4/24/25

**Title: Facing the heat: behavioral and molecular underpinnings of climate hardiness in bumblebees**

Authors: Nastacia L. Goodwin[1], Z Yan Wang[1,2]

Affiliations:
[1]Department of Psychology, University of Washington, Seattle WA
[2] Department of Biology, University of Washington, Seattle WA

Running title: Climate hardiness in bumblebees

Text information
Abstract: 193 words
Introduction: 717 words
Body: 2600 words
Discussion: 329 words
Main Figures: 3
Supplemental Figures: 0
Supplemental Appendix: 0
Supplemental Tables: 1
Supplemental Videos: 0
References: 78

Corresponding Author: Z Yan Wang (zywang22@uw.edu)

Acknowledgements: We would like to sincerely thank Kristen Drummey, Kennedy Omufwoko, and Ashley Baker for their thoughtful comments on our manuscript.

Funding and Disclosure: This work is funded by the University of Washington Department of Psychology, Department of Biology, Royalty Research Fund (ZYW and NLG), and T32 Nutrition, Obesity and Atherosclerosis Training Grant (NLG).

CRediT author statement: NLG: Conceptualization, data curation, writing, visualization, funding acquisition. ZYW: Conceptualization, writing, funding acquisition.

Keywords: Bees, Bumblebees, Climate change, Heat waves, gene expression

ABSTRACT:
Climate change heralds an era of increased heat waves, with an estimated 20-30 additional high heat days per year. While climate change is upon us, we still have little understanding of the organismal impacts of high heat and how to combat them. 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 in three ways: by moving in space or time to remain in a constant environment; by exploiting phenotypic plasticity; or by exploiting evolutionary adaptation. 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 social living systems can buffer against environmental challenges.

In this review, we highlight how the behavioral repertoire of social 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 response to high heat and propose additional studies/analytical 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 and droughts are projected to become more frequent, with an estimated 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 4C to 2.1-2.8C [3]. However, rises above 1.5C are still expected to trigger multiple climactic points of no return, including the collapse of ice sheets in polar regions, and diebacks in the Amazon rainforest and coral reefs [4]. We are hurtling toward a world defined by climactic extremes, and understanding the organismal impacts of high heat, and how to combat them, is an increasingly urgent priority.

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 |

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 broad 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 in three ways: by moving in space or time to remain in a constant environment; by exploiting phenotypic plasticity; or by exploiting 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 social living systems can buffer against environmental challenges. Bumblebees are broadly distributed across diverse climates, making them an ideal model for studying evolutionary and plastic responses to temperature extremes. Like other social 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 [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.

Additionally, the annual life cycle of bumblebees presents a powerful framework for studying the effects of heat stress across generations. Each year, new queens overwinter in solitary diapause before emerging in spring to establish a new colony. As the colony develops, waves of new workers emerge, creating opportunities to experimentally manipulate biotic and abiotic developmental conditions and examine their cascading effects on later cohorts of workers. Toward the end of the colony cycle, the queen produces male drones and future queens (gynes), which enter diapause while the rest of the colony dies. By taking advantage of bumblebee social structure and life history, researchers can learn about both short-term plasticity between cohorts within a colony and longer-term adaptations to thermal stress across colonies.

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 40-55C, for example, bumblebees reach their critical maximum temperature (CTmax) and begin to spasm and then experience complete 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]. Between their preferred temperatures in the mid-20s and their CTmax, bumblebees exhibit a host of behavioral and physiological adaptations that lend them resilience to heat waves compared to solitary insects. In this review, we highlight how the behavioral repertoire of social 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 response 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, which are critical factors for the development of worker bees and the survival of the colony, but their ability to do so has upper thermal limits. In the early days of nest establishment, variation in brood temperature is typically at its highest because the queen must balance brood care with foraging [25]. However, in multiple species, once workers eclose, they assist in regulating nest temperatures within a narrow range of 28-32C and maintaining brood temperatures at 1-2C above ambient conditions (see Kevan et al., 2024 for review) [25,26]. Thermal stability is achieved through several group behaviors. Collective fanning, during which a sizable portion of the community rapidly fan their wings, is one of the primary tools for decreasing nest temperature and $CO_2$ levels. Alternately, bees move on or off of brood en masse to increase or decrease brood temperature respectively. By taking advantage of this collective behavior, bumblebees can maintain larger amounts of brood at acceptable temperatures than solitary bees. Careful maintenance of brood temperature is essential for maintaining worker size and allometry, as discussed below. 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*, the common eastern bumblebee, reduces brood care above 32C and abandons their brood entirely at 36C [25,27], while *B. huntii*

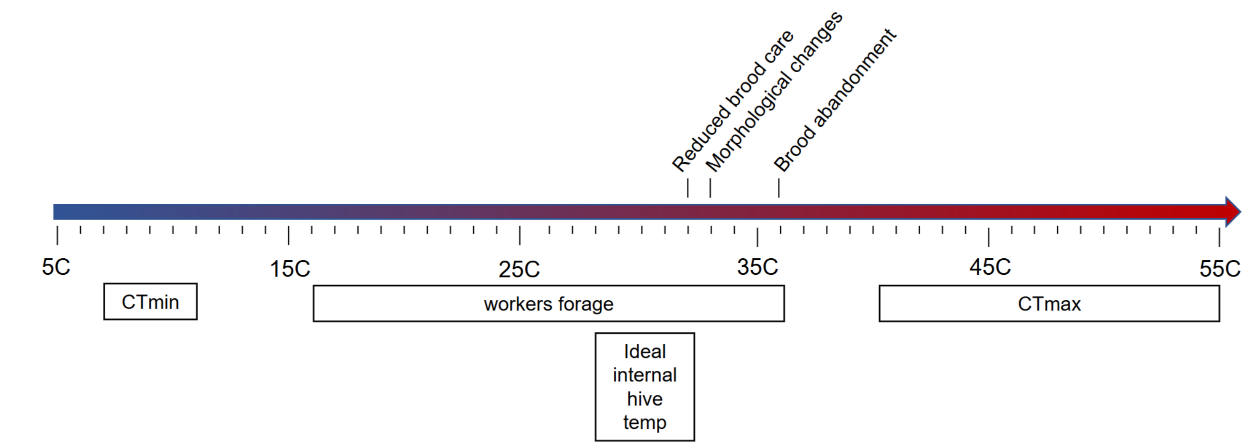


Figure 1. The temperature range for bumblebees extends from CTmin at 7C-10C to CTmax at 40-55C. While bees can inhabit this wide range, foraging typically occurs between 16C and 36C, while hive temperature is maintained between 28C and 32C. Small changes matter, however: between 32C and 36C, we begin to see broad changes such as reduced brood care or abandonment, as well as morphological changes.

exhibits slightly higher thresholds, initiating heat-dissipating behaviors at 38.6C [28]. Higher temperatures also accelerate development, causing multiple bumblebee species to eclose faster at 32C than 29C [29]. These changes in brood care may lead to rippling changes in body size and behavior in future cohorts of workers and gynes, as discussed below. 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.

Closer study of specific bumblebee behaviors in response to heat waves is also warranted. Honeybees are known to physically shield their brood from heat [30,31], to spread liquid around the hive for evaporative cooling purposes, and to shunt heat to their abdomens and vomit hot liquid away from the brood [32] in an effort to thermoregulate the nest. Characterizing these behaviors in bumblebees will allow us to better understand their social and asocial responses to heat stress, and the manner in which this impacts them directly as well as future cohorts and generations.

## BUMBLEBEE SIZE AND SOCIAL ROLES IN A CHANGING CLIMATE

In bumblebees, social roles depend on body size. Under normal developmental temperatures, bumblebees may exhibit up to a 10-fold difference 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]). Larger bees tend to be more efficient at both tasks, making body size a critical factor for colony function [33,34]. High temperatures during development greatly impact worker size, leading to functional impairments in foraging, colony maintenance, and reproductive success.

Climate change threatens this balance as worker bee body size shrinks under high temperatures. Ambient temperatures as low as 33C results in significantly smaller workers [35], which are less efficient at foraging and nest protection, reducing overall colony fitness. In *B. terrestris*, the buff-tailed bumblebee, heat stress at 33C leads to reduced reproductive investment from the queen and her workers, and morphological changes in emerging brood, including smaller body and antennae sized but unchanged tongue and wing sizes [35,36]. These allometric shifts can disrupt plant-pollinator relationships, causing mismatches that may impair foraging or pollinating success [37].

High heat impacts on body size can ripple through both cohorts of workers and future colony cycles. A single heat wave can lead to a cohort of undersized, less effective workers, compounding stress and decreasing colony fitness and longevity. These undersized young may also face higher mortality rates in the face of future heat waves. Smaller bees have lower heat tolerance, losing more water and surviving for shorter periods at Ta = 30C compared to larger bees [38]. While the effects of high heat on gyne size remain unclear, smaller gynes are less likely to survive the winter or forage effectively, and are more likely to be usurped [39]. Ultimately, heat waves are expected to have lasting negative effects on future generations of workers and gynes.

## 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.

Bumblebees exhibit a temperature-dependent foraging efficiency curve, with foraging increasing from 12C to 27C and decreasing at higher temperatures [43]. Heat waves exacerbate this decline, as foraging bees experience elevated thoracic temperatures, particularly when carrying pollen. In *B. impatiens,* every milligram of pollen carried increases thoracic temperatures by 0.07C, pushing foragers closer to their CTmax [42]. At 32C, *B. eximius* show reduced wing beat frequency, shorter flights, and smaller pollen loads compared to foraging at 26C [44]. This likely forces more frequent trips out of the nest, increasing the risk of predation.

Heat waves also disrupt plant-pollinator relationships. At 35C, 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 significantly fewer flowers at 26C versus 21C, and *B. terrestris* foraging at 21C decrease their visits to 26C reared plants by over four times compared to plants raised at 21C [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, but heat stress impairs learning, likely leading to reduced pollen and nectar collection. *B. terrestris* raised at 32C [2] and 33C [47] show significantly fewer proboscis extension responses to sucrose or lights associated with sucrose rewards than bees raised at lower temperatures. Extreme heat waves of 40C further disrupt foraging behavior by reducing antennal responses to floral scents in *B. terrestris* and *B. pascuorum* [48]. Given that bees rely on learned associations, such as recognizing petal microbes on petals with high-quality floral resources [49] and developing color preferences for the flowers [50], 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 [51,52].

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 workers and potentially undersized gynes. 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, primarily containing n-alkanes, alkenes, and methyl-branched alkanes [32]. CHCs are involved in task performance and in the recognition of species, sex, kin, nestmate, and caste [53]. 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 [54,55]. There is evidence to suggest that bumblebee CHCs shift with high heat exposure, which could similarly alter social signaling.

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, and upregulation is metabolically costly. 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 [53,58].

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 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 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, interaction with nucleic acids, and cell death [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, supporting the role of metabolic enzymes in responses to heat stress. 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 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 but not males express HSP70 at heat shock temperatures of 38C or greater [57,61], and *B. eximius* show increases in HSP60, HSP83, and sHSP when kept at 32C versus 26C [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, HSP 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.

Other important genes found in these studies impact cytoskeletal conformation, protein folding and stabilization, chromatin organization, and RNA processing, all of which provide interesting avenues for future targeted studies examining transcriptomic responses to heat stress. One area of differential regulation involves metal processing, including zinc, copper, and iron binding (Table 2). The upregulation of metal-binding proteins, particularly iron-binding proteins, has been implicated in cell preservation during diapause [67]. As insects begin to desiccate or freeze due to extreme temperatures, the metal ion concentrations in their remaining liquid hemolymph increase, leading to increases in oxidative stress unless sequestration occurs [67]. The relationship between metal processing and heat in bumblebees has not been explicitly investigated, but transferrin, which binds iron, was upregulated in heat treated *B. vosnesenskii* but downregulated in *B. terrestris* and not found to be differentially regulated in *B. magnus* [24,57]. The suite of studies in Table 2 suggest that this is an important aspect of cell protection during heat exposure.

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 [68,69]. 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 [70–75]. Many core molecular mechanisms that modulate physiology and behavior are evolutionarily conserved between humans and insects [76], from genes that lay the groundwork for central nervous system development [77], to broad metabolic processing [78], 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.

The recent advances in pose estimation and tracking techniques, as well as automated in-hive filming, will 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 | | | |
| | | 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 | C. 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 | - | 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 | Mice | Uniprot |

1. **IPCC AR6 Working Group 1: Summary for Policymakers**. [date unknown],

2. Gérard M, Amiri A, Cariou B, Baird E: **Short-term exposure to heatwave-like temperatures affects learning and memory in bumblebees**. *Global Change Biology* 2022, **28**:4251–4259.

3. **Report of the Conference of the Parties serving as the meeting of the Parties to the Paris Agreement on its fifth session, held in the United Arab Emirates from 30 November to 13 December 2023. Addendum. Part two: Action taken by the Conference of the Parties serving as the meeting of the Parties to the Paris Agreement at its fifth session | UNFCCC**. [date unknown],

4. Armstrong McKay DI, Staal A, Abrams JF, Winkelmann R, Sakschewski B, Loriani S, Fetzer I, Cornell SE, Rockström J, Lenton TM: **Exceeding 1.5°C global warming could trigger multiple climate tipping points**. *Science* 2022, **377**:eabn7950.

5. Hill GM, Kawahara AY, Daniels JC, Bateman CC, Scheffers BR: **Climate change effects on animal ecology: butterflies and moths as a case study**. *Biological Reviews* 2021, **96**:2113–2126.

6. Schowalter TD: *Insect Ecology: An Ecosystem Approach*. Academic Press; 2022.

7. van Klink R, Bowler DE, Gongalsky KB, Swengel AB, Gentile A, Chase JM: **Meta-analysis reveals declines in terrestrial but increases in freshwater insect abundances**. *Science* 2020, **368**:417–420.

8. Buckley LB, Kingsolver JG: **Functional and Phylogenetic Approaches to Forecasting Species' Responses to Climate Change**. *Annual Review of Ecology, Evolution, and Systematics* 2012, **43**:205–226.

9. Menzel F, Feldmeyer B: **How does climate change affect social insects?** *Current Opinion in Insect Science* 2021, **46**:10–15.

10. Couvillon MJ, Dornhaus A: **Location, location, location: larvae position inside the nest is correlated with adult body size in worker bumble-bees (Bombus impatiens)**. *Proceedings of the Royal Society B: Biological Sciences* 2009, **276**:2411–2418.

11. Foster RL, Brunskill A, Verdirame D, O'Donnell S: **Reproductive physiology, dominance interactions, and division of labour among bumble bee workers**. *Physiol Entomol* 2004, **29**:327–334.

12. Jandt JM, Dornhaus A: **Spatial organization and division of labour in the bumblebee Bombus impatiens**. *Animal Behaviour* 2009, **77**:641–651.

13. Amsalem E, Galbraith DA, Cnaani J, Teal PEA, Grozinger CM: **Conservation and modification of genetic and physiological toolkits underpinning diapause in bumble bee queens**. *Molecular Ecology* 2015, **24**:5596–5615.

14. Beekman M, van Stratum P, Lingeman R: **Diapause survival and post-diapause performance in bumblebee queens (Bombus terrestris)**. *Entomologia Experimentalis et*

*Applicata* 1998, **89**:207–214.

15. Easwaran S, Montell DJ: **The molecular mechanisms of diapause and diapause-like reversible arrest**. *Biochemical Society Transactions* 2023, **51**:1847–1856.

16. Gosterit A, Gurel F: **Effect of different diapause regimes on survival and colony development in the bumble bee, Bombus terrestris**. *Journal of Apicultural Research* 2009, **48**:279–283.

17. Hotchkiss MZ, Forrest JRK, Poulain AJ: **Changes in bumblebee queen gut microbiotas during and after overwintering diapause**. *Insect Molecular Biology* 2025, **34**:136–150.

18. Kim B-G, Shim J-K, Kim D-W, Kwon YJ, Lee K-Y: **Tissue-specific variation of heat shock protein gene expression in relation to diapause in the bumblebee Bombus terrestris**. *Entomological Research* 2008, **38**:10–16.

19. Araújo MB, Ferri-Yáñez F, Bozinovic F, Marquet PA, Valladares F, Chown SL: **Heat freezes niche evolution**. *Ecology Letters* 2013, **16**:1206–1219.

20. Bowler K, Terblanche JS: **Insect thermal tolerance: what is the role of ontogeny, ageing and senescence?** *Biological Reviews* 2008, **83**:339–355.

21. García-Robledo C, Kuprewicz EK, Staines CL, Erwin TL, Kress WJ: **Limited tolerance by insects to high temperatures across tropical elevational gradients and the implications of global warming for extinction**. *Proceedings of the National Academy of Sciences* 2016, **113**:680–685.

22. Hamblin AL, Youngsteadt E, López-Uribe MM, Frank SD: **Physiological thermal limits predict differential responses of bees to urban heat-island effects**. *Biology Letters* 2017, doi:10.1098/rsbl.2017.0125.

23. Hemberger JA, Rosenberger NM, Williams NM: **Experimental heatwaves disrupt bumblebee foraging through direct heat effects and reduced nectar production**. *Functional Ecology* 2023, **37**:591–601.

24. Pimsler ML, Oyen KJ, Herndon JD, Jackson JM, Strange JP, Dillon ME, Lozier JD: **Biogeographic parallels in thermal tolerance and gene expression variation under temperature stress in a widespread bumble bee**. *Sci Rep* 2020, **10**:17063.

25. Vogt FD: **Thermoregulation in Bumblebee Colonies. I. Thermoregulatory versus Brood-Maintenance Behaviors during Acute Changes in Ambient Temperature**. *Physiological Zoology* 1986, **59**:55–59.

26. Kevan PG, Rasmont P, Martinet B: **Thermodynamics, thermal performance and climate change: temperature regimes for bumblebee (Bombus spp.) colonies as examples of superorganisms**. *Front Bee Sci* 2024, **2**.
**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.

27. Couvillon MJ, Fitzpatrick G, Dornhaus A: **Ambient Air Temperature Does Not Predict whether Small or Large Workers Forage in Bumble Bees (Bombus impatiens)**. *Psyche: A Journal of Entomology* 2010, **2010**:536430.

28. Gardner KE, Foster RL, O'Donnell S: **Experimental analysis of worker division of labor in bumblebee nest thermoregulation (Bombus huntii, Hymenoptera: Apidae)**. *Behav Ecol Sociobiol* 2007, **61**:783–792.

29. Tian L, Hines HM: **Morphological characterization and staging of bumble bee pupae**. *PeerJ* 2018, **6**:e6089.

30. Starks PT, Johnson RN, Siegel AJ, Decelle MM: **Heat shielding: a task for youngsters**. *Behavioral Ecology* 2005, **16**:128–132.

31. Starks PT, Gilley DC: **Heat shielding: A novel method of colonial thermoregulation in honey bees**. *Naturwissenschaften* 1999, **86**:438–440.

32. Perez R, Aron S: **Adaptations to thermal stress in social insects: recent advances and future directions**. *Biological Reviews* 2020, **95**:1535–1553.

33. Goulson D, Peat J, Stout JC, Tucker J, Darvill B, Derwent LC, Hughes WOH: **Can alloethism in workers of the bumblebee, *Bombus terrestris*, be explained in terms of foraging efficiency?** *Animal Behaviour* 2002, **64**:123–130.

34. Cnaani J, Hefetz A: **The effect of workers size frequency distribution on colony development inBombus terrestris**. *Ins Soc* 1994, **41**:301–307.

35. Guiraud M, Cariou B, Henrion M, Baird E, Gérard M: **Higher developmental temperature increases queen production and decreases worker body size in the bumblebee Bombus terrestris**. *JHR* 2021, **88**:39–49.

36. Gérard M, Guiraud M, Cariou B, Henrion M, Baird E: **Elevated developmental temperatures impact the size and allometry of morphological traits of the bumblebee Bombus terrestris**. *Journal of Experimental Biology* 2023, **226**:jeb245728.
*Heat exposed brood show size differences, but is this uniform? Gérard et al., exposed Bombus terrestris brood to 33C versus 25C and examined the allometry of resultant bees.Brood exposed to 33C emerged with smaller body sizes and reduced antennae, but similarly sized tongues and wings to brood exposed to 25C. They propose that smaller body size and antennae could impair both individual and colony level fitness.

37. Gérard M, Vanderplanck M, Wood T, Michez D: **Global warming and plant–pollinator mismatches**. *Emerging Topics in Life Sciences* 2020, **4**:77–86.

38. Botsch JC, Daniels JD, Bujan J, Roeder KA: **Temperature influences desiccation resistance of bumble bees**. *Journal of Insect Physiology* 2024, **155**:104647.
*It's a dry heat: Botsch et al., examined the intersection of heat and humidity on Bombus impatiens water retention and survival. Both higher temperatures and lower humidity increase water loss rates, while warmer temperatures decrease survival time. Large individuals survive longer than smaller individuals, and can tolerate more water loss before mortality. From this study, it appears that the smaller bees which eclose after an initial heat wave are at greater risk of dying during future heat waves due to these changes in desiccation rates.

39. Owen RE: **BODY SIZE VARIATION AND OPTIMAL BODY SIZE OF BUMBLE BEE QUEENS (HYMENOPTERA: APIDAE)**. *The Canadian Entomologist* 1988, **120**:19–27.

40. Brian AD: **Division of Labour and Foraging in Bombus agrorum Fabricius**. *Journal of Animal Ecology* 1952, **21**:223–240.

41. Stewart T, Bolton-Patel N, Cresswell JE: **Eating versus heating: a study of the allocation of workers between foraging and nest incubation in bumble bees**. *Ecological Entomology* 2021, **46**:844–855.

42. Naumchik M, Youngsteadt E: **Larger pollen loads increase risk of heat stress in foraging bumblebees**. *Biology Letters* 2023, **19**:20220581.

43. Kenna D, Pawar S, Gill RJ: **Thermal flight performance reveals impact of warming on bumblebee foraging potential**. *Functional Ecology* 2021, **35**:2508–2522.

44. Kuo Y, Lu Y-H, Lin Y-H, Lin Y-C, Wu Y-L: **Elevated temperature affects energy metabolism and behavior of bumblebees**. *Insect Biochemistry and Molecular Biology* 2023, **155**:103932.

45. Descamps C, Jambrek A, Quinet M, Jacquemart A-L: **Warm Temperatures Reduce Flower Attractiveness and Bumblebee Foraging**. *Insects* 2021, **12**:493.

46. Memmott J, Craze PG, Waser NM, Price MV: **Global warming and the disruption of plant–pollinator interactions**. *Ecology Letters* 2007, **10**:710–717.

47. Perl CD, Johansen ZB, Moradinour Z, Guiraud M, Restrepo CE, Wen Jie V, Miettinen A, Baird E: **Heatwave-Like Events During Development Are Sufficient to Impair Bumblebee Worker Responses to Sensory Stimuli**. *Front Ecol Evol* 2022, **9**.

48. Nooten SS, Korten H, Schmitt T, Kárpáti Z: **The heat is on: reduced detection of floral scents after heatwaves in bumblebees**. *Proceedings of the Royal Society B: Biological Sciences* 2024, **291**:20240352.

**Nootnen et al., expose Bombus terrestris and pascuorum to 40C heatwaves with or without high humidity, as well as with or without access to a sugar solution. They then examine depolarization via electroantennograms to five different scents. Workers of both species show decreased responses to floral scents following heat treatment, with no clear recovery pattern after 24h. This manuscript highlights the lasting impacts of heat exposure.

49. Russell AL, Ashman T-L: **Associative learning of flowers by generalist bumble bees can be mediated by microbes on the petals**. *Behavioral Ecology* 2019, **30**:746–755.

50. Raine NE, Chittka L: **No Trade-Off between Learning Speed and Associative Flexibility in Bumblebees: A Reversal Learning Test with Multiple Colonies**. *PLOS ONE* 2012, **7**:e45096.

51. Avarguès-Weber A, Chittka L: **Local enhancement or stimulus enhancement? Bumblebee social learning results in a specific pattern of flower preference**. *Animal Behaviour* 2014, **97**:185–191.

52. Leadbeater E, Chittka L: **The dynamics of social learning in an insect model, the bumblebee (Bombus terrestris)**. *Behav Ecol Sociobiol* 2007, **61**:1789–1796.

53. Ayasse M, Jarau S: **Chemical Ecology of Bumble Bees**. *Annu Rev Entomol* 2014, **59**:299–319.

54. Derstine NT, Villar G, Orlova M, Hefetz A, Millar J, Amsalem E: **Dufour's gland analysis reveals caste and physiology specific signals in Bombus impatiens**. *Sci Rep* 2021, **11**:2821.

55. Jansen J, Pokorny T, Schmitt T: **Disentangling the effect of insemination and ovary development on the cuticular hydrocarbon profile in the bumblebee Bombus terrestris (Hymenoptera: Apidae)**. *Apidologie* 2016, **47**:101–113.

56. Michelutti KB, Soares ERP, Sguarizi-Antonio D, Piva RC, Súarez YR, Cardoso CAL, Antonialli-Junior WF: **Influence of temperature on survival and cuticular chemical profile of social wasps**. *Journal of Thermal Biology* 2018, **71**:221–231.

57. Przybyla K, Martinet B, Michez D, Bocquet M, Askri D, Bulet P: **Differential expression of hemolymph proteins in wild bumblebees provides insights into species-specific impacts of heat stress**. *Physiological Entomology* 2025, **50**:105–117.

*Przybyla et al., expose male Bombus terrestris and magnus bees to 40C until heat stupor. Sampling hemolymph, they perform off-gel bottom-up proteomics and LC-MS/MS analysis to identify a set of 164 shared proteins between species, many of which are differentially expressed following heat exposure. Importantly, their results indicate that B. terrestris displays higher adaptability of its hemolymph proteome in response to heat waves.

58. Granero AM, Sanz JMG, Gonzalez FJE, Vidal JLM, Dornhaus A, Ghani J, Serrano AR, Chittka L: **Chemical compounds of the foraging recruitment pheromone in bumblebees**. *Naturwissenschaften* 2005, **92**:371–374.

59. De Loof A, Schoofs L: **Mode of Action of Farnesol, the "Noble Unknown" in Particular in Ca2+ Homeostasis, and Its Juvenile Hormone-Esters in Evolutionary Retrospect**. *Front Neurosci* 2019, **13**.

60. Shpigler HY, Herb B, Drnevich J, Band M, Robinson GE, Bloch G: **Juvenile hormone regulates brain-reproduction tradeoff in bumble bees but not in honey bees**. *Hormones and Behavior* 2020, **126**:104844.

61. Blasco-Lavilla N, García-Reina A, De la Rúa P: **Mild thermal stress does not negatively affect immune gene expression in the bumblebee Bombus terrestris**. *Apidologie* 2021, **52**:163–173.

62. Kim YH, Kim BY, Kim HS, Kim JM, Qiu W, Yoon HJ, Lee KS, Jin BR: **Elevated developmental temperature affects gene expression and oxidative stress in bumblebee (*Bombus terrestris*) workers**. *Journal of Asia-Pacific Entomology* 2024, **27**:102311.

Kim et al., examined the expression of several genes related to growth, immune response, antioxidants, and chaperones in queens, workers, and male Bombus terrestris. All genes assayed, including heat shock proteins 90 and 83, were significantly increased in workers raised at 32C versus 27C. Hemolymph analysis also revealed that workers reared at 32C had significantly higher reactive oxygen species and iron than those raised at 27C.

63. Quinlan GM, Hines HM, Grozinger CM: **Leveraging Transcriptional Signatures of Diverse Stressors for Bumble Bee Conservation**. *Molecular Ecology* 2025, **34**:e17626.

*Quinlan et al., expose bombus impatiens workers to 38C heat stress, 6C cold stress, starvation, immune challenge, sham-injections, or control conditions. Following extraction of RNA from eviscerated abdomens and thoraces, they perform differential gene expression analysis, gene ontology enrichment analysis, and leverage random forest explainability methods to evaluate the most important gene for stressor differentiation. They then applied their random forest classifiers to transcriptomic data from wild-caught bees, and were able to acutely predict naturally heat-stressed bees.

64. Soo Choi Y, Lee KS, Joo Yoon H, Kim I, Sohn HD, Jin BR: **A bumblebee thioredoxin-like protein gene that is up-regulated by a temperature stimulus and lipopolysaccharide injection**. *Eur J Entomol* 2006, **103**:291–296.

65. Rojas-Pirela M, Andrade-Alviárez D, Rojas V, Kemmerling U, Cáceres AJ, Michels PA, Concepción JL, Quiñones W: **Phosphoglycerate kinase: structural aspects and functions, with special emphasis on the enzyme from Kinetoplastea**. *Open Biol* 2020, **10**:200302.

66. Hu C, Yang J, Qi Z, Wu H, Wang B, Zou F, Mei H, Liu J, Wang W, Liu Q: **Heat shock proteins: Biological functions, pathological roles, and therapeutic opportunities**. *MedComm* 2022, **3**:e161.

67. Storey KB, Storey JM: **Insect cold hardiness: metabolic, gene, and protein adaptation1This review is part of a virtual symposium on recent advances in understanding a variety of complex regulatory processes in insect physiology and endocrinology, including development, metabolism, cold hardiness, food intake and digestion, and diuresis, through the use of omics technologies in the postgenomic era.** *Can J Zool* 2012, **90**:456–475.

68. Vasiliev D: **Implications of Pollinator Biodiversity Decline for Food Security, Economy, and Pollinator Conservation Policies**. *E3S Web Conf* 2021, **259**:01006.

69. Murphy JT, Breeze TD, Willcox B, Kavanagh S, Stout JC: **Globalisation and pollinators: Pollinator declines are an economic threat to global food systems**. *People and Nature* 2022, **4**:773–785.

70. Simons M, Tibbetts E: **Insects as models for studying the evolution of animal cognition**. *Current Opinion in Insect Science* 2019, **34**:117–122.

71. Bridges AD, MaBouDi H, Procenko O, Lockwood C, Mohammed Y, Kowalewska A, González JER, Woodgate JL, Chittka L: **Bumblebees acquire alternative puzzle-box solutions via social learning**. *PLOS Biology* 2023, **21**:e3002019.

72. Bridges AD, Royka A, Wilson T, Lockwood C, Richter J, Juusola M, Chittka L: **Bumblebees socially learn behaviour too complex to innovate alone**. *Nature* 2024,

**627**:572–578.

73. Galpayage Dona HS, Solvi C, Kowalewska A, Mäkelä K, MaBouDi H, Chittka L: **Do bumble bees play?** *Animal Behaviour* 2022, **194**:239–251.

74. Gatto E, Guan C, Christian A, Cutini S, Chittka L, Petrazzini MEM: **An insect's view of a numerosity illusion: a simple strategy may explain complex numerical performance in bumblebees**. 2023, doi:10.1101/2023.08.22.554303.

75. Louâpre P, Alphen JJM van, Pierre J-S: **Humans and Insects Decide in Similar Ways**. *PLOS ONE* 2010, **5**:e14251.

76. Pandey UB, Nichols CD: **Human disease models in Drosophila melanogaster and the role of the fly in therapeutic drug discovery**. *Pharmacol Rev* 2011, **63**:411–436.

77. Bier E, Mcginnis W: **Model Organisms in the Study of Development and Disease**. In *Epstein's Inborn Errors of Development: The Molecular Basis of Clinical Disorders of Morphogenesis*. Edited by Erickson RP, Wynshaw-Boris AJ. Oxford University Press; 2016:0.

78. Schlegel A, Stainier DYR: **Lessons from "Lower" Organisms: What Worms, Flies, and Zebrafish Can Teach Us about Human Energy Metabolism**. *PLOS Genetics* 2007, **3**:e199.