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Title: Facing the heat: behavioral and molecular underpinnings of heat stress in bumblebees

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Table 1. Useful terms and definitions.

Ta	Ambient temperature
Tn	Nest temperature
Tb	Brood temperature
CTmax	Temperature at which insects go into muscle spasms & eventual stupor
Eclose	Emerge from brood
Brood	Offspring enclosed in wax
Gyne	Future queen
Drone	Male
Worker	Non-reproductive daughters of the queen, typically divided into bees who nurse the brood or those who forage for nectar and pollen
Diapause	A hibernation-like state, or period of developmental arrest brought on by cold temperatures in queens over the winter before their reproductive season.
Usurpation	When the queen of one colony overtakes another colony

ABSTRACT:
Climate change heralds an era of increased heat waves. Insects, due to their short generation times and their sensitive ecological requirements, offer a powerful model for studying rapid physiological and behavioral responses to high temperatures. Solitary insects primarily respond to temperature extremes by moving in space or time to remain in a constant environment, or by exploiting phenotypic plasticity evolutionary adaptation. Eusocial insects, however, possess an additional tool in mitigating thermal stress: cooperative group behavior. Here, we discuss how bumblebees (*Bombus*) in particular exemplify the ways in which social living systems can buffer against environmental challenges. We focus on the urgent gap in understanding bumblebee response to high heat and propose additional studies/analytic frameworks to facilitate the identification of conserved behavioral and neural mechanisms to heat stress.

INTRODUCTION

Climate change heralds an era of increased heat waves, with global surface temperatures increasing faster since 1970 than in any other 50-year period in the last 2,000 years [1]. By the mid-21st century, compound heat waves are projected to become more frequent, with 20-30 additional high heat days per year [2]. Emission restrictions under the Paris Agreement have thus far successfully reduced potential global warming by 2050 from 4℃ to 2.1-2.8℃ [3]. However, these rises are still expected to trigger multiple climactic points of no return [4]. We are hurtling toward a world defined by climactic extremes, and understanding the organismal impacts of high heat is increasingly urgent.

Insects, due to their short generation times and their sensitive ecological requirements, offer a powerful model for studying rapid physiological and behavioral responses to high temperatures [5]. The most taxonomically diverse group of organisms on Earth, representing >90% of all animal species [6], insects also provide insight into climate-driven biodiversity shifts. However, terrestrial insect populations have declined by approximately 8.81% per decade between 1960 and 2005 [7], largely due to climate change, agrochemical use, and habitat loss.

Solitary insects primarily respond to temperature extremes by moving in space or time to remain in a constant environment, or by exploiting phenotypic plasticity or evolutionary adaptation [8]. Social insects, however, possess an additional tool in mitigating thermal stress: cooperative group behavior. Here, we discuss how bumblebees (*Bombus*) in particular exemplify the ways in which eusocial living systems can buffer against environmental challenges. Bumblebees are broadly distributed across diverse climates, with greater than 250 species worldwide, making them an ideal model for studying evolutionary and plastic responses to temperature extremes. Like other eusocial insects, the division of labor within the colony enables bumblebees to efficiently allocate resources under stress. For example, some individuals may engage in active thermoregulation while others continue foraging and brood care, preserving colony function and size despite environmental fluctuations (See Table 1 for definitions) [9]. However, unlike ants and honeybees, bumblebees lack the strict age-based division of labor. Instead, early life environmental conditions, including heat exposure and social experience during development, shape adult roles within the colony [10–12]. We can therefore use adult behavioral measures as an outcome measure for early life environmental manipulations.

While previous studies have largely focused on bumblebee adaptations to cold temperatures [13–18], far less is known about their responses/resilience to high temperatures. This is a critical gap in understanding: high heat imposes destabilizing effects on membranes and proteins, making it more difficult for insects to acclimate to heat than to cold [19,20]. At high temperature ranges of 40-55℃, for example, bumblebees reach their critical maximum temperature (CTmax) and begin to spasm before experiencing loss of muscle control [21–24]. Social bees are more resistant to heat and maintain their typical behaviors to a higher thermal limit than solitary bees [22]. However, formal studies of bumblebee thermal tolerance remain limited and heavily biased, towards commercially available species, primarily *Bombus impatiens* and *terrestris*. These species exhibit a host of behavioral and physiological adaptations within their thermal range (between CTmin and CTmax) that likely buffer them against heat waves more effectively than solitary bees. In this review, we highlight how the behavioral repertoire of eusocial insects provides a uniquely suitable lens for studying the impact of climate change on dynamic societies. We focus on the urgent gap in understanding bumblebee responses to high heat and propose additional studies/analytical frameworks to facilitate the identification of conserved behavioral and neural mechanisms to heat stress.

ONE DEGREE MATTERS: HEAT-STRESSED BUMBLEBEES IN THE NEST

Bumblebees rely on social cooperation to maintain stable brood and nest temperatures, but their ability to do so has upper thermal limits. In multiple species, once workers eclose, they assist in regulating nest temperatures within a narrow range of 28-32℃ (see Kevan et al., 2024 for review) [25,26], which is lower than the typical brood temperature maintained by honey bees (34.5 +/- 1.5℃) [27]. Collective fanning, during which a portion of the community rapidly fan their wings, is one of the primary tools for decreasing nest temperature. Bumblebees also adjust brood temperature by move on or away from the brood en masse to increase or decrease brood temperature respectively. However, it remains unknown whether bumblebees use additional thermoregulatory behaviors seen in the honeybee, such as heat shielding [28,29], evaporative cooling via liquid spreading around the hive, or transferring heat to their abdomens and vomiting hot liquid away from the brood [30].

Solitary insects have fewer tools at their disposal for heat protection - primarily individual fanning, burrowing, shading, and evaporative cooling, and are thus more susceptible to heat waves. By taking advantage of this collective behavior, bumblebees can maintain larger amounts of brood at acceptable temperatures than solitary bees. Solitary insects, in contrast, rely solely on individual behaviors such as fanning, burrowing, shading, and evaporative cooling, and are thus presumably more susceptible to heat waves.

Small fluctuations in temperature well below CTmax can also significantly alter behavior and brood care. For example, *B. impatiens* reduces brood care above 32℃ and abandons their brood entirely at 36℃ [26,31], while *B. huntii*

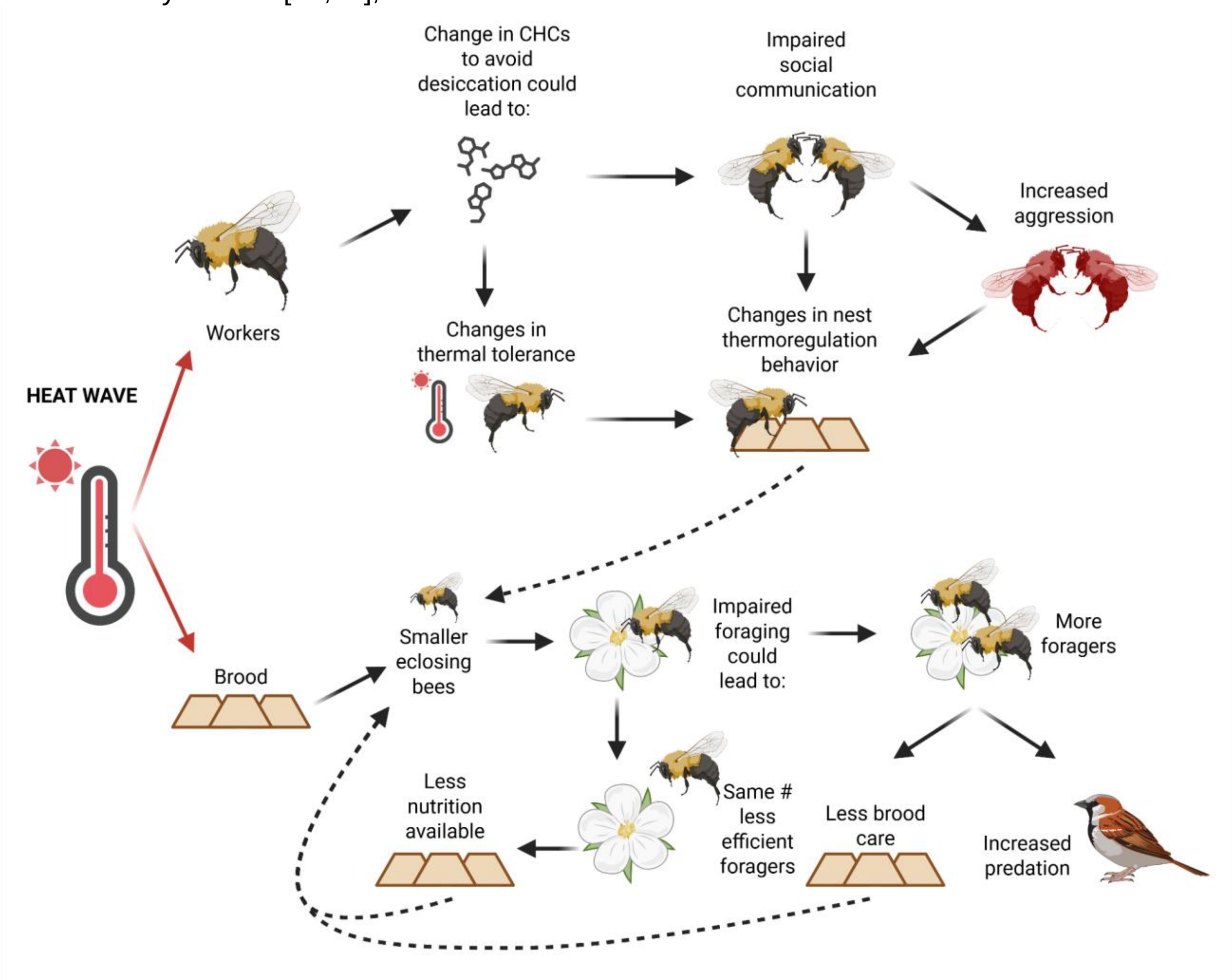

Figure 1. Mechanisms by which a single heat wave may lead to changes in social, thermoregulatory, and nursing behavior in adults and incubating brood. Many of these pathways potentially lead to altered nutrition or thermoregulation of brood, and ultimately changes in bee size and colony fitness.

initiates heat-dissipating behaviors at 38.6℃ [32]. As climate change drives more frequent and intense heat waves, understanding the limits of these thermoregulatory behaviors is crucial for predicting bumblebee resilience and informing conservation efforts.

BUMBLEBEE SIZE AND SOCIAL ROLES IN A CHANGING CLIMATE

Unlike in honeybees, where social roles are primarily determined by age, bumblebee division of labor is strongly influenced by body size. Under typical developmental temperatures, bumblebee workers can vary up to tenfold in mass [33]. Larger bees typically stay on the periphery of the nest and perform foraging behaviors, while smaller bees remain in the center of the nest and perform nursing duties [11,12]. Despite this spatial and functional separation, larger bees tend to be more efficient at both tasks, making body size a key factor in colony performance [33,34].

Exposure to high temperatures during development reduces worker size [35], leading to functional impairments in foraging, nest maintenance, and reproductive success. In *B. terrestris*, heat stress at 33℃ leads to reduced reproductive investment from the queen and her workers [35], as well as morphological changes in emerging brood, including smaller body and antennae sized, while tongue and wing sizes remain unchanged [35,36]. These allometric shifts can disrupt plant-pollinator relationships, causing mismatches that may impair foraging or pollinating success [37].

Smaller bees also exhibit lower heat tolerance, losing water more quickly and surviving for shorter durations at Ta = 30℃ compared to larger bees [38]. Although the effects of high heat on gyne size remain unclear, smaller gynes are less likely to survive the winter, less effective at foraging, and more vulnerable to usurpation [39]. Collectively, these findings suggest that heat waves exert long-lasting negative effects on bumblebee colony fitness by reducing the functional capacity of both current workers and future cohorts of workers and reproductive individuals.

FORAGING THROUGH THE HEAT

Foraging success is critical to bumblebee colony success, with up to ~⅓ of workers dedicated to collecting pollen and nectar [40,41]. Larger bees tend to be more effective foragers, carrying heavier pollen loads and making longer flights than their smaller sisters [42]. However, rising temperatures threaten bumblebee foraging efficiency.

In *B. impatiens,* every milligram of pollen carried increases thoracic temperatures by 0.07℃ , pushing foragers closer to their CTmax [42]. To manage heat stress, honeybees reduce wingbeat frequency and increase stroke amplitude, maintaining foraging efficiency while minimizing heat production [43] Similarly, at 32℃, *B. eximius* foragers show reduced wing beat frequency, shorter flights, and smaller pollen loads compared to conditions at 26℃ [44]. These behavioral adjustments likely require more frequent trips out of the nest, increasing the risk of predation.

Heat waves also disrupt plant-pollinator relationships. At 35℃, plants produce less nectar, and *B. impatiens* perform fewer successful foraging bouts and reduced flower visits [23]. Similarly, *Borago officinalis*, the star flower plant, opens fewer flowers at 26℃ versus 21℃, and *B. terrestris* decrease their visits to plants reared at 26℃ by over four times compared to plants raised at 21℃ [45]. Beyond direct foraging inefficiencies, high heat events can also shift plant blooming times, creating temporal mismatches between pollinators and their floral resources, further threatening colony longevity [46].

Bumblebee associative learning is also essential for successful foraging, and impairments due to heat would likely lead to reduced pollen and nectar collection. *B. terrestris* tested at 32℃ [2] show significantly fewer proboscis extensions to lights associated with sucrose rewards than bees raised at lower temperatures. Extreme heat waves of 40℃ further disrupt foraging behavior by reducing antennal responses to floral scents in *B. terrestris* and *B. pascuorum* [47]. Given that bees rely on learned associations, such as recognizing petal microbes on petals with high-quality floral resources [48] and developing color preferences for the flowers [49], heat-induced cognitive deficits likely decrease foraging efficiency. Furthermore, young bumblebees rely on social learning, observing experienced foragers to identify which flower types are most rewarding, a process that may also be disrupted under high temperatures [50,51].

Impaired foraging efficacy directly impacts the health and size of new workers, threatening colony health and survival. If fewer resources are collected, more workers may need to forage, reducing the number of bees available to care for the brood. Alternatively, if forager numbers remain constant, reduced food acquisition will limit resource distribution within the colony. In either case, developing brood will likely receive inadequate nutrition, leading to smaller young. These size reductions could further diminish foraging capacity, creating a feedback loop of declining colony fitness. Thus, heat stress not only disrupts foraging but also has cascading effects on colony structure, worker production, and long-term colony survival.

CHEMICAL CUES FOR SOCIAL COMMUNICATION

The social interactions essential for colony health are mediated through several factors, including cuticular hydrocarbons (CHCs). Many insects, including bumblebees, maintain a viscous wax of CHCs on their cuticle [30]. CHCs are involved in task performance and in the recognition of species, sex, kin, nestmate, and caste [52]. Slight alterations in their composition may have broad social impacts. For example, CHCs shift to reflect fertility during the competition phase of a colony, leading to increased aggression among workers [53,54]. There is evidence to suggest that bumblebee CHCs shift with high heat exposure, which could similarly alter social signaling.

In honeybees, foragers have a higher alkane composition in their CHCs than nurses, likely due to their exposure to more variable temperatures outside the nest [55]. In wasps, CHC composition changes under high heat, as increasing the proportion of linear versus branched alkanes increases desiccation tolerance [56]. While CHCs and changing chemical profiles during heat waves have not been tested directly in bumblebees, we found two key genes modulated by high heat in the existing literature: APD-3 and farnesol dehydrogenase [57]. The APD-3 protein contributes to hydrophobic cuticular proteins in a manner similar to what has been observed in wasps. Farnesol dehydrogenase is a foraging recruiter [58] and is key to the synthesis of juvenile hormone [59].

APD-3 upregulation likely has similar effects on CHCs as seen in wasps, leading to increased desiccation protection. There are currently no studies examining the impacts of these changes on social signaling, but this is of utmost importance to long term colony survival following heat waves.

General behavioral effects of changes in farnesol and juvenile hormone are more characterized - juvenile hormone is a gonadotropin which controls reproductive development in bumblebees. Increased juvenile hormone decreases protein biosynthesis in the brain, leading to issues with long-term memory and synaptic plasticity [60], which could be related to the associative learning deficits described above. Bumblebees typically also spread farnesol around the hive to recruit other nestmates to forage [52,58].

The downstream effects of an upregulation in farnesol dehydrogenase are unclear. One possibility is that it leads to an increase of farnesol spreading, and thereby foraging. Alternatively, it could lead to increased juvenile hormone, altering the development and fertility of workers. In either case, if CHCs in bumblebees shift in response to heat, social signaling and communication would be drastically altered. Further research is needed to understand how heat-induced changes in CHC composition affect social behavior in bumblebees during heat waves, when maintaining social behaviors in the colony is crucial for survival.

MOLECULAR RESPONSES TO HEAT STRESS IN BUMBLEBEES

Changes in genes related to social behaviors, such as those determining CHC composition, are among the physiological and transcriptomic defenses bumblebees employ against heat waves. Studies examining transcriptomic responses to heat stress in bumblebees are limited but diverse, using different techniques, tissues, sexes, species, and temperature regimes [24,44,57,61–64], lending us a global view of conserved responses to heat stress across conditions. Undirected differential gene expression studies have been conducted in order to assess differentially expressed genes across conditions [24,57,63], while directed studies using targeted qPCR have examined the relative expression levels of select genes [44,61,62,64]. To facilitate comparisons across these studies, we have compiled a list of all genes analyzed or found as differentially expressed across these studies into Supplemental Table 1. All genes replicated across species or studies are summarized in Table 2.

Across these seven studies examining transcriptomic responses to heat, ~550 genes were targeted or identified as differentially expressed, and eight have been replicated across studies (Table 2). Of these 8, phosphoglycerate kinase, Kelch-like protein diablo, and probable cytochrome P450 303a1 are all involved in metabolic and biosynthetic pathways, while protein lethal(2)essential for life-like and ubiquitin-like protein 7 are both involved with protein processing and quality control. In addition to ties to heat, metabolic pathways and protein processing both have behavioral relevance. For example, phosphoglycerate kinase is a glycolytic enzyme, but is also involved in functions unrelated to energy metabolism, including pathogenesis [65]. Kuo et al., 2023 found that energy metabolism in flight muscles is reduced during heat stress, and that the energy metabolic pathway tends to involve anaerobic respiration. Anaerobic metabolism is common in times of low oxygen availability, such as high intensity exercise, and this shift in bees may be due to the intensive muscle activity during fanning. Aerobic versus anaerobic metabolism has not yet been evaluated in low versus high fanners.

Protein lethal(2) essential for life-like encodes heat shock 20 and is a common response to heat. Like many other organisms, bumblebees express heat shock proteins (HSPs) in response to diverse stressors ranging from heat exposure to social isolation. HSPs work to appropriately fold proteins and to degrade misfolded proteins. *B. terrestris* females express HSP70 at heat shock temperatures of 38℃ or greater [57,61], and *B. eximius* show increases in HSP60, HSP83, and sHSP when kept at 32℃ versus 26℃ [44]. While acute upregulation during stressors, including heat shock, works to maintain a healthy proteomic landscape, extended upregulation of HSPs is associated with multiple cancers, neurodegenerative diseases of inappropriate protein aggregation, cardiovascular diseases, and autoimmune diseases [66]. Additionally, while broadly known for their role in protein folding and protection during thermal stress, HSPs may also vary in expression across behavioral castes—potentially reflecting differing physiological demands between nurses and foragers. Foragers, in particular, face compounded thermal loads during flights, and may be especially vulnerable to memory and navigation deficits under heat stress [2], Clarifying the timescale of HSP activation during and following heatwave exposure in bumblebees is essential for understanding the potential long term physiological impacts of climate change.

These studies also find differential gene expression in areas of sensory axon growth, development, and damage, as well as neurogenesis and nervous system development, highlighting the importance of studying the impacts of heat stress on the central nervous system, and the downstream implications for individual and group behavior. With the exception of targeted studies on whole heads, there are no existing studies of bumblebee brains to directly understand the neurobiological impacts of high heat. Thoracic and abdominal tissue responses to heat vary substantially [63], and neural responses presumably do as well.

Ultimately, these datasets are tremendously useful in identifying future avenues of research into heat coping in bumblebees. Implicated genes range from those involved with cellular protection to those regulating behavioral stress responses, highlighting the importance of studying these organisms in their full, cooperative context. Future studies that use consistent experimental conditions and analytical frameworks to examine the impacts of heat-exposure across bumblebee species and generations will shed additional light on behavioral and physiological adaptations selected for in social species.

CONCLUSIONS

Insect responses to the impacts of climate change serve as a canary in the coal mine for understanding the impacts of climate change on human health. Pollinator health and human health are intimately intertwined [67,68]. Bumblebees are emerging as powerful model organisms for studying cognition and behavior, due in part to the evolutionary conservation of core molecular mechanisms that regulate physiology across species [69–74]. Many core molecular mechanisms that modulate physiology and behavior are evolutionarily conserved between humans and insects [75], from genes that lay the groundwork for central nervous system development [76], to broad metabolic processing [77], heightening the translational potential of studying bumblebees.

Bumblebees are generally considered to be obligately social insects whose behavioral and molecular responses to heat stress are increasingly characterized by researchers. Existing literature highlights their susceptibility to small fluctuations in temperature, and the impacts of heat waves on their fanning behavior, foraging behavior, and transcriptomics. Currently, little is known about the social networks, division of labor, propensity for task switching, or reproductive efficacy of these heat-exposed workers or gynes - essential areas of study for understanding how climate change will impact social insect species' cooperative survival. Increasing consistency in protocols, species, worker status, and sexes used in future experiments will further allow us to evaluate replicability across studies.

As a group, bees exhibit a wide range of social structures, ranging from solitary to eusocial. Many "solitary" bees display some degree of social tolerance, for example, two or more females may inhabit the same nest [78]. This social flexibility will likely allow for increased adaptability to climate change. In facultatively social bees, eusociality is expected to become more common as warming climates extend the growing seasons and increase the need for brood and nest cooling behaviors [79]. Going forward, bees with flexible social structures will be especially valuable models for investigating the ecological and evolutionary interplay between sociality and environmental stress.

While the impacts of climate change on insects are typically discussed and studied in terms of average global temperatures, understanding microclimate extremes and latitudinal risk is essential for understanding how insects respond to increasing temperature threats. Although Arctic regions are projected to experience a larger rise in temperature than the tropics, insects living in the tropics may face greater threats. Tropic species are more highly specialized to their current temperature ranges, having evolved in relatively stable climates with little seasonal variability [9], and already operate near their CTmax [8,80]. These factors place tropical insects at greater risk of extinction from climate change driven heat stress than Arctic insects.

Expanding the scope of our research to include novel species, particularly those with diverse life history traits, will broaden our understanding of the physiological and behavioral mechanisms insects use to cope with heat stress.

The recent advances in pose estimation and tracking techniques, as well as automated in-hive filming, will furthermore allow researchers to examine collective behavior in previously inaccessible depth. Individual tracking throughout a lifespan will allow researchers to use techniques such as social network analysis to better understand how bees form and maintain social structures through stressors. These techniques also allow us to pair individual transcriptomic and neurobiological changes back to individual behavior, allowing us an unparalleled window into individual, group, and hive dynamics in the face of heat stress. Understanding these dynamics is not only critical for bumblebee conservation, but may offer deeper insights into how cooperative systems across taxa, including humans, cope with accelerating climate change.

Table 2. Differentially expressed genes that have been replicated across studies and/or across species in heat treated versus control bumblebees. Up = increased in heat treated versus control bees. Dashed in the Kuo column indicate that these genes were not analyzed via qPCR. Empty cells in the Przybla, Pimsler, and Quinlan columns indicate that the gene was not found to be differentially expressed between groups. A list of all surveyed or differentially expressed genes found across all seven studies analyzed can be found in Supplementary Table 1.

	Author	**Kuo**	**Przybla**		**Pimsler**	**Quinlan**	**Go terms**	**Species**	**Database**
	Species	*eximius*	*terrestris*	*magnus*	*vosnesenskii*	*impatiens*			
	Sex	Female	Male		Female	Female			
	Tissue	Flight muscles	Hemolymph		Thoracic muscle	Abdomens and thoraces			
Locus		qPCR	DEG analysis		DEG analysis	DEG analysis			
AKJ80163.1	Acetyl-CoA C-acetyltransferase	-	Up	Up			acetyl-CoA C-acetyltransferase activity	*Bombus lucorum*	Uniprot
LOC105682137	APD-3 protein	-	Down	Down			synaptic vesicle membrane organization, protein transport, vesicle-mediated transport	*Caenorhabditis elegans*	Uniprot
LOC100645857	Centromere-associated protein E isoform X1	-	Down	Down			Microtubule binding	*Xenopus laevis*	Amigo
LOC100746959	Cytosolic non-specific dipeptidase	-	Up	Up			proteolysis	*Bombus impatiens*	Uniprot
LOC100743566	Endothelin-converting enzyme 1	-	Up	Up			Zinc ion binding, peptide hormone processing	*Bombus impatiens*	Uniprot

LOC100742025	Farnesol dehydrogenase	-	Up	Up			Juvenile hormone synthetic process	*Aedes aegypti*	Amigo
LOC100748965	Glucose dehydrogenase uncharacterized protein	-	Up	Up			oxidoreductase activity, acting on CH-OH group of donors	*Bombus impatiens*	Uniprot
LOC100743189, LOC100744090	IQ and ubiquitin-like domain-containing protein	-			Down	Down	flagellated sperm motility	*Bombus impatiens*	Uniprot
LOC100740524	Kelch-like protein diablo	-		Up		Up	proteasome-mediated ubiquitin-dependent protein catabolic process	*Branchiostoma floridae*	Uniprot
LOC100745677	Phosphoglycerate kinase	Down	Down				gluconeogenesis, glycolytic process	*Bombus impatiens*	Uniprot
LOC100749471, LOC105681459	PiggyBac transposable element-derived protein 4-like				Up	Up	no functions identified		Uniprot/ Amigo
LOC100743524	Probable cytochrome P450 303a1				Up	Up	organic acid metabolic process, xenobiotic metabolic process	*Bombus impatiens*	Uniprot
LOC100741712, LOC117152215	Protein lethal(2)essential for life-like	-	Up		Up	Up	protein refolding, response to heat	*Bombus impatiens*	Uniprot

LOC105682067	Sodium/calcium exchanger regulatory protein 1 isoform X1	-	Up	Up			lipid binding	*Bombus impatiens*	Uniprot
LOC100748278	synaptic vesicle 2-related protein	-	-	-	Up	Up	transmembrane transporter activity	*Bombus impatiens*	Uniprot
LOC100643526	Thioredoxin-2	-	Up	Up			protein-disulfide reductase activity	*Bombus terrestris*	Uniprot
LOC100741469	Ubiquitin-like protein 7	-			Up	Up	ubiquitin-dependent protein catabolic process	*Bombus impatiens*	Uniprot
LOC105680201	uncharacterized protein LOC100644182	-	Down	Down			sleep, regulation of synaptic transmission, cholinergic	*Bombus terrestris*	Uniprot
XP_003397850, Formerly XP_020720452.1	V-type proton ATPase catalytic subunit A	-	Up	Up			intracellular iron ion homeostasis	*Mus musculus*	Uniprot

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**This article contextualizes insect responses to microclimates rather than global averages, and speaks to insects' ability to rapidly adapt to heat. Kevan et al., further describe the manners in which insects can respond to heat, and highlights their unimodal, asymmetrical, and leftward-shifted temperature dependence. An extremely well written manuscript, and deeply influential to the writing of our own.

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