J Insect Behav (2014) 27:527–539
DOI 10.1007/s10905-014-9447-3

Impact of Food Availability, Pathogen Exposure, and Genetic Diversity on Thermoregulation in Honey Bees (*Apis mellifera*)

M. Simone-Finstrom · B. Foo · D. R. Tarpy · P. T. Starks

Revised: 27 February 2014 / Accepted: 4 March 2014 /
Published online: 20 March 2014

Abstract Accurate thermoregulation in honey bees is crucial for colony survival. Multiple factors influence how colonies manage in-hive temperature, including genetic diversity. We explored the influence of genetic diversity on thermoregulatory behavior under three conditions: natural foraging, supplemental feeding, and exposure to the fungal pathogen shown to induce a social fever in honey bees. Our data suggest that (1) the degree of genetic diversity expected under normal conditions is not predictive of thermoregulatory stability, (2) the social fever response of honey bees is not a simple stimulus–response mechanism but appears to be influenced by ambient temperature conditions, and (3) a temperature-based circadian rhythm emerges under high nectar flow conditions. Taken together, these data suggest that a richer, context-dependent thermoregulatory system exists in honey bees than previously understood.

Keywords *Ascosphaera apis* · behavioral plasticity

Introduction

Among the social insects, honey bees (*Apis mellifera*) are particularly adept at accurate thermoregulation of the nest environment. Maintaining a consistent hive temperature is

M. Simone-Finstrom · D. R. Tarpy
Department of Entomology, North Carolina State University, Raleigh, NC 27695-7613, USA

M. Simone-Finstrom · D. R. Tarpy
W. M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, NC 27695-7613, USA

B. Foo · P. T. Starks (✉)
Department of Biology, Tufts University, 310 Dana Labs, 163 Packard Avenue, Medford, MA 02155, USA
e-mail: philip.starks@tufts.edu

crucial to the health and survival of the colony, particularly with respect to developing larvae and pupae. Although brood rearing can potentially occur at ambient temperatures ranging from 0 to 40 °C, the temperature range within the brood comb must be maintained between 32 and 36 °C for proper brood development (Seeley 1985; Southwick and Heldmaier 1987). Rearing under suboptimal temperatures can cause workers to exhibit shrivelled wings, brain damage, behavioral abnormalities, and other developmental problems (Tautz et al. 2003; Groh et al. 2004; Basile 2009). Colonies must also be able to survive more extreme temperatures during winter and summer in certain climatic zones (e.g. Southwick 1987; Southwick and Heldmaier 1987). Given these factors, thermoregulation in honey bees has likely experienced significant selection pressure (Jones and Oldroyd 2007). However, there is little information concerning the factors that influence the stability of temperature regulation within a honey bee colony.

Adult worker bees in a hive can perform several behaviors that either increase or decrease the temperature of the nest cavity (reviewed in Stabentheiner et al. 2010). Individuals will cluster or station themselves on brood comb, where they generate heat via shivering if nest temperature is too low. If the in-hive temperature gets too hot, workers can fan hot air out of the nest (Gates 1914) and use evaporative processes by spreading collected water on comb surfaces. One previous study has suggested that highly genetically diverse colonies maintain stable hive temperatures more effectively than colonies with minimal diversity, as indicated by reduced variance in mean brood temperature (Jones et al. 2004).

High levels of genetic diversity within honey bee colonies is the result of hyperpolyandry, extreme multiple mating of the queen. On average, honey bee queens naturally mate with 12 drones, but the range of matings is high (from 4 to 30; Tarpy and Nielsen 2002; Kraus et al. 2004; Tarpy et al. 2004, 2010), which results in workers from as many patrilines (or subfamilies) within a colony. The evolution of hyperpolyandry in honey bees likely has several non-mutually exclusive explanations (reviewed by Palmer and Oldroyd 2000; Smith et al. 2008), including increased diversity of behavioural repertoires enabling colonies to better exploit resources (Robinson and Page 1989) and reducing the impact of diploid male production on colony success (Page 1980). Genetic diversity can influence task allocation among hive workers, which can enhance behavioral traits essential to the growth of a colony (Page et al. 1989, 1995; Fuchs and Moritz 1998). For example, colonies headed by multiple-mated queens have been shown to exhibit greater food storage, comb construction, and population growth than colonies headed by a singly-mated queen (Mattila and Seeley 2007).

In addition, Sherman and colleagues (1998) hypothesized that colony-level genetic diversity might also reduce disease susceptibility, and various subsequent experimental studies have provided support for this hypothesis. For example, Tarpy (2003) inoculated colonies with the causitive fungal agent of the larval disease Chalkbrood (*Ascosphaera apis*) and determined that colonies headed by single-drone inseminated queens had increased levels of variance in infection compared to those headed by multiple-drone inseminated queens. Similar results were found after controlled infections with *Paenibacillus larvae*, the causitive agent of American foulbrood (Seeley and Tarpy 2007). While the mechanisms for these effects are not yet fully elucidated (see Wilson-Rich et al. 2012), it is clear that genetic diversity can help promote hive homeostasis (Oldroyd and Fewell 2007), at least when comparing colonies headed by single- versus multiple-mated queens. The threshold mating frequency to achieve these

potential benefits is currently unknown, although recent evidence indicates that a mating number above 7 males correlates with increased colony survivorship (Tarpy et al. 2013).

While genetic diversity can be viewed as one mechanism of colony-level disease resistance, other mechanisms of social immunity can effectively and directly reduce pathogen and parasite loads (e.g. hygienic behavior, grooming and use of antimicrobial plant resins; reviewed in Wilson-Rich et al. 2009; Evans and Spivak 2010). In fact, thermoregulatory behavior can also play a role in disease resistance. Honey bees mount a behavioral "social fever" against at least one heat-sensitive pathogen (Starks et al. 2000). Specifically, after exposure to *A. apis* fungal spores that cause Chalkbrood, honey bee colonies elevate temperature of the brood comb. *A. apis* spores are ingested by larvae and germinate in the larval gut, showing optimal growth between 33 and 36 °C (reviewed in Aronstein and Murray 2010). Chalkbrood propagates most effectively in larvae that have experienced slight chilling down to 30–32 °C (Bailey 1981; Bailey and Ball 1991; Flores et al. 1996; Vojvodic et al. 2011), and so a small increase in brood temperature within the optimal development range may deter successful germination of the fungus. It remains unclear how genetic diversity, which can promote efficient hive temperature regulation (Jones et al. 2004), may influence a colony's ability to display a fever response.

The aim of this study was to determine the influence of un-manipulated levels of genetic diversity of the thermoregulatory behavior of honey bee colonies under different environmental conditions. To accomplish this, we measured the variance in mean temperature in colonies during periods of natural foraging, high nectar flow, and exposure to the fungal pathogen *A. apis* (Chalkbrood), and then subsequently estimated the natural intracolony genetic diversity through genotyping of nestmates.

Material and Methods

Colony Setup and Experimental Design

In June 2010, at Tufts University, seven two-frame observation hives (inner dimensions: 53×48×5 cm) were each established with ~1,600 honey bees (*Apis mellifera ligustica*) and a newly introduced queen. The upper frame of each observation hive was separated from the lower frame with a queen excluder to restrict brood development to the lower comb (for description of these observation hives see Starks et al. 2005; Siegel et al. 2005). Hives were installed in an enclosure maintained at ~28 °C (but see below) and were allowed to forage naturally throughout the experiment.

The in-hive temperature of each colony was measured using probes (sensitive to 0.1 °C) and recorded with an Omega OMB ChartScan-1400 Portable Data Recorder. Ten Thermistor probes were placed within each colony. Eight of the probes were placed in the lower frame of the observation hives, and two of the probes were positioned in the upper frame. The eight probes in the lower frame were separated into two categories ('outer' and 'inner' brood comb). Inner probes were centralized in the brood area, whereas outer probes were placed on the edges of the brood frame. An additional temperature probe was placed outside each of the observation hives to record the ambient temperature.

Temperatures were monitored for a total of 19 days approximately 6 weeks after colony setup to ensure that all workers were the offspring of introduced queens. After

an initial 3 days of recording in-hive temperatures under natural conditions (no-feed period), each colony's diet was supplemented with 300 mL 1:1 sucrose feed daily for 5 days (supplemental feed period).

Chalkbrood Exposure and Behavioral Fever

To examine the effect of genetic diversity on a colony's ability to mount a fever response, we exposed our honey bee colonies to *Ascosphaera apis*, the causative fungal agent of the larval disease Chalkbrood. Mycelia from a pure culture of *A. apis* (USDA strain: A0015) were grown on a Potato Dextrose Agar + 0.4 % yeast extract plates at room temperature, and spores developed after 3–4 days. Spores from the *A. apis* cultures were isolated, and the fungus was identified morphologically at 40× and 100× magnification using reference images from Chorbinski and Rypula (2003). The isolated spores were suspended in sterile water, and concentrations of spores were estimated using a hemocytometer.

Following the no-feed and supplemental-feed periods, five hives were given approximately 4.2×10^5 spores mixed with 100 ml of 1:1 sucrose syrup feed each day (total: 1.26×10^6 spores/hive) for 3 days. We chose to inoculate five hives and leave two as control colonies to maximize the ability to detect a fever response, given constraints on the maximum sample size and the fact that we expected based on previous research that more variance may exist among the treated hives. Flores and colleagues (2004) showed that a concentration of 1.25×10^6 spores/hive delivered in sucrose water induced a ~60 % mummification of susceptible brood. Two control hives received the same volume of 1:1 sucrose feed during this 3-day period, but without *A. apis* spores added to their feed. All colonies were given supplemental feed (300 ml 1:1 sucrose syrup) daily for 5 days post-inoculation. After this post-inoculation feeding period, in-hive temperatures were monitored for another 3 days with no supplemental feeding.

Analysis of Genetic Diversity

Returning foraging workers were collected after closing the hive entrances of each colony. Individual bees were stored at −20 °C until DNA extraction. DNA was extracted from the abdomen tissue of each specimen using a standard Chelex 100® DNA extraction method (Walsh et al. 1991). Each sample was crushed using minipestles and incubated for 10–15 min at 95 °C in 250 μl of 5 % Chelex 100® solution. Three cycles of incubation were performed. After each period of incubation, the samples were vortexed for 30 s. After the last incubation period, the samples were centrifuged at 13,000 rpm for 5 min. The supernatant of each sample was collected and diluted at a 1:1 ratio with ddH_2O.

The number of subfamilies—and therefore, the mating frequency of each queen—was determined using microsatellite alleles following previously established methods (c.f., Tarpy et al. 2010). Briefly, a genotypic paternity analysis was performed for all samples using two separate multiplex PCR reactions (Plex 1 and Plex 2) in 10 μl total volumes with approximately 100 ng bee DNA, 1x PCR buffer (Takara® without Mg++), 1 mg/ml BSA,1.5 U Taq polymerase, 300 μM dNTP's, and either 1.5 mM (Plex 1) or 1.1 mM (Plex 2) $MgCl_2$. Primer sets in Plex 1, using the unified nomenclature of Solignac et al. (2003), included 2.0–2.5 pM Am061, Am052, Am010, and Am553,

whereas those in Plex 2 included 2.5–3.5 pM of Am043, Am098, Am125, and Am059 (see Delaney et al. 2011).

The reactions were performed using Thermo® Px2 thermocyclers with a 7 min initial denaturation at 95 °C, followed by 30 cycles of 30 s at 95 °C, 30 s at an annealing temperature of 55 °C (Plex 1) or 54 °C (Plex 2), and 30 s at 72 °C, then a final 10 min extension at 72 °C. The PCR products were run on an ABI 3730® DNA Analyzer at the Genomic Sciences Laboratory of North Carolina State University, USA, and the data were analyzed using Genemapper 4.0 (ABI). Loci with poor amplification were excluded from certain colonies, and the data were then analyzed using Colony 1.2 (Wang 2004) to estimate the number of subfamilies in each colony (and therefore the mating frequency of each queen) following Nielsen and colleagues (2003).

Statistical Analysis

Our honey bee facility was maintained at 28 °C for the majority of the project. However, because of a temporary extreme external temperature drop during the inoculation period, the temperature of the field enclosure briefly dropped from 28 to 17 °C. As such, for purposes of data management and analysis, we controlled for this external temperature difference by separating the inoculation period into two periods; period one included the first 26 h of the 'inoculation' period and period two included the remainder of the 'inoculation' period. All statistics were calculated using SPSS for windows (v. 17).

Thermoregulatory Stability and Genetic Diversity Hourly temperature of the inner and outer brood comb were calculated from four probes each, and the variance around the mean hourly temperature was compared against effective paternity frequency. We divided the brood comb into inner and outer areas because the outer brood comb area is most sensitive to temperature change and therefore is a good indicator of thermoregulatory stability (Jones et al. 2004). Given our small colony sample size ($n=7$), we used a Spearman's rho non-parametric correlation test to evaluate the relationship between variance and patriline number. Wilcoxon signed-rank tests were used to determine differences within colonies in hourly variance between inner and outer brood comb measurements.

Supplemental Feeding and Thermoregulation To compare the effect of high nectar flow, we compared the brood-temperature distribution between the no-feed and feed period using the Kolmogorov-Smirov (KS) test. To determine whether there was a change in the colony temperature circadian rhythm, we compared the temperature trend of the no-feed and feed period graphically. We also compared inner brood temperature distribution between the post-inoculation feed and post inoculation no-feed period using the KS test to determine if the temperature regulation would return to the no-feed base line after the colonies were deprived of their supplemental sugar feed.

Pathogen Exposure To examine the response of honey bees to pathogen exposure under conditions of relatively higher, constant ambient temperature, we examined the temperature distributions across all hives inoculated with *A. apis* spores. One sample KS test of normality revealed that temperature distribution across all hives was not normally distributed, therefore we compared the mean hourly temperature of the inner

brood comb from each treatment period (no-feed, feed, inoculation [period 1 and period 2], post-inoculation feed, post-inoculation no-feed) against each other using Kruskal-Wallis H tests, followed by non-parametric post-hoc pair-wise comparisons.

Results

Thermoregulatory Stability and Genetic Diversity

Across our 7 colonies, we found a range of 20–37 matings by queens (Mean ± SD: 27.14±6.31), with the effective paternity frequency within colonies ranging from 8.72 to 37.75 (Table 1). The mean temperature of the brood area across the 4-day unfed period ranged from 34.61 to 35.19 °C (Mean ± SD: 34.89 °C±0.19 °C). We found no significant correlation between genetic diversity and thermoregulatory stability in the inner or outer brood comb (inner: $r=-0.357$, $p=0.432$; outer: $r=0.36$, $P=0.531$; Fig. 1). Variance of the inner comb across all hives was significantly lower than the variance of the outer brood comb in 5 of 7 hives (Wilcoxon signed-rank test: $S\leq 0.01$ for colonies B, D, E, F, G and $S>0.2$ for colonies A and C).

Supplemental Feed and Thermoregulation

For the brood comb, KS comparison between the no-feed and feed periods, and between the post-inoculation feed and post-inoculation no-feed periods, revealed a significant change in the thermoregulation patterns of our colonies ($p<0.0001$ for all). Variance of the inner- and outer-brood comb increased significantly across all 7 hives between the no-feed and feed periods (Wilcoxon signed-rank test; inner: $Z=-2.366$, $S=0.018$; outer: $Z=-2.371$, $S=0.018$). Graphical comparison of temperature regulation patterns showed the emergence of ~24 h cyclic patterns across all 7 hives when fed (Fig. 2).

Table 1 Data from the genetic analysis

Colony	Treatment	Number of loci used	Sample size	Mating number (N_o)	Effective paternity frequency (M_e)	95 % C.I. of M_e
A	*A. apis*	6	86	28	19.34	4.50
B	*A. apis*	6	92	27	14.05	3.99
C	*A. apis*	8	85	33	10.06	5.84
D	Control	5	82	37	37.75	7.19
E	Control	7	85	25	10.23	3.83
F	*A. apis*	5	84	20	14.06	2.76
G	*A. apis*	6	82	20	8.72	2.83

Colonies are presented individually, data on treatment (pathogen exposure), on the loci used in the analysis, and on sample sizes are presented. Results indicate both the observed mating number (N_o) and effective paternity (M_e) of the experimental colonies

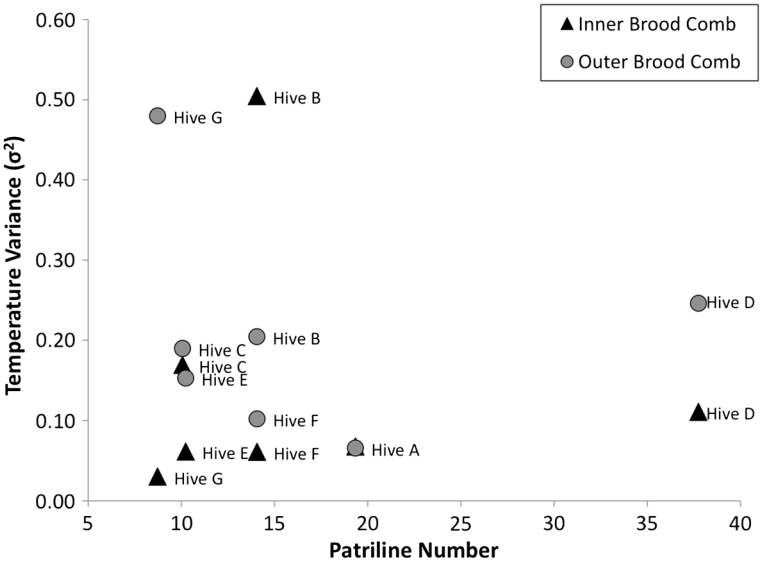

Fig. 1 The relationship between effective paternity frequency and temperature variance for both the inner and outer sections of the brood comb during the 3 day pre-feed period. No clear pattern was seen between genetic diversity, as determined by effective matting number, and thermoregulatory stability for either inner or outer brood comb measurements

Pathogen Exposure and Temperature Response

Kruskal-Wallis H comparison of the mean temperature during different treatment periods revealed a significant difference across all hives. Post-hoc pair wise comparison using Tamhane's test revealed that there was a significant difference between the no-feed and feed period across all hives (all $p<0.0001$; Table 2). However, mean temperature from the feed and the first 26th hours of the inoculation period across all hives were not significantly different (all $p\geq0.062$; Table 2).

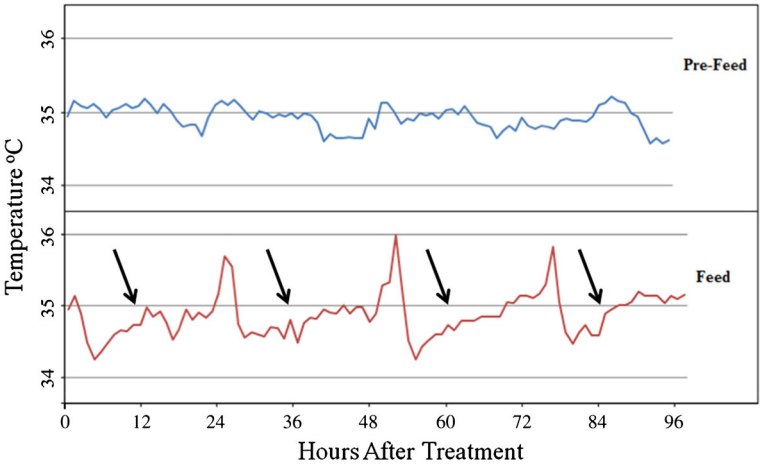

Fig. 2 Comparison of thermoregulation pattern during the pre-feed and feed period in one representative hive. *Arrows* indicate time when feed was provided. Approximately 12 h post-feeding a temperature peak was seen, indicating a circadian rhythm in thermoregulatory behavior based on feeding cycles

Table 2 Tamhane's post-hoc pair wise comparison, period of comparison and *p* values are shown in the table

Hive	Period of comparison	*P* value
A Inner brood comb	No feed and feed	$P<0.0001$
	Feed and inoculation	$P=0.941$
B Inner brood comb	No feed and feed	$P<0.0001$
	Feed and inoculation	$P=0.832$
C Inner brood comb	No feed and feed	$P<0.0001$
	Feed and inoculation	$P=0.110$
D Inner brood comb	No feed and feed	$P<0.0001$
	Feed and sham (control)	$P=0.999$
E Inner brood comb	No feed and feed	$P<0.0001$
	Feed and sham (control)	$P=0.788$
F Inner brood comb	No feed and feed	$P<0.0001$
	Feed and inoculation	$P=0.440$
G Inner brood comb	No feed and feed	$P<0.0001$
	Feed and inoculation	$P=0.062$

Temperatures changed significantly in response to feeding, but were not influenced by fungal spores or between two feeding periods as seen in the control colonies (D and E)

Discussion

Our data, collected on naturally mated queens, suggest that the degree of genetic diversity expected under normal conditions is not predictive of thermoregulatory stability, that the social fever response of honey bees is not a simple stimulus–response mechanism but is rather influenced by ambient temperature conditions, and that a temperature-based circadian rhythm emerges under high nectar flow conditions. Taken together, these data suggest a rich, context-dependent thermoregulatory system exists in honey bees.

The ability to maintain stable temperature within the hive is an important colony-level behavior because consistent brood temperature is essential for proper development of honey bee larvae and pupae. Since thousands of honey bees all function within a single colony unit, large groups of workers together regulate hive temperature (Stabentheiner et al. 2010). As with other tasks, the ability for an individual to be directly involved in thermoregulation is likely related to the worker's sensitivity to task stimulus (Graham et al. 2006). Given that task threshold sensitivity is often patriline/subfamily specific (i.e., there is a genetic component to the trait), we predicted that genetically diverse colonies (with multiple patrilines) would show less variance in hive temperature (Jones et al. 2004; Graham et al. 2006).

Jones and colleagues (2004) were able to show a difference in thermoregulation ability of genetically diverse versus genetically uniform colonies. However, genetically uniform colonies (one patriline) are extremely unusual (although not absent) in nature; queens do not typically mate with fewer than 8 males (Tarpy and Nielsen 2002; Wilson-Rich et al. 2012), and mating frequencies in the current study ranged from 20 to 37. At these natural levels of polyandry, there seems to be no difference in a colony's ability to maintain stable in-hive temperatures. It has been suggested that the fitness

benefits to multiple mating asymptote around 10 mates (Rueppell et al. 2008). Here, we provide some empirical evidence that the additional adaptive benefits are fairly negligible above 8 effective matings, at least in regard to thermoregulatory abilities, which corresponds to the finding that colony over-wintering survivorship correlates with effective matings higher than 7 (Tarpy et al. 2013).

Given the need for stability of in-hive temperature, it was somewhat surprising to find that honey bee colonies experience a food-based circadian rhythm. During periods of food supplementation, colony-level circadian rhythms adjusted to our feeding protocol. Hive temperature typically peaked after sucrose feeding and reached the nadir ~12 h after the feed (Fig. 2). Graphical comparison of the thermoregulation pattern between the no-feed and feed periods show a drastic difference in temperature patterns (Fig. 2).

Many organisms have evolved circadian clocks that are sensitive to external time cues derived from the daily rotation of the earth. By synchronizing physiological functions to specific changes in light and temperature, organisms can anticipate environmental change such as seasonal transitions. The ability to predict change and response appropriately can thus increase the likelihood of survival (Edery 2000). Of course, not all clocks are set to light or temperature, as some cues relate to resource availability. Fuller and colleagues (2008) observed that a "food-related clock" can take precedence over the "light-based" circadian pattern. It appears that a "food-related clock" can help animals switch their sleep and wake cycles to maximize access to food-related resources. Indeed, there are a few studies documenting circadian pattern of O_2 consumption in wintering bees and diurnal rhythms of metabolic rate and locomotor activity in active honey bee colonies. These circadian rhythms seem to be associated largely with day and night cycles (Southwick 1982; Kronenberg and Heller 1982), although individuals can be entrained to temperature (Moore and Rankin 1993) and foragers can show behavioral rhythmicity to food resources (e.g., Frisch and Aschoff 1987; reviewed in Moore 2001). However, to date, there has been no previous indication of circadian rhythms operating at the colony level (Moore 2001), as it appears that younger bees are arrhythmic due to constant brood maintenance and foragers generally follow their own individual patterns of behavior. Even with circadian "sleep" rhythms it appears that different worker behavioral castes follow different patterns (Klein et al. 2008; Eban-Rothschild and Bloch 2012). Furthermore a typical entrainment pattern to food sources results in increased activity (and perhaps temperature) before the feeding period so that it peaks at the onset of feeding (e.g., Frisch and Aschoff 1987). However, we saw that temperature peaked 12 h after feeding, which indicates that another mechanism is likely involved.

The mechanism by which this food-based colony-level circadian rhythm is regulated remains unclear. Given that honey bees follow other metabolic models seen in eukaryotic animals and the fitness benefits derived from a circadian rhythm that can be used to predict food access, it should not be surprising that honey bees can utilize a food-related circadian rhythm (Hou et al. 2010). Alternatively, it is possible that the increase in hive temperature is simply due to an increase in metabolic activity following food consumption or storage. Similarly bees that receive nectar are more likely to engage in cell-heating behavior after ingestion (Basile et al. 2008) and this could cause an increase in comb temperature (e.g., Kleinhenz et al. 2003; Humphrey and Dykes 2008). Although we would predict this response to occur more quickly since these bees often immediately partake in cell heating. Furthermore, since a relatively small proportion (~20 %) of bees

are likely involved in receiving the supplemental feed (Seeley 1995), if it follows typical food transfer behavior, this explanation cannot fully explain the change in temperature.

An additional alternative explanation is that the greater intake of resources stimulates comb production and that the temperature peak could theoretically be related to wax production. This too is an unlikely explanation, as previous studies have shown that after receiving sugar syrup, colonies take ~2 days to start to increase comb construction (Pratt 2004), which is greater than the first cycle that we show here. Future studies will investigate the robustness of this rhythm as well as the mechanisms to explain these results.

Previous research has shown that honey bees can manipulate hive temperature in response to the presence of particular pathogens. Exposure to the fungal pathogen that causes chalkbrood (*Ascosphaera apis*) can trigger a behavioral "social fever" (Starks et al. 2000). Starks and colleagues (2000) showed that colonies will mount a preventive fever response to prevent larvae from chilling and developing symptoms, suggesting that the presence of spores could cause the initiation of the fever response. While it is possible that we neglect to show a fever response here due to it being masked by the rhythmical increase in temperature due to feeding, this would have also presumably existed in the previous study (Starks et al. 2000) that utilized the same inoculation methods. In contrast to the present study, where ambient temperature was largely maintained at 28 °C, Starks and colleagues (2000) tested the fever response at an ambient temperature range from 12 to 35 °C. Given that our colonies failed to show any significant changes in thermoregulation patterns after exposure to *A. apis* spores, we speculate that in addition to spore recognition there is a temperature threshold above which the behavioral fever response is not necessary. This finding suggests that the presence of spores alone may not trigger the initiation of the behavior. It is possible that individuals involved in the fever response are able to detect and respond to infected (but non-pathological) larvae, as appears to be the case in honey bees performing hygienic behavior (Swanson et al. 2009).

Given that we did not detect a fever response, it is possible that the levels of genetic diversity seen in this experiment do not further influence behavioral fever. These results, however, should be tested at decreased ambient temperatures and with lower levels of intracolony diversity. Such a test is needed to reconcile the results of Wilson-Rich and colleagues (2012), which suggest that colony-level physiological immunity is not impacted by the natural range of colony-level genetic diversity, and Tarpy (2003) who showed that highly genetically diverse colonies are on average better able to fend off chalkbrood infection than are genetically poor colonies. As the results of Tarpy (2003) likely reflect differences in behavioral immunity rather than physiogical immunity, exploring the fever response of hives displaying a greater range of genetic diversity is essential. It is still hypothesized that at extremely low levels of genetic diversity, colonies would be less likely to effectively mount a behavioral fever at lower ambient temperatures.

While hyperpolyandry is likely to have effects on multiple aspects of individual and colony fitness, it appears that there is a threshold above which the benefits are negligible. We have provided empirical evidence that the level of genetic diversity seen in typical honey bee colonies is sufficient for proper in-hive temperature stability and that higher levels of genetic diversity appear to have little effect on this trait. Surprisingly, we determined that honey bee colonies experience food-related circadian rhythms with respect to thermoregulation. Previously, it was not thought that circadian

rhythms operate at the colony-level, but were restricted to individual regulation. Finally, our data suggest a thermal limit above which behavioral fever is not displayed, further highlighting the context-dependent richness of honey bee thermoregulation.

Acknowledgments We would like to thank Joel Caren for assisting with the genotyping of the colonies. This study was supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number 2007-02281, and a National Science Foundation REU site award to Tufts University (DBI-0649190).

References

Aronstein KA, Murray KD (2010) Chalkbrood disease in honey bees. J Invertebr Pathol 103:S20–S29
Bailey L (1981) Honey bee pathology. Academic, London
Bailey L, Ball BV (1991) Honey bee pathology. Academic, London
Basile R (2009) Thermoregulation and resource management in the honeybee (Apis mellifera). Ph.D. Dissertation. University of Wurzburg, Germany
Basile R, Pirk CWW, Tautz J (2008) Trophallactic activities in the honeybee brood nest–heaters get supplied with high performance fuel. Zoology 111:433–441
Chorbinski P, Rypula K (2003) Studies on the morphology of strains Ascosphaera apis isolated from chalkbrood disease of the honey bees. Vet Med 6:1–12
Delaney DA, Keller JJ, Caren JR, Tarpy DR (2011) The physical, insemination, and reproductive quality of honey bee queens (Apis mellifera). Apidologie 42:1–13
Eban-Rothschild A, Bloch G (2012) Circadian rhythms and sleep in honey bees. In: Galizia GC, Eisenhardt D, Giurfa M (eds). Honeybee neurobiology and behavior: a tribute to Randolf Menzel. Springer, Dordrecht, The Netherlands, pp 31–46
Edery I (2000) Circadian rhythms in a nutshell. Physiol Genomics 3:59–74
Evans JD, Spivak M (2010) Socialized medicine: individual and communal disease barriers in honey bees. J Invertebr Pathol 103:S62–S72
Flores JM, Ruiz JA, Ruz JM, Puerta F, Bustos M, Padilla F, Campano F (1996) Effect of temperature and humidity of sealed brood on chalkbrood development under controlled conditions. Apidologie 27:185–192
Flores JM, Gutierrez I, Puerta F (2004) A comparison of methods to experimentally induce chalk brood disease in honey bees. Span J Agric Res 2:79–83
Frisch B, Aschoff J (1987) Circadian rhythms in honeybees: entrainment by feeding cycles. Physiol Entomol 12:41–49
Fuchs S, Moritz RFA (1998) Evolution of extreme polyandry in the honeybee Apis mellifera L. Behav Ecol Sociobiol 9:269–275
Fuller P, Lu J, Saper C (2008) Differential rescue of light- and food- entrainable circadian rhythms. Science 320:1074–1077
Gates B (1914) The temperature of the bee colony. Bull US Dep Agric 96:1–29
Graham S, Myerscough MR, Jones JC, Oldroyd BP (2006) Modelling the role of intracolonial genetic diversity on regulation of brood temperature in honey bee (Apis mellifera L.) colonies. Insect Soc 53: 226–232
Groh C, Tautz J, Rossler W (2004) Synaptic organization in the adult honey bee brain is influenced by brood-temperature control during pupal development. Proc Natl Acad Sci 101:4268–4273
Hou C, Kaspari M, Vander Zanden H, Gillooly J (2010) Energetic basis of colonial living in social insects. Biol Lett 107:3634–3638
Humphrey JAC, Dykes ES (2008) Thermal energy conduction in a honey bee comb due to cell-heating bees. J Theor Biol 250:194–208
Jones J, Oldroyd B (2007) Nest thermoregulation in social insects. Adv Insect Physiol 33:153–191
Jones J, Myerscough M, Graham S, Oldroyd B (2004) Honey bee nest thermoregulation: diversity promotes stability. Science 305:402–404
Klein BA, Olzsowy KM, Klein A, Saunders KM, Seeley TD (2008) Caste-dependent sleep of worker honey bees. J Exp Biol 211:3028–3040
Kleinhenz M, Bujok B, Fuchs S, Tautz J (2003) Hot bees in empty broodnest cells: heating from within. J Exp Biol 206(23):4217–4231

Kraus FB, Neumann P, van Praagh J, Moritz RFA (2004) Sperm limitation and the evolution of polyandry in the honeybee (Apis mellifera L.). Behav Ecol Sociobiol 55:494–501
Kronenberg F, Heller C (1982) Colonial thermoregulation in honey bees (Apis mellifera). J Comp Physiol B 148:65–76
Mattila H, Seeley T (2007) Genetic diversity in honey bee colonies enhances productivity. Science 317:362–364
Moore D (2001) Honey bee circadian clocks: behavioral control from individual workers to whole-colony rhythms. J Insect Physiol 8:843–857
Moore D, Rankin MA (1993) Light and temperature entrainment of a locomotor rhythm in honeybees. Physiol Entomol 18:271–278
Nielsen R, Tarpy DR, Reeve HK (2003) Estimating effective paternity number in social insects and the effective number of alleles in a population. Mol Ecol 12:3157–3164
Oldroyd BP, Fewell JH (2007) Genetic diversity promotes homeostasis in insect colonies. Trends Ecol Evol 22:408–413
Page R (1980) The evolution of multiple mating behavior by honey bee queens (Apis Mellifera L.). Genetics 96:263–273
Page RE, Robinson GE, Calderone NE, Rothenbuhler WC (1989) Genetic structure, division of labor, and the evolution of insect societies. In: Breed MD, Page RE (eds) The genetics of social evolution. Westview, Boulder, pp 15–30
Page RE, Robinson GE, Fondrk MK, Nasr ME (1995) Effects of worker genotypic diversity on honey bee colony development and behavior (Apis mellifera). Behav Ecol Sociobiol 36:387–396
Palmer K, Oldroyd B (2000) Evolution of multiple mating in the genus Apis. Apidologie 31:235–248
Pratt SC (2004) Collective control of the timing and type of comb construction by honey bees (Apis mellifera). Apidologie 35:193–205
Robinson G, Page R (1989) Genetic basis for division of labour in an insect society. In: The genetics of social evolution. Westview Press, Boulder, pp 61–88
Rueppell O, Johnson N, Rychtar J (2008) Variance-based selection may explain general mating patterns in social insects. Biol Lett 4:270–273
Seeley TD (1985) Honeybee ecology: a study of adaptation in social life. Princeton Univ. Press, Princeton
Seeley TD (1995) The wisdom of the hive. Harvard University Press, Cambridge
Seeley TD, Tarpy DR (2007) Queen promiscuity lowers disease within honeybee colonies. Proc R Soc 274:67–72
Sherman PW, Seeley TD, Reeve HK (1998) Parasites, pathogens, and polyandry in social hymenoptera. Am Nat 131:602–610
Siegel AJ, Hui J, Johnson RN, Starks PT (2005) Mobile insulator units: honey bee workers as living insulation. Insect Soc 52:242–246
Smith CR, Toth AL, Suarez AV, Robinson GE (2008) Genetic and genomic analyses of the division of labour in insect societies. Nat Rev Genet 9:735–748
Solignac M, Vautrin D, Loiseau A, Mougel F, Baudry E, Estoup A, Garnery L, Haberl M, Cornuet J-M (2003) Five hundred and fifty microsatellite markers for the study of the honeybee (*Apis mellifera* L.) genome. Molec Ecol Notes 3:307–311
Southwick E (1982) Metabolic energy of intact honey bee colonies. Comp Biochem Physiol 71:277–281
Southwick EE (1987) Cooperative metabolism in honey bees: an alternative to antifreeze and hibernation. J Therm Biol 12(2):155–158
Southwick EE, Heldmaier G (1987) Temperature control in honey bee colonies, precise social cooperation permits adaptation to temperate climates. Bioscience 37:395–399
Stabentheiner A, Kovac H, Brodschneider R (2010) Honeybee colony thermoregulation–regulatory mechanisms and contribution of individuals in dependence on age, location and thermal stress. PLoS ONE 5(1). doi:10.1371/journal.pone.0008967
Starks PT, Blackie CA, Seeley TD (2000) Fever in honeybee colonies. Naturwissenschaften 87:229–231
Starks PT, Johnson RN, Siegel AJ, Decelle MM (2005) Heat-shielding: a task for youngsters. Behav Ecol 16: 128–132
Swanson JAI, Torto B, Kells SA, Mesce KA, Tumlinson JH, Spivak M (2009) Odorants that induce hygienic behavior in honeybees: identification of volatile compounds in chalkbrood-infected honeybee larvae. J Chem Ecol 35:1108–1116
Tarpy DR (2003) Genetic diversity within honeybee colonies prevents severe infections and promotes colony growth. Proc R Soc B 270:99–103
Tarpy DR, Nielsen D (2002) Sampling error, effective paternity, and estimating the genetic structure of honey bee colonies (Hymenoptera: Apidae). Ann Entomol Soc Am 95:513–528

Tarpy DR, Nielsen R, Nielsen DI (2004) A scientific note on the revised estimates of effective paternity frequency in Apis. Insect Soc 51:203–204

Tarpy DR, Caren J, Delaney D, Sammataro D, Finley J, Loper G et al (2010) Mating frequencies of Africanized honey bees in the South Western USA. J Apic Res 49:302–310

Tarpy DR, vanEngelsdorp D, Pettis JS (2013) Genetic diversity affects colony survivorship in commercial honey bee colonies. Naturwissenschaften 100:723–728

Tautz J, Maier S, Rossler W, Brockmann A (2003) Behavioral performance in adult honey bees is influenced by the temperature experienced during their pupal development. Proc Natl Acad Sci 100:7343–7347

Vojvodic S, Jensen AB, James RR, Boomsma JJ, Eilenberg J (2011) Temperature dependent virulence of obligate and facultative fungal pathogens of honeybee brood. Vet Microbiol 149:200–205

Walsh PS, Metzger DA, Higuchi R (1991) Chelex(r) 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTech 10:506–513

Wang JL (2004) Sibship reconstruction from genetic data with typing errors. Genetics 166:1963–1979

Wilson-Rich N, Spivak M, Fefferman NH, Starks PT (2009) Genetic, individual, and group facilitation of disease resistance in insect societies. Annu Rev Entomol 54:405–423

Wilson-Rich N, Tarpy DR, Starks PT (2012) Within- and across-colony effects of hyperpolyandry on immune function and body condition in honey bees (Apis mellifera). J Insect Physiol 58:402–407