The Influence of Sugar Concentration on Bumblebee Thermoregulation: Implications Under Climate Change

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Abstract

Bumblebees have the physiological ability to actively transfer heat from their thorax to their abdomen as a means of managing heat excess. This ability to thermoregulate enables them to occupy a wide range thermal environments. Bumblebees feature an active physiological heat transfer mechanism that is well documented, but the extent to which the sugar concentration of nectar influences the effectiveness and limitations of this mechanism is understudied.

I asked if sugar concentration mediates this thermoregulatory mechanism in *Bombus melanopygus,* the black-tailed bumblebee. I found that under heat stress thermoregulatory ability increased after bees consumed highly concentrated sugar solutions. Plants grown under high heat or drought have been shown to produce nectar with low sugar concentrations and both temperature and drought are predicted to increase under climate change. This study evaluates nutritional and climactic factors that may influence the decline of bumblebee populations from a physiological perspective. Investigating how energy resources affect bumblebee thermoregulatory process will enhance our understanding of how bumblebees will adapt to a changing climate.

Introduction

Bumblebees (Apidae) are cosmopolitan insects that serve as global pollinators. They are heterothermic and require a constant supply of readily available energy to maintain an optimal temperature relative to that of their surroundings (Heinrich, 1993).

In a classic experiment on *Bombus vosnesenskii*, Bernd Heinrich (1976) investigated the physiological mechanisms for thermoregulatory heat dissipation. He inserted electrical thermocouple probes into bees to measure temperature fluctuations together with dorsal and diaphragm activity. When heat was applied to the thorax of a live bee, its dorsal vessel and ventral diaphragm produced coordinated pulsations that stimulated the circulatory system to actively pump hemolymph across the petiole into the abdomen. The counter-current pumping of heated hemolymph from the thorax to the abdomen enabled the bee to reduce thoracic temperature by means of convective heat dissipation via the ventral side of its abdomen. When heat was applied to the thorax of recently dead bees or bees with an obstructed petiole there was only a minor transfer of heat to the abdomen -- likely due to passive heat transfer. Heinrich elegantly demonstrated that bumblebees have the physiological ability to actively transfer heat from their thorax to their abdomen as a means of managing excess body heat (Heinrich 1976).

The ability to thermoregulate enables bumblebees to occupy a wide range thermal environments including tropical, desert and arctic zones (Potapov et al., 2014; Roberts & Harrison, 1999; Stone et al., 1999). Most metabolic heat is produced in the thorax of flying insects where the wing muscles are located (McCallum et al., 2013; Heinrich,

1993) The physiological regulation of thoracic temperature is essential for activating and maintaining flight in bumblebees (Gilmour & Ellington, 1993; Mapalad, Leu, & Nieh, 2008). Insect flight is energetically expensive and requires a high metabolic rate which in turn produces a large amount of heat byproduct. A bumblebee in flight is able to produce enough internal heat to forage in cooler conditions compared to non-heterothermic insects (Heinrich 1993). If ambient temperatures are high and if/or a flying bee is subject to an external heat source (such as solar radiation) a bumblebee must dissipate heat through its abdomen to maintain the narrow range of temperature where it will not overheat. In this paper I will focus on the counter-current heat exchange mechanism present in bumblebees. This highly adaptive thermoregulatory process is critical to bumblebee thermoregulation and brood incubation (Heinrich, 1975b; Heinrich 1993).

The main energy source for adult foraging bees is primarily simple carbohydrates (sugars) found in nectar (Crailsheim, 1993; Paoli et al., 2014). Flowers in nature will have nectar that varies from about 10% to 80% sugar (Heinrich, 1975a). Most bee species will visit flowers with a wide range of nectar concentrations. However, lab experiments show that when given a choice, *Bombus spp.* prefer highly concentrated nectar (Ashbacher, in prep; Konzmann & Lunau, 2014). Highly concentrated nectar has more energetic value per unit weight than less concentrated nectar. Optimal foraging theory states that pollinator foraging patterns should be dictated by the quality of floral resources. A pollinator must balance the energy it gains from consuming food with the energy it loses foraging for food (Charnov, 1976). The consumption of highly concentrated viscous nectar would allow a bee forage for pollen for longer periods of time before having to feed at another flower.

The active heat transfer mechanism of bumblebees is well documented (Heinrich, 1976; Heinrich, 1993; Chown et. al, 2004), but the extent to which nutritional quality influences the effectiveness and limitations of this mechanism is understudied. Thoracic temperatures increase for the bumblebee *Bombus wilmatta* in response to higher sugar concentrations (Nieh et al., 2006). Similar responses were found for two other Apidae bees (Nieh & Sánchez, 2005; Schmaranzer & Stabentheiner, 1988; Underwood, 1991), suggesting this phenomenon may be common among all Apidae members. Given the effect of sugar concentration on thoracic temperature, it follows that sugar concentration could also affect thermoregulatory capacity. No study to date has examined the interaction between nutritional quality, thermoregulatory ability, and heat stress, a scenario that bumblebees are likely to be exposed to in the coming years.

Global temperatures are predicted to rise in the coming decades, while the American west in particular is possibly facing an unprecedented mega-drought (Cayan et al., 2007; Cook et al., 2015; Rahmstorf et al., 2012). High water and heat stress, typical of drought have the potential to decrease flower abundance and the sugar concentration of floral nectar (McLaughlin & Boyer, 2004; Wyatt et al., 2016; Carroll et al., 2008). Energy resources regulate many essential processes for bees (McCallum et al., 2013; Nieh et al., 2006; Nieh & Sánchez, 2005) and temperatures often limit the activity of bees (Cooper et al., 1985; Vicens & Bosch, 2000). Thus the physiological regulation of body temperature is a vital process that will determine a bees thermoregulatory ability to adapt and function in its environment.

A decline of nectar sugar concentration is a plausible outcome of increasing temperatures and decreasing water inputs. Climate mediated temperature shifts could lead to a decline of bumblebee populations by exposing them to a dangerous combination of higher radiative temperatures and lower quality resources (Al-Ghzawi et al., 2009; Asif & Shaheen, 2011; Hegland et al., 2009). Here I investigate to what extent the quality (sugar concentration) of nectar influences thermoregulation in bumblebees. I assessed the ability of *Bombus melanopygus (*Nylander, 1848*)* to transfer excess heat from its thorax to its abdomen after the consumption of various sugar solutions. The overarching question of this study is: Do *Bombus melanopygus* individuals that consume low quality nectar thermoregulate less efficiently than individuals that consume high quality nectar? Specifically, I hypothesize that *Bombus melanopygus* individuals that consume more dilute sugar solutions will transfer excess heat from their thorax to their abdomen at lower rates than those that consume more highly concentrated sugar solutions.

Materials and Methods

<u>Experimental Species</u>

Bombus melanopygus, the black-tailed bumblebee can be found throughout most of California, Oregon, Washington, British Colombia and Alaska. It is also found in the highland deserts of Arizona, New Mexico, Utah and Nevada. Populations of *B. melanopygus* are found sporadically in the northern great planes and populations occur as far north as the Artic circle (Hatfield *et al.* 2014). *B. melanopygus* forages on a wide variety of flowers (Hatfield *et al.* 2014) and inhabits an assortment of habitat types including agricultural and urban areas. It’s size ranges from 12 to 17mm.

Trial preparation:

I caught bees during the Spring of 2016 at the UCSC arboretum in Santa Cruz, CA. Each day I captured 5-10 *B. melanopygus* workers and quickly transported them to the lab for testing. I misted bees with water and individually held them in a glass chamber for one hour without access to food. Misting encouraged bees to remain active as they dried off. Constant movement enabled them to consume any relictual nectar in their crop, thereby expending surplus energy left from free foraging in the field. After one hour, bees were placed in feeding chambers and haphazardly assigned a known sugar solution treatment presented in an artificial flower. Artificial flowers were constructed from a small piece of sponge and local bee pollen. Sugar solutions were deionized water mixed with Karo® syrup. I mixed fresh sugar solutions daily and I confirmed sugar concentrations with a handheld refractometer (Eclipse® Brix 50 low volume; Xylem, Lawrenceville, GA).

Nectar Quality Treatments:

I presented bees with one of five sugar concentrations: 0%, 15%, 35%, 55%, 75% (n=16 per treatment). All five treatments were tested each trial day in haphazard fashion with up to two replicates per treatment per day. A total of 80 bees were used for this experiment. Each treatment was replicated 16 times with a new bee each time.

Bees fed freely until they stopped on their own. After feeding they were relaxed for 30 seconds with a mild dose of ethyl acetate after which they were then fastened to a Styrofoam pad by cross-pinning around their petiole. A piece of aluminum sheeting was then positioned around the petiole to shield the abdomen from the heat application to the thorax. Thus, I ensured that subsequent recorded abdominal temperatures were the result of active heat transfer from the thorax and not the result of radiative heat or passive heat transfer.

Heat application:

I applied heat to the thorax of bees with a blue heat lamp (25 watt incandescent bulb) positioned 2 inches away from the thorax. Heat was continuously applied to the thorax over a period of 5 minutes. Thoracic (T_{th}) and abdominal (T_{ab}) temperatures were simultaneously recorded every 30 seconds using a pair of infrared temperature thermometers.

Analysis

Results were analyzed using R statistical software (R Foundation for Statistical Computing, 2014). I calculated the difference between T_{th} and T_{ab} as (T_d). I used T_d as a metric for evaluating heat transfer for bees, with higher values indicating a greater temperature difference between the thorax and abdomen and thus less heat transfer. Our response variable was the average difference between T_{th} and T_{ab} (T_d) from 30 seconds to 5 minutes for each trial. Percent sugar treatment was our explanatory variable. Trial was treated as a random factor in a linear mixed effects model using a two-way analysis of variance (Anova) with type III sums of squares. I used Tukey tests to evaluate specific differences between individual treatments, and to determine saturation points for each treatment by comparing T_d at each 30 second interval of the trials.

Results

Higher sugar concentrations increased the thermoregulatory ability (lower T_d) of live bees experiencing heat stress (Fig. 1, $F=96.34_{(4,60)}$ $p < 0.001$). Sugar concentration did not influence baseline thoracic temperatures ($F=1.45_{(4,75)}$, $P=0.23$) and individual bee baseline thoracic and abdominal temperatures were similar (± °C). T_d increased for the first 30 seconds of each trial and then stabilized, regardless of treatment (Fig. 2, $F=2.88_{(19,50)}$, p=.008).

Bees that consumed the least concentrated sugar solutions, 0% and 15%, had the highest T_d values and T_d did not differ between them (Tukey p=0.67). T_d then decreased with each subsequent rise in sugar concentration (35%-75%) indicating that T_{th} and T_{ab} were more similar in temperature. The 75% sugar treatment had the lowest average T_d (2.88 °C) while 0% sugar treatment had the highest (4.83 °C).

Thoracic temperature in dead bee controls continuously increased for the duration of heat trials, however there was not a corresponding rise in abdominal temperature. Bees that were alive were able to eventually maintain a constant T_d regardless of sugar concentration, while dead bees showed no ability to maintain a continuous T_d. As a result, T_d was highest for dead bee controls compared to live bees (Fig. 1,Tukey p=0.67)

Discussion

Heat transfer from thorax to abdomen of *B. melanopygus* was greatest for bees that had consumed highly concentrated sugar solutions. Thus, bumblebees should thermoregulate most efficiently under heat stress after consuming highly concentrated floral nectar. Studies show that plants grown under high heat or drought conditions produce dilute nectar (Pacini et al., 2003; Scaven & Rafferty, 2013). Temperature and drought are predicted to increase in the coming decades(Cayan et al., 2007). Consequently, under a climate change scenario, bumblebees may be trapped in a "two-sided climate vise" where higher temperatures increase their energetic needs while simultaneously reducing the quality of energy available to them in nectar.

Energy rewards serve as the co-evolutionary currency that maintain plant-pollinator interactions (McCallum et al., 2013; Mitchell et al., 2009). The consumption of highly concentrated viscous nectar would allow a bumblebee to maximize its energy intake and thus forage most efficiently. Highly concentrated floral nectar would in theory be optimal for maintaining high energy expenditure activities such as foraging, brood incubation and thermoregulation. However, bumblebees would find it increasingly difficult to fulfill their energetic needs as nectar quality declines with climate stress.

Transferring heat from the thorax to the abdomen is a vital physiological process that enables bumblebees to adapt to a given thermal environment (Heinrich, 1975b; Heinrich, 1993). Thermoregulation is also essential for reproduction as it is an indispensable aspect of brood incubation (Heinrich 1993). The results of this study show that sugar concentration plays a role in regulating the counter-current heat exchange mechanism in bumblebees. Bumblebees with only access to dilute nectar will experience a reduction in convective heat dissipation from their abdomen and may be more likely to overheat over a shorter period of time. Furthermore, bumblebees also experience a reduced ability to incubate a vigorous brood; larval development is largely influenced by diet (Burkle & Irwin, 2009; Kim & Thorp, 2001) and requires an active transfer of heat analogous to the thermoregulatory process. A Bumblebee's ability to forage, thermoregulate, and incubate the brood is limited by nectar concentration which in turn is sensitive to temperature and moisture inputs. Accordingly, changing these inputs will have a direct impact on processes crucial to bumblebee livelihood.

Bumblebees are important pollinators of native plants and have been shown to be exceptional pollinators of a number of crops (Rao & Stephen, 2010). They are keystone species that maintain global biodiversity and support vital ecosystem function in most terrestrial systems(Memmott et al., 2007; Parmesan, 2006). Evidence suggests climate-mediated declines in pollinator diversity (Kluser & Peduzzi, 2007; Potts et al., 2010), and also parallel declines in many of the flowering plants that rely on them to be pollinated (Miller-Struttmann et al., 2015). Furthermore, as their habitat contracts, bumblebees are failing to migrate northward to cooler temperatures prompting dramatic species loss along the southern reaches of their habitat (Kerr et al., 2012). Pollinator decline could have a large scale impact on the natural environment and world food production (Hegland et al., 2009; Potts et al., 2010). The relationship between climate change and a decline in bumblebee species has been established (Kerr et al., 2012; Miller-Struttmann et al., 2015). This study illustrates nutritional and climactic factors that may influence the decline of bumblebee populations from a physiological perspective.

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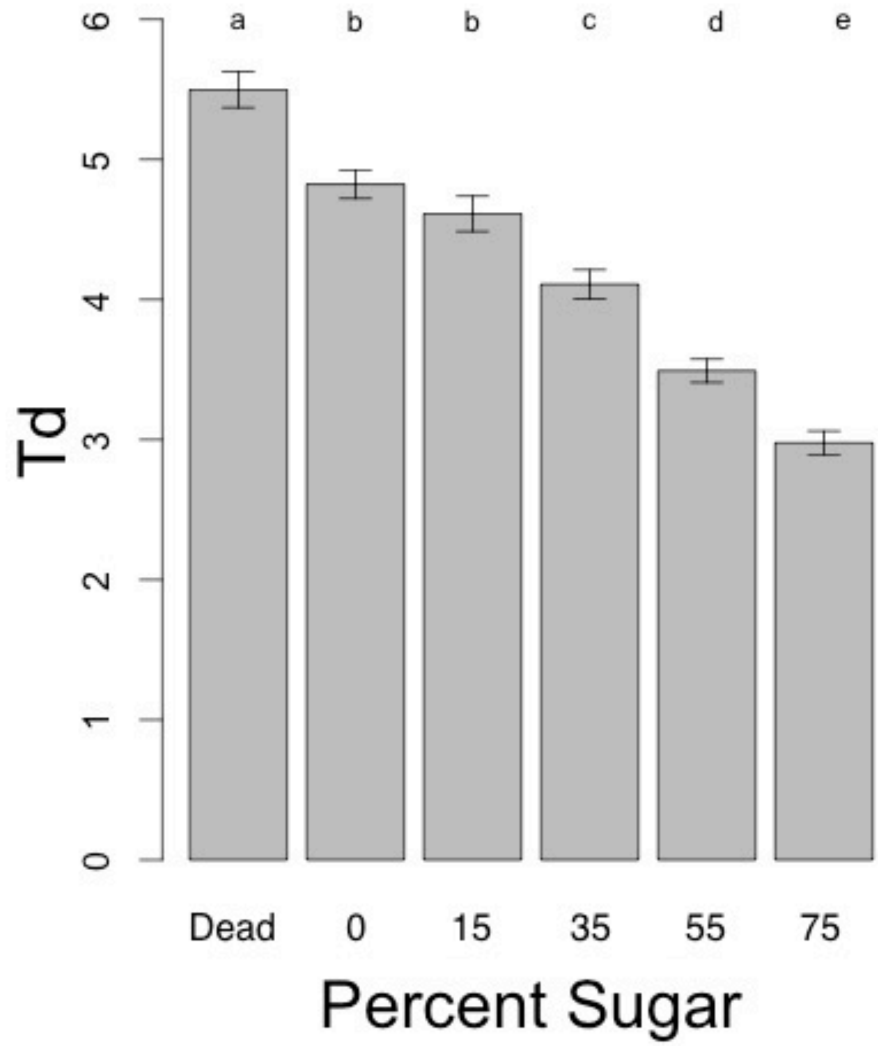

Figure 1. T_d of bumblebees consuming sugar solutions (0%, 15%, 35%, 55%, 75% glucose). Error bars indicate ±1 SE. Data collected in Santa Cruz County, CA.

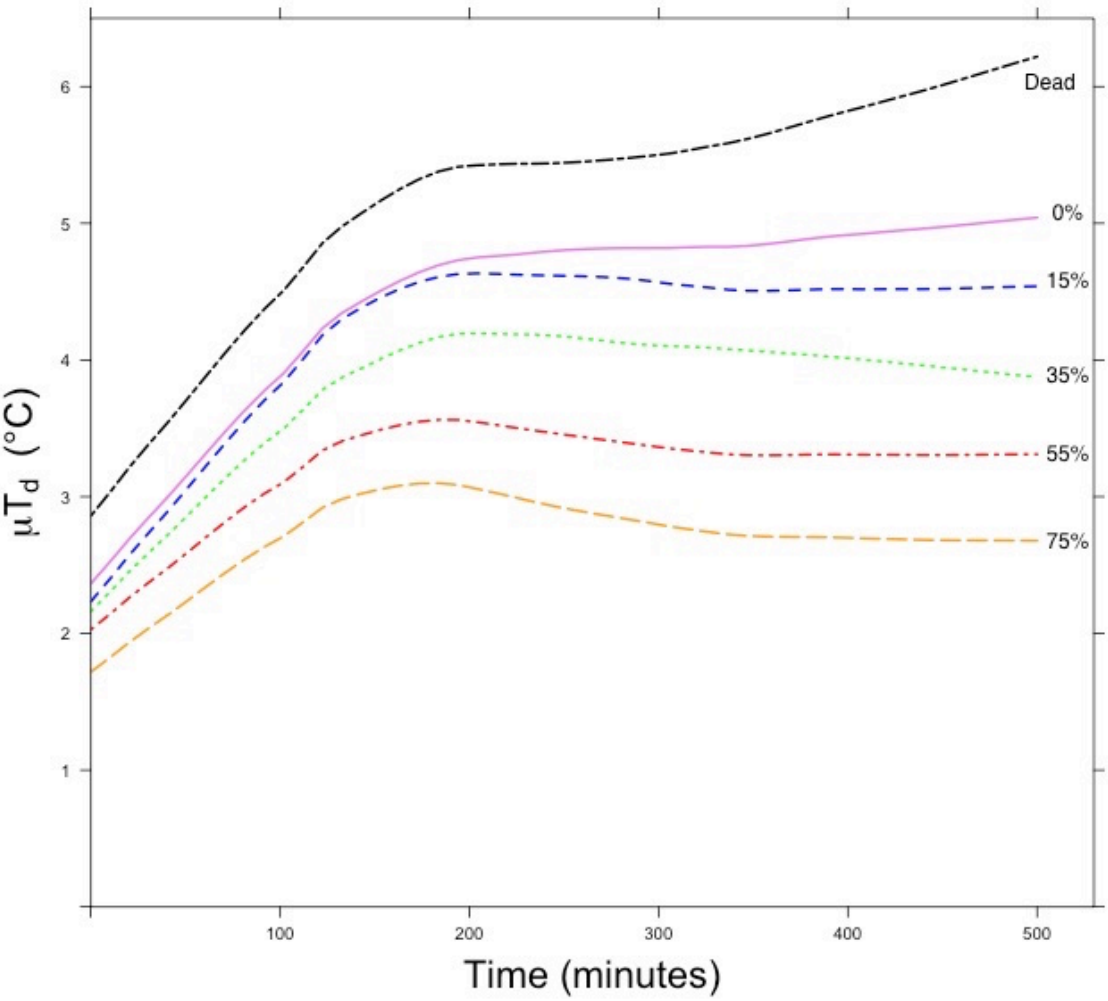

Figure 2. LOESS model fit for T_d of bees consuming sugar solutions over a period of five minutes. Error bars indicate ±1 SE. Data collected in Santa Cruz County, CA.

References:

Al-Ghzawi, A. A.-M., Zaitoun, S., Gosheh, H., & Alqudah, A. (2009). Impacts of drought on pollination. *Archives of Agronomy and Soil Science*, *55*(February 2015), 683–692.

Asif, M., & Shaheen, T. (2011). Alternative Farming Systems, Biotechnology, Drought Stress and Ecological Fertilisation. *Media*, *6*(1), 39–76.

Burkle, L., & Irwin, R. (2009). Nectar sugar limits larval growth of solitary bees (Hymenoptera: Megachilidae). *Environmental Entomology*, *38*(4), 1293–1300.

Carroll, A. B., Pallardy, S. G., Galen, C., American, S., & Pallardy, G. (2008). Drought Stress , Plant Water Status , and Floral Trait Expression, *88*(3), 438–446.

Cayan, D. R., Maurer, E. P., Dettinger, M. D., Tyree, M., & Hayhoe, K. (2007). Climate change scenarios for the California region. *Climatic Change*, *87*(1 SUPPL).

Charnov, E. L. (1976). Optimal foraging, the marginal value theorem. *Theoretical Population Biology*. http://doi.org/10.1016/0040-5809(76)90040-X

Cook, B. I., Ault, T. R., & Smerdon, J. E. (2015). Unprecedented 21st century drought risk in the American Southwest and Central Plains. *Science Advances*, *1*(February), 1–7. http://doi.org/10.1126/sciadv.1400082

Cooper, P. D., Schaffer, W. M., & Buchmann, S. L. (1985). Temperature Regulation of Honey Bees (Apis mellifera) Foraging in the Sonoran Desert. *J. Exp. Biol.*, *114*, 1–15.

Crailsheim, K. (1993). Glucose Utilization During Flight of Honeybee (Apis mellijkra) Workers , Drones and Queens, *39*(1), 959–967.

Hegland, S. J., Nielsen, A., L??zaro, A., Bjerknes, A. L., & Totland, ??rjan. (2009). How does climate warming affect plant-pollinator interactions? *Ecology Letters*, *12*(2), 184–195.

Heinrich, B. (1975a). Energetics of pollination.

Heinrich, B. (1975b). Thermoregulation in bumblebees. *Journal of Comparative Physiology*, *96*(2), 155–166.

Heinrich, B. (1976). Heat exchange in relation to blood flow between thorax and abdomen in bumblebees. *The Journal of Experimental Biology*, *64*(3), 561–85.

Kerr, J. T., Pindar, A., Galpern, P., Packer, L., Potts, S. G., Roberts, S. M., … Gall, L. F. (2012). Climate change impacts on bumblebees converge

across continents, *349*(6244), 177–180.
Kim, J. yoon, & Thorp, R. W. (2001). Maternal investment and size-number trade-off in a bee, Megachile apicalis, in seasonal environments. *Oecologia*, *126*(3), 451–456.
Kluser, S., & Peduzzi, P. (2007). Global pollinator decline: a literature review. *UNEP/GRID Europe*, (September), 10 pp.
Konzmann, S., & Lunau, K. (2014). Divergent rules for pollen and nectar foraging bumblebees - A laboratory study with artificial flowers offering diluted nectar substitute and pollen surrogate. *PLoS ONE*, *9*(3).
Mapalad, K. S., Leu, D., & Nieh, J. C. (2008). Bumble bees heat up for high quality pollen. *The Journal of Experimental Biology*, *211*(Pt 14), 2239–42.
McCallum, K. P., McDougall, F. O., & Seymour, R. S. (2013). A review of the energetics of pollination biology. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, *183*(7), 867–876.
McLaughlin, J. E., & Boyer, J. S. (2004). Sugar-responsive gene expression, invertase activity, and senescence in aborting maize ovaries at low water potentials. *Annals of Botany*, *94*(5), 675–689.
Memmott, J., Craze, P. G., Waser, N. M., & Price, M. V. (2007). Global warming and the disruption of plant-pollinator interactions. *Ecology Letters*, *10*(8), 710–717.
Miller-Struttmann, N. E., Geib, J. C., Franklin, J. D., Kevan, P. G., Holdo, R. M., Ebert-may, D., … Galen, C. (2015). Functional mismatch in a bumble bee pollination mutualism under climate change, *78*(2010), 75–78.
Mitchell, R. J., Irwin, R. E., Flanagan, R. J., & Karron, J. D. (2009). Ecology and evolution of plant-pollinator interactions. *Annals of Botany*, *103*(9), 1355–1363.
Nieh, J. C., León, A., Cameron, S., & Vandame, R. (2006). Hot bumble bees at good food: thoracic temperature of feeding Bombus wilmattae foragers is tuned to sugar concentration. *The Journal of Experimental Biology*, *209*(Pt 21), 4185–4192.
Nieh, J. C., & Sánchez, D. (2005). Effect of food quality, distance and height on thoracic temperature in the stingless bee Melipona panamica. *The Journal of Experimental Biology*, *208*(Pt 20), 3933–3943.
Pacini, E., Nepi, M., & Vesprini, J. L. (2003). Nectar biodiversity: a short review. *Plant Systematics and Evolution*, *238*(1), 7–21.
Paoli, P. P., Donley, D., Stabler, D., Saseendranath, A., Nicolson, S. W., Simpson, S. J., & Wright, G. A. (2014). Nutritional balance of essential

amino acids and carbohydrates of the adult worker honeybee depends on age. *Amino Acids*, *46*(6), 1449–1458.
Parmesan, C. (2006). Ecological and Evolutionary Responses to Recent Climate Change, 637–671.
Potapov, G. S., Kolosova, Y. S., & Gofarov, M. Y. (2014). Zonal distribution of bumblebee species (hymenoptera, apidae) in the North of European Russia. *Entomological Review*, *94*(1), 79–85.
Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Global pollinator declines: Trends, impacts and drivers. *Trends in Ecology and Evolution*.
R Foundation for Statistical Computing. (2014). R: a language and environment for statistical computing. Vienna, Austria: R Development Core Team.
Rahmstorf, S., Foster, G., & Cazenave, A. (2012). Comparing climate projections to observations up to 2011. *Environmental Research Letters*, *7*(4), 044035.
Rao, S., & Stephen, W. P. (2010). Abundance and diversity of native bumble bees associated with agricultural crops: The Willamette valley experience. *Psyche*, *2010*.
Roberts, S., & Harrison, J. (1999). Mechanisms of thermal stability during flight in the honeybee apis mellifera. *The Journal of Experimental Biology*, *202 (Pt 11*, 1523–33. Retrieved from
Scaven, V. L., & Rafferty, N. E. (2013). Physiological effects of climate warming on flowering plants and insect pollinators and potential consequences for their interactions, *59*(3), 418–426.
Schmaranzer, S., & Stabentheiner, A. (1988). Variability of the thermal behavior of honeybees on a feeding place. *Journal of Comparative Physiology B*, *158*(2), 135–141.
Stone, G. N., Gilbert, F., Willmer, P., Potts, S., Semida, F., & Zalat, S. (1999). Windows of opportunity and the temporal structuring of foraging activity in a desert solitary bee. *Ecological Entomology*, *24*(2), 208–221.
Underwood, B. A. (1991). Thermoregulation and energetic decision-making by the honeybees Apis cerana, Apis dorsata and Apis laboriosa. *J. Exp. Biol.*, *157*, 19–34.
Vicens, N., & Bosch, J. (2000). Weather-dependent pollinator activity in an apple orchard, with special reference to Osmia cornuta and Apis mellifera (Hymenoptera:Megachilidae and Apidae). *Physiological and Chemical Ecology*, *29*(3), 413–420.
Wolkovich, E. M., Cook, B. I., Allen, J. M., Crimmins, T. M., Betancourt, J.

L., Travers, S. E., … Cleland, E. E. (2012). Warming experiments underpredict plant phenological responses to climate change. *Nature*, *485*(7399), 18–21.

Wyatt, R., Broyles, S. B., & Derda, G. S. (2016). Environmental Influences on Nectar Production in Milkweeds (Asclepias syriaca and A . exaltata) Author (s): Robert Wyatt , Steven B . Broyles and Gregory S . Derda Published by : Botanical Society of America , Inc .