


DOES NOT
CIRCULATE

Thermal Reaction Norms of Metabolic Scope in Madagascar Hissing Cockroaches

A Senior Thesis Presented to
The Faculty of the Department of Biology,
The Colorado College

By
Kelsey Wise
Bachelors of Arts Degree in Biology
21st day of May, 2012



Dr. Emilie Gray
Primary Thesis Advisor



Dr. Ralph Bertrand
Secondary Thesis Advisor

COLORADO COLLEGE LIBRARY
COLORADO SPRINGS, COLORADO

Introduction

The effects of current climate change on species distributions and survival make up an area under intense investigation. Recent models suggest that the global average surface temperature will increase by 1.8 to 4°C by the year 2100 (Solomon et al., 2007). The ecological impact of such warming is already evident in the effects seen on species fitness (Post et al., 1997), range shifts (Parmesan and Yohe, 2003), species interactions (Hofstetter et al., 2007), and community structure (Sagarin et al., 1999). Ectotherms are particularly sensitive to environmental cues reflecting changes in temperatures because their body temperatures are directly dependent on the ambient temperature around them. They are adapted physiologically and behaviorally to either tolerate thermal stresses or find a way to escape them (Lann et al., 2011). Acclimation is a process where an ectotherm biochemically, physiologically, or behaviorally conforms to its environment. Migration, on the other hand, is an insect response where the animal attempts to find a suitable environment despite temporal fluctuations in a single home range. This includes seasonal movements, such as from the upper slopes of the Sierra Nevada down to California's Central Valley by the convergent ladybird beetle (*Hippodamia convergens*), as well as the transcontinental movement of a monarch butterfly (*Danaus plexippus*) (Gullan and Cranston, 2010).

Physiological limits arise when certain abiotic conditions of an organism's environment become stressful, reducing fitness to the extent that organisms fail to

reproduce and survive (Hoffman, 2009). Ambient temperature is a major abiotic condition that has a significant effect on animal fitness. When ambient temperature changes, it can jeopardize the survival of animals if they are not biologically capable of coping with this change, thus correlating thermal tolerance and fitness. Fitness can be reduced over time by affecting the organism's energy levels, interactions with conspecifics or members of other species, or hindering it from finding food.

There has been heightened attention to both the ways in which thermal tolerance evolves and the rate at which this can take place (Chown et al., 2004). Harmful temperatures are thought to result from insufficient aerobic capacity of mitochondria at low temperatures, and a mismatch between excessive oxygen demand by mitochondria and insufficient oxygen uptake and distribution by ventilation and circulation at high temperatures (Chown et al., 2004). Essentially, this means that animal metabolism is limited at both low and high temperatures. In nature, temperature will rarely climb to a lethal level, however, it may rise to the point where there are still detrimental effects on an individual.

If exposed to stressful conditions, insects may respond in different ways depending on the time-scale of the stress. Animals respond acutely to an unpredictable or rapid stress, through acclimation to a more predictable or longer-term stress, and finally, through evolution to stresses that are maintained over multiple generations. As global temperatures rise, perhaps certain ways various organisms will react and adjust differently, which could ultimately lead either to one of two options:

extinction or adaptation. For a species to endure environmental changes over a long period of time, genetic adjustments through natural selection, genetic drift, and mutation would need to occur. This process would encompass the elimination of unfavorable traits, the retention of beneficial traits, or the appearance of new favorable mutations.

Acute stress requires an immediate response from animals. An example of an acute stress could be an animal being attacked by a predator, or an animal being subjected to a heat wave or a particularly cold night. Acute stress can be paralleled with the 'fight or flight' response, which is when an animal reacts to threats with a general discharge of the sympathetic nervous system, allowing it to prepare for fleeing or a fight. Within the context of climatic changes, the 'flight' aspect is similar to an animal's migration to a friendlier environment. For example, as mentioned in Almeida's study, modern climate change has affected stink bug performance of two pentatomid species, causing them to move northwards and to higher elevations over the last half-century (Almeida et al., 2010). Species with a large geographical range should be less affected by temperature changes, but problems may arise when species that migrate up mountains ultimately have no higher place to go. Most likely the species would then have to adapt to prevent extinction.

An animal that is not able to escape its environment would need to adjust to environmental stresses through phenotypic plasticity, where an animal undergoes metabolic changes and acclimates. Different species have different degrees of

plasticity. While some animals are not able to easily adjust to varying environmental conditions, others have genes that code for a spectrum of enzymes that work at varying temperatures. Levels of plasticity often have much to do with the environment an animal lives in. If the environment has temperatures that fluctuate frequently, an animal is more likely to have higher plasticity than an animal that is used to a constant climate year-round. On a larger scale, phenotypic plasticity is thought to play an important role in organismal diversification and evolutionary innovation (Moczek, 2010). Much analysis concerning the evolutionary consequences of environmental variation is underway to further examine how organisms resist the effects of temperature extremes over many generations, whether this is by evolving increased resistance to unfavorable conditions or by evolving acclimation abilities that allow facultative increases in resistance (Berrigan and Hoffman, 1998). A better understanding of the metabolic adjustments of insects to temperature variation will provide another tool to predict how insects are adjusting to global temperature changes.

Changes in temperature are thought to have significant effects on metabolic rate (Terblanche et al., 2009). Metabolic activity, a measurement of energy used by an animal per unit time, is of central importance to ecology and physiology. Several factors affect metabolic rate, some of which are size, activity, and ambient temperature (Terblanche et al., 2009). The biochemistry of an organism only allows a specific temperature range for the enzymes to work most efficiently. A temperature too low does not fuel metabolic reactions of an organism with sufficient energy, and

the lack of appropriate speed of these reactions prevents survival of the organism. As the temperature increases, enzymes are able to operate at a quicker pace and the metabolic activity increases as well. Ultimately, there will be a temperature that begins to denature proteins, and this results in a plateau of the metabolic rate, as well as eventual death.

The extent to which temperature change over a period of time affects the metabolic rate of ectotherms is undergoing a polarizing debate. The 'Biochemical Adaptation' standpoint argues that adaptation at the macromolecular level can compensate for low body temperatures, while the 'Hotter is Better' hypothesis states that species living at colder temperatures can't evolutionarily overcome the kinetically depressing effects of low temperature, concluding that a cold-adapted species will have lower metabolic scope, and in turn, fitness, than a warm-adapted species.

One way to test these hypotheses is to examine the metabolism of animals acclimated at different temperatures at various acute temperatures. Two important components of insect metabolism, that must be tested individually, are the resting metabolic rate (RMR) and the maximal metabolic rate (MMR). The RMR signifies the minimal metabolic rate an animal requires to function at rest. This includes maintaining respiratory function, brain and nervous system function, posture, and basic needs of resting tissue. The MMR is the highest an animal's metabolic rate can reach. The MMR is reached by stress, excitement, or strenuous activity. The difference between MMR and RMR represents the metabolic scope. Temperature

governs RMR through its effects on rates of biochemical reactions (Gillooly et al., 2001). MMR, like RMR, is speculated to increase with temperature. However, there may be a critical temperature where MMR plateaus as it reaches the point of maximal rate of enzymatic reactions. As this happens, the metabolic scope, or difference between RMR and MMR, could decrease. Essentially, as temperatures increase, the amplitude available for physiological activity may decrease. Unlike RMR, the Q_{10} of the MMR has undergone minimal investigation, as the correlation between MMR and temperature does not display a clear pattern. This indicates that temperature isn't the only factor that increases metabolic rate of an animal undergoing a stress.

In this study, we test the 'Hotter is Better' physiological hypothesis by acclimating Madagascar hissing cockroaches (*Gromphadorhina portentosa*) to several temperatures and measuring metabolic rate, aerobic scope, and running speed as fitness indicators. By measuring CO₂ release rate of the insects at a range of temperatures, we hope to elucidate the effect of thermal acclimation on aerobic scope. The running speeds of animals at different thermal acclimations should provide additional data in support or rejection of the 'Hotter is Better' hypothesis. These findings will allow further understanding of the relationship between RMR and MMR and the effects of temperatures on aerobic scope.

Methods

Animals And Environment

Twelve medium-sized, sub-adult Madagascar hissing cockroaches (*Gromphadorhina portentosa*) were used for experimentation. The cockroaches were obtained from a lab colony at Colorado College. The cockroaches were divided into 2 groups of 6 individuals each, identified by marking the thorax with dots using white-out (Sanford Corp. Bellwood Il). The cockroaches for each group were numbered from 1 to 6. One group of cockroaches (group A) was maintained at 22°C while the other group (group B) was maintained at 28°C, both on a 12L:12D day/night cycle. Both groups were stored in equal-sized plastic containers, watered daily, and fed dog food, apples, lettuce, and cricket feed three times a week. The animals were left to acclimate for about a month.

Measurement of Metabolic Rates

We measured the resting and maximal metabolic rates (RMR and MMR respectively) of each individual cockroach at 16, 22, 28, 34, and 40°C. Metabolic rates were determined by measuring the rate of CO₂ release (V_{CO_2}). Insects were sealed in a metabolic chamber of volume 22 mL, located inside a Peltier-effect temperature control cabinet (Pelt-5, Sable Systems, Las Vegas, NV). The chamber was perfused with air scrubbed of CO₂ and H₂O by passage through 2 silica gel columns and 1

Drierite/Ascarite column. Flow rate was maintained at 200 mL/min using a Side-Trak flowmeter (Sierra Instruments, Monterey, CA) controlled by a mass flow-controller (Sable Systems, Las Vegas, NV). A thermocouple connected to a TC2000 meter (Sable Systems, Las Vegas, NV) was inserted in the tubing immediately upstream of the chamber. The rate of CO₂ release was then measured each second using an infrared gas analyzer (Li-Cor, Lincoln, NE). Data were recorded and analyzed using ExpeData software (Sable Systems, Las Vegas, NV).

Individual cockroaches were sealed in a chamber and then left in the dark for about 40-60 minutes to measure the RMR. When the gas exchange was cyclic, RMR was calculated by taking the lowest continuous average that included a set of ventilation cycles. When gas exchange was not cyclic, the lowest continuous 5-10 minute average was used as RMR.

Following measurement of RMR, we shook the chamber three times for 1 minute, separated by 2 minute intervals to elicit maximal metabolic activity of the insect. To avoid changing the temperature inside the Peltier cabinet and prevent light from getting in, the metabolic chamber was connected to a stick, which was taped to the chamber beforehand and extended out of a hole in the cabinet. After the first 2 shake intervals, the animal was left alone for 2 minutes. Following the third interval, the animal was left alone for 3 minutes before the chamber was disconnected, and a final baseline reading was obtained. An example of a typical run can be seen in Figure 1.

All individuals were weighed at the beginning and the end of the experiment to standardize metabolic rate results.

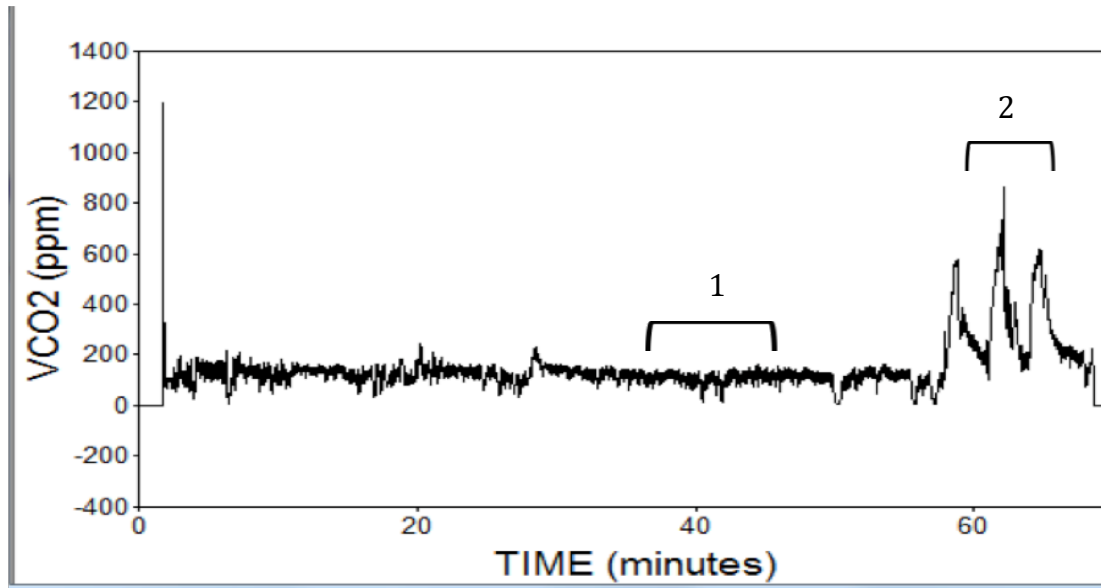


Figure 1: To determine RMR we chose a stable 5-10min portion of the insect's gas exchange pattern once it had been in the chamber for at least 30min (1). The three peaks to the right result from the three shaking bouts, and the highest running average VCO₂ over a 30-second period in this region was used to determine MMR (2).

Running Speed Test

Cockroaches acclimated to 16, 22, and 28° for about a month were left to equilibrate with the ambient temperature within a running arena located inside a water-filled tank maintained at either 15, 23 or 28°C, then prodded to run as fast as they could. Speeds were recorded and analyzed using video software (iHome, Rahway, NJ).

Results

RMR and MMR

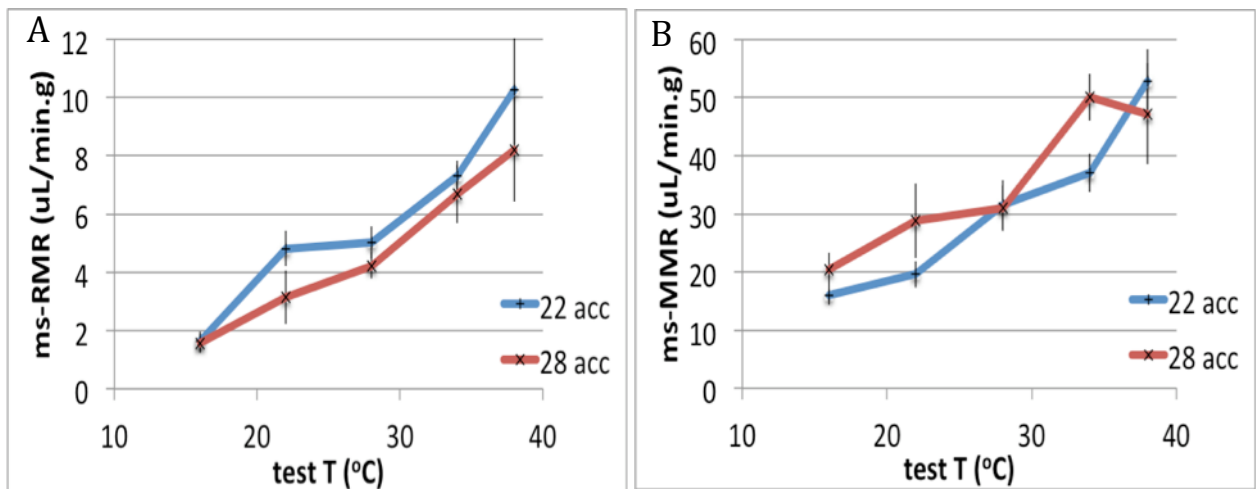


Figure 2: (A) RMR at the 5 test temperatures (16, 22, 28, 34, and 40°) with the blue line representing the 22° acclimated group and red line representing the 28° acclimated group. (B) MMR at the 5 test temperatures (16, 22, 28, 34, and 40°) with the blue line representing the 22° acclimated group and red line representing the 28° acclimated group. Bars represent standard error.

For both acclimation groups, both RMR and MMR increased with temperature (Fig. 2). The Q_{10} of the RMR of the 22° acclimated cockroaches was $2.45 \pm SE$, while the Q_{10} of the MMR of the 28° acclimated cockroaches was $2.41 \pm SE$. The Q_{10} values of the MMR for the cockroaches were $8.52 \pm SE$ and $10.35 \pm SE$ (for 22° acclimated and 28° acclimated, respectively). Although differences observed between acclimation groups were not significant ($p=0.061$ for RMR and $p=0.148$ for MMR) the warm acclimated group showed a left-shifted RMR but a right-shifted MMR relative to the cold acclimated group.

The metabolic scopes of each acclimation group at the various acute temperatures can be seen below in Figures 3. Both the warm- and cold-acclimated groups displayed an increase in metabolic scope with increasing test temperatures. However, the warm-acclimated group had, on average, a higher metabolic scope than the cold-acclimated group ($p < 0.05$).

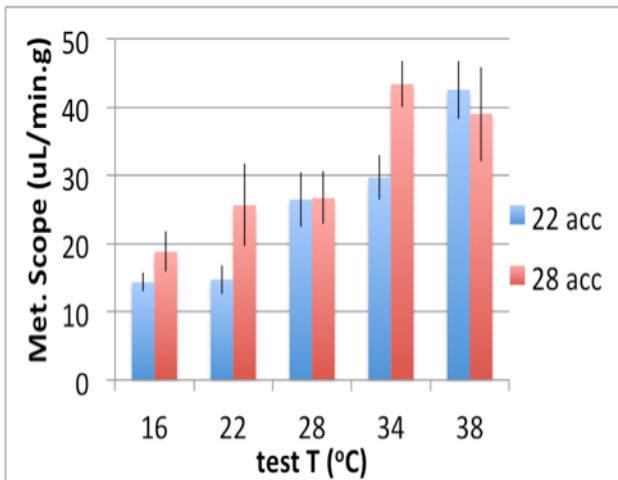


Figure 3: Average metabolic scopes of the 22° and 28° groups at respective acute temperatures.

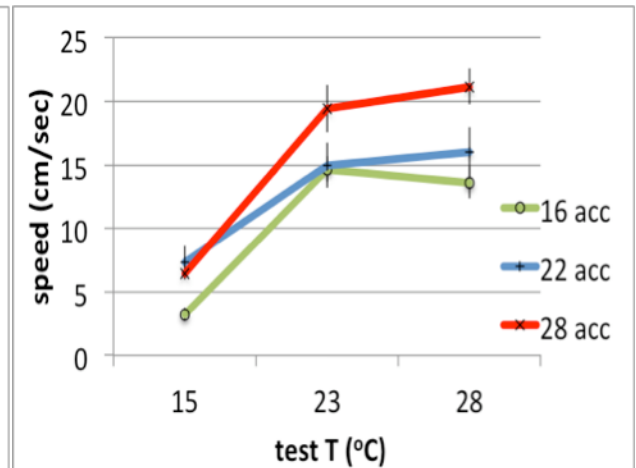


Figure 4: Average running speed (cm/s) of the 3 acclimated groups at 3 test temperatures (16, 22, and 28°).

By using a 2-way ANOVA for the average running speed of cockroaches at the three different acclimation temperatures (Fig. 4), we determined that the higher the test temperature, the faster all animals were able to run ($p < 0.001$). In addition, the higher the animal's acclimation temperature, the faster they were able to run ($p < 0.001$). Finally, we found that there was no effect of acclimation on how the animals respond to acute temperature change ($p = 0.21$).

Discussion

Ectothermic animals that are subjected to a new thermal environment for an extended time period can compensate for this change by adjusting their metabolism. However, the extent to which these compensatory biochemical changes can counteract the thermodynamic effects of the shift in body temperature has been subject to debate. When the animal's body temperature is lowered, can it eventually compensate for the slowing of internal biochemical processes? This question is the foundation for the 'Hotter is Better' hypothesis, which states that organisms acclimated to higher temperatures show increased maximal performance and is based on the premise that biochemical changes cannot compensate fully for the thermodynamic effects of lowered temperature.

Metabolic scope and running speed are both components that logically are thought to influence the fitness of an organism. A higher metabolic scope allows an organism to conserve energy when inactive (lower RMR), in order to save energy for reproduction. On the other end, a higher MMR allows animals' enzymes to work at higher rates than cold acclimated animals in order to, for example, evade a predator.

When comparing 22 and 28°C acclimated animals, we found the RMR of the warm-acclimated group to be right-shifted compared with that of the cold-acclimated group. Terblanche et al. (2005) conducted research that paralleled ours regarding resting metabolic data, as they found that the RMR of cooler acclimated adult *G.*

morsitans morsitans was left-shifted compared with that of the warmer acclimated group. This trend implies that cold-acclimated animals must exert more biochemical energy, at each temperature for their enzymes to be as active as warm-acclimated animals.

Metabolic scope, on the other hand, is an area of research that has been minimally investigated. When we compared the thermal reaction norms of maximal metabolic rate (induced by shaking the animals), we found that the cold-acclimated group was right-shifted compared to the warm-acclimated group. The resultant metabolic scope of the warm acclimated group was higher than that of the cold acclimated group, providing support for the 'Hotter is Better' hypothesis. In addition, the warm acclimated insects performed better in all conditions of the running speed test, which also supported the 'Hotter is Better' hypothesis.

The 'Hotter is Better' hypothesis proposes that genotypes or species with comparatively high optimal temperatures also have relatively high maximal performance or fitness (Hamilton, 1973; Bennett, 1987; Savage et al., 2004; Frazier et al., 2006). It is based on the thermodynamic argument that, on a biochemical level, enzyme kinetics are faster as the external temperature increases (up to a critical limit). Evidence suggests that enzymes may never be able to compensate in cooler temperatures and match the kinetic rate of corresponding enzymes that exist at warmer temperatures, supporting the notion that metabolic activity is temperature-dependent. Also, a larger metabolic scope, which was the extent of

phenotypic plasticity displayed in our warm-acclimated experimental group, further supports the 'Hotter is Better' hypothesis. When an animal is at rest, the ability to maintain a lower metabolic rate is important for conserving energy that will be used for reproduction. On the contrary, a higher metabolism allows an animal to have speed and therefore evade a predator, increasing the individual's chances of producing viable offspring. On a larger scale, population growth rate would be affected by altering acclimation temperatures, because lower enzyme activity as a result of lower temperatures leads to relatively lower individual growth and development rates, and thus relatively longer development and generation times (Kingsolver and Huey, 2008). Longer development time increases generation time, which may decrease fecundity or mating success and ultimately decrease population growth.

While metabolic scope and running speed can be considered valid representations of fitness, it would ultimately be most interesting to study the effect of thermal acclimation on life long fecundity, as fecundity success is the ultimate gauge of fitness. If the species being examined is divided into cold-adapted and warm-adapted populations, the 'Hotter is Better' hypothesis would be supported if the population growth rate increases with increasing temperature. Long-term results would show decreased population growth over time in the colder environment. From these results, connections to broad scale ecological patterns could be inferred.

The possible connections between physiology and macroecology are plentiful and contrasting, though most of these ideas are just beginning to develop (Clarke, 2003). These proposals ultimately involve the relationship between temperature and the cost of living. Periods of climate change in geographical history are believed to have altered the fitness and even survival of different species, particularly rapid climate changes, because of the biochemical instability it can cause on individuals (Clarke, 2003). By fine-tuning our understanding of temperature change on metabolism at a small scale and how organelles, organ systems, and individual organisms are affected, we can start to formulate broad connections between physiology and ecology at a population level, the area with highest uncertainty. Understanding how populations of organisms respond to environmental cues is an important topic that will allow us to link the range of an organism and the role of ecological physiology at the assemblage scale. These answers may give clues as to how species, and perhaps sub-species populations, will react to long-term climate change, and ultimately fill the holes in the biochemical-ecological bridge.

Literature Cited

- Almeida R. P. P., S. S. Prado, K. Y. Hung, and M. P. Daugherty. 2010. Indirect Effects of Temperature on Stink Bug Fitness via Maintenance of Gut-Associated Symbionts. *Appl. Environ. Microbiol.* 76(4): 1261-1266.
- Bennett, A.F. 1987. Evolution of the control of body temperature: is warmer better? In *Comparative Physiology: Life in Water and on Land* (P. Dejours, C.R. Taylor and E.R. Weibel, eds.), pp. 421–431. Padova, Italy: Liviana Press.
- Berrigan D. and A. A. Hoffman. 1998. Correlations between measures of heat resistance and acclimation in two species of *Drosophila* and their hybrids. *Biological Journal of the Linnean Society* 64: 449-462.
- Chown S. L., C. J. Klok, and B. J. Sinclair. 2004. Upper thermal tolerance and oxygen limitation in terrestrial arthropods. *J. Exp. Biol.* 207: 2361-2370.
- Clarke, A. 2003. Costs and consequences of evolutionary temperature adaptation. *Trends in Ecology and Evolution* 18(11): 573-581
- Frazier, M., Huey, R.B. and Berrigan, D. 2006. Thermodynamics constrains the evolution of insect population growth rates: 'warmer is better'. *Am. Nat.*, 168: 512–520.
- Gillooly, J. F., J. H. Brown, G. B. West, V. M. Savage, and E. L. Charnov. 2001. Effects of Size and Temperature on Metabolic Rate. *Science* 293: 2248-2251
- Gullan P. J. and P. Cranston. 2010. *The Insects: An Outline of Entomology*. Fourth edition Wiley-Blackwell: Oxford, UK
- Hamilton, W.J. 1973. *Life's Color Code*. New York: McGraw-Hill.
- Hoffman A. A. 2009. Physiological climatic limits in *Drosophila*: patterns and implications. *J. Exp. Biol.* 213: 870-880.
- Hofstetter R.W., T.D. Dempsey, K.D. Klepzig, and M.P. Ayres. 2007. Temperature-dependent effects on mutualistic, antagonistic, and commensalistic interactions among insects, fungi and mites. *Community Ecol.* 8:47
- Kingsolver, J. G. and Huey, R. B. 2008. *Evolutionary Ecology Research* 10: 251-268

- Lann C. L., O. Reux, N. Serain, J. J. M. Van Alphen, P. Vernon, and J. Van Baaren. 2011. Thermal tolerance of sympatric hymenopteran parasitoid species: does it match seasonal activity? *Physiological Entomology* 36: 21-28.
- Moczek A. P. 2010. Phenotypic plasticity and diversity in insects. *Phil. Trans. R. Soc. B.* 365: 593-605.
- Parmesan C. and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421: 37-42
- Post E., N. C. Stenseth, R. Langvatn, and J.M. Fromentin. 1997. Global climate change and phenotypic variation among red deer cohorts. *Proc. Boil. Sci.* 264: 1317-1324
- Sagarin R. D., J. P. Barry, S. E. Gilman, and C. H. Baxter. 1999. Climate-related change in an intertidal community over short and long time scales. *Ecol. Monogr.* 69: 465-490.
- Savage, V.M., Gillooly, J.F., Brown, J.H., West, G.B. and Charnov, E.L. 2004. Effects of body size and temperature on population growth. *Am. Nat.*, 163: 429-441.
- Solomon S., D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller, 2007. Summary for Policymakers. In: *Climate Change 2007: the physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press, Cambridge, United Kingdom
- Terblanche J. S., S. Clusella-Trullas, J. A. Deere, B. J. Van Vuuren, and S. L. Chown. 2009. Directional Evolution of the Slope of the Metabolic Rate-Temperature Relationship Is Correlated with Climate. *Physiological and Biochemical Zoology* 82(5): 495-503.
- Terblanche J. S., C. J. Klok, and S. L. Chown. 2005. Temperature-dependence of metabolic rate in *Glossina morsitans morsitans* (Diptera, Glossinidae) does not vary with gender, age, feeding, pregnancy or acclimation. *Journal of Insect Physiology* 51(8): 861-870.