dowel with a full-spectrum light at the top. Males voluntarily run toward the light when placed on the pole. They were allowed to run at least 5 cm before starting measurement; running speed was calculated as time it took a male to travel the next 10 cm. After the initial running speed measurement (run 1), the first mating trial was conducted (mating trial 1, “preheat treatment”). Each male was allowed to mate with three different females, 1 h each. Running speed was measured immediately after this first mating trial (run 2), and males and females were then held overnight at 4°C . On day 2, running speed was measured (run 3), and all males were exposed to a heat treatment (36°C , 3 h) in controlled-temperature, lighted incubators (146E low-temperature incubator, Fisher Scientific, Asheville, NC). After heat treatment, males were held for 1 h before running speed was measured again (run 4). Males were then allowed to mate with three additional females, 1 h each (mating trial 2, “postheat treatment”). Running speed was measured a final time (run 5), after which males were weighed and flash-frozen at -80°C for biochemical analysis.

Recovery of Mating Activity after Heat Stress

Male beetles were collected, returned to the laboratory, and acclimated to mild laboratory conditions for 3 d (16L : 8D, $20^\circ/4^\circ\text{C}$). Males were treated for 4 h at either 36° or 20°C (control). After heat treatment/control, males were allowed to mate with four different females, 1 h each, and mating frequencies per female were quantified. Males were weighed and flash-frozen at -80°C for biochemical analysis.

Effect of Prior Mating on Running and Subsequent Mating Frequency

To determine whether copious mating would reduce subsequent mating activity or running speed, male beetles were acclimated to mild laboratory conditions for 3 d (16L : 8D, $20^\circ/4^\circ\text{C}$) and then held for 4 h at 20°C with either three females or no females (control). Males were then allowed to mate with four different females, 1 h each, as described above, and mating frequencies per female were quantified. Running speed was measured before and after the four female mating trials.

Measuring Mating Activity

Each male was placed in a petri dish with a single female and fresh willow leaves. Every 4–5 min, a male was scored as mating (copulating with the female) or not mating: walking, feeding, sitting away from the female, or sitting on the female’s back (mate guarding). At least 12 observations were made for each

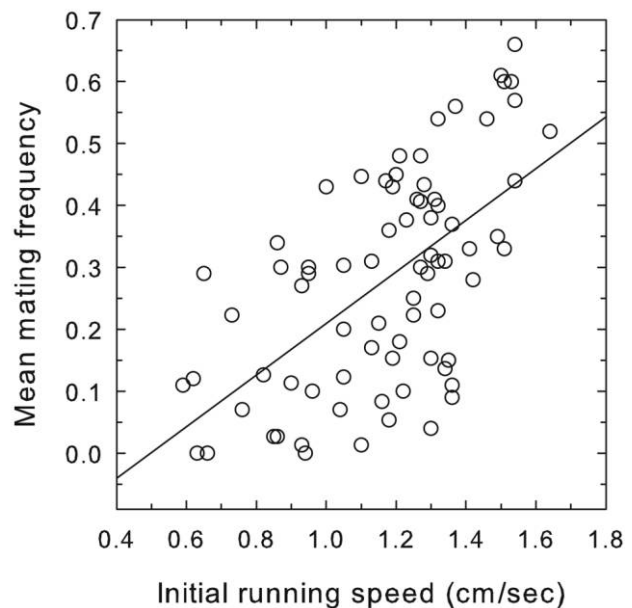


Figure 4. Relationship between male mating frequency and running speed. Data shown are running speed measured immediately after field collection versus the mean mating frequency of that male with three consecutive females prior to laboratory heat treatment ($n = 77$; $Y = 0.4X - 0.2$; $R^2 = 0.36$, $F_{1,76} = 41.4$, $P < 0.0001$).

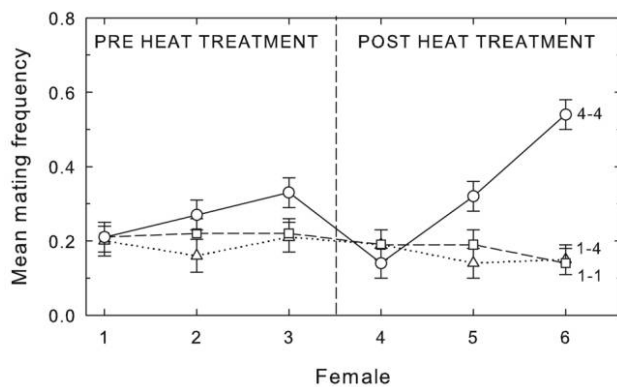


Figure 5. Male mating frequency before and after heat treatment varies among *Pgi* genotypes. Data shown are least squares means (\pm SE) of mating frequency of three *Pgi* genotypes (1-1, 1-4, 4-4; $n = 33, 35, 12$). Males were held with six different females for 1-h mating trials (three each before and after heat treatment, which is indicated as a dashed vertical line).

male-female pair during a 1-h period. For all experiments, mating frequency was calculated as the number of times males were observed copulating with the female, divided by the total number of observations made during the hour. For experiments examining relationship between mating and running, number of times the male copulated with the female and duration of each mating event was measured. Mating duration was calculated as follows. Exact start time of each mating trial was noted. If a male was observed mating in that trial and then in the next trial, it was assumed that he was mating through the entire first trial. If he was observed not mating in the next trial, it was assumed that he stopped mating in the middle of that trial. For example, a male observed mating at the beginning of four consecutive trials, each 5 min in length, and then observed not mating the following (fifth) trial mated for 18.5 min.

Data Analyses

All statistical analyses were conducted in JMP Pro 10.0 (SAS, Cary, NC) with the exception of Fisher's exact tests, which were calculated using web-based freeware (<http://www.langsrud.com/fisher.htm>). Direct relationships between running speed and mating activity were assessed using regression analysis. Effects of repeated running and mating, effects of heat treatment on subsequent mating, and effects of prior mating on running speed and subsequent mating were analyzed using repeated-measures ANCOVA, with treatment (heat treatment, three female treatments) as the main effect, male body mass as the covariate, and mating frequency with individual females as the repeated measure (1-6 for running vs. mating, 1-4 for effects of heat treatment and prior mating). Hourly mean temperatures at the field sites were determined from the Hoboware raw data file (Onset Computer) for the period behavioral observations were made in June 2008, as well as for field collections in 2006 and 2007. Enzyme genotype of abdomen tissue was determined

by starch gel electrophoresis using established protocols (Rank 1992). Tissue levels of a 70-kD inducible Hsp70 were measured for thorax using quantitative western blots (Rank et al. 2007).

Results

Field Temperatures

During the mating season (2008), air temperatures were relatively mild and consistent from day to day (fig. 1). Air temperatures increased from sunrise until midday; maximal daytime temperatures did not exceed 25°C (at 1400 hours), and minimal nighttime temperature never dropped below 4°C (at 0500 hours). There was one night where minimum temperatures were atypically warm (about 8° vs. 5°C) and one day where it was unusually cool at noon. While beetle body temperature is routinely 3°–4°C above air temperature during the day, the relationship between air and body temperature is linear at all air temperatures; that is, there is little evidence of behavioral thermoregulation in this small ectotherm (Dahlhoff et al. 2000). Field temperature data reported here confirm that beetle body temperature was highest when behavioral observations were made and that no extreme thermal conditions occurred during field behavioral observations.

Behavior in Nature

During peak mating season, 46% of adults were observed in mating pairs, copulating or mate guarding. The majority of paired males (fig. 2A) were mating (e.g., copulating), whereas unpaired males were sitting or running (fig. 2B). Unpaired males ran more and fed less than unpaired females (fig. 2B, 2D; $\chi^2 = 58.1$, $df = 488$, $P < 0.0001$), and paired males were not observed feeding (fig. 2A, 2B; $\chi^2 = 19.2$, $df = 273$, $P < 0.0001$). Behavior of unpaired beetles that were first observed running differed between sexes (fig. 3). Females never ran for more than 8 min and never mated after running. In contrast,

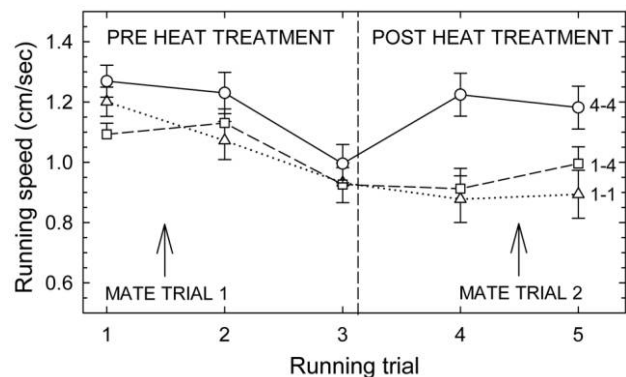


Figure 6. Mating activity does not reduce male running speed before or after heat treatment. Data shown are least squares means (\pm SE) of running speeds of three *Pgi* genotypes (1-1, 1-4, 4-4; $n = 33, 35, 12$) before and after mating treatments (arrows) and heat treatment (dashed vertical line).

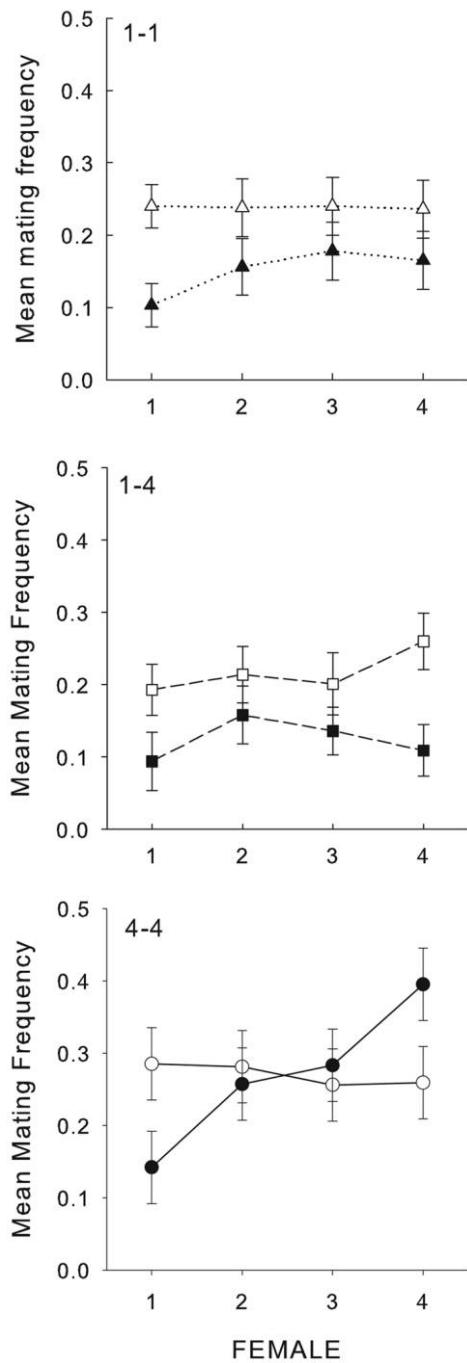


Figure 7. Recovery of mating activity after heat stress differs between *Pgi* genotypes. Data are least squares means (\pm SE) of mating frequencies for heat-treated (filled symbols) and control (open symbols) males allowed to mate with four different females after treatment. Sample sizes (control, heat treatment) are as follows: *Pgi* 1-1: $n = 39, 39$; *Pgi* 1-4: $n = 47, 45$; *Pgi* 4-4: $n = 20, 20$.

15% of males ran continuously for more than 15 min, and an additional 40% of males directly engaged in mating-related behavior (mating, mate guarding, or fighting) after running (mating-related vs. non-mating-related behavior; two-tailed

Fisher's exact test, $P = 0.002$). Fighting was the most common male behavior observed after running (fig. 3); fights lasted an average of 3.5 min.

Mating Frequency versus Running Speed

Males that ran fastest immediately after field collection had the highest mating frequency during the first set of mating trials, females 1–3 (fig. 4; $Y = 0.4X - 0.2$; $R^2 = 0.36$, $F_{1,76} = 41.4$, $P < 0.0001$). Males mated zero to two times in their 1 h with a female; less than 5% of males mated more than twice per female, and 36% of males did not mate. The average duration of each pretreatment mating event was 16.7 ± 1.3 min, which declined to 13.0 ± 1.4 min posttreatment. Mating frequency and duration differed among *Pgi* genotypes after heat treatment and was highest for 4-4 males at the end of mating trials (fig. 5; multivariate analysis of variance [MANCOVA]; Wilks's λ , *Pgi* genotype \times mating trial: $F_{10,144} = 3.0$, $P = 0.02$). Mating duration was higher for 4-4 males (21.0 ± 2.4 min) than 1-1 or 1-4 males after heat treatment (9.5 ± 1.9 and 9.4 ± 1.6 min, respectively; MANCOVA: $F_{4,150} = 5.0$, $P = 0.009$). *Pgi* 4-4 males also ran faster than 1-1 or 1-4 males after heat treatment (fig. 6; MANCOVA: *Pgi* genotype \times running trial: $F_{8,142} = 2.8$, $P = 0.03$). Mating did not reduce running speed, either before or after heat treatment (fig. 6; $P > 0.5$ for all comparisons). Males held with three females (versus nonmated controls) did not mate less frequently with four consecutive females or run slower after mating ($N = 30$ for three-female treatment, controls: $P > 0.5$ for all comparisons).

Recovery of Mating Activity after Heat Stress

All heat-treated males mated less with their first female than males held at 20°C (fig. 7; MANCOVA: treatment \times *Pgi* genotype \times mating trial: $F_{6,392} = 3.1$, $P = 0.03$). Mating frequencies of heat-treated *Pgi* 1-1 and 1-4 males were consistently lower than control males for all four trials (fig. 7, top, center), whereas mating frequency of *Pgi* 4-4 heat-treated males was higher than that of controls by the end of mating trials (fig. 7, bottom). Heat-treated males had significantly slower running speeds (0.9 ± 0.06 vs. 1.3 ± 0.05 cm/s) and higher Hsp70 expression (33.2 ± 1.6 vs. 7.4 ± 1.5 ng Hsp70/g thorax soluble protein) than control males (effect of heat treatment, one-way ANOVA, running speed: $F_{1,167} = 9.6$, $P = 0.002$; Hsp70: $F_{1,167} = 21.4$, $P < 0.0001$).

Discussion

Running speed and mating frequency are important components of male reproductive success in scramble competition mating systems, where males seek out and copulate with as many different females as possible. Results presented here suggest that running is an important component of male mating success in Sierra Nevada populations of the leaf beetle *Chrysomela aeneicollis* and provide one of the first experimental demonstrations of the differential effects of heat stress on male

mating success. They also show that copulation itself does not reduce running speed or subsequent mating activity but that ecologically relevant thermal stress reduces the ability of males to perform energetically demanding activities associated with mating and that these effects differ among *Pgi* genotypes.

In nature, males engaged in energetically demanding behaviors associated with mating more often than sitting or eating, the most common female behaviors. When a running male found a female, he mated with her, fought with the resident male for the opportunity to mate with her, or sat on her back, which drastically reduced his ability to feed. Energy expended running to seek mates can be considerable in scramble competition mating systems (Lane et al. 2009, 2011), but male willow beetles do not seem to be prevented from running and mating as much as possible when exposed to mild climatic conditions in nature. Males sometimes fight with intruders while paired with a female, though this fighting does not appear to increase subsequent mating success (Rank et al. 2006). Running is therefore the most energetically demanding activity conducted by male willow beetles during mating season that will increase reproductive success. While we were not able to measure the total number of male mating events in nature as a function of his running speed, we were able to do so in the laboratory; males that ran fastest mated most, and even copious prior mating did not reduce running speed or subsequent mating activity.

Air temperatures during mating season in mid-June 2008 were relatively mild and fairly consistent from day to day; the diurnal temperature profile shows a slow increase in air temperature from sunrise until midday, dropping into evening; freezing temperatures were never observed. Our prior studies have shown that beetle body temperature linearly tracks air temperature (Dahlhoff et al. 2000), meaning behavioral thermoregulation is limited and thermal conditions that induce physiological stress are sometimes experienced during mating season (Neargarder et al. 2003; Dahlhoff et al. 2008). During this experiment, temperatures never reached extreme highs (or lows) that may compromise mating activity in other years. It appears that daytime temperatures were high enough to induce active behavior but mild enough to minimize physiological stress. Future studies may allow us to more directly observe responses of stressful conditions in nature, as climatic conditions in the Sierra are becoming more extreme as the result of climate change (Hayhoe et al. 2004).

Contrary to the expectation that there would be a negative trade-off between mating and running, we found that males that ran fastest mated the most and repeated mating, or the opportunity to mate with many females, did not result in slower running speeds. In addition, mating frequency did not decline after prior, repeated copulations with females. This is similar to lizards, where males with high sprint speeds have greatest reproductive success, although they frequently run to chase off intruder males (Garland et al. 1990; Husak et al. 2008). We also found no effect of body size; small males were not faster as in other scramble competition systems (Schulte-Hostedde and Millar 2002). Taken together, this suggests that there may

not be an energetic trade-off between running and mating when conditions are mild. It is also possible that faster males mated more often than slow ones due to female choice—a fast male is a “sexy” male; prior studies suggest some evidence of female willow beetles choosing to mate with males who won fights (Rank et al. 2006). Alternatively, faster males may be in better physiological condition than slower males, a pattern that has been shown for other insect species (Lovegrove 2004; Clusella-Trullas et al. 2010; Lachenicht et al. 2010). Either way, fast males mate more and may therefore have a higher reproductive success than slow, less attractive males.

Changes in mating frequency and running speed after exposure to a heat treatment differed among *Pgi* genotypes. Mating frequency, mating duration, and running speed of *Pgi* 4-4 males were all higher at the end of running/mating trials than those of 1-1 and 1-4 males. Furthermore, *Pgi* 4-4 males that were acclimated to mild laboratory conditions recovered mating activity after heat stress to a greater extent than males possessing *Pgi*-1, though heat treatment reduced running speed and increased Hsp70 expression for all males. This result may be due to natural selection operating directly on *Pgi*, leading to differences in flow of glucose through glycolysis during activity. It could also be due to selection operating on genes linked to *Pgi*, either directly or functionally. For example, significant variation in another metabolic gene, *mtCOII*, has been recently observed in Sierra willow beetles. Larval development rate is fastest when *Pgi* 4-4 larvae also have a *COII* haplotype frequent in the south but lowest when *Pgi* 4-4 larvae have a northern mitochondrial haplotype (S. Heidl, unpublished observations). This concordant pattern may be due to effects of glycolytic activity on later steps in cellular metabolism, as *Pgi* (nuclear) and *COII* (mitochondria) are not physically linked. However, other unidentified genes may be physically linked to *Pgi* and their variation may be part of what is causing variant thermal phenotypes in willow beetle populations, with *Pgi* a marker for this variation. Either way, these data suggest that males homozygous for the electrophoretically slower *Pgi*-4 allele are better able to recover mating activity after thermal stress than individuals possessing the faster *Pgi*-1 allele.

These results are consistent with studies of several species of butterflies, which found that individuals possessing electrophoretically slow *Pgi* alleles had higher flight activity and (in some cases) mating success after exposure to high temperatures than individuals possessing faster *Pgi* alleles (Watt et al. 1985; Klemme and Hanski 2009; Niitepold et al. 2009; Karl et al. 2010; Mitikka and Hanski 2010). These data are also congruent with our earlier studies of Sierra willow beetles, where we show that individuals homozygous for *Pgi*-4 allele are more tolerant of extreme or repeated thermal stress than individuals possessing *Pgi*-1 allele (Neargarder et al. 2003; McMillan et al. 2005; Rank et al. 2007; Dahlhoff et al. 2008). Temperature selection at *Pgi* is also consistent with allele frequency variation in nature; *Pgi*-1 is most frequent in populations living in northern, colder sites and *Pgi* allele 4 in southern, warmer sites; alleles 1 and 4 are in relatively equal frequency in populations intermediate in geography and climate (Rank 1992; Dahlhoff et al. 2000). The

presence of adaptive variation in *Pgi* and other genes may become critical to survival of many species, as extreme thermal conditions, which are the result of accelerating climate change, become more frequent in montane and other fragile habitats.

Acknowledgments

We sincerely thank the director and staff of University of California's White Mountain Research Station for providing laboratory and housing facilities during experiments. We also thank Steve Gordon and Beth Sabo for help with field observations, Ben Virzi for assisting C.A.D. and D.H. with the laboratory acclimation experiments, and Gavin McCann for providing invaluable assistance with heat shock protein analyses. We especially thank Dr. Christopher Wheat and three anonymous reviewers for insightful comments on an earlier version of this manuscript. This research was supported by the National Science Foundation (0844404/06 to E.P.D. and N.E.R.), a Santa Clara University Research Grant (E.P.D.), and a Faculty Student Research Assistant Program Award (C.A.D., M.M., and E.P.D.). Fieldwork described here was conducted in the John Muir Wilderness, Inyo National Forest (permit WMD09005P). Insect collections were conducted in accordance with California Department of Fish and Wildlife (Scientific Collecting Permit 004330).

Literature Cited

- Boggs C.L. and D.D. Murphy. 1997. Community composition in mountain ecosystems: climatic determinants of montane butterfly distributions. *Glob Ecol Biogeogr Lett* 6:39–48.
- Brown W.J. 1956. The New World species of *Chrysomela* L. (Coleoptera: Chrysomelidae). *Can Entomol* 88:1–54.
- Chown S.L. and S. Nicolson. 2004. Insect physiological ecology: mechanisms and patterns. Oxford University Press, Oxford.
- Clusella-Trullas S., J.S. Terblanche, and S.L. Chown. 2010. Phenotypic plasticity of locomotion performance in the seed harvester *Messor capensis* (Formicidae). *Physiol Biochem Zool* 83:519–530.
- Dahlhoff E.P., S.L. Fearnley, D.A. Bruce, A.G. Gibbs, R. Stoneking, D.M. McMillan, K. Deiner, J.T. Smiley, and N.E. Rank. 2008. Effects of temperature on physiology and reproductive success of a montane leaf beetle: implications for persistence of native populations enduring climate change. *Physiol Biochem Zool* 81:718–732.
- Dahlhoff E.P. and N.E. Rank. 2000. Functional and physiological consequences of genetic variation at phosphoglucose isomerase: heat shock protein expression is related to enzyme genotype in a montane beetle. *Proc Natl Acad Sci USA* 97:10056–10061.
- . 2007. The role of stress proteins in responses of a willow leaf beetle to environmental temperature variation. *J Biosci* 32:477–488.
- Dillon M.E., L.R.Y. Cahn, and R.B. Huey. 2007. Life history consequences of temperature transients in *Drosophila melanogaster*. *J Exp Biol* 210:2897–2904.
- Fasolo A.G. and R.A. Krebs. 2004. A comparison of behavioural change in *Drosophila* during exposure to thermal stress. *Biol J Linn Soc* 83:197–205.
- Feder M.E. 2010. Physiology and global climate change. *Annu Rev Physiol* 72:123–125.
- Feder M.E., T. Garland Jr., J.H. Marden, and A.J. Zera. 2010. Locomotion in response to shifting climate zones: not so fast. *Annu Rev Physiol* 72:167–190.
- Garland T., Jr., E. Hankins, and R. Huey. 1990. Locomotor capacity and social dominance in male lizards. *Funct Ecol* 4:243–250.
- Gibert P., R.B. Huey, and G. Gilchrist. 2001. Locomotor performance of *Drosophila melanogaster*: interactions among developmental and adult temperatures, age, and geography. *Evolution* 55:205–209.
- Haag C.R., M. Saastamoinen, J.H. Marden, and I. Hanski. 2005. A candidate locus for variation in dispersal rate in a butterfly metapopulation. *Proc R Soc B* 272:2449–2456.
- Hayhoe K., D. Cayan, C.B. Field, P.C. Frumhoff, E.P. Maurer, N.L. Miller, S.C. Moser, et al. 2004. Emissions pathways, climate change, and impacts on California. *Proc Natl Acad Sci USA* 101:12422–12427.
- Hochachka P.W. and G.N. Somero. 2002. Biochemical adaptation: mechanism and process in physiological evolution. Oxford University Press, New York.
- Hoffmann A., J. Sorensen, and V. Loeschcke. 2003. Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. *J Therm Biol* 28:175–216.
- Huey R.B. and J.G. Kingsolver. 1993. Evolution of resistance to high temperature in ectotherms. *Am Nat* 142(suppl.):S21–S46.
- Husak J.F., S.F. Fox, and R.A. Van Den Bussche. 2008. Faster male lizards are better defenders not sneakers. *Anim Behav* 75:1725–1730.
- Kallioniemi E. and I. Hanski. 2011. Interactive effects of *Pgi* genotype and temperature on larval growth and survival in the Glanville fritillary butterfly. *Funct Ecol* 25:1032–1039.
- Karl I., K.H. Hoffmann, and K. Fischer. 2010. Food stress sensitivity and flight performance across phosphoglucose isomerase enzyme genotypes in the sooty copper butterfly. *Pop Ecol* 52:307–315.
- Karl I., S.A. Janowitz, and K. Fischer. 2008. Altitudinal life-history variation and thermal adaptation in the copper butterfly *Lycaena tityrus*. *Oikos* 117:778–788.
- Karl I., T. Schmitt, and K. Fischer. 2008. Phosphoglucose isomerase genotype affects life-history traits and cold stress resistance in a copper butterfly. *Funct Ecol* 22:887–894.
- . 2009. Genetic differentiation between alpine and lowland populations of a butterfly is related to *Pgi* enzyme genotype. *Ecography* 32:488–496.
- Klemme I. and I. Hanski. 2009. Heritability of and strong single gene (*Pgi*) effects on life-history traits in the Glanville fritillary butterfly. *J Evol Biol* 22:1944–1953.

- Lachenicht M.W., S. Clusella-Trullas, L. Boardman, C. Le Roux, and J.S. Terblanche. 2010. Effects of acclimation temperature on thermal tolerance, locomotion performance and respiratory metabolism in *Acheta domesticus* L. (Orthoptera: Gryllidae). *J Insect Physiol* 56:822–830.
- Lane J.E., S. Boutin, M.R. Gunn, and D.W. Coltman. 2009. Sexually selected behaviour: red squirrel males search for reproductive success. *J Anim Ecol* 78:296–304.
- Lane J.E., S. Boutin, J.R. Speakman, and M.M. Humphries. 2011. Energetic costs of male reproduction in a scramble competition mating system. *J Anim Ecol* 79:27–34.
- Loeschcke V., R.A. Krebs, J. Dahlgaard, and P. Michalak. 1997. High-temperature stress and the evolution of thermal resistance in *Drosophila*. *Exp Suppl (Basel)* 83:175–190.
- Lovegrove B.G. 2004. Locomotor mode, maximum running speed, and basal metabolic rate in placental mammals. *Physiol Biochem Zool* 77:916–928.
- Mahroof R., B. Subramanyam, and P. Flinn. 2005. Reproductive performance of *Tribolium castaneum* (Coleoptera: Tenebrionidae) exposed to the minimum heat treatment temperature as pupae and adults. *Entomol Exp Appl* 98:626–633.
- Marden J.H. and R.A. Rollins. 1994. Assessment of energy reserves by damselflies engaged in aerial contests for mating territories. *Anim Behav* 48:1023–1030.
- McMillan D.M., S.L. Fearnley, N.E. Rank, and E.P. Dahlhoff. 2005. Natural temperature variation affects larval survival, development and Hsp70 expression in a leaf beetle. *Funct Ecol* 19:844–852.
- Michalak P., I. Minkov, A. Helin, D.N. Lerman, B.R. Bettencourt, M.E. Feder, A.B. Korol, and E. Nevo. 2001. Genetic evidence for adaptation-driven incipient speciation of *Drosophila melanogaster* along a microclimatic contrast in “Evolution Canyon,” Israel. *Proc Natl Acad Sci USA* 98:13195–13200.
- Mitikka V. and I. Hanski. 2010. Pgi genotype influences flight metabolism at the expanding range margin of the European map butterfly. *Ann Zool Fenn* 47:1–14.
- Mitton J.B. 1997. Selection in natural populations. Oxford University Press, New York.
- Musolin D.L. 2007. Insects in a warmer world: ecological, physiological and life-history responses of true bugs (Heteroptera) to climate change. *Glob Change Biol* 13:1565–1585.
- Neargarder G.G., E.P. Dahlhoff, and N.E. Rank. 2003. Variation in thermal tolerance is linked to phosphoglucose isomerase genotype in a montane leaf beetle. *Funct Ecol* 17:213–221.
- Niitepold K. 2010. Genotype by temperature interactions in the metabolic rate of the Glanville fritillary butterfly. *J Exp Biol* 213:1042–1048.
- Niitepold K., A.D. Smith, J.L. Osborne, D.R. Reynolds, N.L. Carreck, A.P. Martin, J.H. Marden, O. Ovaskainen, and I. Hanski. 2009. Flight metabolic rate and Pgi genotype influence butterfly dispersal rate in the field. *Ecology* 90:2223–2232.
- Partridge L. and M. Farquhar. 1983. Lifetime mating success of male fruitflies (*Drosophila melanogaster*) is related to their size. *Anim Behav* 31:871–877.
- Rank N.E. 1992. A hierarchical analysis of genetic differentiation in a montane leaf beetle (*Chrysomela aeneicollis*). *Evolution* 46:1097–1111.
- Rank N.E., D.A. Bruce, D.M. McMillan, C. Barclay, and E.P. Dahlhoff. 2007. Phosphoglucose isomerase genotype affects running speed and heat shock protein expression after exposure to extreme temperatures in a montane willow beetle. *J Exp Biol* 210:750–764.
- Rank N.E. and E.P. Dahlhoff. 2002. Allele frequency shifts in response to climate change and physiological consequences of allozyme variation in a montane insect. *Evolution* 56:2278–2289.
- Rank N.E., K. Yturralde, and E.P. Dahlhoff. 2006. Role of contests in the scramble competition mating system of a leaf beetle. *J Insect Behav* 19:699–716.
- Saastamoinen M. and I. Hanski. 2008. Genotypic and environmental effects on flight activity and oviposition in the Glanville fritillary butterfly. *Am Nat* 171:701–712.
- Saastamoinen M., S. Ikonen, and I. Hanski. 2009. Significant effects of Pgi genotype and body reserves on lifespan in the Glanville fritillary butterfly. *Proc R Soc B* 276:1313–1322.
- Sartori M., L. Keller, A.G.B. Thomas, and L. Passera. 1992. Flight energetics in relation to sexual differences in the mating behavior of a mayfly, *Siphonurus aestivalis*. *Oecologia* 92:172–176.
- Schulte-Hostedde A.I. and J.S. Millar. 2002. Effects of body size and mass on running speed of male yellow-pine chipmunks (*Tamias amoenus*). *Can J Zool* 80:1584–1587.
- Stevenson R.D. 1985. Body size and limits to the daily range of body-temperature in terrestrial ectotherms. *Am Nat* 125:102–117.
- Watt W.B. 1983. Adaptation at specific loci. II. Demographic and biochemical elements in the maintenance of the *Colias* Pgi polymorphism. *Genetics* 103:691–724.
- . 1992. Eggs, enzymes, and evolution: natural genetic variants change insect fecundity. *Proc Natl Acad Sci USA* 89:10608–10612.
- Watt W.B., P.A. Carter, and S.M. Blower. 1985. Adaptation at specific loci. IV. Differential mating success among glycolytic allozyme genotypes of *Colias* butterflies. *Genetics* 109:157–175.
- Watt W.B., R.C. Cassin, and M.S. Swan. 1983. Adaptation at specific loci. III. Field behavior and survivorship differences among *Colias* Pgi genotypes are predictable from *in vitro* biochemistry. *Genetics* 103:725–729.
- Watt W.B., C.W. Wheat, E.H. Meyer, and J.F. Martin. 2003. Adaptation at specific loci. VII. Natural selection, dispersal and the diversity of molecular-functional variation patterns among butterfly species complexes (*Colias*: Lepidoptera, Pieridae). *Mol Ecol* 12:1265–1275.
- Zizzari Z.V. and J. Ellers. 2011. Effects of exposure to short-term heat stress on male reproductive fitness in a soil arthropod. *J Insect Physiol* 57:421–426.